AN EVALUATION OF MACROINVERTEBRATE-BASED BIOMONITORING AND ECOTOXICOLOGICAL ASSESSMENTS OF DETERIORATING ENVIRONMENTAL WATER QUALITY IN THE SWARTKOPS RIVER, SOUTH AFRICA

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OGHENEKARO NELSON ODUME

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ABSTRACT

Freshwater resources are increasingly subject to pollution because of escalating human population growth, accompanied by urbanisation, industrialisation, and the increased demand for food. Consequently, freshwater quality, and aquatic ecosystem structure and function have been severely impaired. The Swartkops River, which drains an urbanised and industrialised catchment in the Eastern Cape of South Africa, is no exception. An integrated environmental water quality (EWQ) approach is needed to measure the impacts of deteriorating water quality on its aquatic ecosystem structure and function to sustain these vital ecosystem-attributes. In this study, an integrated EWQ approach, which included i) analysis of water physico-chemical variables; ii) macroinvertebrate-based family-level taxonomic- and traits-based community analysis; iii) Chironomidae species-level taxonomic- and traits-based community analysis; iv) Chironomidae deformity-based sub-lethal analysis; and v) experimental investigation of long-term wastewater effluent effects, using model stream ecosystems, were applied to investigate environmental water quality in the Swartkops River. One upstream reference site and three downstream sites in the Swartkops River were monitored over a period of three years (August 2009 – September 2012).

The family-level taxonomic community responses based on the South African Scoring System version 5 (SASS5) and a newly developed Swartkops multimetric index indicated very poor river health conditions for the three downstream sites, compared with the good condition of the upstream site. The Chironomidae species-level responses in the three downstream sites provided evidence of differences in biotic impairments, which were not evident with the family-level taxonomic data at these sites, thus highlighting the importance of species identification in freshwater biomonitoring.

The family-level traits-based approach (TBA) showed that macroinvertebrates with gills and lungs were more abundant at the upstream site, decreasing markedly at the downstream sites. The relative abundance of macroinvertebrates relying on aerial and tegument respiration increased at the downstream sites compared with the upstream sites. The results of the family-level TBA highlighted the inextricable link between the traits-based approach (TBA) and taxonomic identification, clearly showing that the TBA is additional to, and not an alternative to, taxonomic recognition because important traits, e.g. reproductive cannot be used at a coarse taxonomic identification. A novel chironomid species traits-based *functional*

strategies approach developed in this study, based on species combining similar sets of traits, proved sensitive in diagnosing the main abiotic water physico-chemical stressors. The functional traits responded predictably to deteriorating water quality and provided an adaptive and mechanistic basis for interpreting chironomid species occurrences at the four sampling sites, providing insight into why certain chironomid species occurred at one site but not at the other.

Chironomid deformities provided evidence of sub-lethal in-stream biological response to deteriorating water quality. A newly developed deformity-based *extended toxic score index* proved sensitive, enabling the discrimination of the sampling sites, indicating that a biomonitoring tool based on sub-lethal effects could be used to assess the effects of deteriorating water quality before it reached lethal levels.

Empirical evidence based on the taxonomic, traits and sub-lethal responses suggested that the changes in macroinvertebrate community structure were caused chiefly by the discharge of wastewater effluents into the river. This was supported by the model-stream ecosystem results indicating significant effects of effluents on the macroinvertebrate community structure, similar to the observed in-stream responses. The model stream results indicated that improved physico-chemical effluent quality compliance after 50% effluent dilution did not significantly reduce the effects of the effluent on the macroinvertebrate communities, showing that ecologically-based methods rather than physico-chemical measures alone are necessary to assess effluent quality.

Finally, the results of the multi-criteria approach were integrated to propose tools to manage environmental water quality in the Swartkops River, and the benefits of the study were highlighted in the context of biomonitoring in South Africa.

TABLE OF CONTENTS

LIST OF TABLES (SHORT TITLES)

LIST OF FIGURES (SHORT TITLES)

LIST OF APPENDICES

ABBREVIATIONS AND ACRONYMS

ANOSIM	Analysis of similarity
ANOVA	Analysis of variance
APHA	American Public Health Association
ASPT	Average score per taxon
AUSRIVAS	Australian River Assessment System
BMWP	Biological monitoring working party
BOD	Biochemical oxygen demand
CCA	Canonical correspondence analysis
CID	Community incidences of deformities
CPOM	Coarse particulate organic matter
COD	Chemical oxygen demand
DBI	Dragonfly biotic index
DCA	Detrended correspondence analysis
DDT	dichlorodiphenyltrichloroethane
DEEEP	Direct Estimation of Ecological Effects Potential
DO	Dissolved oxygen
DWA	Department of Water Affairs
DWAF	Department of Water Affairs and Forestry
ECOM	Environment community analysis
EC	Electrical conductivity
ECP	Eastern Cape Province
EPT	Ephemeroptera – Plecoptera – Trichoptera
ET	Ephemeroptera-Trichoptera
ETOC	Ephemeroptera – Trichoptera – Odonata- Coleoptera
ETSI	Extended toxic score index
EWQ	Environmental water quality

FEPA	Freshwater ecosystem priority areas
FFG	Functional feeding groups
FNS	Functional strategy
FPOM	Fine particulate organic matter
GA	General Authorisation
GSM	Gravel sand and mud
HFC	Habitat filtering concept
HSD	Honestly significant difference
HTC	Habitat template concept
IBI 12	Index of Biotic Integrity 12
IHAS	Integrated Habitat Assessment System
IQRs	Inter-quartile ranges
ISAD	Index of severity of antennal deformation
ISLD	Index of severity of ligula deformation
IWRM	Integrated water resources management
LHT	Life-history tactics
LLT	Left lateral teeth
MAR	Mean annual runoff
MM1	Multimetric index
MT	Median teeth
NCPM	National Physico-Chemical Monitoring Programme
NMDS	Non-metric multidimensional scaling
NWA	National Water Act
NWRS	National Water Resources Strategy
PAST	Paleontological statistics
PES	Present ecological states

POPs	Persistent organic pollutants
PRC	Principal response curve
RCC	River continuum concept
RDM	Resource directed measures
RHP	River Health Programme
RHTC	River-habitat template concept
RIVPACS	River Invertebrate Prediction and Classification System
RLT	Right lateral teeth
RQOs	Resource quality objectives
SASS5	South African System version 5
SDC	Source directed controls
SIC	Stone-in-current
SIMPER	Similarity percentage
SOMI	Serra dos Órgãos Multimetric Index
SOOC	Stone-out-of-current
SPEAR	Species at risk
SSD	Species sensitivity distribution
TBA	Traits-based approach
TIN	Total inorganic nitrogen
TSI	Toxic score index
TWQR	Target water quality range
UCT	University of Cape Town
VIF	Variance inflation factor
WFD	Water framework directives
WSA	Water Service Act
WSRS	Water Services Regulation Strategy
WWTW	Wastewater treatment works

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DEDICATION

This project is dedicated to all the less privileged in the society as a token that no matter the hurdles, determination and perseverance could take you above the sky.

CHAPTER 1: GENERAL INTRODUCTION AND LITERATURE REVIEW

This chapter provides the rationale for undertaking this study through a critical literature review of existing knowledge, and the need to manage the Swartkops River water quality. It begins with an introduction highlighting global water quality situations and then focuses on the Swartkops River. It provides a comprehensive review of water resource management in South Africa, and then focuses on the rationale for the approaches used in this study, which include water chemistry (river and effluent physicochemical quality), macroinvertebrate-based biomonitoring (deformity screening, taxonomic, and traits-based approaches) and ecotoxicology (experimental investigation of treated wastewater effluent effects on macroinvertebrate communities in model-stream ecosystems). The chapter concludes with the significance of the study, aim, objectives, guiding research questions and the thesis structure.

1.1 Introduction

A sustainable supply of appropriately clean and safe quality water is critically important to the survival of all living organisms, the maintenance of aquatic ecosystem integrity and to socio-economic development (Hassan *et al.,* 2005; UN-Water, 2008; 2011; 2012). However, freshwater resources are increasingly subject to pollution because of rapid human population growth, accompanied by urbanisation, industrialisation, agricultural activities and the increased demand for food (Pettigrove and Hoffmann 2003; Hassan *et al.,* 2005; UN, 2006; UN-Water, 2011). There is a growing global concern about deteriorating water quality caused by pollution from human settlements, agricultural and industrial activities. This is exacerbated by climate change, which threatens to cause major alterations to the global hydrological cycle, and current ecosystem function. Human activities and industrialisation over the past half century have elevated levels of both organic and inorganic chemicals in aquatic ecosystems (Hassan *et al.,* 2005). The toxicities and mode of biological interaction of most of these chemicals are unknown, thereby heightening uncertainty about the extent of ecological risks exposure to these chemicals pose to freshwater ecosystem sustainability. With the current estimates of three billion more people and the quadrupling of world

economy by the year 2050 (UN, 2004; 2011a), the demand for and consumption of freshwater resources would increase significantly, as would the consequent impact on freshwater ecosystem structure and function.

The increased pollution of freshwater ecosystems has negatively affected their capacity to provide clean and reliable sources of freshwater, to maintain the natural hydrological cycle and biological food webs, and to provide food and to recycle nutrients (Hassan *et al.,* 2005; UN-Water, 2011; de Groot *et al.,* 2012; Schneiders *et al.,* 2012). Consequently, the water quality and quantity needed for biodiversity, for further economic and social development, as well as natural ecosystem functioning and provision of ecosystem services has been severely compromised (Jewitt, 2002; Hassan *et al.,* 2005; de Groot *et al.,* 2012). Therefore, the achievement of sustainable freshwater resource management and conservation of biological diversity, whether at global, national or at catchment scales, requires a deep understanding of factors influencing water quality and of ecosystem changes, and the ability to quantify them reliably and accurately.

Although Africa has abundant surface freshwater resources with 17 large rivers, each with a catchment of over 100 000 km^2, and more than 160 lakes larger than 27 km^2, 75% of the continent's total surface water is concentrated in eight major river basins, with the Congo River basin alone providing 50% (PACN, 2010). The disparity in water resources availability between sub-regions means that most of Africa's population live under conditions of water scarcity (Falkenmark, 1989; Ashton, 2002; Donkor, 2003; PACN, 2010). Most countries in Africa, particularly those in the northern and southern regions, suffer from chronic or acute water scarcity occasioned by pollution, extreme temperatures, high evaporation rates, as well as by rainfall patterns that are variable, erratic and unpredictable (Ashton, 2002; Donkor, 2003). Water scarcity, coupled with pollution problems, may hamper further socio-economic development and the continent's ability to feed her growing human population.

In most parts of Africa, freshwater resources are inadequately monitored, thereby hampering the development of indicators that decision-makers and water-resource managers need to assess progress towards sustainable water resource use (Hassen *et al.,* 2005; PACN, 2010; UN-Water, 2011). Current population growth rates (estimated at about 2.5% annually for sub-Saharan Africa), changes in demography and water-use patterns place heavy pressure on already stressed freshwater resources (PACN, 2010; UN, 2011b). Inadequate water quality monitoring tools, inappropriate management policies and lack of coordination among

relevant agencies have exacerbated this situation (Oyebande, 2001; Swatuk, 2005; Lankford and Dickinson, 2007).

Pollution is a major cause of water quality problems in Africa (PACN, 2010). Over 80% of wastewater is discharged, untreated, into water bodies. In addition, many industrial and agrochemicals such as metals, pesticides, fertilizers, hormones, and pharmaceuticals partially end up in freshwater resources thereby altering water quality and ecosystem integrity (Nyenje *et al.*, 2010; UN-Water, 2011). The escalating water quality problems and the increasingly negative impacts on freshwater ecosystem function and biological diversity will be difficult to curtail unless robust and efficient water quality monitoring and management tools are urgently developed.

South Africa depends mainly on surface freshwater resources for most of its socio-economic development. The country is located in a predominantly semi-arid to arid region of Africa, with an annual rainfall of about 450 mm, and a total mean annual runoff (MAR) of approximately 50 x 10^9 m^3, including Lesotho and Swaziland (Pitman, 2011; DWA, 2013). This total mean annual runoff (MAR) amounts to only about 50% of the mean annual flow of the Zambezi River, and 3% of the Congo River (Pitman, 2011). The country also experiences a comparatively high evaporation rate, from 1100 mm to 3000 mm, which is considerably higher than the mean annual rainfall of about 450 mm. These factors indicate that South Africa has limited water resources. Industrialisation, a growing human population, and residents' legitimate expectation of an increased standard of living, have put enormous pressure on the already stressed freshwater resources to an extent that almost all large rivers in the country are already over-exploited (Smith-Adao *et al.*, 2011; DWA, 2013).

Deteriorating water quality is threatening ecosystem sustainability, the well-being of riparian communities that rely on these resources, and further economic growth (Van der Merwe-Botha, 2009). Although there are several sources of pollution, a major contributor to the deteriorating biotic and water quality is the discharge of wastewater effluents into receiving rivers and streams (Odjadjare and Okoh, 2010; Van Vuuren, 2011). Wastewater effluent discharges have led to elevated nutrient levels in many freshwater bodies, with ecological consequences such as algal blooms, and excessive growth of cyanobacteria, particularly members of the *Microcystis* spp. and *Anaboena* spp., as well as of macrophytes (de Villiers and Thiart, 2007; van Ginkel, 2011). Other ecological effects include disruption of ecological processes, fish kills, histological and morphological alterations within both fish and

invertebrate fauna, changes in faunistic composition, and general biodiversity loss (de Villiers and Thiart, 2007; Oberholster *et al.,* 2008; Odume, 2011; van Ginkel, 2011; Marchand *et al.,* 2012).

Consequently, to minimise the impact of discharged effluents on receiving freshwater bodies, in 2008, the South African Department of Water Affairs (DWA) introduced an incentive-based green-drop certification programme (to be discussed in detail later), which seeks to regulate the management and operation of all wastewater treatment works to ensure final effluent quality compliance. Although some progress has been made since the introduction of the programme (DWAF, 2009; DWA, 2011a), the water quality of most effluent-receiving rivers and streams in the country continue to deteriorate. This suggests either a widespread non-compliance of effluent quality (DWA, 2011a), or that compliance may not guarantee effluent-receiving water resource protection at the desired level. The increased deterioration of water quality caused by effluent discharges may stem from the current method of measuring effluent compliance, which considers only chemical and microbiological variables, or may suggest a lack of in-depth understanding of the link between ecological processes and the impact of discharged effluents on them. **Therefore, to ensure that discharged wastewater effluent has a minimal ecological impact on receiving water resources, it is necessary to develop ecologically-based approaches for measuring and monitoring effluent quality, and quantifying effects on in-stream biota to inform management decision.**

One such effluent-receiving river in South Africa is the Swartkops River, which is located in the Eastern Cape Province. The river is an important regional ecological asset, supporting an estuary that ranks eleventh most important in term of biodiversity in South Africa (DWAF, 1996a; Enviro-Fish Africa, 2009; 2011). The estuary is home to many bird and fish species and is considered the most important estuary in the Eastern Cape (DWAF, 1996a; Taljaard *et al.,* 1998). It also serves as an important recreational site for activities such as swimming, angling, boating and bait-digging. The upper catchment of the river supplies water for agricultural requirements and resources within its catchment are utilised for several socio-cultural purposes, including baptism by churches, spiritual rituals and harvesting medicinal plants (DWAF, 1996a; 2003). However, several urban and industrial areas drain into the river, as does wastewater effluent discharges, run-off from informal settlements, agricultural lands, surrounding road and rail networks, and industrial sites, severely influencing the water

quality (Odume, 2011). Although there are several point and diffuse sources of pollution responsible for deteriorating water quality in the Swartkops River, wastewater discharges, which constitute over 50% of the downstream river flow (i.e. below Uitenhage) during low flow periods, are the major contributors to the observed elevated nutrient levels, metal concentrations, faecal contamination, excessive algal and macrophyte growth (DWAF 1996a; Taljaard *et al.*, 1998; Binning, 1999; Bornman and Klages 2005; de Villiers and Thiart, 2007; Odume, 2011).

Therefore, this study applied an integrated environmental water quality approach, including water chemistry (river and effluent physicochemical quality), macroinvertebrate-based biomonitoring (deformity screening, taxonomic, and trait-based approaches) and ecotoxicology (systematic investigation of effluent effects on in-stream organisms in model stream ecosystems). The results of these approaches are analysed and integrated to present proposed tools for managing environmental water quality in the Swartkops River system.

The rest of this chapter provides a critical literature review of water resource management in South Africa, the National Water Act and the Water Services Act, as well as the National Water Resource Strategy, and the National Water Services Regulation Strategy. It also provides a detailed review of the triad principles of environmental water quality, starting with water chemistry, followed by macroinvertebrate-based biomonitoring, including deformity screening, taxonomic and traits-based approaches, and finally, the use of the model stream ecosystem as the ecotoxicological approach adopted in this study. The chapter concludes with the rationale and significance of the study, aim, objectives, guiding research questions and thesis structure.

1.2 Water Resource Management in South Africa

Water resource management in South Africa is anchored in the principles of equity and sustainability clearly captured in the slogan, "Some for all forever" (Palmer *et al.*, 2002; DWA, 2013). Striving towards achieving these two fundamental principles, within the context of limited water resources and a growing demand, has been the driving factor behind all water-resource policies and programmes in South Africa. The National Department of Water Affairs, as the custodian of water resources, bears the responsibility for realising these two principles. Consequently, two pieces of legislation, i.e. the National Water Act (NWA,

No. 36 of 1998) and the Water Services Act (WSA, No. 108 of 1997), were formulated to provide the legal framework for sound, equitable and sustainable water resource and related-services management in South Africa.

1.2.1 The South African National Water Act (NWA, No. 36 of 1998) and the Water Services Act (WSA, No. 108 of 1997)

Managing and protecting water resources, as well as providing water services and sanitation, are at the heart of democratic South Africa. The NWA and the WSA are the two pieces of legislation that regulate the protection of water resources and the provision of water services. The NWA recognises sustainability and equity as central guiding principles in the protection, use, development, conservation, management and control of water resources (NWA, 1998). It places a premium on basic human and environment water needs and emphasises that the use and development of water resources in all aspects, including quality, quantity and reliability of supply, must be sustainable in the long term (NWA, 1998; King and Pienaar, 2012). The NWA embraces the overarching principles of "Integrated Water Resources Management (IWRM)" by taking into account the multi-faceted social (including gender), economic, environmental issues, and the need for trans-boundary cooperation and coordination in managing South Africa's water resources. Chapter 2 of the NWA provides for a National Water Resource Strategy (NWRS), which sets out clearly defined steps, strategies, plans, guidelines and procedures to achieve the purpose of the NWA and mandate the minister to review the NWRS at intervals of not more than five years (NWA, 1998).

The WSA on the other hand, guarantees right of access to a basic water supply and sanitation, including the treatment of wastewater before discharge into receiving water bodies, treatment that ensures sufficient water and an environment that is not detrimental to human health and well-being (WSA, 1997). The WSA recognises access to water supply and sanitation as human rights. It also provides for establishing water service institutions and intermediaries, and for developing a regulatory framework for the water services sector. Consequently, the Water Services Regulation Strategy (WSRS) (DWAF, 2008a) clearly spelt out strategies and guidelines to regulate the water and sanitation services sector in achieving the overarching goals of the WSA. While the WSA made provision to deliver water-related services, including the treatment of wastewater by municipalities, the NWA provides for the protection of water resources, and the control and regulation of environmental stressors that result from providing water services.

1.2.2 The National Water Resource Strategy (NWRS) and the Water Services Regulation Strategy (WSRS)

The second National Water Resource Strategy (DWA, 2013) provides strategies and guidelines to achieve the objectives of the NWA. To achieve the interrelated objectives of equity and sustainability enshrined in the NWA, an approach for managing South African water resources was adopted that introduces measures to protect them by setting objectives for desired conditions of the resource, and imposing limits to avoid unacceptable exploitation. This approach comprises two complementary strategies: the Resource Directed Measures (RDM) and the Source Directed Controls (SDC).

Resource Directed Measures are directed at protecting and using the water resource itself sustainably, in terms of quality, quantity, overall health, ecological integrity, as well as riparian habitat (DWA, 2013). A national water resource-classification system, determination of Ecological Categories, determination of the Reserve, and setting of Resource Quality Objectives (RQOs) are the four components of the RDM. The classification system ensures long-term ecological integrity and continuing water use for socio-economic development (DWAF, 2008b). Water resources are classified into three management classes: Class I (a resource with no noticeable or with minimal human impacts); Class II (a resource slightly or moderately impacted by human activities with little deviation from natural conditions); and Class III (a resource with significant impacts resulting in serious deviation from natural conditions) (DWAF, 2008b; DWA 2010). The Ecological Categories are classified as A, B, C, D while degraded systems may be in categories E and F (Kleynhans and Louw, 2008, DWA, 2013). The Reserve provides for the quality, quantity and reliability of a supply of water required for basic human needs, and for the functioning of aquatic ecosystems (King and Pienaar, 2011; DWA, 2013). The basic human need Reserve provides for the essential needs of individuals such as water for drinking, preparing food and for personal hygiene. The ecological Reserve relates to the quality and quantity of water required to protect and maintain the aquatic ecosystems of the water resource (DWAF, 2008c; King and Pienaar, 2011). Resource Quality Objectives (RQO) provide quantitative and qualitative descriptions of the physical, biological and chemical attributes that characterise the desired level of protection of a water resource as defined by its management class (DWA, 2011b; DWA, 2013). The RQO capture the management class and the ecological needs determined in the

reserve into measurable objectives that give direction on how a water resource should be managed (DWA, 2011b; King and Pienaar, 2011).

Used in combination with RDM, the source directed controls (SDC), define, impose limits, and restrict the use of water resources to achieve the desired levels of protection. The SDC are designed primarily to control water-use activities at the source of impact through tools such as licenses, registrations, authorisations and permits (DWA, 2013). These tools usually reflect best national management practices, site-specific requirements and/or source-related requirements. Source Directed Controls are the essential links between protecting water resources and regulating their use (DWA, 2013).

On the other hand, the Water Services Regulation Strategy (WSRS) clarifies the requirements and duties placed on water-service providers and institutions to protect consumers from potential water-related detrimental services (DWAF, 2008a). The development of the strategy embraced a multi-faceted and pragmatic approach directed mostly at risk mitigation, punitive and incentive-based regulation, as well as compliance monitoring in managing water and sanitation infrastructures. The incentive-based regulation, introduced in 2008 by the Department of Water Affairs, encompassed two initiatives: the blue-drop certification programme for regulating and managing drinking water quality; and the green-drop certification programme for regulating and managing wastewater quality (DWAF, 2009).

The green-drop certification programme requires a comprehensive understanding of wastewater treatment works (WWTWs), and introduces key indicators for effectively and efficiently managing wastewater treatment works, and their effluent discharges. The programme uses nine sets of criteria for evaluating performance and recognising excellence. Thirty percent of these criteria relate to effluent quality compliance (i.e. compliance with standards as contained in general authorisation, license, or special permit); while 70% relate to operational processes, quality assurance and asset management procedures, as follows:

- Process control, maintenance and management skill: 10%;
- Wastewater quality monitoring programme: 10%;
- Wastewater sample analysis (credibility): 5%;
- Submission of wastewater quality results: 5%;
- Wastewater quality failures response management: 10%;
- Local regulation: 5%;
- Wastewater treatment facility capacity: 10%;

- Wastewater asset management: 15%

Wastewater quality compliance forms an important component of the green-drop certification programme, which seeks to minimise potential human and ecological health risks and hazards associated with effluent discharges into receiving water bodies (Adewumi and Olanrewaju, 2011). Since the introduction of the green-drop certification programme, effluent quality has improved nation-wide (DWA, 2011a). However, this has not sufficiently translated into improved in-stream water quality and resource protection of effluent-receiving rivers and streams (Oberholster *et al.,* 2008; Osode and Okoh, 2009; Marchand *et al.,* 2012). The continuing deteriorating water quality in effluent-receiving rivers may stem from non-compliance, current methods of measuring and monitoring effluent quality compliance, or effluent quality compliance may not guarantee resource protection at the desired level. It could also reflect a lack of thorough understanding of the long-term effects of effluent on the structure and function of the biotic communities that comprise receiving water bodies, or, it could simply mean that most effluent-receiving water bodies are highly urbanised, hence several other point and diffuse sources contribute significantly to their deteriorating biotic integrity and water quality. **Consequently, in addition to chemical and microbiological methods, it remains pertinent to investigate both biomonitoring and ecotoxicological approaches for evaluating long-term effluent effects on biotic communities, by developing methods that closely model in-stream natural conditions (**Scherman *et al*., 2003, Palmer *et al.,* 2004a**).**

Source directed controls tools such as licenses, general authorisation and special permits, which stipulate limits for physico-chemical and microbiological variables to monitor for compliance by water services providers, and the green-drop certification requirements of the wastewater quality component of effluent discharges form an important link between the NWA and the WSA (Figure 1.1). Figure 1.1 provides a summary of water resources management in South Africa in the context of this study and highlights key areas that are investigated in this study.

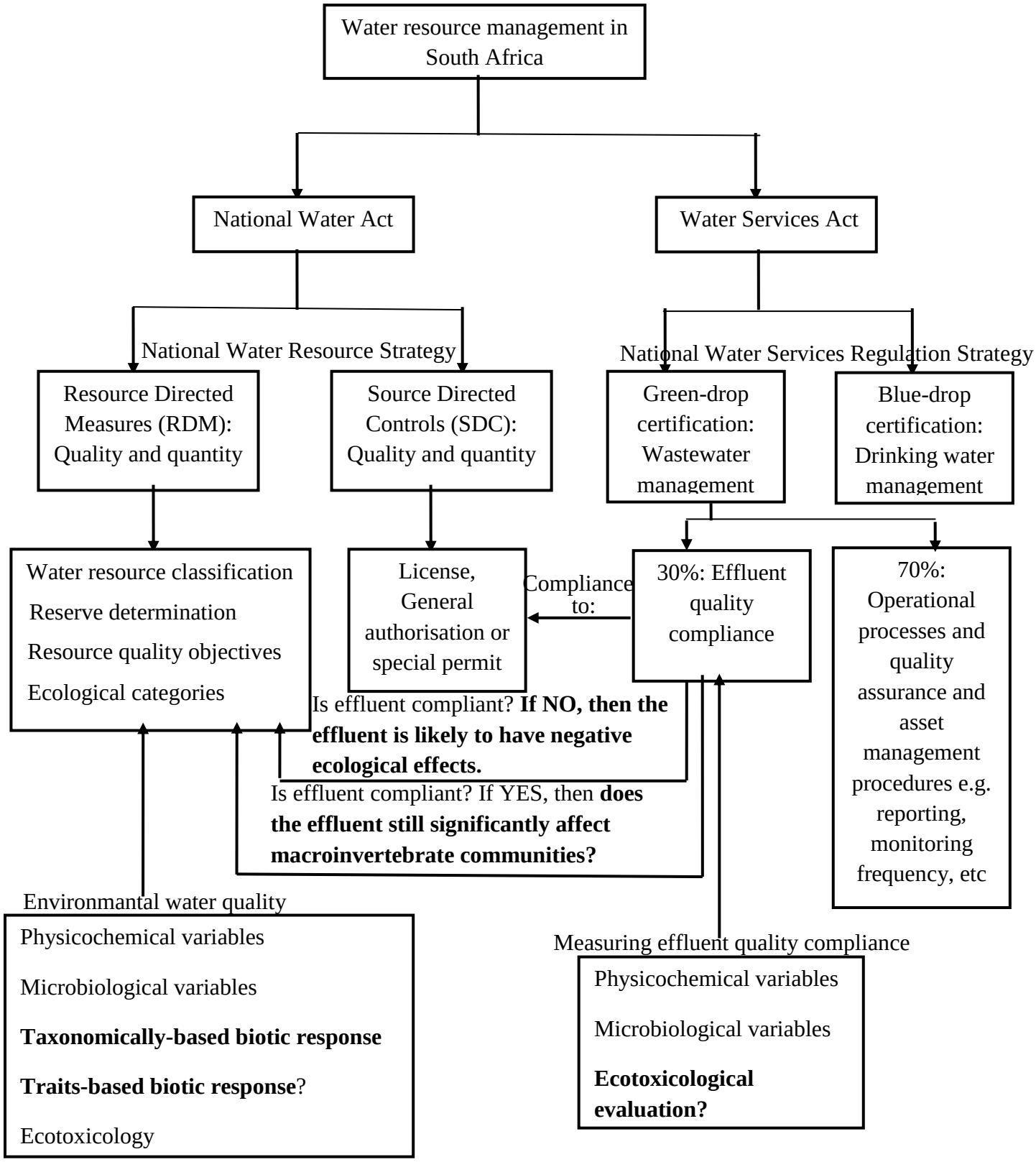


Figure 1.1: Summary of areas of investigation in this study (in bold) in relation to water resource management in South Africa (Macroinvertebrate traits investigated include body size, respiration, body shape, locomotion, food and feeding habit, biotope preference, and haemoglobin in chironomids. The model-stream ecosystem approach was used for ecotoxicological evaluation. Chapter references: taxonomically-based biotic response (Chapters 3 and 6), Traits-based biotic response (Chapters 4 and 5) effluent effects (Chapter 7)

1.3 Environmental Water Quality

Determining pollutant concentrations (both dissolved and suspended) in aquatic ecosystems, understanding their effects on instream organisms, and understanding biotic responses to pollutants are necessary to accurately determine the water quality components of the ecological Reserve, and to set robust resource quality objectives. An integrated approach, environmental water quality (EWQ), which links the chemical, physical and radiological characteristics of a water resource to the responses of instream biota, ecosystem function and processes has been developed (Scherman *et al.*, 2003, Palmer *et al.,* 2004a). The EWQ approach combines water physico-chemistry, biomonitoring and ecotoxicology information to manage instream water quality. Water physico-chemistry involves measuring and analysing physical and chemical variables to indicate water quality without taking into account effects of the measured variables on biological organisms. Biomonitoring uses resident biota to provide information on aquatic ecosystem health without providing a cause-effect relationship, while ecotoxicology experimentally evaluates effects of specific toxicants on aquatic biota, adding the identifying potential for causal linkages. Combining water physico-chemistry, biomonitoring and ecotoxicology provide an integrated approach to managing environmental water quality. This study was undertaken within the framework of the EWQ approach.

1.3.1 Water physico-chemistry

Water physico-chemistry describes the physical and chemical attributes and characteristics of the aquatic ecosystem. Human activities such as agriculture, domestic and industrial wastewater discharges, environmental engineering, and natural factors including geology and soils, hydrology, seasonal patterns, geomorphology, climate and weather can heavily influence water physico-chemistry (Palmer *et al.,* 2004a; PACN, 2010). Changes to water physico-chemistry by natural processes and by human activities can adversely affect ecosystem structure and function. It therefore remains important to measure, analyse and monitor water physico-chemical variables in a water quality study.

Water physico-chemical monitoring is the traditional approach to controlling pollution and managing water quality (Palmer *et al.,* 2004a). It helps water-resource managers to measure and analyse the concentrations of pollutants, determine their fate and transport, as well as their persistence in the aquatic environment. This approach, through the National Physico-

Chemical Monitoring Programme (NCPM), is widely and routinely use in South Africa to monitor the water quality status of rivers and streams (Hohls *et al.,* 2002).

Although this approach forms an important component in managing water quality, it has a number of limitations. The analytical expense and expertise, and the extensive number of chemicals dissolved and suspended in water, make exhaustive measuring and monitoring chemicals uneconomical and practically impossible. Analysis of single substances does not account for the effects of chemical interactions in mixtures. Furthermore, measuring only physico-chemical variables cannot provide important information on ecosystem health because the effects of chemical pollutants, their by-products, as well as their synergistic and antagonistic interaction on aquatic biotic communities cannot be fully assessed using this approach alone (Rosenberg and Resh, 1993; Bonada *et al.,* 2006). From a perspective of managing water quality, this approach only represents the time and location at which samples are collected, missing events in between, which may be critical to ecosystem health (Palmer *et al.,* 2004a). In view of the above limitations, and in line with the goal of conserving and sustaining water resources, biomonitoring (i.e. biological monitoring) provides the opportunity for detecting ecological impairments, and measuring both taxonomic and functional diversity, which are important components of the aquatic ecosystem. Biomonitoring therefore provides information needed to conserve biodiversity, e.g. the Dragonfly Biotic Index (DBI) developed for prioritising and assessing site conditions for conservation purposes (Simaika and Samways, 2009) and the South African Scoring Systerm version 5 (SASS5) for monitoring river health (Dickens and Graham, 2002). In addition, biomonitoring provides an integrated measure of water quality by integrating the multiple effects of stressors, including chemical (e.g. metal pollution, reduced oxygen), physical (e.g. habitat degradations) and biological (e.g. predation effects) (Rosenberg and Resh, 1993; Extence *et al.,* 2013; Marzin *et al.,* 2012).

1.3.2 Biomonitoring

Biomonitoring is one of the approaches that contributes to determining the ecological Reserve, setting the RQOs and classifying water resources in South Africa. Biomonitoring relies on the sound understanding that resident biota, i.e. plants, algae, animals and microorganisms provide an indication of ecosystem health, and it is a well-established discipline in freshwater ecology (Rosenberg and Resh, 1993; Bonada *et al.,* 2006; Choi *et al.,* 2011; Marzin *et al.,* 2012; Szöcs *et al.,* 2012). Changes in sub-organismal endpoints (e.g.

gene mutation, cell alteration, histopathology), changes in individual species, population, community and ecosystem structure are also used to infer water quality conditions, which then inform appropriate management actions. At the heart of biomonitoring is the search for and identification of suitable biological indicators (i.e. bioindicators), whose presence or absence, abundance and diversity, and behaviour reflect environmental conditions (Bonada *et al.,* 2006). Consequently, over the years, many studies have used bioindicators such as fish, diatoms, algae and macroinvertebrates to assess water quality (Pace *et al.*, 2012; Virtanen and Soininen, 2012).

However, among the bioindicators, macroinvertebrates are arguably the most widely used group of organisms in biomonitoring (Bonada *et al.*, 2006; Murphy *et al.,* 2013). Their wide application in biomonitoring can be attributed to their ubiquitous occurrence, abundance and diversity. In addition, they can be easily collected and identified to the family level, though species level identification requires more time, and for some taxa may not be possible. They have a huge species richness that offers a wide spectrum of environmental responses and they are relatively sedentary, representing local conditions. Furthermore, they provide an indication of environmental conditions over varying times; they are differentially sensitive to a variety of pollutants and consequently, capable of a graded response to stress. They also serve as a critical pathway for transporting and utilising energy and matter in the aquatic ecosystem (Wallace and Webster, 1996; Bonada *et al.,* 2006). Because of these advantages of macroinvertebrates over other indicator groups, many macroinvertebrate-based biomonitoring approaches have been developed.

1.4 Macroinvertebrate-based biomonitoring

Freshwater macroinvertebrates spend at least part of their lifecycles in the aquatic environment and are large enough to be seen unaided (Rosenberg and Resh, 1993). Depending on the goal of the biomonitoring, they can be monitored for changes in population, community, growth rate and cohorts (Rosenberg and Resh, 1993). They can also be monitored for bioaccumulation of pollutants, as well as for morphological and biochemical changes in cells, tissues, organs and systems (Rosenberg and Resh, 1993; Faria *et al.,* 2006; Al-Shami *et al.,* 2011; Pelletier *et al.,* 2012).

A growing interest in macroinvertebrate-based biomonitoring to managing water resources has led to the development of different approaches. These approaches include single biotic

indices such as the Biological Monitoring Working Party (BMWP) and the South African Scoring System version 5 (SASS5) (Walley and Hawkes, 1996; Dickens and Graham, 2002). The multimetric indices e.g. the Index of Biotic Integrity 12 (IBI 12) and the Serra dos Órgãos Multimetric Index (SOMI) (Böhmer *et al.,* 2004; Baptista *et al.,* 2007); the multivariate predictive techniques e.g. the Australian River Assessment System (AUSRIVAS) and the United Kingdom's River Invertebrate Prediction and Classification System (RIVPACS, UK) (Turak *et al.,* 2004; Jorgenson *et al*., 2005).

A multivariate predictive technique evaluates river health condition by comparing biota at a site to those expected to occur in the absence of human disturbances (Norris and Hawkins, 2000; Bonada *et al.,* 2006). A predictive model is constructed using reference sites' biotic communities, and correlating the community to natural environmental variables using multivariate statistics to predict expected communities at impacted sites (Turak *et al.,* 2004). A multimetric approach on the other hand, combines metrics representing several aspects of macroinvertebrate ecology (e.g. structure, function and processes) to indicate river health (Lücke and Johnson, 2009; Sanchez-Montoya *et al.,* 2010; Moya *et al.,* 2011). Bonada *et al*. (2006) assessed the utilities, strengths and weaknesses of both approaches using a set of 12 criteria in three categories: rationale, implementation and performance. Out of the 12 criteria evaluated, the multivariate predictive approach satisfies nine, while the multimetric approach fulfils 10. Both the multivariate predictive models and multimetric approaches have gained popularity among researchers, water-resource managers and decision makers; because they are perceived to be superior to a single biotic index approach (Karr and Chu, 2000; Norris and Hawkins, 2000; Klemm *et al*., 2002; Baptista *et al.,* 2007; Moya *et al.,* 2011).

However, in South Africa, the macroinvertebrate-based single biotic index, the South African Scoring System (SASS), is widely used to assess water quality (Dickens and Graham, 1998; Fouche and Vlok, 2010; Mantel *et al.,* 2010a; 2010b; Odume 2011). It is an index based on the presence of selected families of aquatic macroinvertebrates and their perceived sensitivity or tolerance to water quality impairment (Chutter, 1998). There have been several modifications and improvements to the index, culminating in the current version 5 (SASS5) (Dickens and Graham, 2002). In the index, macroinvertebrate families are awarded scores based on their perceived/ or known sensitivity ranging from 1 - 15 in increasing order of sensitivity to deteriorating water quality. The SASS5 results are expressed both as an index score and as an average score per recorded taxon (ASPT). The index has been rigorously

tested both for its regional and nation-wide application (Dallas, 1997; 2004; Vos *et al.,* 2002). It has become a key biomonitoring tool within the South African National River Health Programme (RHP), and it is continuously gaining popularity among aquatic researchers, because it is easy and simple to operate, and requires no sophisticated equipment. It is inexpensive, and the results are usually rapidly interpreted, and conveyed to water-resource managers. The index provides river health condition, and sampling is usually not destructive, unless representative samples are taken to the laboratory for analysis (Davies and Day, 1998; Dickens and Graham, 2002).

Although SASS5 is widely use to assess water quality in South Africa, the index was not intended for an in-depth ecological study as it only provides a rapid bioassessment of water quality condition (Dickens and Graham, 2002). Consequently, developing approaches aimed at elucidating in-depth ecological effects of deteriorating water quality is necessary to meet the goal of conserving and sustaining freshwater resources in South Africa.

1.4.1 A Multimetric Approach

Metrics are measurable aspects of functional, structural or other processes of a biological assemblage, and the value of a metric change in response to human-induced perturbation in an aquatic ecosystem (Barbour *et al.,* 1995; Ofenböck *et al.,* 2004). A good biological metric responds only to a human-induced impact, without a corresponding response to natural variations such as seasonality and inter-annual changes (Ofenböck *et al.,* 2004). The different aspects of macroinvertebrate ecology that are combined to develop multimetric indices are diversity, richness, composition, abundance, functional feeding groups and biotic indices (Bonada *et al.,* 2006; Baptista *et al.,* 2007). The combination of metrics provides an integrated picture of ecosystem health, but their selection needs a careful consideration to avoid redundancy (Ofenböck *et al.,* 2004; Odume *et al.,* 2012a).

The first multimetric approach was developed in the United States of America to assess fish integrity (Karr, 1981). However, macroinvertebrate-based multimetric indices are now widely used in other parts of the world for assessing water quality and general river health condition (Klemm *et al.,* 2002; Böhmer *et al.,* 2004; Camargo *et al.,* 2004; Ofenböck *et al.,* 2004; Baptista *et al.,* 2007). In Germany, for example, the Index of Biotic Integrity 12 (IBI 12), based on 12 sets of metrics, is used to assess water quality in all stream types. The IBI 12 was found to be a very sensitive index, enabling the detection of impairments, particularly of

effects of wastewater effluent discharges (Böhmer *et al.,* 2004). Baptista *et al.* (2007) developed a similar multimetric index: the Serra dos Órgãos Multimetric Index (SOMI) for assessing water quality in south-east Brazil. These authors found that the index was a very sensitive, robust and an easy-to-use tool for routine biomonitoring of streams and rivers. More recently, Mondy *et al.* (2012) developed a macroinvertebrate-based multimetric index (the I_2M_2) for assessing French stream ecological conditions. According to these authors, the index fulfils the requirements of the European Water Framework Directives (WFD). These authors found the I_2M_2 to be a robust and sensitive tool for detecting both water quality impairments, e.g. organic pollution and nutrient inputs, and habitat degradations such as degradation of in-stream vegetation and hydrological instability. Similar indices have been developed for assessing stream conditions in the Netherlands (Vlek *et al.,* 2004), in Belgium (Gabriels *et al.,* 2010) and in Bolivia (Moya *et al.,* 2011).

A useful step in developing a multimetric approach is to consider the performances of metrics with respect to stream types or the stressor of interest (Gray and Delaney, 2008; Lücke and Johnson, 2009; Simaika and Samways, 2011). Although the multimetric approach has received considerable attention elsewhere, the method is still not used to assess water quality in South Africa. Identifying useful metrics suitable for assessing the Swartkops River water quality, and applying a multimetric approach that combines these metrics can provide a robust and an integrated assessment of water quality condition in the river system.

The need to develop simple and low-cost tools, requiring minimal resources for sampling, sorting and identifying taxa, is critically considered during biomonitoring method development. Consequently, most tools developed to date, particularly those in South Africa, are at the family level of taxonomic resolution (Walley and Hawkes, 1996; Dickens and Graham, 2002; Thirion, 2008). Protecting functional biodiversity is a core goal of sustainability, and identifying taxa to only family level may not provide the required information on biodiversity, as many important species (e.g. rare, endemic, endangered, pollution tolerant or sensitive) would have been aggregated into one family; limiting the interpretive power of the data and the resultant understanding of the implications of biodiversity in an aquatic ecosystem. Secondly, tools developed at genus and species level of taxonomic resolutions are likely to be more precise in indicating both biotic and water quality impairments than those developed at the family level (Resh and Unzicker, 1975; Lenat and Resh, 2001; Schmidt-Kloiber and Nijboer, 2004). The assumption for aggregating species

into families in most biomonitoring tools is that species and genera of the same family are presumed to have similar ecological requirements and would therefore respond in similar patterns to human-induced perturbations. However, since the systematics of macroinvertebrates and definition of species are not based on functional characteristics, species and genera within the same family may have different ecological requirements and would thus respond differently to water quality impairment (Schmidt-Kloiber and Nijboer, 2004).

Lenat and Resh (2001) demonstrated the differences between species-level and family-level biotic indices. These authors compared the classification of sites by a genus/ species-level biotic index to a family-level biotic index and concluded that about 40% and 20% of sites, respectively, identified as excellent and poor by a family-level index were inaccurate. Classifying sites erroneously as illustrated above, could have significant implications for managing water quality. From the foregoing arguments, it therefore remains important to identify genus and species to capture as much biodiversity as possible, and accurately assess water quality.

McGeoch, (1998) listed some important factors to consider when searching for appropriate bioindicators for biomonitoring. These factors include taxonomic richness (i.e. number of species in the taxonomic group), representativeness of species in all habitats and biotopes in the ecosystem to be assessed, and availability of appropriate sampling methods and reliability of species. A useful bioindicator should readily reflect environmental conditions, effects of environmental change at various scales, and be an appropriate surrogate for other taxa (McGeoch, 1998; Simaika and Samways, 2011). The macroinvertebrate family Chironomidae fulfils all of these considerations and its use in this study will enable an exploration of its bioindication potentials, taxonomic diversity as well as functional ecology at the species/ genus level of taxonomic resolutions. Since chironomid diversity can match or even exceed that of other aquatic insect groups combined (Harrison, 2003; Ferrington Jr., 2008; Heino and Paasivirta, 2008), studying them in relation to assessing deteriorating water quality is an important step to developing a species-level biotic index in South Africa.

1.4.2 Chironomidae as bioindicator organisms

Among aquatic macroinvertebrates, larvae of the family Chironomidae are unarguably one of the most diverse groups (Armitage *et al*. 1995; Cranston, 1996; Porinchu and MacDonald,

2003; Ferrington Jr., 2008). They occur in most aquatic habitats, often dominating the species richness of samples taken from any stream (Armitage *et al*., 1995; Cranston, 1996; Harrison, 2003; Ferrington Jr., 2008). A recent review of global diversity of Chironomidae revealed that 4 147 species in 339 genera are unambiguously aquatic in their immature stages (Ferrington Jr., 2008). Species of the family Chironomidae exhibit an extraordinary ecological range, inhabiting all forms of habitats and biotopes, and with a wide-range of tolerances/sensitivities to pollution (Porinchu and MacDonald, 2003; Adriaenssens *et al.,* 2004; Ferrington Jr., 2008; Wright and Burgin, 2009; Odume and Muller, 2011). They are an important component of the aquatic food web and they play vital ecological function, including recycling of nutrients by accelerating decomposition of leaf litter through fragmentation. They transport energy and matter by serving as a link between primary producers and secondary consumers (Armitage *et al*. 1995).

The extraordinary ecological range, huge species richness and diversity of Chironomidae, make them a good object of study for biomonitoring of rivers and streams. Consequently, several studies elsewhere have use them to biomonitor freshwater ecosystems (Diggins and Stewart, 1998; Janssens de Bisthoven and Gerhardt, 2003; Mousavi *et al*., 2003; Adriaenssens *et al*., 2004; Janssens de Bisthoven *et al*., 2005, Carew *et al.,* 2007; Wright and Burgin, 2009; Cortelezzi *et al.,* 2011; Luoto, 2011). Chironomid communities were applied as bioindicators for monitoring metal contamination in the Buffalo River, New York, with their mean density decreasing with increasing metal contamination (Diggins and Stewart, 1998). They have also been used to monitor the effects of thermal pollution where their diversity declined compared to neighbouring unpolluted streams (Janssens de Bisthoven and Gerhardt, 2003). Mousavi *et al.* (2003) investigated the changes in diversity and structure of Chironomidae communities along a gradient of metal contamination and reported that species such as *Procladius* spp., *Tanytarsus* spp., and *Chironomus anthracinus* appeared to be good indicators of metal contamination. Chironomidae's role as indicators of organic and nutrient enrichment was examined by Adriaenssens *et al*. (2004) who reported that certain chironomid species were indictors of good quality water, nutrient and organically enriched streams, while others were indifferent to changes in water quality. Wright and Burgin (2009) also noted that chironomid species were sensitive to both organic and metal pollution in the upper Grose River catchment. Cortelezzi *et al.* (2011) investigated the response of Chironomidae assemblages at three study sites in a stream with predominantly different anthropogenic impacts: agricultural inputs at Site 1, textile discharges at Site 2, and wastewater discharges

and metallurgical activities at Site 3. These authors reported that different chironomid species dominated each of these sites despite their proximity to one another, which seemed to indicate that different species of Chironomidae respond to specific types of pollutants. In South Africa, however, knowledge of Chironomidae as object of freshwater quality study is scarce (Harrison, 2003; Odume, 2011; Odume and Muller, 2011).

To use species of Chironomidae successfully as bioindicators in South Africa's freshwater ecosystems, it is important to understand their preference for, and relationship with specific biotopes. Knowledge of Chironomidae preferences for specific biotopes is important for conservation purposes and interpretation of chironomid-based bioassessment results because rivers and streams are characterised by patchiness (Palmer *et al.*, 1991a). Patchiness is governed at the reach scale by factors such as water velocity, hydraulics, substratum characteristics, channel width as well as canopy cover which provide a diverse-range of biotopes, which shape the structure of benthic communities (Palmer *et al*., 1991a; Dallas 2007a; Heino and Paasivirta, 2008). Therefore, accurately interpreting bioassessment results needed for conserving biodiversity and quantifying effects of pollution requires an understanding of the relationship between Chironomidae as bioindicator species and specific biotopes.

Several studies have considered these relationships (Principe *et al.*, 2008; Syrovátka *et al*., 2009; Rosa *et al.*, 2011; Epele *et al.*, 2012). Principe *et al.* (2008) investigated the structure of Chironomidae assemblages in the Chacancharava River basin (Córdoba, Argentina) and reported the highest species richness for complex habitat (biotope) units. Syrovátka *et al*. (2009) examined the role of substrate and hydraulic characteristics in shaping the distribution of chironomid larvae within a river reach. They identified four chironomid communities: i) communities occurring at patches with minor currents and high amounts of particulate organic matter, ii) those occurring at habitats with low current and mineral substrates, but sometimes covered with fine particulate organic matter, iii) those occurring in runs and riffles with mineral substrates, and iv) those occurring in runs and riffles with aquatic vegetation. Different species dominated the different communities, suggesting species preferences for specific biotopes and substrates. Epele *et al.* (2012) examined the relationship between Chironomidae and different habitats (biotopes) in two low-order Patagonia streams. These authors found that of the 35 taxa recorded, 14 exhibited a strong affinity for a particular substrate, with structurally complex macrophytes supporting more taxa, than less complex

ones. Earlier, Rosa *et al.* (2011) had stated that environmental heterogeneity promotes high chironomid species richness and diversity. Although in South Africa, few studies have considered the relationships between macroinvertebrates and biotopes (Palmer *et al.* 1991a; Dallas, 2007a), **knowledge of chironomid preference for, and relationship with specific biotopes, which should inform the design of a chironomid-based bioassessment technique, is completely lacking. Hence, this study investigates the relationship between the Swartkops River Chironomidae and selected biotopes**.

Nevertheless, biomonitoring techniques that take cognisance only of community taxonomic structure without recognising the role of function in shaping structure are likely to produce incomplete knowledge of river health because freshwater ecological integrity comprises both structural and functional integrity. Thus, most freshwater resource management goals refer to protecting, restoring and maintaining both components (Gessner and Chauvet, 2002; Kefford *et al.,* 2012a). Macroinvertebrate functional traits help elucidate patterns and distribution of community structure, and confer both resistance and resilience on them. For example, food shortage in an ecosystem is likely to have more impact on a selective feeder than a non-selective feeder. Consequently, altered functional ecology may affect both pattern and distribution of community structure (Bunn *et al.,* 1999). Although both structural and functional ecology are linked, they describe different aspects of the community (Rosenberg and Resh, 1993; Merritt *et al.,* 1996; Kefford *et al.,* 2012). To provide a complete picture of an ecosystem health, it is necessary to assess both structure and function because assessing community structure alone may yield an incomplete knowledge of mechanisms driving observed patterns (Gessner and Chauvet, 2002; Clapcott *et al.,* 2010; Kefford *et al.,* 2012). Current macroinvertebrate-based biomonitoring approaches in South Africa do not incorporate aspects of functional ecology because community structure is presumed as surrogate for function. However, if the overarching goal of sustainability is to be met, it is important to develop biomonitoring methods that incorporate both structural and functional aspects of macroinvertebrate communities. Empirical evidence suggests that some macroinvertebrate traits (e.g. biological, behavioural, ecological traits) are associated with ecological function, providing an opportunity for investigating ecosystem function (Statzner *et al.,* 2001; de Bello *et al.,* 2010). Studies on macroinvertebrate biological and ecological traits in relation to river health are lacking in South Africa, but such studies could provide an avenue for understanding functional ecology, with the potential to complement community structural analysis for assessing river health (Statzner *et al.,* 2001; Poff *et al.,* 2006). In

addition, since water resource-managers are often interested in identifying causes of deteriorating water quality, traits, which are mechanistically linked to environmental factors, could be diagnostic of the stressors responsible for the observed changes (Pollard and Yuan, 2010). Therefore, it is crucial to investigate macroinvertebrate (including chironomid) ecological and biological traits, and their application to biomonitoring South Africa's freshwater resources.

1.5 Macroinvertebrate traits-based approach in biomonitoring: the habitat template and habitat filtering concepts

Traits are measurable functional attributes (e.g. physiological, morphological, behavioural or ecological) of a species, which may confer resistance, resilience, vulnerability or adaptability on an individual in an ecosystem (Poff *et al.,* 2006; Baird *et al.,* 2008; Verberk *et al.,* 2008; Menezes *et al.,* 2010; Statzner and Bêche, 2010). Thus, the sum total of a species' traits is the product of natural selection of the environment within which a species evolved (Southwood, 1977; Townsend and Hildrew, 1994). Development in theoretical ecology, particularly the formulation of the habitat template concept (HTC) (Southwood, 1977), its adaptation to freshwater habitat (Townsend and Hildrew, 1994), and the formulation of the habitat filtering concept (HFC) (Poff, 1997) stimulated interest in the use of species traits for biomonitoring.

The habitat template concept (HTC) (Southwood, 1977; 1988) is the primary theoretical underpinning of the traits-based approach in biomonitoring. It predicts that in theory, species traits evolve in response to their environmental demands, and that these demands (i.e. characteristics of the habitat), select certain traits in an individual, while eliminating others. Consequently, where habitat characteristics are similar, the composition of species traits selected or favoured should be similar, even across distant eco-regions where species taxonomic composition differs (Southwood, 1977). Townsend and Hildrew (1994) later extensively adapted the Southwood concept to rivers and streams. Based on autecology, Townsend and Hildrew (1994) predicted trends in species traits in two-dimensional habitat gradients (i.e. temporal heterogeneity and spatial heterogeneity). Temporal heterogeneity was defined as the frequency and magnitude of deviation caused by disturbance in environmental variables (habitat characteristics) away from long-term averages; spatial heterogeneity was viewed as the provision of "refugia" in the face of disturbances. Spatial heterogeneity therefore acts as a buffer against a disturbance of any magnitude in the habitat. Townsend and Hildrew (1994) then further predicted that species traits that confer resilience or resistance on

the individual organism dominate a habitat with high temporal heterogeneity (i.e. high disturbances: in both frequency and magnitude) and low spatial heterogeneity (i.e. low capacity to provide refugia) that act as a buffer against disturbances. The Townsend and Hildrew (1994) concept is anchored on the assumption that autecological processes govern species, population and community dynamics and distributions. However, although the present study is aligned with the Townsend and Hildrew (1994) concept and focuses primarily on autecological processes as the driver of species composition in the Swartkops River, recognising that synecological processes such as predator-prey interaction, competition and parasitism will also shape community compositions.

The habitat template concept (Southwood 1977; Townsend and Hildrew, 1994) led to the understanding that local biotic communities are shaped by environmental conditions at multiple scales, acting as filters eliminating undesirable biological and ecological traits from the individual organisms. Upon this understanding, Poff (1997) presented the HFC demonstrating how regional-scale processes (i.e. both biotic and abiotic conditions) act hierarchically to influence functional attributes (i.e. species traits) of local stream communities. These landscape (i.e. regional-scale) processes acting as filters impose constraints on the biological communities, such that species with appropriate combinations of traits survive to form local communities. Increased stress (i.e. harsher habitat characteristics) reduces the number of traits that are favoured. For example, Lamouroux *et al.* (2004) studied the effects of multiple-scale filters (i.e. filters at the microhabitat, reach and basin levels) and noted that, in agreement with the Poff (1997) concept, these filters influenced the functional community of the studied stream macroinvertebrates, but the influences were strongest with the microhabitat filters. If the prevailing environmental factors are known, the HFC and the HTC provide a mechanistic basis for predicting biotic composition.

The strong theoretical framework for linking and predicting trait-environment relationship stimulated ecologists to investigate and develop traits-based approaches (TBA) in biomonitoring (Usseglio-Polatera *et al.,* 2000; Doledec and Statzner, 2008; Pollard and Yuan, 2010; Statzner and Beche, 2010). Poff *et al.* (2006), Vieira *et al.* (2006) and Culp *et al.* (2011) enumerated some advantages of the TBA over the taxonomically-based approaches as follows:

i) The TBA mechanistically links biotic responses to environmental conditions, resulting in improved sensitivity and diagnosis of causes of impairment;

ii) It is stable across large spatial scales;

iii) It is more seasonally and inter-annually stable compared to taxonomic metrics;

iv) It reflects ecosystem function (e.g. functional feeding groups, mobility etc) rather than just taxonomic identity;

v) It is cost-efficient as some traits accurately reflect environmental conditions at coarser taxonomic resolutions (e.g. genera and families) reducing costs of identifying taxa to species;

vi) It responds to specific environmental stressors and therefore has a potential for disentangling the impacts of multiple stressors.

1.5.1 Developments and applications of traits-based approach (TBA) in biomonitoring

The earliest attempts at testing the river habitat template and investigating the potential of TBA came from the upper Rhône River system in France (Chevenet *et al.*, 1994; Dolédec and Chessel, 1994; Usseglio-Polatera and Tachet, 1994; Dolédec *et al.*, 1999). These authors related the HTC, species traits and species richness by investigating trends in species traits within spatial and temporal heterogeneity using fuzzy coding, correspondence and co-inertial analyses. Although the results of these earliest studies generally supported the predictions of the habitat template, not all species traits investigated matched the predictions because, in reality, the relationship between any given habitat and species traits is complex. The complex nature of the relationship could be attributed to factors such as trait syndromes (trais that are evolutionarily linked: Nylin and Gotthard (1998); Menezes *et al.*, (2010)), correlations and trade-offs, which make it difficult for a complete match of the habitat template predictions. For example, Usseglio-Polatera and Tachet (1994) examined the trends of 17 species traits of Ephemeroptera and Plecoptera according to the predictions of the habitat template, and found that trends in species traits were significantly correlated to two habitat gradients (the spatial and the temporal heterogeneities). However, out of the species traits examined, only six (minimum age at reproduction, potential longevity, descendant per reproductive cycle, voltinism, body size and body flexibility) matched the temporal heterogeneity predictions. Nevertheless, when Dolédec *et al.* (1999) examined the patterns of six ecological traits and 15 biological traits of invertebrates along the Rhône River system, in reaches influenced by different human impacts: organic pollution, industrial pollution and habitat alterations, the

biological traits proved more responsive to human impacts than the ecological traits, indicating the utility of the TBA in biomonitoring.

The development of the species at risk (SPEAR) model, that combines different categories of macroinvertebrate traits to evaluate the effects of pesticides on freshwater ecosystems marked an important milestone in the use of species traits in biomonitoring (Liess and Von der Ohe, 2005). In the SPEAR model, macroinvertebrate taxa were grouped according to their vulnerability to pesticides based on their ecological and biological traits: sensitivity to toxicants, generation time, migration ability, and presence of aquatic stages during time of maximum pesticide application (Liess and Von der Ohe, 2005; Beketov *et al.,* 2009). The SPEAR model proved to be a sensitive stressor-specific tool (Liess and Von der Ohe, 2005; Beketov *et al.,* 2009). Because the SPEAR was based on species traits that are mechanistically related to pesticides, and not based on taxonomic attributes such as composition, richness, diversity and abundance, it is less affected by seasonality, and natural biotic community variations (Beketov *et al.,* 2008a; Beketov *et al.,* 2009). These factors made it applicable across wide spatial scales (Beketov *et al.,* 2008a; Beketov *et al.,* 2009). Liess and Beketov *et al.* (2011) used a similar approach to investigate traits-based vulnerability of invertebrates' long-term exposure to low concentrations of the insecticide thiacloprid in outdoor mesocosm experiments. Vulnerability was characterised using two trait sets: sensitivity to pesticide and voltinism. Based on these traits, these authors established four macroinvertebrate groups: i) non-sensitive multivoltine, ii) non-sensitive univoltine, iii) sensitive multivoltine and iv) sensitive univoltine. Their approach detected long-term pesticide effects at a level 1 000 times more sensitive than the convention principal response curve (PRC) approach. These studies illustrated the usefulness of the TBA if developed based on a mechanistic understanding between trait occurrence and stressor(s).

The TBA is gaining popularity in biomonitoring of freshwater ecosystems (Demars *et al.,* 2012; Feio and Dolédec, 2012; Gamito *et al.,* 2012; Mondy *et al.,* 2012). Biomonitoring studies in Europe (Dolédec *et al.,* 1999; Gayraud *et al.,* 2003; Dolédec and Statzner, 2008), in North America (Poff *et al.,* 2006; 2010; Wooster *et al.,* 2012), in Australia and New Zealand (Kefford *et al.,* 2012) and in the neotropical region (e.g. Tomanova *et al.,* 2008), investigated the TBA under single prevailing or multiple anthropogenic stressors. Gayraud *et al.* (2003) assessed the potential of using invertebrate traits for biomonitoring large rivers in Europe. Their results indicated that invertebrate traits significantly discriminated river reaches

according their level of impairment, suggesting that the TBA is sensitive and applicable across a large spatial scale. Dolédec and Statzner (2008) used invertebrate traits to examine the effects of metal contamination and cargo ship traffic on selected large European rivers. According to these researchers, the TBA performed better in correctly assigning river reaches as least impacted or most impacted. Wooster *et al* (2012) investigated taxonomic metrics and 18 biological traits under multiple stressors (summer water withdrawal and channelization) scenarios, and later compared their ability to disentangle effects of the different stressors. These authors reported that both taxonomic metrics and biological traits detected ecological change but only the biological traits were able to identify the main driver of the observed ecological change, indicating the potential of traits to disentangle effects multiple co-occuring impacts. Response of species traits to anthropogenic climate change and their usefulness in predicting invertebrate vulnerability to future climate change has been investigated (Poff *et al.,* 2010). Kefford *et al.* (2012) used the traits of insect taxa in south-east Australia to demonstrate that protecting species does not always protect biological traits; yet, some of these traits not protected are directly related to ecosystem function and services. The implication of this study is that it is necessary to assess both structure and function in monitoring river health and in assessing ecological risk.

Apart from their use in developing new TBA, traits have been incorporated into existing taxonomically-based approaches (Feio and Dolédec, 2012; Mondy *et al.,* 2012). Feio and Dolédec (2012) integrated invertebrate traits into an existing Portuguese AUSRIVAS-type predictive multivariate model. Integrating traits into the model enhanced its capacity to detect ecological change. Mondy *et al.* (2012) developed a multimetric index that incorporated some aspects of invertebrate biological traits, which improved the index robustness and sensitivity, correctly identifying 82% of impaired reaches, and improving detection of nitrogen compound and organic micropollutants effects by 17% and 35%, respectively. The ease with which trait information can be integrated into existing methods emphasises the value of developing new biomonitoring methods that incorporate trait information in South Africa.

1.5.2 Biological traits and ecosystem function

Managing river health requires protecting both the structural and the functional integrity of the ecosystem. Certain species traits can be linked to ecosystem function (Merritt *et al.,* 1996; Statzner *et al.,* 2001; Haybach *et al.,* 2004; Spooner and Vaughn, 2008; de Bello *et al.,* 2010). Traits such as the functional feeding groups (FFG) for example, can be related to ecosystem

function, including resource acquisition and processing, nutrient cycling and energy transfer in the aquatic food web (Wallace and Webster, 1996; Vieira *et al.,* 2006). Further, top-down control (localised regulation of the abundance of lower trophic level organisms by usually higher trophic level organisms e.g. predators) is indirectly assessed as the ratio of all FFGs to predators (Merritt *et al.,* 1996).

In addition, combining certain traits provides information on ecosystem processes and function. For instance, biomass turnover or changes in secondary productions are reflected by a combination of body size, adult life span, emergence, number of eggs per reproductive cycle and voltinism (Huryn and Wallace, 2000). Spooner and Vaughn (2008) studied eight freshwater mussel species and noticed that the ecological trait, thermal preference, was directly related to resource assimilation and ecological function such as nutrient excretion and benthic-pelagic coupling. Mussel species with a high thermal tolerance at higher temperature (35^{o}C) were reported to have an increased resource assimilation and nutrient excretion (Spooner and Vaughn, 2008). Therefore, assessing traits can provide an indirect measure of ecosystem functional integrity. However, not all traits have a demonstrable link with ecosystem function, consequently, selecting and applying them for biomonitoring purposes needs careful consideration in line with their structural and functional usefulness.

Although TBA in biomonitoring represents an advance in freshwater ecology, testing the habitat template concept, which forms the theoretical underpinning of the approach, reveals that species trait trends in spatio-temporal heterogeneity are often apparent (Usseglio-Polatera and Tachet, 1994; Dolédec *et al.,* 1999). However, traits did not always correspond to the details of the predictions of the template (Usseglio-Polatera and Tachet, 1994; Dolédec *et al.,* 1999; Usseglio-Polatera *et al.,* 2000). The non-total conformity of traits to the predictions of the habitat template indicates that the habitat does not act in a uniform pattern in shaping species traits, but unexpected traits may occur along the two prediction axes. This and other factors present challenges to the use of traits for biomonitoring purposes.

1.5.3 Challenges in using traits information for biomonitoring

Several challenges face ecologists interested in using traits in biomonitoring. Understanding them is critical to developing appropriate and robust traits-based tools.

Traits trade-offs, syndromes, correlations and strategies

The earliest attempts to test the habitat template revealed that traits trade-offs were one of the confounding factors that resulted in the non-total match of the habitat template predictions, as investments in one sets of traits leave fewer resources available to the others (Usseglio-Polatera *et al.*, 2000; Menezes *et al.,* 2010). As species diversity increases, trait trade-offs, particularly those related to ecological function increase in parallel, leading to the occurrence of unexpected traits not matching prevailing environmental conditions (Usseglio-Polatera *et al.,* 2000; Kneitel and Chase, 2004).

Correlations among traits occurring in syndromes (trait sydromes are traits that are linked together by evolution) result in trait redundancies complicating the interpretations of trait-environment relationship, posing additional problem to using traits for biomonitoring (Usseglio-Polatera *et al.,* 2000; Poff *et al.,* 2006). Poff *et al.* (2006) demonstrated inter-correlations between traits of North American insect taxa in which correlations closely relate to phylogenetic relationships. Evolutionarily conserved traits, co-occurring in related taxa, may hinder applying the TBA on a wide-scale because traits would link closely to taxonomic identity (Poff *et al.,* 2006; Vieira *et al.,* 2006). Consequently, Poff *et al.* (2006) advocated the use of labile traits (uncorrelated or unlinked traits, not phylogenetically constrained) for biomonitoring. The authors then provided a conceptual model for identifying them. Usseglio-Polatera *et al.,* (2000) used multivariate statistical techniques to investigate traits syndromes among French invertebrate taxa and noted that ecological traits such as habitat preferences were less phylogenetically constrained than biological traits such as morphology and life history.

Although Poff *et al.* (2006) and Vieira *et al.* (2006) have indicated that uncorrelated and phylogenetically unconstrained traits are best suited for biomonitoring, in reality, species traits should not been seen as independent entities but as a complex of adaptations co-evolved to enable organisms adapt to their environment (Verberk *et al.,* 2008; Menezes *et al.,* 2010). Recognising correlated traits (although some of them become redundant) based on known functional relationships or strategies, which enable a species to adapt to a range of stressors, may provide well-informed mechanistic relationships between traits and environmental variables (e.g. water quality) (Verberk *et al.,* 2008). Based on life-history theory, Verberk *et al.* (2008) provided an approach that aggregates functionally related traits into life-history strategies enabling the survival of organisms in specific environments. These authors

identified four possible strategies: dispersal, synchronisation, reproduction, and developmental trade-off. Since there are numerous species traits among aquatic invertebrates, aggregating them into useful strategies and relating these strategies to known environmental variables could provide a better way of using species traits for biomonitoring, particularly if the study objective is not to diagnose the effects of multiple co-occuring stressors.

Biological traits and taxonomic resolution

One of the arguments for the use of traits is the reduced cost in comparion to taxonomic identification (Vieira *et al.* 2006), the question then arises as to what level of taxonomic resolution can traits reliably be applied? There are conflicting views in the literature (Gayraud *et al.,* 2003; Poff *et al.,* 2006; Beketov *et al.,* 2009). Poff *et al.* (2006) argued that the use of a family-level traits result in the loss of useful characteristic trait information, especially among families that possess a high diversity of traits, descriptive of their huge species and generic richness. The authors supported this by further arguing that several North American species and genera within the limnephild, baetid, heptageneiid and perlid families have unique functional niches. Poff *et al.* (2006) then concluded that genus-level trait information would be more sensitive in detecting subtle ecological changes.

However, Gayraud *et al.* (2003) investigated whether taxonomic resolutions affect the usefulness of traits to reflect human-induced impacts in large European rivers and found that identifying taxa to species, genera or families has little effect on the utility of traits for biomonitoring. These authors concluded that identifying taxa to genera or families was sufficient for biomonitoring large European rivers. In addition, a recent test of the traits-based SPEAR (species at risk) method revealed that the explanatory power of the family-level SPEAR method was not significantly different from the species-level method and that the family-level SPEAR was as sensitive as the species-level SPEAR (Beketov *et al.,* 2009). The bulk of evidence in the literature suggests that genus- or family-level trait information is adequate, and identifying species is not necessary for the TBA in freshwater biomonitoring (Dolédec *et al.,* 1998; 2000; Gayraud *et al.,* 2003; Beketov *et al.,* 2009).

Availability of traits data and information

A major challenge to the use of invertebrate traits for biomonitoring, particularly in Africa, is the low availability of trait data and information. Although considerable research efforts have gone into compiling and developing trait databases in Europe, North America and Australia

(Usseglio-Polatera *et al.,* 2000; Poff *et al.,* 2006; Vieira *et al.,* 2006; Statzner *et al.,* 2007; Liess *et al.,* 2008; Schäfer *et al.,* 2011), trait information on Afro-tropical stream invertebrate species is still scattered in the literature. This reflects the low popularity of the TBA among African freshwater ecologists, with only a few studies focusing on functional feeding groups (Palmer *et al.,* 1993a; 1993b; Uwadiae, 2010). Overcoming this challenge would require coordinated research efforts across institutions and regions.

Trait measurements

The lack of consistency in describing, categorising and measuring traits, among researchers poses a major drawback to the use of traits in biomonitoring (Stearns, 1976; Poff *et al.,* 2006; Verberk *et al.,* 2008; van den Brink *et al.,* 2011). For example, Verberk *et al.* (2008) uses the term "life-history strategies" to describe sets of co-evolved and interlinked traits that enable organisms to adapt to their environment, while Sterns (1976) uses the term "life-history tactics" to describe the same concept. Although Verberk *et al.* (2008) argued that life-history tactics only referred to traits related to reproduction, while the term "strategies" was adopted to indicate broader coverage of other traits related to reproduction, synchronisation, development and dispersal, such inconsistencies in terminology among researchers may pose problems to the use of trait information for biomonitoring. Van den Brink *et al.* (2011) therefore emphasised the importance of a framework for uniformly and consistently defining, describing, categorising and measuring traits to realise their full potentials in biomonitoring.

Despite the above-mentioned challenges, the TBA holds promise in freshwater biomonitoring**. Therefore, this study identifies useful traits and develops a TBA suitable for managing water quality and assessing functional ecology in the Swartkops River.**

The same traits occurring among individuals of a species or population (e.g. the configuration of the mentum in chironomids) may vary among the individuals within the species or population (Culp *et al.*, 2011). Such traits variation among individuals of a specified group could be cause by exposure to environmental stressors or reflect biological and ecological differences such as development, growth and feeding rates among the individuals within the group (Culp *et al.*, 2011). These traits variations could be morphological, genotypic or life history characteristics, and in some cases, they have been used for biomonitoring (Janssens de Bisthoven *et al.,* 1998; Lewis *et al.,* 2012). In Chironomidae larvae, for example, morphological variations in the configuration of the larval mouthparts of individual

chironomids were used to investigate the effects of pollutants in the aquatic ecosystem (Janssens de Bisthoven *et al.,* 1998; Di Veroli *et al.,* 2012a). Morphological variations, which depart from the normal configurations of the mouthparts of individual chironomids, have been termed "deformities" (Warwick, 1985; Nazarova *et al.,* 2004). The screening of deformities in Chironomidae larvae represents an in-stream sub-lethal response to environmental stressors and may therefore serve as an early warning indicator of deteriorating water quality. **Therefore, this study employs the screening of morphological deformities in chironomids as an additional biomonitoring tool to assess deteriorating water quality in the Swartkops River.**

1.6 Morphological deformity in Chironomidae larvae

There is a growing need to develop biomonitoring tools that can detect sub-lethal effects of pollutants on in-stream organisms (Di Veroli *et al.,* 2012a). Such biomonitoring tools, in addition to relying on presence, absence and abundance, also measure sub-lethality, serving as early warning indicator of biotic deterioration in "mildly" polluted sites or streams. Furthermore, at "heavily" polluted sites or streams, from which sensitive taxa have disappeared, the question remains whether tolerant taxa at such sites or streams, are sub-lethality influenced that can affect their ecological function. Morphological deformity not only represents a sub-lethal response, but is also an ecologically relevant fitness endpoint (Janssens de Bisthoven *et al.,* 1998; Cortelezzi *et al.,* 2012; Di Veroli *et al.,* 2012a; 2012b; Odume *et al.,* 2012b). For example, mouthpart deformities in Chironomidae larvae directly relate to ecological traits such as feeding, development and emergence rate (Janssens de Bisthoven *et al.,* 1998), as well as to ecosystem function such as top-down control.

Hamilton and Saether (1971) were among the earliest researchers who noted aberrant mouthparts and thickening of the body wall in chironomids, sampled from Lake Erie and Lake Ontario in Canada. Since then, several other studies employed deformity screening as a biomonitoring tool for assessing deteriorating water quality and sediment toxicity (Warwick 1985; Lenat, 1993; Vermeulen, 1995; Janssens de Bisthoven *et al.,* 1998; Nazarova *et al.,* 2004; MacDonald and Taylor, 2006; Ochieng *et al.,* 2008; Cortelezzi *et al.,* 2012; Di Veroli *et al.,* 2012a; 2012b). For example, Lenat (1993) applied *Chironomus* mentum deformities to investigate the effects of toxicity and organic loading in North Carolina streams, while Janssens de Bisthoven *et al.* (1998) examined the relationship between deformities and larval development rates, growth and emergence and reported that, in some instances, normal larvae

were often larger than deformed larvae. These authors further noted that, in most cases, normal larvae survived better and emerged earlier than deformed larvae, suggesting a slower development rate of deformed larvae.

Structures usually examined for morphological deformities in Chironomidae larvae include the mentum, mandible, ligula, paraligula, pecten epipheryngis and antenna (Warwick, 1985; Janssens de Bisthoven and Gerhardt, 2003; Bhattacharyay *et al.* 2005; Di Veroli *et al*., 2008). For instance, Jeyasingham and Ling (2000) used antennal deformities as sub-lethal responses to arsenic and organic pollution in two New Zealand lakes. Similarly, Janssens de Bisthoven and Gerhardt (2003) also used antennal deformities to examine the effects of chemical and thermal pollution and noted elevated level of deformities compared to the unpolluted rivers. Similarly, Bhattacharyay *et al.* (2005) reported significantly higher levels of antennal deformities in the metal polluted River Damodar in India. However, of all the structures, the mentum has provided the greatest amount of information on sediment toxicity and on deteriorating water quality (Lenat, 1993; Jeyasingham and ling, 2000; Nazarova *et al.,* 2004; MacDonald and Taylor, 2006; Ochieng *et al.,* 2008; Di Veroli *et al*., 2012a; 2012b). The mentum seems to have a wider pollution response range, it can easily be prepared for examination, and deformities quantified rapidly (Odume, 2011). The mentum was used to evaluate the effects of agricultural activities and effluent discharges in selected streams and a lake in north Nova Scotia, Canada (MacDonald and Taylor, 2006). The authors reported high incidences of mentum deformities at the mixing zone, where the discharged effluent mixes with stream water. Ochieng *et al.* (2008) also reported significantly higher incidences of mentum deformities from Lake Victoria in Uganda.

Several field and laboratory studies implicated metals as the main agents of observed chironomid larval deformities (Lenat, 1993; Diggins and Stewart, 1998; Martinez *et al.*, 2002; Jassen de Bisthoven and Gerhardt, 2003; Nazarova *et al.* 2004; Bhattacharyay *et al*., 2006; Martinez *et al.,* 2006; Dias *et al.* 2008; Ochieng *et al.,* 2008). For example, Diggins and Stewart (1998) noted that increased chironomid larval deformities corresponded with increased levels of trace element concentrations in the Buffalo River, New York. Similarly, in the Coeur d'Alene River, Martinez *et al.* (2002) reported a significant correlation between incidences of deformities and metal concentrations, including arsenic, cadmium, copper, lead and zinc. Although metals are extensively implicated as causal agents of deformities, in Vermeulen (1995) review, it was noted that several other agents could also induce

deformities in Chironomidae larvae. Other causal agents include DDT (Madden *et al.* (1992), 4-n-nonylphenol (Meregalli *et al.*, 2001), acid mine drainage (Janssens de Bisthoven *et al.*, 2005), and organic pollution (Servia et al., 1998; 2000; MacDonald and Taylor 2006). For example, Meregalli *et al.* (2001) reported significantly high incidences of mentum deformities in *Chironomus riparius* exposed to 4-n-nonylphenol. Janssens de Bisthoven *et al.* (2005) also reported that chironomid larvae at sites affected by acid mine drainage had high incidences of deformities.

A growing interest among researchers for the use of chironomid larval morphological deformities for assessing and monitoring deteriorating water quality and sediment toxicity led to the development of deformity-based indices. A simple and widely used index for quantifying community incidences of deformities (CID) is the percentage of deformed individual to the total number of larvae sampled. Reference levels of deformities were estimated using fossil analysis to range from zero to 0.8% (Warwick, 1980). However, several real-time field-based studies in mildly polluted sites estimated reference levels of community incidences of deformities to range from zero to 8% and a site having a value above 8% is considered significantly polluted (Warwick 1985; Vermeulen, 1995; Nazarova *et al.*, 2004; Ochieng *et al.*, 2008). Nevertheless, since ecological conditions vary between regions, applying such arbitrarily determined reference deformity levels for all studies is questionable. It therefore remains important that each study objectively determine its reference value, taking account of prevailing ecological conditions.

Warwick (1985) developed the pioneering index, the index of severity of antennal deformation (ISAD), which is base on the apparent severity of deformities in the antennae of *Chironomus* larvae. This index was later extended to the ligula of *Procladius* i.e. index of severity of ligula deformation (ISLD) (Warwick, 1991). However, these two indices are highly technical (i.e. requiring that operators are extremely familiar with the configuration of the antenna and ligula, with a very difficult scoring system for observed deformities), limiting their applications. A more widely used index is the Lenat's toxic score (Lenat, 1993). The toxic score index (TSI) considers the severity of deformities in the mentum of *Chironomus* larvae. The author classified deformities in the mentum into three classes according to their apparent severity. These classes include Class I: larvae having slight deformities, which were difficult to distinguished from breakage or abrasion of the teeth; Class II: larvae having severe and clear deformities including extra teeth, fused teeth, missing teeth, large gaps and

distinct asymmetry; Class III: larvae having severe deformities including at least two Class II types of deformity (Lenat, 1993). The TSI assigned higher weighting to severe deformities and is expressed as:

$$\text{TSI} = \frac{[\text{No. of Class I} + 2(\text{No. of Class II}) + 3(\text{No. of Class III})]\text{X } 100}{\text{Total No. of larvae}}$$

Other authors have successfully used the TSI for biomonitoring organic pollution (e.g. Servia *et al*., 2000; Al-Shami *et al.*, 2011). However, a limiting factor of the TSI is that it is based on only the *Chironomus* genus, thereby ignoring the responses of other chironomid genera. The rationale for restricting the index to only *Chironomus* could be related to its frequent abundance in freshwater ecosystems, and the ease with which deformities can be quantified. However, the genus *Chironomus* does not always occur in sufficient numbers (at least 25 individuals), particularly at less impacted sites (Odume, 2011). Therefore, the index cannot be use for biomonitoring sites that do not have sufficient numbers (at least 25 individuals) of *Chironomus*.

Warwick (1988) and Burt (1998) hypothesised that each chironomid genus responds within a range of pollutant concentrations, and consequently, chironomid genera would be differentially sensitive in expressing deformities. This hypothesis was supported by the work of Odume *et al.* (2012b) who reported differences in the level of deformities among chironomid genera collected from the same sites. In addition, apart from sensitivity of a taxon, which may influence its expression of deformities, other factors such as niche inhabited and history of previous exposure, whether a taxon have developed capacity to adapt to a particular stressor may also influence expression of deformities in chironomid larvae. **Therefore, the present study builds on the TSI by developing an extended toxic score index (ETSI) that take account of the tolerance of chironomid species to deteriorating water quality in the Swartkops River.**

Although current biomonitoring approaches can adequately measure the impact of deteriorating water quality on biotic communities, they cannot concretely establish cause-effect relationship because of their heavy reliance on statistical inferences. To be definitive in deciding on appropriate remedial and mitigation measures in polluted water bodies, it is important to establish an evidence-based cause-effect relationship. Aquatic ecotoxicology fills this gap by providing empirical cause-effect evidence of the relationship between stressors and biota through experimental manipulations.

1.7 Aquatic ecotoxicology

Protecting water resources requires a thorough understanding of pollutant or toxicant effects on aquatic ecosystems by manipulating experiments to establish an evidence-based cause-effect relationship. Aquatic ecotoxicology provides data needed to explore a cause-effect relationship between stressors and biota (Rand, 1995; Mensah *et al.,* 2012). Traditionally, the ecological effects of environmental stressors (e.g. chemicals) on aquatic biotic communities are assessed using single species tests in the laboratory. Depending on the duration of exposure and the endpoints measured, these tests are termed acute or chronic. Acute toxicity tests are short-term tests that last for 48 h or 96 h, usually measuring mortality as an endpoint (Mensah *et al.,* 2011). Chronic toxicity tests last longer, and in addition to long-term mortality, sub-lethal effects such as growth, reproduction, changes in behaviour, enzymatic activities, deformities, histological alterations, are measured as endpoints. Many of these single-species acute and chronic toxicity tests have been standardised, and are widely use in managing water resources (Warne, 2001; Warne *et al.,* 2004; Moiseenko, 2008).

Although these tests are widely used in managing water resources, they are unable to provide direct community or ecosystem level effects, and they therefore rely heavily on laboratory to field extrapolations by applying safety assessment factors or using the species sensitivity distribution (SSD) approach (Schmitt-Jansen *et al.,* 2008; Jesenská *et al.,* 2011). **To reduce the uncertainty associated with extrapolating single-species tests, and to obtain more ecologically relevant and realistic assessments, this study employed the multispecies model-stream ecosystem approach to assess experimentally wastewater effluent effects on macroinvertebrate communities**. The multispecies model-stream ecosystem approach occupies an intermediate space between field biomonitoring studies and the traditional single-specie laboratory-based approach. If reasonably controlled, manipulated and replicated, they can simulate community and even ecosystem effects that can be linked to field-based observations (Ledger *et al.,* 2009; O'Brien *et al.,* 2010). While the single-species approach offer high degree of precision, repeatability and simplicity, the model-stream ecosystem approach represents a compromise between these factors, and their high environmental realisms and their ability to accurately, predict community effects (Buikema and Voshell, 1993; Ledger *et al.,* 2009). Therefore, the multispecies model-stream ecosystem approach used in this study focuses more on the ecological basis underpinning aquatic ecotoxicology than the toxicological aspects that emphasised a dose-response relationship.

Both ecologists and ecotoxicologists have successfully used model-stream ecosystems to study effects of toxicants on biota (Culp *et al.*, 2000; Dube *et al.*, 2005; Cox, *et al.*, 2007; Liess and Beketov 2011; Berghahn *et al.*, 2012; Choung *et al.*, 2013). Model-stream ecosystems are termed mesocosms or microcosms depending on their sizes and locations (Odum, 1984; Buikema and Voshell, 1993; Hill *et al.*, 1994; Palmer *et al.*, 1996; Belanger *et al.*, 1997). For example, Odum (1984) defined mesocosms as outdoor experimental streams bounded and partially closed, which closely simulate the natural conditions. Buikema and Voshell (1993) use volume as a factor for differentiating between microcosms and mesocosms, referring to microcosms as experimental streams (usually indoor) with a volume equal or less than 10 m^3 and mesocosms as those (usually outdoor) having a volume greater than 10 m^3. Hill *et al.* (1994) on the other hand, defined mesocosms as experimental streams that are more than 15 m long and microcosms as those that are shorter. However, Belanger (1997) review revealed that increased physical sizes of experimental streams did not correspond to increased biological complexity. Since the goal of a multispecies model-stream ecosystem is to achieve an adequate ecological realism irrespective of size, the terms ”microcosm” or “mesocosm” were not used in this study. Instead, this study adopted the term “model-stream ecosystems” as the appropriate terminology.

Model-stream ecosystems have several advantages over conventional single species toxicity tests. They enable the simulation of natural conditions, offering a high degree of environmental realism and enabling complex biophysical interaction (Buikema and Voshell, 1993; Petersen *et al.*, 1999; Wong *et al*., 2004). They enable the researcher to evaluate direct effects of pollutants at higher biological organisation such as population, community and even ecosystem levels (Buikema and Voshell, 1993; Petersen *et al.*, 1999; Pettigrove and Hoffmann, 2005; Colville *et al.*, 2008; Choung *et al.*, 2013). Moreover, they enable the study of biotic interaction and community dynamics and measurement of indirect ecosystem effects (Buikema and Voshell, 1993; Wong *et al* 2004; Beketov *et al* 2008b; Ledger *et al.*, 2009; Beketov and Liess, 2011; Spooner *et al.*, 2012).

Most studies using model-stream ecosystems focus on investigating effects of single chemicals adopting the regression approach without replication (Liess and Beketov, 2011). The lack of replication has been justified by several workers who argue that variability within system units is more important than variability between systems (Lamberti and Steinman, 1993). The underlying assumptions of the regression approach are that, at the beginning of the experiment, model streams are similar then develop progressively in a similar pattern and

therefore the measured response is dose-dependent (Wong *et al.*, 2004). However, replication is important to enhance the power of detecting effects, and to capture variability that is not related to dose effects, but which could be useful for interpreting results (Culp *et al.,* 1996; 2000; Wong *et al.,* 2004). **Therefore, the replication approach was followed in this study to test among other things, whether significant biological variability exist between systems of the same treatment.**

The use of model-stream ecosystems to understand toxicant effects on biotic communities has received little attention in South Africa compared to technologically advanced countries (Palmer *et al.,* 1996; 2004b; DWAF, 2000; Williams *et al.,* 2003; Kefford *et al.,* 2004). The few studies that have employed the method focused on understanding toxicant effects on single species, thereby having similar limitations as single-species toxicity tests (DWAF, 2000; Kefford *et al.,* 2004). For example, Muller and Palmer (2002) applied model streams to evaluate the effects of artificial effluent on indigenous mayfly species collected from the Vaal River system. The experiment was based on a single species, measuring mortality over 96 h. Using model streams in this way does not enable the measurement of direct community effects or complex biological interaction. **Therefore, because the Swartkops River received wastewater effluent discharges, developing multispecies model-stream ecosystems that enable measuring long-term effects of effluents on macroinvertebrate community structure could provide an exploration of cause-effects relationships between the wastewater effluent discharges and the river's biota. It is an ecologically-based approach that enables a thorough understanding of effects of wastewater effluents at higher biological organisation and the results of the approach can complement the current chemistry-based method of measuring effluent quality compliance in South Africa**.

In South Africa, the Direct Estimation of Ecological Effects Potential (DEEEP) approach is proposed as a complement to the chemistry-based approach for measuring potential effluent effects (Slabbert, 2004). The DEEEP approach encompasses several tests evaluating the potential ecological effects of discharged effluents on receiving water bodies. These include oxygen depletion potential, analysed as chemical oxygen demand (COD) and 5-day biochemical oxygen demand (BOD_5), effluent persistence potential and mutagenicity, and toxicity tests (Slabbert, 2004). The toxicity tests prescribed in the DEEEP approach are both single-species acute and chronic tests involving organisms from different trophic levels (i.e. fish, invertebrates and algae). Because the toxicity tests specified in the DEEEP approach are

all single-species tests, they have the inherent limitations associated with the single-species tests (Buikema and Voshell, 1993). **Therefore, in this study, multispecies model-stream ecosystems were developed to investigate macroinvertebrate community responses to long-term wastewater effluent effects and the results were interpreted in the context of observed field-based biomonitoring results. This enables an exploration of cause-effect relationship between the Swartkops River biota and the discharges of wastewater effluents.**

1.8 Rationale and Significance of the Study

From the foregoing literature review, it is clear that, to achieve the overarching principles of sustainability and equity enshrined in the South African National Water Act, it is important to develop novel tools for quantifying in-stream biotic deterioration occasioned by water quality impacts and understanding the effects of discharged wastewater effluents on receiving water-biotic integrity. Given that wastewater effluent discharges contribute to deteriorating water quality of many rivers, including the Swartkops River, an integrated, multi-criteria approach was employed to better understanding how wastewater effluent discharges contributed to biotic deterioration in the Swartkops River, which drains an urban and industrial catchment. This involved developing new biomonitoring tools and multispecies model-stream ecosystem approach to deepen an understanding of the effects of deteriorating water quality on the macroinvertebrate community structure and function of the river. Understanding the community effects of discharged wastewater effluent would provide a solid conceptual framework for managing environmental water quality in the Swartkops River. This study contributes to this using: i) a newly-developed Chironomidae deformity-based sub-lethal index; ii) family-level taxonomic and traits-based community analysis iii) species-level taxonomic and traits based community analysis iv) and experimental investigation using the model stream ecosystem.

1.9 Aim and Objectives of the Study

1.9.1 Aim

The overall aim of this study therefore was to undertake an evaluation of macroinvertebrate-baseed biomonitoring and ecotoxicological assessments of deteriorating water quality in the Swartkops River.

The following objectives were set to achieve this overall aim.

1.9.2 Objectives

i) To investigate the impacts of deteriorating environmental water quality on macroinvertebrate communities in the Swartkops River, using family- and chironomid species-level taxonomically- and family-level traits-based biomonitoring approaches;

ii) To develop a novel chironomid traits-based approach (TBA) for biomonitoring the Swartkops River, with potential for use in other rivers receiving wastewater effluent discharges;

iii) To develop a chironomid deformity-based sub-lethal biomonitoring tool for monitoring the Swartkops River with potential for use in other river systems;

iv) To develop a model-stream ecosystem method to investigate and understand long-term effects of wastewater effluent on macroinvertebrate community structure;

v) To integrate the multi-criteria approach to propose tools for managing environmental water quality in the Swartkops River.

The objectives listed above, lead to the following guiding research questions:

- What are the impacts of deteriorating water quality on macroinvertebrate communities in the Swartkops River?
- What macroinvertebrate traits can be use for biomonitoring in an effluent-receiving river, and how can they contribute to managing water resources in South Africa?
- Does the model-stream ecosystem approach enable exploration of cause-effect relationship between the Swartkops River biotic deterioration and wastewater effluents?
- How would the model-stream ecosystem approach contribute to protecting effluent-receiving **river resource?**

1.10 Thesis Structure

Chapter 1: This general introduction and literature review chapter contains the rationale for undertaking this study having done extensive review of existing literature. It concludes with the aim, objectives, guiding research questions and thesis structure.

Chapter 2: This is a general materials and methods chapter describing the study area, sampling sites and protocols, methods and approaches used, and statistical analyses.

Chapter 3: This is the first results chapter in which a critical evaluation of the taxonomically-based family-level SASS5 and multimetric index approach in relation to river health was provided. The chironomids species-level response to deteriorating water quality was explored and insight gained critically discussed. The chapter addresses aspects relating to the family- and chironomid species-level taxonomically-based approach in Objective 1.

Chapter 4: The results for the family-level traits-based approach were presented and discussed in this chapter. The chapter addresses aspects relating to the family-level traits-based approach in Objective 1.

Chapter 5: In this chapter, a novel chironomid traits-based approach was developed and used as a biomonitoring tool in the Swartkops River. The results provided adaptive and mechanistic basis for interpreting chironomid species response to deteriorating water quality in the Swartkops River. Objective 3 was addressed in this chapter.

Chapter 6: Deformities in the mentum of chironomid species larvae were screened as sub-lethal instream response to deteriorating water quality in the Swartkops River. This chapter presents the results and discussion of the deformities. It addresses Objective 4 of this study.

Chapter 7: The results of macroinvertebrate community response to treated wastewater effluent effects in the model-stream ecosystems were presented and discussed in this chapter, thereby addressing Objective 5 of this study.

Chapter 8: This is a concise discussion of all results from previous chapters and their evaluation and integration to propose tools for managing water quality in the Swartkops River.

CHAPTER 2: GENERAL MATERIALS AND METHODS

The aim of this chapter was to provide a description of the study area and sampling sites, common methods, procedures and approaches used in this study. Methods applicable only to specific chapters are described in the relevant chapter(s). This chapter describes the general materials and methods employed in this study. It begins with a thorough description of the study river system and human-induced changes to the catchment that influence water quality. Detailed descriptions of the sampling sites, sampling protocols, and of macroinvertebrate taxonomic indices and metrics applied for water quality monitoring are provided. A critical discussion of selected macroinvertebrate traits, and the rationale for selecting them is also provided in the chapter. The method employed for mounting chironomid larvae and screening the mentum for deformities is carefully presented. The chapter concludes with a description of the methods used to assess the habitat integrity of the Swartkops River, collect and preserve water samples, and to measure water physico-chemical variables, followed by a discussion on the statistical analyses and approaches used. The underlying assumptions and principles for each statistical analysis are critically discussed. In discussing the statistical analyses and approaches used, references were made to chapter(s) where the methods are applied in the study. Subsequent chapters provide a brief discussion of specific methods.

2.1 Study area description

The Swartkops River has a catchment of about 1 555 km^2, and is located in the Eastern Cape Province of South Africa. It originates in the foothills of the Groot Winterhoek Mountains, meandering across structurally weak and shale-filled synclines in the upper catchment, and largely gravel-and-stone flood plains of poorly consolidated rocks of the Uitenhage Group in the lower catchment, before discharging into the Indian Ocean at Algoa Bay, about 18 km north of the city of Port Elizabeth (Heydorn and Grindley, 1986; Fromme, 1988; Odume, 2011). The two main tributaries of the Swartkops River, the Kwazunga River to the north and the Elands River to the southwest, originate in the Groot Winterhoek Mountains and descend to the flood plains to form a confluence just above Uitenhage at Kruisrivier forming the Swartkops River (Figure 2.1). The Elands River, which is approximately 46 km in length, is much drier than the Kwazunga River, with multiple, narrow and well-watered ravines

(Heydorn and Grindley, 1986; Haigh, 2002). Two smaller tributaries, the Brak and Chatty Rivers, originate in the plains north of the city of Port Elizabeth and form a confluence with the Swartkops River below Kruisrivier. Overall, the Swartkops River drains a main catchment M10, sub-divided into four quaternary catchments: M10A (Kwa-Zunga River), M10B (Elands River), M10C (Swartkops River: Elands Confluence), and M10D (Swartkops River: Despatch River Mouth) (Haigh, 2002).

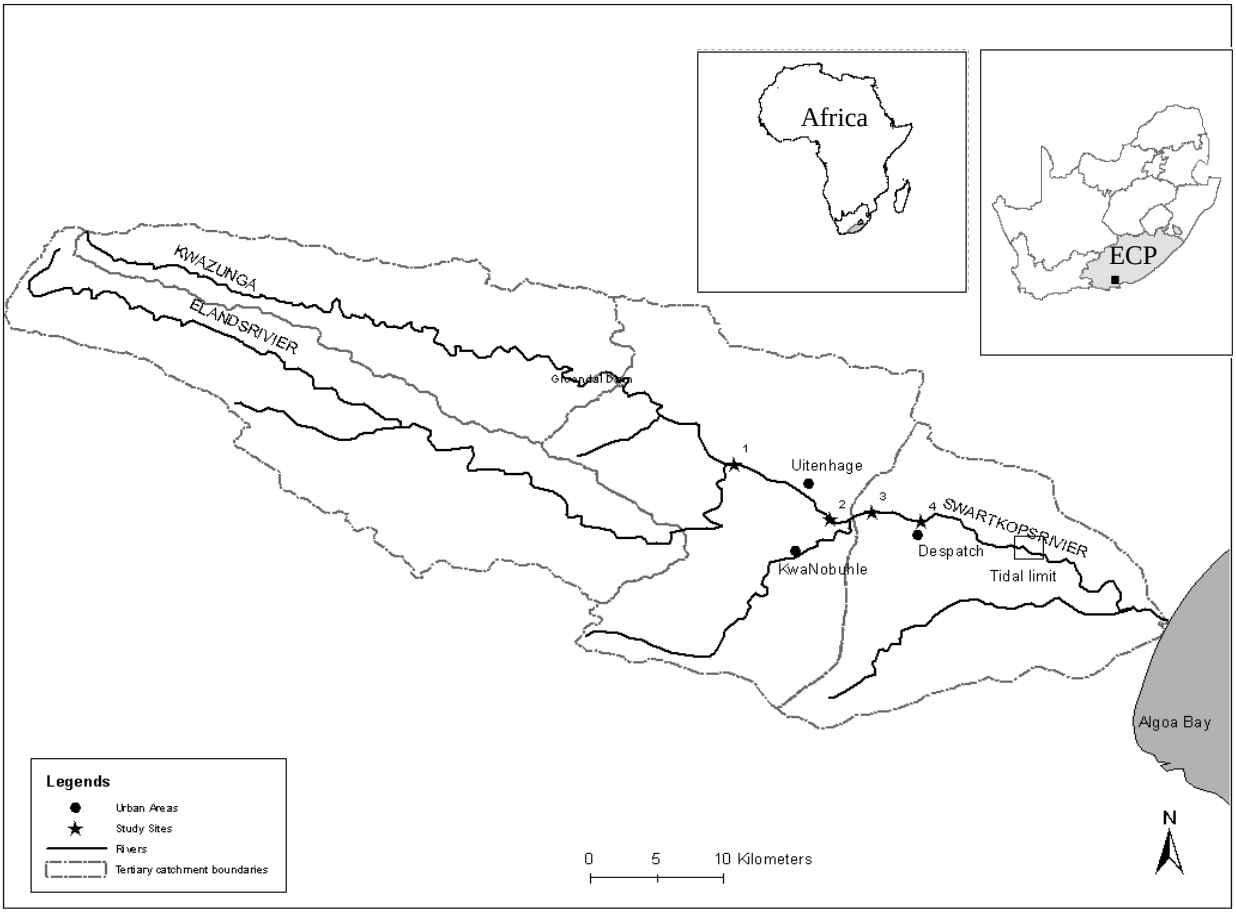


Figure 2.1 Map of study area showing location of sampling sites in the Swartkops River, Abbreviation: ECP = Eastern Cape Province.

The river is an important ecological and socio-cultural asset. Ecologically, the river supports a permanently open estuary that has the third largest inter-tidal salt mash of South African estuaries (Enviro-Fish Africa, 2009; 2011). The estuary is home and breeding ground to many important bird and fish species, and it is considered the most important estuary in the Eastern Cape, ranking eleventh most important in South Africa in terms of biodiversity (DWAF, 1996a; Enviro-Fish Africa, 2009; 2011). During summer, over 3 500 birds can be present along the estuarine salt marsh, but in winter, when the migratory waders and terns

depart after breeding, the numbers can drop to as low as 1 200. Of the over 190 birds species identified in the Swartkops Estuary, five are common: *Larus dominicanus* (Cape Gull), *Pluvialis squatarola* (Grey Plover), *Numenius phaeopus* (Wader), *Sterna hirundo* (Common Tern) and *Calidris ferruginae* (Curlew Sandpiper) (Enviro-Fish Africa, 2009; 2011). Socio-culturally, resources within the Swartkops River catchment are utilised for several socio-cultural purposes, including church baptism, spiritual rituals and cleansing, and medicinal plant harvesting. The estuary is an important recreational site for swimming, boating, angling, and bait digging. In addition to its ecological and socio-cultural significance, the river supplies water for irrigational requirements in its upper catchment.

Geologically, the Swartkops River catchment is mainly of marine, estuarine and fluvial origin (DWAF, 1996a; Maclear, 1995). The upper Cretaceous shale and mudstones, which are overlaid by marine sedimentary deposits in the upper catchment, and by various alluvial deposits on the flood plains, are deposited into a trough formed by the Table Mountain Group quartzite and the Bokkeveld Group shale (Heydorn and Grindley, 1986; Fromme, 1988). The river, in its upper catchment, drains the quartzite of the Table Mountain Group, whereas below the confluence of the Eland and the Kwa-Zunga, it flows through weakly consolidated and easily erodible Cretaceous shale of the Uitenhage Group (Fromme, 1988; DWAF, 1996a; Haigh, 2002; Odume, 2011). The Cretaceous shale are inter-bedded with sandstone and mud stone, greenish-grey slate, siltstones and sandstones, and thinly bedded greenish-grey mudstones and siltstones. The sandstone and mudstone are of the Enon formation (marine, estuarine and fluvial origin), whereas the greenish-grey slate, siltstones and sandstones are of the Kirkwood formation (fluvial origin), and the thinly bedded greenish-grey mudstones and siltstones are of the Sundays River formation, which are of marine origin (DWAF 1996a; Bornman and Klages, 2005). Because of the weak consolidated and easily erodible nature of the geology in the low-lying floodplains, the river below the confluence has eroded its way into the sedimentary deposit of the Uitenhage Group (Maclear, 1995). The water quality implication of this geology, which is mainly of marine origin, is naturally high levels of dissolved salts in the low-lying region of the river.

The upper mountainous catchment of the Swartkops consists mainly of rocks with shallow soils unsuitable for agriculture (DWAF 1996a). However, soils in the lower floodplains, which are derived mainly from the Uitenhage Group, are deep and well suited for agriculture (Haigh, 2002). The main soil types in this part of the catchment are Swartland with Hutton

and Mispah, with a texture characterised mainly by fine sand, loam and clay of consolidated dune sands and lime-rich sandy clay, which are easily erodible (DWAF, 1996a).

Generally, the climate is warm and temperate with a mean daily maximum temperature of about 6 ^{o}C in July and 27 ^{o}C in January (van Eeden and van Jaarsveld, 2006). The rainfall in the Swartkops River catchment varies between the regions, with rainfall decreasing from the upper regions to the low-lying floodplains (DWAF, 1996a; Heydorn and Grindley, 1986). The catchment receives rain throughout the year, with a mean annual rainfall between 300 mm – 750 mm (DWAF, 1996a; Haigh, 2002). Rainfall usually peaks between the months of June and October. Although the rainfall pattern in the catchment could be described as evenly distributed, flooding usually occurs because of heavy precipitation over several days. Mean annual evaporation rate in the catchment is about 1 700 mm, considerably higher than the mean annual rainfall (DWAF, 1996a). However, other catchments to the west are more prone to aridity (DWAF, 1996a; Haigh, 2002). In terms of run-off, the Kwa-Zunga/ Swartkops systems alone contributes about 0.3% of the total annual run-off in South Africa, but the entire catchment has a mean annual run-off of approximately 84.2 $\times 10^{6}$ m^{3} (Bate *et al.*, 2004).

Bushveld and succulent thicket dominate the natural vegetation in the low-lying region of the Swartkops catchment, whereas the upper catchment is dominated by mountainous fynbos (DWAF 1996a; Kleynhans *et al.*, 2005). However, alien invasive vegetation has impacted greatly on the integrity of the catchment. The dominant exotic invaders include gum trees (*Eucalyptus* spp), wattle (*Acacia* spp), cluster pine (*Pinus* spp) and prickly pear (*Opuntia* spp) (Haigh, 2002; Odume *et al.*, 2012a). These exotic plants promote bank instability and sedimentation. They also reduce stream flow by using up available water. In the Swartkops River system itself, particularly at Uitenhage and Despatch, there are extensive and dense growths of common reed (*Phragmites australis*), water hyacinth (*Eichhornia crassipes*) and Kariba weed (*Salvinia molesta*) (Odume *et al.*, 2012a). However, during floods, these weeds are washed away, clearing the river system (*Personal observation*). The presence of water hyacinth, Kariba weed and other invasive species in the river affect both the recreational and aesthetic value of the river, and its in-stream physical habitat integrity.

The Swartkops River catchment is highly urbanised and industrialised, with a greater portion of the municipal areas of Uitenhage, Despatch, KwaNobuhle, Ibhayi and part of Port

Elizabeth all within the river catchment (Binning and Baird, 2001). Because of the urbanised and industrialised nature of the catchment, several sources of pollution, including wastewater effluent discharges, run-off from informal settlements, agricultural farms, surrounding road and rail networks, and industrial sites, severely influence the water quality of the river (Odume, 2011; Odume and Muller, 2011; Odume *et al.*, 2012a).

2.1.1 Anthropogenic influences on the Swartkops River

The Swartkops River flows through pristine and inaccessible mountainous areas in its upper catchment, but in the lower catchment, the river is subject to several human-induced impacts that influence its ecological integrity (Bornman and Klages, 2005; Odume, 2011). The main contributors to the deteriorating water quality in the Swartkops River are discharges from wastewater treatment works (WWTWs). There are three WWTWs in the river's catchment and these are the Kelvin Jones WWTW in Uitenhage, the Despatch WWTW in Despatch, and the KwaNobuhle WWTW in KwaNobuhle. Of these three WWTWs, only the Kelvin Jones is located within the sampling sites and is therefore the focus of this study. It has a design capacity of 24 ML/ day, but currently operates at 18.4 ML/ day, releases over 6 822 500 m^3 per annum into the Swartkops River (DWAF, 1996a) at Site 3 in this study, see site descriptions. The Kelvin Jones wastewater treatment work treats all the wastewater from Uitenhage and since the upgrade to the work in 2008; it operates without being over capacitated (Nelson Mandela Bay Municipality, 2008). Although wastewater effluent discharges influence the water quality of the Swartkops River, they provide for the flow requirements of the lower Swartkops below Uitenhage, contributing approximately 50% of downstream river flow (i.e. below Uitenhage) (Taljaard *et al.*, 1998).

Wool processing factories and the tannery industry in Uitenhage are potential sources of indirect pollution of the Swartkops River. Although effluents from these activities are discharged into evaporation ponds, it is believed that they still influence the water quality of the river due to seepage (Binning, 1999). For example, Binning (1999) estimated that about 50 000 to 100 000 litres of wash water from wool processing factories in Uitenhage seeps into the river. The Transnet Cuylor Manor Plant in Uitenhage discharges its effluent into settling dams where it is treated with alum before further discharging into the Swartkops River. Furthermore, storm-water canals, which often carry untreated wastewater and litter, flow into the river. Diffuse sources of pollution come mainly from agricultural activities and run-off from informal settlements and surrounding roads and rail networks. About 15% of the

catchment is subject to crop cultivation and livestock farming, particularly poultry, contributing to in-stream nutrient levels because of run-off from farmlands (DWAF, 1996a; de Villiers and Thiart, 2007). Overall, the combined discharges from point sources and run-off from diffuse sources have substantially contributed to the generally poor water quality and biotic integrity of the river (Odume *et al.,* 2011).

The geology of the Swartkops River catchment made it an attractive site for sand and gravel mining. Several sand and gravel mining activities take place in the catchment, particularly at Uitenhage and Despatch, and in some cases, on the riparian zone of the river. Sand and gravel mining may have several ecological consequences, which may manifest in the instream and riparian habitats (Bornman and Klages, 2005). Instream effects include modifying channel morphology, altering stream roughness and general hydraulics, sedimentation due to bank instability and bed coarsening. Riparian habitat effects include destroying riparian vegetation and canopy cover. Since the riparian zone plays important role in pollution and erosion control, destroying it may have deleterious effects on instream habitats and biota (Padmala *et al.,* 2008). Altering the instream physical and riparian habitats can also have water quality effects, including increased suspended sediments, turbidity, and mobilisation of adsorbed metal ions due to sediment agitation. These effects can alter the natural biological processes by causing changes that favour some species over others and could cause overall decline in biological diversity (Padmala *et al.,* 2008). The mining activities taking place on the Swartkops River catchment have altered the river morphology at both Uitenhage and Despatch. There is evidence of sedimentation at Despatch due to stockpiling, and destruction of both in-stream and riparian habitats due to bulldozing and excavation at both Uitenhage and Despatch.

Several man-made obstructions along the Swartkops River and its tributaries modify the natural flow of the river. The Groendal Dam, with a storage capacity of approximately 12×10^6 m^3, is the most important in terms of its size and potential effects on downstream flow (Fromme 1988; Bate *et al.,* 2004). However, because the storage capacity of the dam is small compared to the mean annual run-off (14% of mean annual run-off), it has little overall effect on downstream river flow (Heydorn and Grindley, 1986). In addition, below the Groendal Dam, there are at least four causeways along the river length, and some of these form artificial pools, impeding the river flow. Culverts in the river course also cause increased water velocity.

2.1.2 Sampling sites

The study was undertaken seasonally at four selected sites over a period of three years, between August 2009 and September 2012. Site 1 (33^{o} $45^{'}$ 08.4" S, 25^{o} $20^{'}$ 32.6" E), situated upstream of Uitenhage, was selected to represent the reference or least impacted site, in agreement with the recommendations of Reynoldson *et al.* (1997) and Dallas (2000), and was also based on expert judgement. The reference site is described as the least disturbed site in terms of physical, chemical and biological characteristics (Reynoldson *et al.*, 1997). Since the reference site was use as a benchmark for interpreting results, and organising other sites into ecological categories that reflect deviation from the reference assemblages, caution was exercised and several factors considered in selecting the site. These factors include the availability and diversity of macroinvertebrate habitats and biotopes, the extent of surrounding and instream impacts, the water chemistry, the site's accessibility and location within the same ecoregion level II as the other sampling sites. Within the accessible areas in the Swartkops River, this site represents the best available condition in the river in terms of the factors mentioned above (Figure 2.2).

It was important to select a reference site within the same ecoregion level II as other sampling sites because geo-morphological and instream conditions naturally differ between different ecoregions and this may lead to natural variation in macroinvertebrate communities sampled from sites in different ecoregions (Kleynhans *et al.,* 2005; Dallas and Day, 2007). However, within an ecoregion, there are relative similarities in both biotic and abiotic components of the ecosystem and thus, macroinvertebrates collected at sites within the same ecoregion should be naturally similar (Kleynhans *et al.,* 2005).

Figure 2.2: Site 1 in the Swartkops River, showing the biotopes sampled.

The Groendal Dam is located about 16.4 km upstream of this site and it may have some impacts on the site's macroinvertebrate communities. Sites downstream of a dam have been reported to have preponderance of Ecnomidae, Hydropsychidae, Hydraenidae, and a significant drop in species diversity of Ephemeroptera, Plecoptera and Trichoptera (EPT) taxa (Bredenhand and Samways, 2009). However, considering the factors mentioned above for selecting the site, its use, as a reference site in this study was unavoidable.

Site 2 (33^{o} 47$^{'}$ 29.0" S, 25^{o} 24$^{'}$ 26.4" E) is in the industrial city of Uitenhage, where surrounding impacts include run-off from roads and informal settlements, livestock farming and other agricultural practices. Between 2009 and 2010, part of the river reach at the site was covered with water hyacinth and other aquatic weed, but flooding in 2011 cleared them. Although there is evidence of moderate habitat modification on the riparian zone, all sampling biotopes were adequately represented (Figure 2.3). This site is located upstream of the discharge point of the Kelvin Jones WWTW.

Figure 2.3: Site 2 in the Swartkops River at Uitenhage, showing the biotopes sampled

Site 3 (33^{o} 47' 11.8" S, 25^{o} 25' 53.97" E) is further downstream, but also within the industrial city of Uitenhage, where surrounding impacts include industrial and wastewater effluent discharges, run-off from road and rail networks, and agricultural activities. The site is just downstream of effluent discharge point from the Kelvin Jones WWTW in Uitenhage. Therefore, the main difference between this site and Site 2 is the discharges of wastewater effluents from the Kelvin Jones WWTW. Between 2009 and 2010, nearly the entire river reach at this site was covered with water hyacinth, common reed and Kariba weed, but flooding in 2011 cleared them. There is evidence of moderate riparian zone and in-stream habitat modifications, but all sampling biotopes were adequately represented, except for the mud biotope, which was not adequately represented (Figure 2.4).

Figure 2.4: Site 3 in the Swartkops River at Uitenhage, showing the biotopes sampled on the left and extensive growth of aquatic weeds on the right. Aquatic weeds were cleared by floods in 2011.

Site 4 (33^{o} 47$^{'}$ 34.0"S, 25^{o} 27$^{'}$ 58.7"E) is in Despatch and is further downstream of Site 3, where the Kelvin Jones WWTW discharges effluent into the river. The site was also impacted by municipal run-off, as well as by sand and gravel mining in its riparian zone. There is evidence of the impacts of the wastewater effluent discharges of the Kelvin Jones on the water quality and benthic community structure at the site (Odume *et al.*, 2012a). A small access road with a culvert is built across the site, modifying the river flow. Extensive riparian habitat degradation due to the sand and gravel mining activities on the riparian zone is evident. Site 4 was covered with a thick growth of aquatic weeds between 2009 and 2010, but in 2011, they were cleared by floods. The instream sampling habitats were generally adequate and good, with limited gravel biotope (Figure 2.5). Although Site 4 is not as polluted as Site 3, it would have been good to select another site further downstream to monitor for potential system recovery. However, the tidal limit at Perseverance between the estuary and the freshwater section is only a short distance downstream of Site 4. Consequently, it was not possible to select a fifth site further downstream because of likely estuarine effects on both water chemistry and biological communities. The physical characteristics of the four sampling sites are summarised in Table 2.1

Figure 2.5: Site 4 in the Swartkops River at Despatch, showing the biotopes sampled on the left and the instream condition after floods in 2011 on the right.

Table 2.1 Physical characteristics of the four sampling sites in the Swartkops River. Note that grass includes reeds and shrubs include trees.

	Site			
Physical characteristics	**1**	**2**	**3**	**4**
Water Velocity (m/s)	0.232 – 0.433	0.2 – 0.743	0.316 – 0.9	0.3 – 1.009
Approximate width (m)	>5 - 10	>5 - 10	>5 - 10	>5 – 10
Approximate depth (m)	0.21 -0.56	0.14 – 0.63	0.07 – 0.37	0.07 – 0.67
Percent Left ban cover (rocks and vegetation)	81 - 95	81 – 95	>95	>95
Percent right bank cover (rocks and vegetation)	81 - 95	51 – 80	>95	>95
Riparian vegetation	Grass and shrubs	Grass	Mostly grass and shrubs	Mostly grass
Impacted with wastewater effluent discharges?	No	No	Yes (site immediately downstream of WWTW).	Yes (site further downstream of WWTW).

2.2 Sampling macroinvertebrates

Over a period of three years, macroinvertebrates were seasonally sampled in late August (spring) and late November (summer) 2009, March (autumn) and July (winter) 2010, September (spring) and December (summer) 2011, April (autumn) and September (spring) 2012. The second sets of winter samples to be collected between June and July 2012 were not collected because the river was flooded. Instead, samples were collected in September 2012 (additional spring samples). Macroinvertebrates were collected using a kick net (300 x 300 mm frame, 1000 µm mesh) according to the South African Scoring System version 5 protocol (SASS5) (Dickens and Graham, 2002). On each sampling event, macroinvertebrates were sampled from three distinct biotope groups: stones (stones-in-and-out-of-current), vegetation (marginal and aquatic vegetation), and sediment (gravel, sand and mud, GSM). The stone-in-current (SIC) includes pebbles and cobbles (2 – 25 cm), and boulders greater than 25 cm situated in current that prevents the deposition of fine silt or sediments. Stone-out-of-current (SOOC) includes pebbles and cobbles, and boulders in pools that allows the deposition of fine silt and sediments. Marginal vegetation includes vegetation growing on the edge of the river and fringing into the river, whereas aquatic vegetation was largely submerged in the main river course. Gravels are small stones usually less than 2 cm in diameter, while sand and mud are smaller, less than 2 mm and 0.06 mm respectively. The three distinct biotope groups are hereafter referred to as stone, vegetation and GSM, unless otherwise stated.

The sampling protocol requires collecting only one sample per biotope group. However, on each sampling occasion at each of the sampling sites, three replicate samples were collected per biotope, making nine samples per site per sampling season (or event). Consequently, a total of 72 samples (i.e. three samples x three biotope x eight sampling events) were collected per site during the study period. However, at some of the sites, three replicate samples could not be collected because of biotope inadequacy during some of the sampling events. Replicate samples were collected in order to assess the representativeness of a single sample as provided for by the SASS5 protocol, and to enable robust statistical analyses of the data to reduce errors inherent with inadequate replications.

Sampled macroinvertebrates were tipped into a white rectangular tray, half-filled with river water, and macroinvertebrate families identified on site using identification keys by Gerber and Gabriel (2002). The identified families were recorded on a SASS5 sheet together with

their abundance estimates. Once field identification was completed, samples were preserved in 70% ethanol, transported to the laboratory for sorting, mounting, further identification and abundance counts. To ensure quality assurance and control with regard to field identification, macroinvertebrates collected per biotope at each sites were identified for a maximum of 15 minutes, but identification was stopped if no new taxa was seen. The initial field identifications were undertaken as part of the SASS5 protocol, but laboratory identifications served to confirm field identifications. Chironomid larvae were sorted, mounted according to Odume and Muller (2011), and identified as far as practically possible under an Olympus compound microscope (B X 51) using the keys described by Wiederholm (1983), Cranston (1996) and Harrison (2003). Section 2.4 provides further details on chironomid larvae mounting procedure.

2.3 Macroinvertebrate-based taxonomic indices applied for assessing river health

Two main categories of taxonomic indices were applied in this study. These include a single biotic index approach, the South African Scoring System version 5 (SASS5) and a multimetric index approach that integrates multiple taxonomic metrics.

2.3.1 The South African Scoring System version 5

The South African Scoring System version 5 (SASS5) is a rapid bioassessment index based on the presence or absence of selected families of aquatic macroinvertebrates and their perceived sensitivity or tolerance to changes in water quality (Chutter, 1998; Dickens and Graham, 2002). Macroinvertebrate families are awarded scores based on their perceived sensitivity or tolerance in the range of 1 – 15 in increasing order of sensitivity to deteriorating water quality. The results are expressed both as an index score i.e. SASS5 score and as an average score per recorded taxon (ASPT) value. The SASS5 score is calculated by summing the scores of all recorded families, while the ASPT value is obtained by dividing the total SASS5 score by the number of families recorded. The ASPT value, compared to the SASS5 score, is more stable and consistent spatio-temporally and provides a more accurate reflection of river health condition (Dickens and Graham, 2002). According to Dickens and Graham (2002), in addition to assessing water quality, SASS can be used to assess emerging water quality problems, development impacts, ecological state, and spatio-temporal trends, and contributes information to determining ecological Reserve.

Since lotic ecosystems are naturally heterogeneous, variability in biotic composition is inherent, especially between systems in different eco-regions characterised by differing geology, soils, and geomorphology, and substratum, hydraulic and hydrological conditions (Kleynhans *et al.,* 2005; Dallas and Day 2007). To avoid the confounding effects of these natural variations in interpreting SASS results, Dallas (2007b) developed guidelines for interpreting SASS5 data that account for geographical and longitudinal variations (geo-morphological zones). The guidelines stipulate range of SASS5 scores and ASPT values indicating different ecological categories reflective of water quality conditions for the upper and lower areas of each geo-morphological zone. The Swartkops River is within the southern eastern coastal belt (lower zone) and the ranges of SASS5 scores and ASPT values for this zone were applied in this study to interpret SASS5 data (Chapter 3).

Table 2.2: Range of SASS5 scores and ASPT values indicative of the different ecological category and water quality for the southern eastern coastal belt lower zone eco-region (Dallas, 2007b).

Ecological category	**Water quality category name**	**Description**	**Range of SASS5 scores**	**Range of ASPT values**
E/F	very poor	seriously/ critically modified	< 62.9	< 5
D	poor	largely modified	63 - 81.9	5.1 - 5.3
C	fair	moderately modified	82 - 99.9	5.4-5.9
B	good	largely natural with few modifications	100 - 148.9	6.0-7.0
A	natural	unmodified	149 - 180	7.1-8

2.3.2 Taxonomic multimetric-based approach

The multimetric approach combines a range of metrics and indices representing different aspects of macroinvertebrate taxonomic measures to assess river health. The approach is perceived to be superior to a single biotic index approach because it integrates information from the individual, population, community and even the ecosystem levels to provide a river health assessment (Baptista *et al.,* 2007; Mondy *et al.,* 2012). In this study, 10 metrics in four categories i.e. abundance (number of individuals), composition (relative abundance), richness

(number of taxa), and diversity selected based on their discriminating efficiency in the Swartkops River were further investigated to enable the development of a Swartkops River multimetric index (Table 2.3; Chapter 3). The 10 selected metrics proved sensitive to deteriorating water quality in the Swartkops River, distinguishing between reference and impaired sites (Odume *et al.*, 2012a).

Table 2.3: Selected taxonomically-based macroinvertebrate metrics of abundance, community composition, richness and diversity applied to the macroinvertebrate data collected in the Swartkops River. Metrics defined and selected based on the work of Odume *et al.* (2012a). + indicates that the metric increases with deteriorating water quality, and – indicates that the metric decreases with deteriorating water quality Abbreviation of metrics: ETOC (Ephemeroptera-Trichoptera-Odonata-Coleoptera), ET (Ephemeroptera-Trichoptera).

Metrics	**Definition**	**Predicted response to deteriorating water quality**
Abundance measures		
Trichoptera abundance	Absolute number of individuals in Trichoptera taxa	-
Measures of composition (relative abundance)		
Percent Chironomidae + Oligochaeta	Percentage of individuals in Chironomidae + Oligochaeta taxa relative to entire sample	+
Percent Trichoptera	Percentage of individuals in Trichoptera taxa relative to entire sample	-
Percent ETOC	Percentage of individuals in Ephemeroptera, Trichoptera and Odonata relative to entire sample	-
Richness measures		
ETOC richness	Absolute number of taxa in Ephemeroptera, Trichoptera, Odonata and Coleoptera	-
ET richness	Absolute number of taxa in EPT	-
Diversity measures		
Simpson diversity	Weighted towards the abundance of	-

index	commonest families (Ogbeibu, 2005)	
Shannon diversity index	Information statistic index which takes account of the contribution of individual taxa to the diversity while assigning greater weight to most dominant taxa (Ogbeibu, 2005)	-
Margalef's family richnes index	Accounts for both number of taxa and individuals and is independent of sample size (Ogbeibu, 2005)	-
Equitability (or evenness)	Measures the relative even distribution of abundance of taxa within a sample (Clarke and Warwick, 1994)	-

The selected metrics include two diversity indices, i.e. the Shannon diversity index and the Simpson diversity index. The Shannon diversity index is an information statistics index that reflects the abundance of individual taxon, but still assigns greater weight to the commonest taxa. The index is a function of both evenness and species richness. The Simpson index is a dominance index whose value is seriously weighted towards the abundance of the commonest taxa (Clarke and Warwick, 1994; Gray and Delaney, 2008). Although combining metrics reduces the weaknesses of the individual metric, in selecting and combining them, caution must be exercised to avoid redundancy. The multimetric approach was investigated to develop a multimetric index for the Swartkops River biomonitoring, and to ascertain whether additional, useful ecological information and insight can be gained to compensate for the extra time and effort.

2.4 Screening Chironomidae community for mentum deformities

In the laboratory, sorted chironomid larvae were kept in 70% ethanol, and then mounted for taxonomic identification and deformity screening. To mount the larvae, they were transferred from the 70% ethanol into a 10 ml specimen pill vial containing 10% (w: v) cold potassium hydroxide (KOH) solution and were left in the solution for about 10 – 20 minutes to clear (Warwick, 1988; Odume and Muller, 2011). After clearing, larvae were dehydrated using 96% ethanol and finally in absolute ethanol. Dehydrated larvae were transfer into a solution of xylene for about 10 minutes, and the entire larva was removed from the xylene, placed on

a mounting slide containing a drop of Canada balsam. Under a dissecting microscope, and using fine forceps and needles, the head capsule, ventral side up, was dissected from the body segment and placed on a different mounting slide with the same label as the corresponding body segment. Using forceps, a cover slip was placed over the head capsule with the ventral side still up. A gentle rotary force was applied carefully to flatten the head capsule and expose the mouthparts (Odume and Muller, 2011). Slides were left for about five to eight days to air dry. Mounted specimens were then examined for deformities in the mentum under an Olympus compound microscope (B X 51) equipped with an Altra 20 soft imaging system digital camera. The ligula, instead of the mentum, was screened for deformities in the family Tanypodinae, as the mentum is reduced and inconspicuous among members of the Tanypodinae. All specimens with broken mentum and ligula, or having ambiguous deformity types were considered normal. Only specimens with clearly defined deformity types extracted from various sources e.g. Lenat (1993); Janssens de Bisthoven *et al.* (1998); Martinez *et al.* (2002;2004); Ochieng *et al.* (2008); Odume *et al.* (2012b) were considered deformed. The separated body segments were mounted separately to aid identification. Magnifications between X10 and X40 were used, depending on the size of the specimen being examined. Image analysis and photos were taken using the software analySIS Five soft imaging system.

During the course of the work, it was realised that the mounting medium, Canada balsam, required longer processing time and took longer to air dry. Given the number of specimens examined, a decision was made in 2011 to reduce the processing time. Consequently, Canada balsam was replaced with Euparal as the mounting medium of choice, shortening the processing time by eliminating the xylene stage. Euparal also takes less time to air dry, usually between 24 hours and 96 hours.

The chironomid mentum is a double-walled sclerotized and usually toothed medioventral plate of the head capsule posterior to other mouthparts (Armitage *et al.,* 1995) (Figure 2.6). The two walls of the mentum are the median ventral wall (ventromentum) and the dorsal wall (dorsomentum). The mentum also has the ventromental plates, which are lateral or posterolateral expansions (Armitage *et al.,* 1995). The configuration of the teeth on the mentum and ligula were screened for deformities. Figure 2.6 shows a typical *Chironomus* mentum, while Table 2.4 provides the arrangement of teeth in the mentum and ligula of selected chironomid species encountered in this study.

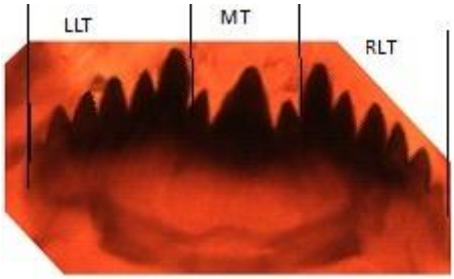


Figure 2.6: A typical *Chironomus* sp. mentum showing the arrangement of teeth. Abbreviations: MT = median teeth, LLT = left lateral teeth and RLT = right lateral teeth.

Table 2.4 Arrangement of teeth in the mentum and ligula in selected chironomid genera encountered during the study. Trifid is defined as having three distinct median teeth as in Figure 2.6 above. Bifid is having two distinct median teeth and laterally crenate median tooth have notches on both sides giving it appearance of three teeth that are not distinct. Summarised from Wielderholm (1983).

Chironomidae Genus	**No. of median teeth (mentum)**	**No. of teeth (ligula)**	**No. of paired lateral teeth (mentum)**
Chironomus	1 trifid	N/A	6
Dicrotendipes	1 laterally crenate	N/A	6
Polypedilum	2	N/A	7
Kiefferulus	1 trifid	N/A	6
Tanytarsus	1 simple, bifid or trifid	N/A	5
Tanypus	N/A	5	N/A
Ablabesmyia	N/A	5	N/A
Clinotanypus	N/A	5 – 7	N/A
Cricotopus	1	N/A	6, rarely 5or 7
Orthocladius	1	N/A	Usually 6, sometime 7-9
Virgatanytarsus	1	N/A	5
Cardiocladius	1	N/A	5

Rheotanytarsus	1 usually crenate laterally	N/A	5
Macropelopia	N/A	5	
Paratrichocladius	1	N/A	6

2.5 Selected macroinvertebrate traits

Macroinvertebrate traits selected for this study are in two broad categories: biological traits and ecological traits. Biological traits are inherent functional characteristics of an organism, reflecting its physiological requirement, morphology, and life history attributes (Vieira *et al.*, 2006). Ecological traits are derived from an understanding of the relationship between the organism and its environment, reflecting its habitat preference, behavioural response and other aspects it employs to adapt to its environment (Vieira *et al.,* 2006). Both sets of traits are capable of providing a mechanistic explanation between a species and its environment, but biological traits are generally phylogenetically constrained, whereas ecological traits are less constrained (Usseglio-Polatera *et al.,* 2000; Poff *et al.,* 2006; Vieira *et al.,* 2006). In selecting traits for this study, several factors were considered and these include:

- a clear demonstrable mechanistic relationship between the trait and the studied stressors or environmental variables;
- the ease of measuring the trait;
- availability of the trait information in the literature;
- the trait's relationship to some adaptive aspects (e.g. resistance and resilience) of the organism or species;
- the role of the trait in the organism's ecological function.

2.5.1 Selected biological traits

The purpose of this subsection is to describe the selected biological traits and the rationale for their selection.

Body size

Animal body size is an important feature that constrains ecological and life-history traits of species (Pianka, 1970; Blanckenhorn, 2000; Hui and McGeoch, 2006). It is an integrative 'taxon-free' classification of individuals, which may provide clues to understanding species' interaction with their environment, and the underlying processes affecting community assemblages (Robson *et al.,* 2005; Siqueira *et al.,* 2008). Changes in the pattern of body-size distribution may reflect the effects of human disturbances, changes in habitat complexity, productivity and biological interactions (Robson *et al.,* 2005; Allen *et al.,* 2006). The Swartkops River receives several contaminants leading to increased dissolved solids, and with organisms exposed to these contaminants through external contact and food ingestion, body size is a critical adaptive feature as many organisms at the polluted sites may seek to maintain a body size that would reduce the ratio of body surface area to volume.

Several metrics exist for quantifying an animal's body size e.g. weight, width, biomass, etc (Robson *et al.,* 2005), but body length was used in this study as a measure of body size. Since many aquatic macroinvertebrates seek to maximize body size to improve adult fitness and reproductive success, Robson *et al.* (2005) argued that body length is the most appropriate measure of body size for most aquatic macroinvertebrates as it is functionally connected to locomotion, predation, and habitat architectures. For these reasons, body length was chosen over other metrics.

The body length of between 10 and 30 individuals of each macroinvertebrate family (including chironomids at the species level) at each sampling site during each sampling season was measured from the tip of the head to the abdominal tip, exclusive of terminal appendages under a dissecting microscope. Measurements were undertaken for only the late juvenile (where the adults is non-aquatic; third and fourth instar larvae for the chironomids) to obtain maximum length. Microscope measurements were converted to length in millimetres using the following formula:

$$\text{Final length} = \frac{\text{Microscope measuremnt x } 10}{\text{Magnification}}$$

Respiratory traits

Direct effects of wastewater effluent discharges in receiving rivers and streams (e.g. the Swartkops River) are reduced dissolved oxygen concentrations, and increased concentration of turbidity due to increased suspended solids (Odume and Muller, 2011). In effluent-impacted rivers and streams, respiratory traits are critical determinants of persistence (both resistance and resilience) because of their direct mechanistic relationships with prevailing environmental conditions. For example, decreased dissolved oxygen concentration will favour organisms having special respiratory mechanisms and apparatuses such as a specialised siphon for breathing atmospheric oxygen, and the presence of oxygen-transporting pigment e.g. haemoglobin. In addition, exposure to increased suspended solids poses the risk of gill clogging to organisms using external gills. To analyse how respiratory traits responded to deteriorating water quality in the Swartkops River macroinvertebrates, the following traits were selected: gills, tegument, spiracles, and specialised breathing siphons, lungs and haemoglobin. Information on macroinvertebrate families respiratory traits was obtained through observation, expert opinion and several published and unpublished literature sources (e.g. Franquet, 1999; Day *et al.,* 2001; 2003; Day and de Moor, 2002a; 2002b; Gerber and Gabriel, 2002; De Moor *et al.,* 2003a; 2003b; Viera *et al*, 2006; Stals and de Moor, 2007; Van Kleef 2010). Haemoglobin is not a direct respiratory apparatus; it was included because of its adaptive importance among the chironomids.

Mobility

Since streams can be considered as a mosaic of patches, with some of these patches acting as refugia during disturbances (Townsend and Hildrew, 1994), type of mobility can determine whether a species can move into refugia, and then re-colonise and re-establish when conditions become favourable. Therefore, high mobility that facilitates macroinvertebrate to access refugia is expected to confer resilience on species (Ladle and Ladle, 1992; Townsend and Hildrew, 1994; Gjerløv *et al.,* 2003). To assess whether there is a pattern in the type of locomotion of macroinvertberates between those in the upstream reference site and downstream sites, sampled macroinvertebrates were analysed based on whether they were climbers, crawlers, sprawlers, swimmers, skaters and burrowers based on observation and literature (e.g. Merritt *et al.,* 1996; Gerber and Gabriel, 2002; Tomanova *et al.,* 2008).

Body shape

Although body shape is not directly related to pollution, they have direct connection with water velocity. The presence of causeways along the Swartkops River, and effluent discharges can alter its normal water velocity. Macroinvertebrate body shapes were resolved into the following classes: streamlined, flattened, spherical, and cylindrical based on observation and literature sources (e.g. Day *et al.,* 2001; 2003; Day and de Moor, 2002b; Gerber and Gabriel, 2002; Tomanova *et al.,* 2008).

2.5.2 Selected ecological traits

The purpose of this subsection is to describe the selected ecological traits and the rationale for selecting them.

Preferred food and feeding habits

The kind of food materials consumed by macroinvertebrates, and the mechanisms by which these food materials are obtained are important ecological traits related to macroinvertebrate ecological function and ecosystem processes (Wallace and Webster, 1996). For example, understanding the food and feeding habits of stream macroinvertebrates provides insight into important ecological function and processes such as nutrient cycling and energy flow. In this study, macroinvertebrate families were assigned to different functional feeding groups: shredders, collector-gatherers, collector-filterers, scrappers (grazers, brushers) and predators, based on observed feeding behaviour. The feeding habits described in Palmer (1991) and Palmer *et al*. (1993b) complemented the information obtained during feeding habits observation (this experiment in described in details in Chapter 4).

Functional feeding groups are defined based on the mechanism by which food material is obtained (Cummins and Klug, 1979). Shredders feed mainly by chewing or mining detrital coarse particulate organic matter (CPOM) such as wood and non-woody materials e.g. leaves, needles, buds etc, while the collectors feed mainly on detrital fine particulate organic matter (FPOM) either by filtering FPOM in transport (collector-filterers), or gathering substances deposited on sediments (collector-gatherers) (Cummins and Klug, 1979). The scrapers graze upon (i.e. shear-off) food materials particularly periphyton attached to surfaces. Predators are adapted to the capture of living prey. Macroinvertebrates were also assigned preferred food

classified as fine particulate organic matter (FPOM), coarse particulate organic matter (CPOM), plant materials including algae and animal materials (Chapter 4).

Biotope preference

Macroinvertebrate families and chironomid-species biotope preferences were determined using the Pearson's point-biserial correlation coefficient that indicates the association between biota and the selected biotopes: stones, GSM and vegetation (Chapters 3 and 4).

2.6 Assessing habitat integrity of the Swartkops River

Since freshwater biophysical habitat structure is an important factor that shapes the distribution of benthic communities, it was important to assess the quality and diversity of the physical habitat at the sampling sites. Specifically, at the reach scale where this study was undertaken, habitat factors such as biotope availability, and hydraulic conditions such as water velocity, depth and, substrate, channel width and morphology govern the diversity, abundance and distribution of benthic community structure (Palmer *et al.,* 1991, Dallas 2007a, Demars *et al.,* 2012; Epele *et al.,* 2012). To provide a measure of the habitat integrity at the sampling sites, an integrated habitat assessment system (IHAS) developed to complement the interpretation of SASS results was used (McMillan, 1998; Ollis *et al.,* 2006). The IHAS is based on a 100-point scoring system, divided into instream sampling habitat (55 points) and stream condition (45 points). The sampling habitat section provides a measure of the quality and adequacy of instream macroinvertebrate habitats including stone-in-current (20 points), vegetation, both marginal and aquatic (15 points) and other habitats such as stone-out-of-current, gravel, sand, mud, bedrocks, and algal presence (20 points). The stream conditions section provides questions that probe the physical characteristics of the stream including water depth, stream width, water velocity, as well as the surrounding impacts, which may influence instream condition. An IHAS assessment was conducted at each sampling site over the study period by visual inspection, and by completing the IHAS form answering the questions on the form. IHAS is a completely subjective exercise depending largely on the assessor's experience and expertise. To reduce the inherent subjectivity, upon completion of the IHAS exercise by one member of the field team, another member independently conducted the assessment, and both results compared and averaged.

2.7 Measurement of water physicochemical variables

Concurrent with macroinvertebrate sampling, water physico-chemical variables were measured at the four sampling sites over the study period. On site, sub-surface mid-channel dissolved oxygen (DO), electrical conductivity (EC), turbidity, temperature and pH were measured using Cyberscan DO 300, Cyberscan Con 300, Orbeco-Hellige 966, mercury-in-glass thermometer and Cyberscan pH 300 metres, respectively.

To analyse for nitrate-nitrogen (NO_3-N), nitrite-nitrogen (NO_2-N), ammonium-nitrogen (NH_4-N), orthophosphate-phosphorus (PO4-P), total inorganic nitrogen and five-day biochemical oxygen demand (BOD_5), mid-channel water samples were collected in acid-washed 250 ml plastic bottles, but BOD_5 samples were collected in acid-washed sterilised 500 ml glass bottles. Water samples were collected facing upstream, allowing no head space (APHA *et al.*, 1971). Collected water samples were transported to the laboratory in ice-filled cooler box, and then preserved at 4 ^{o}C until chemical analysis. All chemical analyses were undertaken within 24 hours of sample collection.

Nitrate-nitrogen (NO_3-N) and NO_2-N were analysed according to Velghe and Claeys (1983) on a Shimadzu mini 1240 spectrophotometer at 388nm and APHA *et al.* (1971) method number 354.1 on a Biotek micro plate reader at 540 nm respectively. For nitrate analysis, samples collected between 2011 and 2012 were analysed according to Ondrus (1996) on a Biotek micro plate reader at 540 nm. The Ondrus (1996) method was quicker, but it gave similar results to the Velghe and Claeys (1983) method. Orthophosphate-phosphorus (PO4-P) and NH_4-N were analysed using Spectroquant® phosphate and ammonium concentration test kits (catalogue numbers 1.14848.0001 and 1.14752.0001 respectively) according to manufacturer's instructions on a Biotek micro-plate reader at 660 nm. Total inorganic nitrogen (TIN) concentration was obtained by summing the individual concentrations of NO_3-N, NO_2-N and NH_4-N (Palmer *et al.*, 2005). All nutrient analyses were conducted on filtered water samples using Whatman GF/F filter paper. Five-day biochemical oxygen demand (BOD_5) was analysed according to APHA (1992). To obtain representative samples and to reduce variability, all analyses were undertaken in triplicate and then averaged.

2.7.1 Chlorophyll *a:* phytoplankton and periphyton analyses

To analyse for chlorophyll *a* phytoplankton, 200 ml river water was filtered through a Whatman GF/F filter paper using a filtering pump unit. The filter paper was then placed in a

10 ml tube containing 8 ml 90% acetone covered with aluminium foil to prevent the tubes from direct sunlight. In the laboratory, the tubes containing the filter papers and acetone were centrifuged at 1500 rpm for 15 minutes, and then analysed fluorometrically using a Turner Design 10-AU digital fluorometer according to Arar and Collins (1997). Analyses were undertaken in triplicate and then averaged.

To analyse for chlorophyll *a* periphyton, three stones were picked randomly from the river bed and a premeasured circular ring of 6.16 cm^2 was placed on a flat surface of the collected stones, and the area within the ring scraped using a scraper. The scraped stone area was rinsed with distilled water onto a beaker, filtered using a Whatman GF/F filter paper, and then the filter paper placed in a 10 ml tube containing 8 ml 90% acetone. In the laboratory, the tube containing the filter paper and acetone was centrifuged at 1500 rpm for 15 minutes, and then analysed fluorometrically using a Turner Design 10-AU digital fluorometer, according to Arar and Collins (1997). Triplicate analyses were undertaken then averaged.

2.7.2 Selected metal analyses

Water samples for metal analysis including zinc (Zn), manganese (Mn) copper (Cu), lead (Pb) and chromium (VI) (Cr^{+6}) were collected monthly (five different occasions) between November 2012 and April 2013 and transported to InnoVenton Analytical, a SANAS (South African National Accreditation System) accredited laboratory for analysis. Apart from Cr^{+6}, metals analysed using inductively coupled plasma spectrometry according to EPA (1984) EPA method number 200.7. Cr^{+6} was analysed using Merck chromate test kit.

2.8 Statistical analyses

The purpose of this section is to give a brief overview of the major statistical analyses applied in this study. Subsequent chapters (i.e. Chapters 3-7) discuss how specific statistics were used to analyse specific data. Prior to analysing the data in the different statistics software employed in this study, data were captured in Excel (Microsoft 2007 Office) and exported or copied to relevant statistical software packages.

2.8.1 Box-and-whisker plots

Box-and-whisker plots, here after referred to as box plots displayed and enabled visualisation of summary statistics, including quartiles, medians, non-outlier ranges, outliers, and extreme values (Baptista *et al.*, 2007; Zuur *et al.*, 2007). Box plots were used to explore the seasonal

stability of selected macroinvertebrate metrics (Table 2.3) at Site 1 (i.e. the reference site) (Chapter 3). It was used in Chapter 5 to visualised discrimination of sites based on the chironomid traits and in Chapter 6 based on chironomid deformities. Box plots were constructed using the Statistica software package version 9.

2.8.2 Kruskal -Wallis test

The Kruskal-Wallis test is a non-parametric univariate counterpart of parametric analysis of variance (ANOVA), which can be used to compare the means and medians between two or more samples (Ogbeibu, 2005). The Kruskal-Wallis multiple comparison test was used to ascertain significant differences between sampling biotopes and sites in terms of their SASS5 scores and ASPT values (Chapter 3). In Chapters 4 and 5, selected macroinvertebrate family-level traits and chironomid species-level traits were tested for significant differences between sites using Kruskal-Wallis multiple comparison test. The Kruskal-Wallis test was conducted using the Statistica software package version 9.

2.8.2 Analysis of variance

Analysis of variance (ANOVA) is a parametric statistics that compares the means between two or more samples. One-way ANOVA was used to test for differences ($P < 0.05$) in the means of the measured water physico-chemical variables between the four sampling sites (Chapter 3). When ANOVA indicated significant differences, a post-hoc test, the Tukey's Honestly Significant Different (HSD) test was computed to indicate sites that differed. In Chapter 6, ANOVA was used to test for significant differences between the incidences of mentum deformities observed at the four sampling sites, whereas in Chapter 7, the test was applied to investigate whether effluent physico-chemical variables differed between the control and effluent-treated streams. It was used used in Chapter 7 to test for significant differences in the abundance of important taxa enabling the discrimination of the controls and effluent treated streams.

Prior to using ANOVA, the basic assumptions of normality and homogeneity of variance were investigated using the Shapiro-Wilk test and the Levene's test, respectively. When it appeared that assumptions were violated, data were transformed. Water physico-chemical data were logarithmically transformed, but were normalised if assumptions were still not met. Mentum deformity data were arcsine transformed. Arcsine transformation is best suited for

proportional data or data expressed as percentages (Ogbeibu, 2005). ANOVA was conducted using the Statistica software package version 9.

2.8.3 Simple correlations

Correlation is used to describe simultaneous change of random variables that are not functionally dependent on each other, but the correlation coefficient *r-value* is used as a measure of dependency (Marques de Sá and Frias, 2007). Since highly correlated variables convey redundant information, the Spearman's rank correlation was used to test redundancy between the selected macroinvertebrate taxonomic metrics (Chapter 3). When two or more variables were highly correlated (Spearman $r > 0.075$, $p < 0.05$), only one of such variables was retained for further analysis. The Spearman's rank correlation method is a non-parametric statistic that uses ranks instead of the actual values and makes no assumption of normality (Ogbeibu, 2005). Correlation was undertaken using SPSS version 16.0.

2.8.4 Multiple linear regression and generalised linear modelling

Regression analysis, unlike correlation, describes dependency between two or more variables by a means of functional relationship (Zuur *et al.*, 2007). Regression models therefore contain both the response (i.e. dependent) variable and the explanatory (i.e. independent) variable. It was used to elucidate relationship between chironomid deformity incidences, and analysed water physico-chemical variables and metal concentrations (Chapters 6). Prior to adopting the final multiple regression model, data were explored to determine whether the assumptions of normality, homogeneity and independence were met (Zuur *et al.*, 2007). To ascertain normality of the data, a histogram of the residuals was plotted, and to ascertain the homogeneity, the residuals were plotted against the explanatory variables. Noticeable patterns, either increasing or decreasing residual values along the explanatory variable axis indicated violation of homogeneity (Zuur *et al.*, 2007). Assumption of independence was tested using autocorrelation analysis. Where these assumptions were not met, deformity data were arcsine transformed. To avoid a single outlier observation of any of the explanatory variables from confounding the model, the Cook's distance statistics was used to determine outlier observation. The Cook's distance measures the beta value with or without an observation, with a Cook's distance for outlier observations deviating from the rest (Marques de Sá and Frias, 2007). The best subset model was obtained by forward selection method determined by the *F* statistics maximisation criterion (Marques de Sá and Frias, 2007). To

achieve this, explanatory variables were added to the model step-wisely, starting with the variable with the highest *F* statistics value, followed by the next variable with the highest *F* statistics value. This process continues until the model contains all significant explanatory variables. Multi-co-linearity of explanatory variables was determined using the variance inflation factor (VIF) analysis. Multiple linear regressions were undertaken using the Statistica software package version 9.

2.8.5 The kernel curve

In analysing body-size distribution of species in a community, an important statistical issue of ecological relevance is the number of size classes into which the distribution of sizes is divided (Robson *et al.*, 2005). Choosing size classes arbitrarily can affect the shape of the community body-size distribution, which may affect results and interpretations. To avoid the use of arbitrarily predetermined body-size classes, the kernel curve (Havlicek and Carpenter 2001) was used to examine the body-size distribution of chironomid communities (Chapter 5). Briefly, the kernel curve has several components, including the lump regions, gap regions, lump area and gap area (Havlicek and Carpenter, 2001). Based on the textural discontinuity hypothesis, the lumps should correspond to body size ranges with many species, while the gap regions correspond to body size ranges with few species (Holling, 1992; Havlicek and Carpenter, 2001). Chironomid body-size classes were determined based on the kernel curve fitted on to a histogram. The kernel curve was computed using Paleontological Statistics (PAST) software package version 2.17 (Hammer, 2012).

2.8.6 Analysis of similarity (ANOSIM) and similarity percentage (SIMPER) analysis

Analysis of similarity (ANOSIM) is a multivariate non-parametric permutation procedure that detects differences based on rank similarity matrix between defined sample groups prior to sampling (Clarke and Warwick, 1994). The ANOSIM procedure produces a global *R* test statistic, which compares the similarities between replicates within a site or season to those from other sites or seasons. The global *R* value ranged between zero and one, with one indicating that all replicates within sites or season were more similar than those from other sites or seasons, and a value close to zero indicating that no differences existed between sites or seasons, with similarities between and within site replicate samples being almost equal (Clarke and Warwick, 1994). One-way ANOSIM procedure was applied to ascertain whether significant differences existed chironomid communities at the four sampling sites. When

significant differences were detected, pair-wise comparisons were undertaken to indicate sites that differed (Chapter 3). The one-way ANOSIM was also applied to macroinvertebrate communities collected from the model stream ecosystems to elucidate whether significant differences existed between the control streams, 50% effluent streams and the 100% effluent streams per sampling occasion (Chapter 7). The ANOSIM procedure makes no prior assumptions about the data and is more suited for community data with zeros compared to the corresponding MANOVA technique. Because the robustness of the procedure depends on the number of replications, replicates samples collected per sites in the river over the study period were not pooled during analysis. Replicate samples per model stream per sampling event were also not pooled. The spread of the global *R*-values was used to determine the significance of the observed global *R*-value at a significant level not above 5%. The contributions of taxa to the dissimilarity matrices were indicated using the analysis of similarity percentage, SIMPER (Clarke and Warwick, 1994). Both ANOSIM and SIMPER were computed using Primer 5 version 5.2.9 (Clarke and Warwick, 1994).

2.8.7 Ordinations

An ordination is a diagrammatic representation of samples in the form of a map, usually in two or three dimensions, in which the distances among samples represent the degree of similarity or dissimilarity of their community structure (Clarke and Warwick, 1994). Nearer samples are often more similar in community structure than distant samples. The following ordination techniques were applied in this study.

Non-metric multidimensional scaling (NMDS)

A NMDS is an ordination method that uses ranks to measure similarity instead of absolute distances and is based on numerical optimisation (Zuur *et al.*, 2007). To ascertain whether mostly sites or seasons shaped chironomid species distributions, the NMDS based on Bray-Curtis similarity index was used to elucidate patterns of chironomid community structure at the four sampling sites during the sampling seasons (Chapter 3). Prior to the ordination, chironomid species data were log (x +1) transformed. A NMDS was applied because it is better at preserving the high-dimensional structure with only few axes (Clarke and Warwick 1994, Zuur *et al.*, 2007). The analysis calculates the distance matrices, and then visualises them in a low dimensional configuration such that samples closer to each other are more similar than samples far apart on the ordination diagram. A stress level between < 0.05 and

0.1 indicates good ordination (Zuur *et al.,* 2007). NMDS was computed using Primer 5 version 5.2.9 (Clarke and Warwick, 1994).

Canonical correspondence analysis (CCA)

CCA is a multivariate ordination technique that elucidates the relationships between biological communities and environmental variables (ter Braak and Verdonschot, 1995). It assumes unimodal relationships between species and environmental variables (Zuur *et al.,* 2007). CCA is used to determine important environmental variables responsible for structuring biological community. In this study, CCA was applied to examine the correlations between species and measured water physicochemical variables (Chapters 3). In Chapter 3, it was also used to elucidate the relationship between macroinvertebrate family-level metrics with water physico-chemical variables. Chironomid traits were also related with water physico-chemical variables using CCA (Chapter 5). Before using a CCA, it is important to determine whether the unimodal assumption is met using a detrended correspondence analysis (DCA). Prior to using CCA for any of the data analysis, they were tested for unimodality using DCA. If the gradient length of the first axis of the DCA was greater than 3.0 it means that, the data met the unimodal assumption of CCA (ter Braak, 1995; Li *et al.,* 2012). Water physico-chemical variables exhibiting high multi-co-linearity ($r > 0.8$) were eliminated from the CCA model. Ecological importance of multi-co-linear variables was used to decide on which of such variables to eliminate or to retain. Both CCA and DCA were computed using Environment Community Analysis 1.33 package (ECOM) (Pisces conservation Ltd, 2000).

CHAPTER 3: RELATING MACROINVERTEBRATE TAXONOMIC COMMUNITIES TO ENVIRONMENTAL ATTRIBUTES IN THE SWARTKOPS RIVER

Biomonitoring in South Africa is based on taxonomic identification, usually to the family level, such as in the South African Scoring System (SASS) (Dickens and Graham, 2002) and occasionally, species diversity and richness. The South African Scoring System, a biotic index approach, is the primary macroinvertebrate-based biomonitoring tool that contributes information towards determining the water quality component of the ecological Reserve and setting Resource Quality Objectives (RQOs) for freshwater resources in South Africa (DWAF, 2008c). This chapter addresses the question of whether integrating SASS with other macroinvertebrate-based family-level metrics that correlate strongly with water quality could improve the sensitivity of the index approach to differences in water quality. To this end, a Swartkops multimetric index of five metrics was developed and compared with the SASS alone results for assessing river health. Since several authors e.g. Lenat and Resh (2001), Schmidt-kloiber and Nijboer (2004) have advocated the use of species-level data in freshwater biomonitoring, the response of a species-rich family, the Chironomidae, was investigated to ascertain whether species identification can improve the interpretative power of the family data in assessing deteriorating water quality in the Swartkops River. The chapter provides a rationale for each of the biomonitoring approaches and highlights the need to improve existing biomonitoring methods to take account of context-specific implications. It also provides evidence of the usefulness of chironomid species-level data in freshwater biomonitoring.

3.1 Introduction

Developing appropriate biomonitoring approaches that can accurately reflect that state of biotic deterioration as a result of pollution can make a valuable contribution to sustainable freshwater resource management (Bonada *et al.,* 2006; Thirion, 2008; Masese *et al.,* 2013). In South Africa, the South African Scoring System (SASS) is the most widely used macroinvertebrate-based biomonitoring tool for assessing river health (Dickens and Graham, 2002; Fouche and Vlok, 2010; Mantel *et al.*, 2010b). SASS is based on the presence of

selected families of aquatic macroinvertebrates and their perceived sensitivity to water quality impairment, and its results are expressed as both an index score i.e. SASS score and as an average score per recorded taxon i.e. ASPT value (Chutter, 1998; Dickens and Graham, 2002). The index is a simple, low-cost tool requiring minimal resources for sampling, sorting and identifying taxa to family level of taxonomic resolution, and it has undergone several modifications resulting in its current version 5, i.e. SASS5 (Dickens and Graham, 2002).

Although SASS5 is widely used to assess water quality in South Africa, this chapter poses the question whether integrating the index with other macroinvertebrate-based metrics with a strong correlation with water quality could improve the sensitivity of the index approach to discerning differences in water quality in the Swartkops River. To this end, a Swartkops multimetric index, which included SASS (SASS5 score and ASPT value) and three other metrics (ET richness i.e. Ephemeroptera-Trichoptera, percent Chironomidae + Oligochaeta and Simpson diversity), was developed and compared with the SASS5 only results for assessing river health in the Swartkops River. The multimetric index approach is perceived to be superior to the single biotic index approach such as SASS5 because it integrates several metrics representing individual-, population-, and community-level taxonomic measures, improving the interpretative power of the index approach for assessing biological responses to deteriorating water quality (Baptista *et al.* 2007; Moya *et al*. 2011). Therefore, developing a multimetric index at the family level of taxonomic resolution would enable a direct comparison of the results of both indices (the multimetric index and the SASS5). Both approaches were reviewed in Chapter 1, section 1.4.

The biotic index SASS5 and the multimetric approach introduced above are at the family level of taxonomic resolution. Since protecting taxonomic diversity at the species level is a principal goal of sustainable freshwater resource management, identifying taxa to family level may not provide a sufficiently accurate assessment of pollution effects on biodiversity, as many species are aggregated into one family (Lenat and Resh, 2001). This could limit the interpretative power of the data in terms of biodiversity implications (Lenat and Resh, 2001; Schmidt-kloiber and Nijboer, 2004). Species within a family can have different functional and ecological requirements, and would therefore likely be affected differently by environmental attributes (Schmidt-kloiber and Nijboer, 2004). Lenat and Resh (2001) emphasised the importance of species-level information in freshwater biomonitoring by demonstrating the differences between a family- and a species-level indices in indicating

water quality conditions. These authors concluded that several sites were erroneously classified by a family-level index and that a species-level index made it possible to discriminate between sites with slight differences in water quality impairment. Therefore, in this study, species of the family Chironomidae were identified to provide a better understanding of their biodiversity status, and to investigate the usefulness of the species-level relative to the family-level data in terms of biotic and water quality deterioration in the Swartkops River.

Ideally, all species should be identified to provide a comprehensive picture of deteriorating water quality on taxonomic biodiversity, but this would be an onerous task considering the sparse taxonomic knowledge of Afro-tropical macroinvertebrate species and the cost implications of sorting and identifying all species. However, McGeoch, (1998) listed taxonomic richness (number of species in the taxonomic group), representativeness of species in all habitats and biotopes, and availability of appropriate sampling methods as important factors to consider when searching for bio-indicators. The macroinvertebrate family Chironomidae is inarguably one of the most diverse groups of aquatic insects, occurring in most aquatic habitats and biotopes, and often dominating the species richness of macroinvertebrate samples (Armitage *et al*. 1995; Cranston, 1996; Porinchu and MacDonald, 2003; Ferrington Jr., 2008). Their extraordinary ecological range enables them to inhabit streams of varying water quality (Adriaenssens *et al.,* 2004; Wright and Burgin, 2009; Odume and Muller, 2011). Therefore, the chironomids were selected to use in investigating the usefulness of species-level data in biomonitoring of the Swartkops River by relating chironomid species to environmental attributes (water physico-chemical variables and biotopes) with a view to elucidating their responses to deteriorating water quality, and their association with selected biotopes. Identifying species in a species-rich family such as Chironomidae may provide an acceptable compromise between a coarse taxonomic identification and the onerous task of comprehensive species identification.

A critical review of the family Chironomidae in freshwater biomonitoring is presented in Chapter 1, section 1.4.2. This chapter addresses part of objective one stated in Chapter 1*: to investigate the impacts of deteriorating environmental water quality on macroinvertebrate communities in the Swartkops River, using family- and chironomid species-level taxonomically- and family-level traits-based biomonitoring approaches* through the following sub-objectives: i) to provide a physico-chemical characterisation of the Swartkops River; ii)

to develop a Swartkops multimetric index, and compare it to the well-established South African Scoring System version 5 for assessing river health; iii) to relate chironomid species to water physico-chemical variables in order to identify specific indicator species; iv) to relate chironomid species to selected biotopes, with a view to identifying chironomid community types based on their preferred biotopes.

Publications based on this chapter:

Odume, O.N and Muller, W.J. (2011). Diversity and structure of Chironomidae communities in relation to water quality differences in the Swartkops River. *Journal of Physics and Chemistry of the Earth*: 36: 929-938.

Odume, O.N., Muller, W.J., Arimoro, F.O. and Palmer, C.G (2012). The impact of water quality deterioration on macroinvertebrate communities in the Swartkops River, South Africa: a multimetric approach. *African Journal of Aquatic Science*: 37(2): 191-200.

3.2 Materials and methods

3.2.1 Measurement of water physico-chemical variables and metals

Water physico-chemical variables including dissolved oxygen, five-day biochemical oxygen demand (BOD_5), electrical conductivity (EC), turbidity, temperature, pH, nitrate-nitrogen (NO_3-N), nitrite-nitrogen (NO_2-N), ammonium-nitrogen (NH_4-N), total inorganic nitrogen (TIN) and orthophosphate-phosphorus (PO_4-P) were analysed at each sampling site during each sampling occasion as described in Chapter 2, sections 2.8. Selected metals including manganese (Mn), chromium (VI) (Cr^{+6}), copper (Cu), zinc (Zn) and lead (Pb) were analysed on five sampling occasions at each of the sampling sites between November 2012 and April 2013 as described in Chapter 2, section 2.7.2.

Concurrent with water physicochemical analysis, chlorophyll *a* phytoplankton and periphyton analyses were undertaken as described in Chapter 2, section 2.7.1. One-way ANOVA was used to test for significant differences ($P < 0.05$) in the means of the measured water physico-chemical variables, selected metals, and chlorophyll *a:* phytoplankton and periphyton concentrations between the four sampling sites. When ANOVA indicated statistically

significant differences, a post-hoc test, the Tukey's Honestly Significant Difference (HSD) test was used to indicate sites that differed. Prior to using ANOVA, the data (physico-chemicals, metals and chlorophyll *a*) were ascertained for normality and when this assumption was violated, they were log (x+1) transformed to approach the assumption of normality (Chapter 2, section 2.8.2).

3.2.2 The South African Scoring System version 5 (SASS5)

Macroinvertebrate samples collected at the four sampling sites (Chapter 2, section 2.2) were analysed seasonally in terms of the SASS5 (SASS5 scores and ASPT values) to provide a SASS5-based river health assessment of the Swartkops River. In SASS5, macroinvertebrate families are awarded scores reflecting their perceived sensitivity to deteriorating water quality in the range of 1–15, with score 1 indicating the least sensitive and 15 the most sensitive macroinvertebrate families. The SASS5 score is calculated by summing the scores of all the recorded taxa, while the ASPT value is obtained by dividing the total SASS5 score by the number of taxa recorded. The ranges of SASS5 scores and ASPT values for the different ecological categories A, B, C, D, and E/F indicative of very poor, poor, fair, good and natural water quality provided in Table 2.2 Chapter 2, section 2.3 were used to interpret the SASS5 results to provide a SASS-based river health assessment.

South African Scoring System version 5 and selected biotopes

During each sampling event (Chapter 2, section 2.2) macroinvertebrates were collected from three distinct biotopes (stones, vegetation, and gravel, sand and mud GSM) as described in Chapter 2, section 2.2. The Kruskal-Wallis multiple comparison test was used to indicate significant differences in community structure based on SASS5 scores and ASPT values between the selected biotopes. Detailed descriptions of the biotopes and the statistical analyses have been provided in Chapter 2.

3.2.3 Developing a Swartkops multimetric index (MMIswartkops)

The Swartkops multimetric index (MM1swartkops) was developed using macroinvertebrate taxonomic metrics, which have been identified as indicating sensitivity of the macroinvertebrate community in the Swartkops River, i.e. they were strongly correlated with water physico-chemical variables and enabled discrimination of Site 1 from the downstream Sites 2, 3 and 4 (Table 2.3, Chapter 2, section 2.3.2). First, the metrics were tested for

seasonal stability and redundancy, using the macroinvertebrate assemblages collected at Site 1 (reference site) only. The assemblages at Site 1 were used for these analyses to avoid confounding effects of pollution on seasonal variation of metrics. To test metrics for seasonal stability, box plots were used to assess metric variations between seasons and the Kruskal-Wallis test was used to assess the statistical significance of the variation ($P < 0.05$). Only metrics not significantly different between the seasons were considered seasonally stable, and were then subjected to a redundancy test using the Spearman's rank correlation test ($r > 0.75$; $P < 0.05$). When two or more metrics were redundant (Chapter 2, section 2.8.3), only one of such redundant metrics was retained for integration into the multimetric index.

Second, the retained metrics were integrated into a multimetric index. Integrating the metrics into a multimetric index requires standardising the numeric values of each metric because the different metrics e.g. ET richness and percent Chironomidae + Oligochaeta are based on different numeric scales (Table 2.3, section 2.3.2). The numeric values of the retained metrics were standardised by dividing the range of metric values into three possible scores for each metric according to the method described by Baptista *et al.* (2007). To standardise the numeric values of the metrics, the minimum, lower quartile (25%), mid-quartile (50%), upper quartile (75 %) and maximum values of each metric for the Site 1 (reference site) assemblage distribution were calculated and used as the basis for scoring the numeric values of metrics at Sites 2, 3 and 4. For metrics expected to increase in numeric value with increasing pollution (Table 2.3, Chapter 2), if the numeric value at Sites 2, 3 and 4 (impacted sites) was lower than the upper quartile (75%) of the Site 1 assemblage distribution, it was scored 5, and if it was between the upper quartile and maximum value of the Site 1 assemblage it was scored 3. A score of 1 was awarded to the metric value if it was greater than the maximum value of that metric for the Site 1 assemblage.

For metrics expected to decrease in numeric value with increasing deteriorating water quality (Table 2.3, section 2.3.2), if the numeric value at the impacted site was greater than the lower quartile (25%) of that metric for the Site 1 assemblage distribution it was scored 5, and if it was between the minimum and the lower quartile of the metric for the Site 1 assemblage, it was scored 3. A score 1 was awarded if the value was less than the minimum value for the Site 1 assemblage for that metric (Baptista *et al.,* 2007). Therefore, depending on the expected response of the metric to pollution, thresholds based on the appropriate quartiles were established for each metric using the Site 1 assemblages so that a score 5 indicates that the numeric value of the metric does not deviate from those at Site 1, a score 3 indicates a

moderate deviation from Site 1, and a score 1 indicates the greatest deviation from the numeric value at Site 1.

Third, after standardising the metric values using the scoring system described above, the Swartkops multimetric was then computed by summing scores of the component metrics and the final index value range quadrisected as described in Baptista *et al.* (2007) into four ecological categories B, C, D, and E/F indicative of good, fair, poor and very poor water quality. Ecological category A indicating pristine (natural) water quality was omitted because the macroinvertebrate assemblages used in developing the index did not come from a pristine condition, but from one that was minimally impacted. The Swartkops multimetric index-based river health assessment of Sites 2, 3 and 4 was then compared with the SASS5-based river health assessment to ascertain whether additional, useful insight was gained to compensate for the extra time and effort.

The canonical correspondence analysis (CCA) was used to relate the individual metrics integrated into the Swartkops multimetric index to water physico-chemical variables. Prior to the CCA analysis, the metrics were subjected to a detrended correspondence analysis (DCA), which revealed a gradient length > 3.0, indicating that the data met the unimodal assumption of CCA (see Chapter 2, section 2.8.7 for a detailed explanation of the method).

3.2.4 Species-level taxonomically-based response: chironomid communities in the Swartkops River

Differences between the sampling sites and seasons, based on chironomid species compositions

Chironomid larvae from the macroinvertebrate samples collected at the four sampling sites over the study period were sorted, mounted and identified as described in Chapter 2, sections 2.2 in order to provide a species-level assessment. To ascertain whether chironomid species distributions were shaped mostly by sites or seasons, the non-metric multidimensional scaling (NMDS) was used to elucidate patterns of chironomid community structure at the four sampling sites during the sampling seasons (Chapter 2, section 2.8.7). The multivariate analysis of similarities (ANOSIM) was used to indicate significant differences between the sampling sites in terms of their chironomid species compositions, while the similarity percentage analysis (SIMPER) was used to indicate species contributing to the observed dissimilarities between the sampling sites (Chapter 2, section 2.8.6).

Relating chironomid species to water physicochemical variables and identifying water quality indicator species

The chironomid species were related to water physico-chemical variables using canonical correspondence analysis (CCA). Prior to the CCA analysis, the chironomid species data were subjected to a detrended correspondence analysis (DCA), which revealed a gradient length > 3.0 indicating that the data met the unimodal assumption of CCA (see Chapter 2, section 2.8.7 for a detailed explanation of the method). Based on the CCA analysis, chironomid species tolerances to deteriorating water quality were determined using the method developed by Murphy *et al.* (2013), which assigns tolerance scores to species based on their relative position along the first axis of the CCA. The species coordinates on Axis 1 of the CCA ordination were extracted and then used to calculate the inter-species distances (differences), starting with the two most distant species on Axis 1. The calculated distances were then converted to percent distances and the species ranked in tenth percentile order, with the most pollution-tolerant species ranked as 10 and the least as 1.

An indicator species analysis that corrects for unequal numbers of replications at the sampling sites was undertaken to ascertain whether its results would support the CCA results. The usefulness of the indicator species analysis depends on the number of replicates; thus, all replicate samples were used without pooling. The indicator species analysis produces a species-indicator value index that reflects the predictive value of the species as a bio-indicator (Dufrêne and Legendre, 1997; De Cáceres and Legendre 2009). The indicator value index has two components A and B, defined respectively as specificity and fidelity. Component A is the positive predictive value of the species for the prevailing water quality at the particular site, whereas the B component expresses the sensitivity of the species to the prevailing water quality condition, that is, the probability of finding the species among replicate samples taken from the site. The statistical significance of the indicator value was tested using the Monte Carlo permutation test with 999 permutations at *alpha* = 0.05.

Relating chironomid species to selected biotopes

To analyse chironomid species-biotope association with a view to identifying community types based on their preferred biotopes, the Pearson's point-biserial correlation coefficient was computed to determine the association between chironomid species and the selected biotopes (stone, vegetation, and GSM) at Site 1. The Pearson's point - biserial coefficient

indicates the degree of species preference for a given biotope, and it can indicate both negative (when a species tends to avoid a particular biotope) and positive correlations (De Cáceres and Legendre, 2009; De Cáceres, 2013). To avoid the confounding effects of pollution on the chironomid species-biotope associations, the analysis was undertaken only for the Site 1 assemblages. Species occurring in less than five replicate samples over the study period were eliminated from the analysis. The statistical significance of the association was tested using the Monte Carlo permutation test with 999 permutations at *alpha* = 0.05. The Pearson's point-biserial correlation coefficient and the indicator species analyses were computed using the Indicspecies package version 1.6.7 within the R version 3.0.0 software environment (De Cáceres, 2013; R Core Team, 2013).

3.3 Results

3.3.1 Water physico-chemical variables

The mean, standard deviation and range of each of the measured water physico-chemical variables at the four sampling sites during the study period are shown in Table 3.1. With the exception of pH and temperature, one-way ANOVA indicated that the mean concentrations of the measured variables differed significantly between the sampling sites ($P < 0.05$). The highest dissolved oxygen (DO) concentration was recorded at Site 2 and the lowest at Site 3. The Tukey's HSD post-hoc test revealed that the mean DO concentration was significantly lower at Site 3 than at Sites 1 and 2, but it did not indicate statistically significant differences between Sites 3 and 4, and between Sites 1, 2 and 4 (Table 3.1). The overall highest DO concentration (9.48 mg/l) was recorded at Site 2 during autumn 2010, whereas the overall lowest concentration (0.9 mg/l) was obtained at Site 4 in spring 2009 (Figure 3.1a). Generally, DO concentrations were mostly higher at Site 2 during the study period and were in most of the sampling events lowest at Site 3, downstream of the discharge point of the Kelvin Jones Wastewater Treatment Work (WWTW).

Although pH and temperature were not statistically significantly different between the sampling sites, the highest pH and temperature values were at Site 2 in spring 2011 and in autumn 2010, respectively (Figure 3.1a). The Tukey's HSD post-hoc test showed that the mean EC concentration was significantly lower at Site 1 than at the rest of the sampling sites. The highest fluctuation in EC concentration was at Site 2 with a minimum concentration of

30 mS/m in spring 2009 and a maximum concentration of 460 mS/m in winter 2010 (Figure 3.1a).

Table 3.1: Mean ± standard deviation and range (in parenthesis) of the measured water physico-chemical variables (n = 8) in the Swartkops River during the study period (August 2009 – September 2012). P and F values are indicated by ANOVA. Different superscript letters per variable across sites indicate significant differences ($P < 0.05$) revealed by Tukey HSD post-hoc test. The same superscript letter between sites per variable indicates no significant differences ($P > 0.05$).

Variable	**Site 1**	**Site 2**	**Site 3**	**Site 4**	***P* value**	***F value***
Dissolved oxygen (mg/l)	6.99 ± 1.15^{a} (4.73 – 9.5)	7.4 ± 1.52^{a} (5.53 – 9.48)	3.19 ± 1.47^{b} (1.81 – 6.36)	4.81 ± 3.01^{ab} (0.9 – 8.31)	0.001	7.18
pH	6.53 ± 1.11 (4.69 – 7.75)	7.37 ± 1.11 (5.69 – 8.99)	7.29 ± 0.42 (6.56 – 7.9)	7.27 ± 0.56 (6.31 – 8.01)	0.201	1.65
Temperature (^{o}C)	17.48 ± 5.46 (7.31 – 24.0)	17.27 ±7.17 (6.11 – 27.3)	20.88 ± 3.29 (14.3 – 25.2)	18.9 ± 4.14 (12.2 – 24.0)	0.415	0.98
Electrical conductivity (mS/m)	32.45± 17.74^{a} (8.23 – 62.0)	160.75±146^{b} (30 – 460)	262.51± 76.14^{b} (154.8 – 333)	259.63±56.28^{b} (171 – 354)	0.000	22.57
Turbidity (NTU)	5.3 ± 2.22^{a} (3.0 – 10.1)	6.33 ±2.44^{a} (3.0 – 11.2)	72.7 ± 102.36^{b} (10.5 – 320)	7.08 ± 8.06^{a} (2.2 – 26)	0.000	15.67
BOD_5 (mg/l)	4.62 ± 1.45^{a} (2.16 – 6.86)	8.25 ±4.33^{ab} (4.58– 16.68)	14.54 ± 3.57^{c} (8.32 – 20.62)	11.77±5.28^{bc} (2.24– 22.94)	0.002	13.50
Nitrate - nitrogen (NO_3-N) (mg/l)	0.09 ± 0.05^{a} (0.03 – 0.19)	0.62 ± 0.96^{ac} (0.03 – 2.93)	1.96 ± 1.77^{bc} (0.41 – 6.15)	3.08 ±2.47^{b} (0.47 – 7.90)	0.000	11.68
Nitrite - nitrogen (NO_2-N) (mg/l)	0.02 ± 0.02^{a} (0.002– 0.6)	0.07 ± 0.08^{a} (0.006– 0.21)	0.24 ± 0.13^{b} (0.05 – 0.49)	0.22 ± 0.15^{b} (0.03 – 0.43)	0.00	9.22
Ammonia - nitrogen (NH_3-N) (mg/l)	0.08 ± 0.11^{a} (0.003– 0.34)	0.33 ± 0.60^{a} (0.02 – 1.79)	7.36 ± 6.93^{b} (0.33 – 23.38)	4.09 ± 3.0^{b} (1.05 – 10.0)	0.000	22.10
Total inorganic nitrogen (TIN) (mg/l)	0.171 ± 0.22^{a} (0.039– 0.49)	1.567 ± 2.26^{a} (0.17 - 4.92)	9.56 ± 6.85^{b} (2.33– 24.44)	7.40 ± 3.52^{b} (1.59 -12.79)	0.000	36.29
Orthophosphate - phosphorus (PO_4-P) (mg/l)	0.03 ± 0.01^{a} (0.01 – 0.05)	0.86 ± 0.52^{b} (0.12 – 1.65)	6.72 ± 4.00^{c} (0.95 – 11.98)	4.86 ± 3.19^{c} (0.53 – 9.61)	0.000	24.48

Nevertheless, the two sampling sites downstream of the discharge point of the Kelvin Jones WWTW had significantly higher EC concentrations during most of the sampling occasions (Figure 3.1a). Turbidity was consistently higher at Site 3 throughout the sampling seasons (Figure 3.1b). The mean turbidity concentration was statistically significantly different from the remaining three sampling sites ($P < 0.05$). There were no significant variations in turbidity between Sites 1, 2 and 4.

Five-day biochemical oxygen demand (BOD_5) showed seasonal variations at the four sampling sites (Figure 3.1b). At Site 1, the lowest BOD_5 concentration was recorded in winter (2009) and the highest in summer (2009). At Sites 3 and 4, the highest BOD_5 values were obtained in autumn (2010) and at Site 2 in winter (2010) (Figure 3.1b). The Tukey's HSD post-hoc test indicated that the mean BOD_5 concentrations were significantly higher at Sites 3 and 4 than at Site 1, and that no significant differences existed between Sites 2, 3 and 4, and between Sites 1 and 2 (Table 3.1).

There were increased nutrient (NO_3-N, NO_2-N, NH_4-N, TIN and PO_4-P) concentrations at Sites 3 and 4, which are downstream of the discharge point of the WWTW. Specifically, the concentrations of NO_2-N and NH_3-N were significantly elevated at Sites 3 and 4 compared to Sites 1 and 2, which are upstream of the discharge point of the WWTW. The mean concentrations of these nutrients did not differ significantly between Sites 1 and 2, and between Sites 3 and 4 (Table 3.1). Throughout the study period, the concentrations of NO_3-N were generally low at Site 1 with moderate increases at Site 2, which were not significantly different from the mean concentration at Site 1 (Table 3.1). With the exception of summer (2009) and spring (2012), NO_3-N was generally higher at Site 4 during the study period, and the overall highest concentration of 7.90 mg/l was recorded during autumn (2012).

During the study period, there were marked increases in the concentrations of orthophosphate-phosphorus (PO_4-P) at Sites 3 and 4 relative to Sites 1 and 2 (Figure 3.1c). Statistically, the mean concentrations at Sites 3 and 4 differed significantly from those recorded at Sites 1 and 2 ($P < 0.05$). Orthophosphate-phosphorus concentrations were generally low at Site 1 and were significantly lower than those recorded at the remaining three sites.

Generally, most of the water physico-chemical variables indicated that the water quality at Sites 3 and 4 were more impaired than at Sites 1 and 2, with the greatest impairment observed

at Site 3, situated immediately downstream of the discharge point of the WWTW. Although the water physicochemical variables indicated that Site 2 was less impaired than Sites 3 and 4, the mean concentrations of EC and PO_4-P were significantly elevated at Site 2 relative to Site 1.

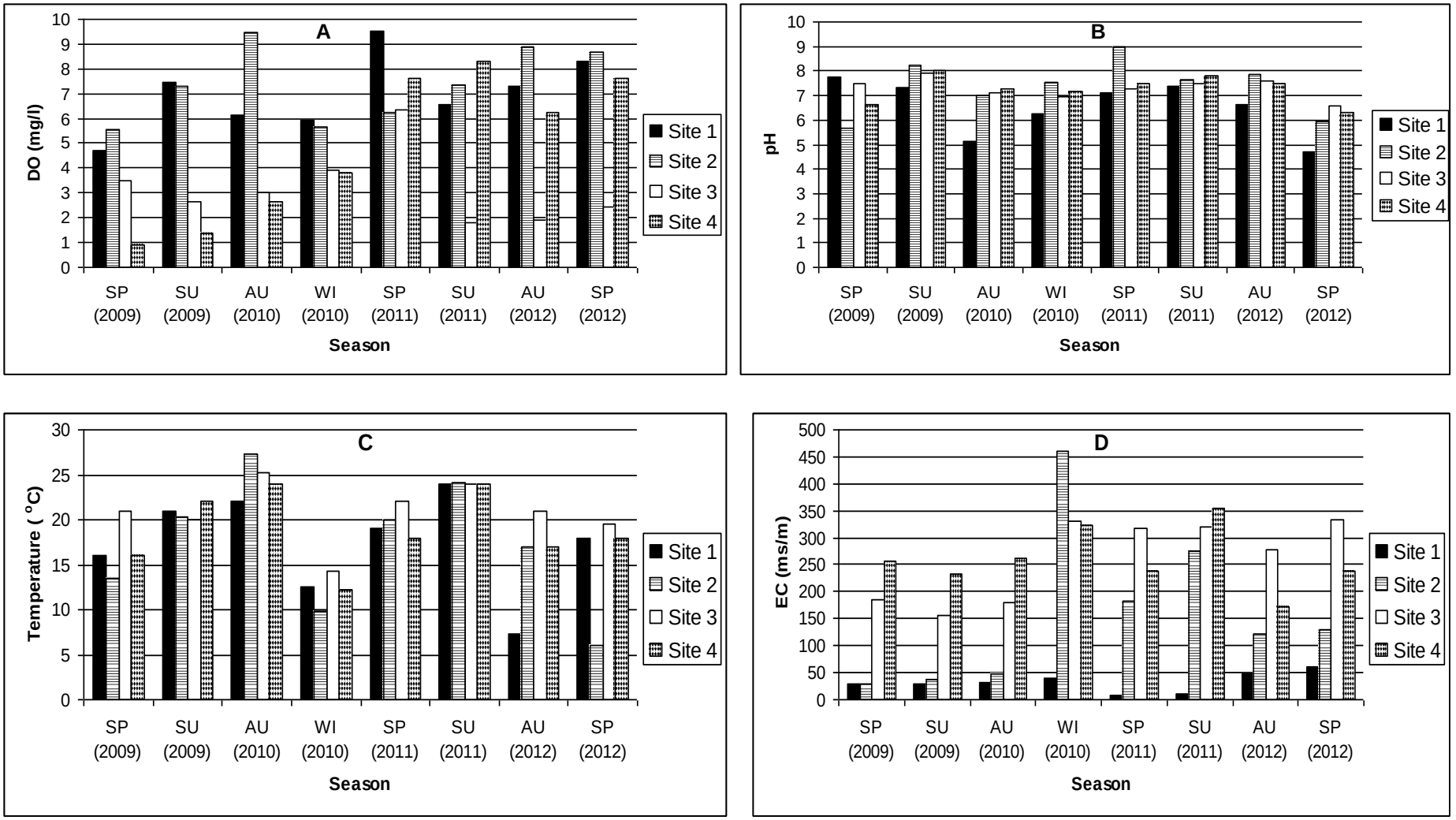


Figure 3.1a: Seasonal variability of the concentrations of dissolved oxygen (DO) (A), pH (B), temperature (C) and electrical conductivity (EC) (D) between the four sampling sites in the Swartkops River during the study period (August 2009–September 2012).

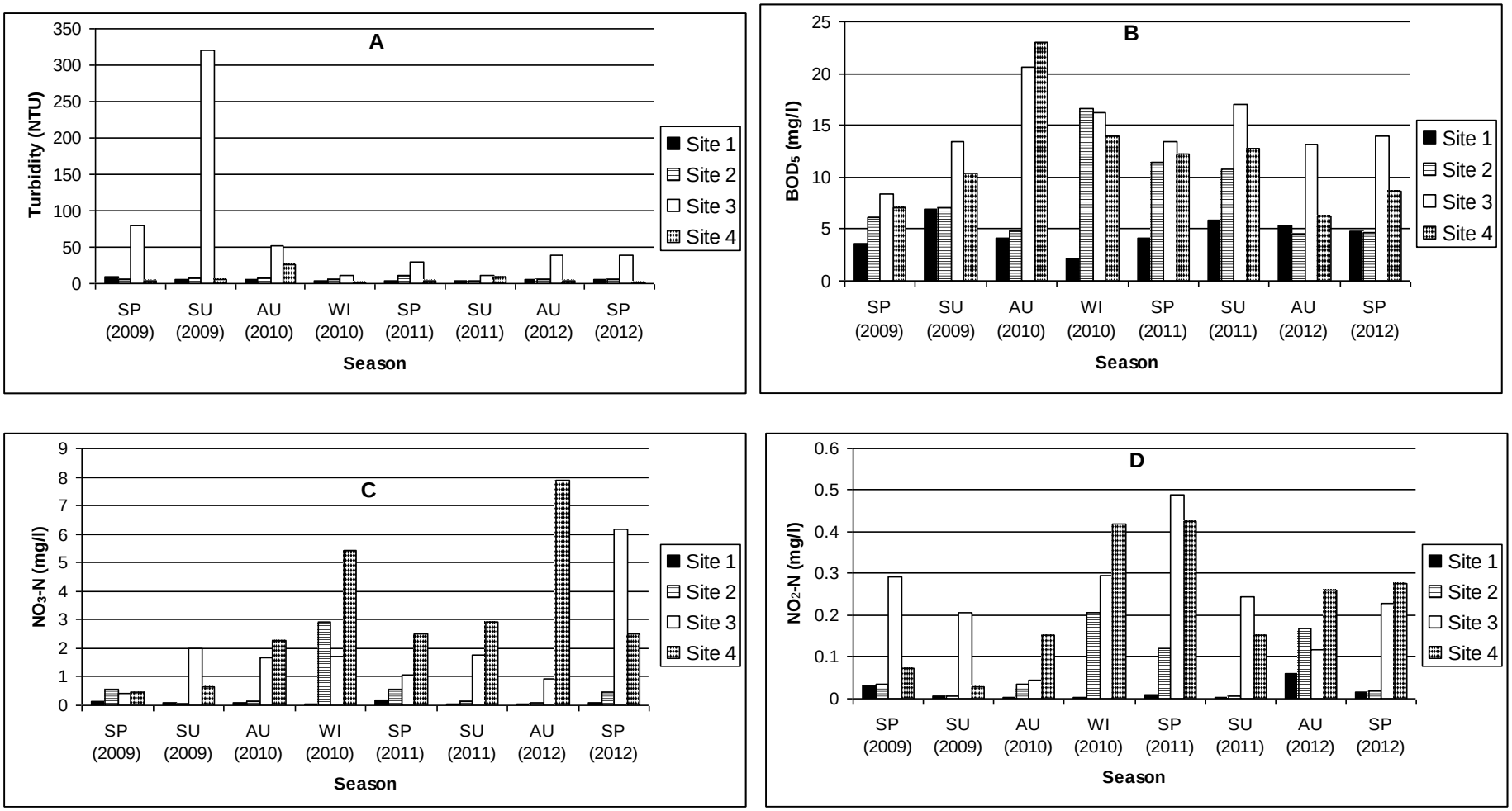
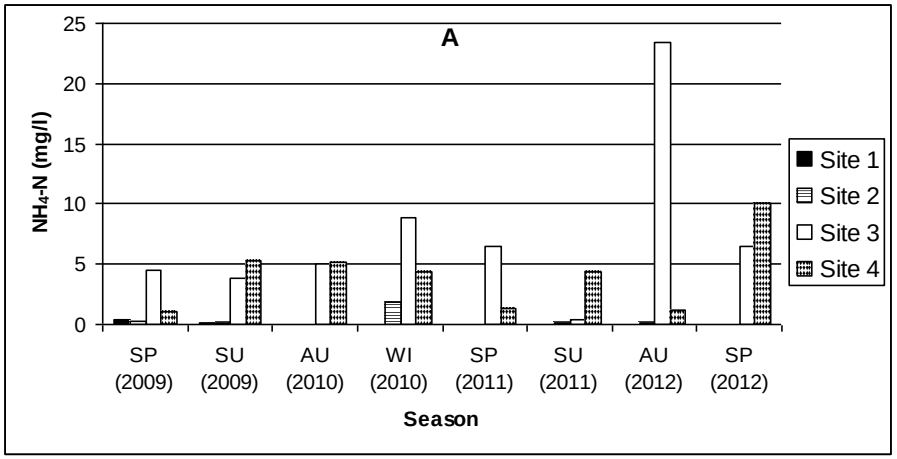
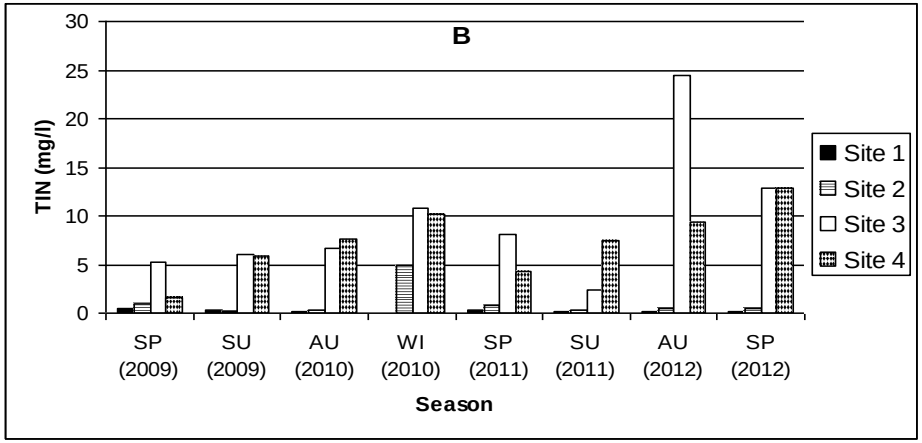


Figure 3.1b: Seasonal variability of the concentrations of turbidity (A), five-day biochemical oxygen demand (BOD5) (B), nitrate-nitrogen (NO3-N) (C) and nitrite-nitrogen (NO2-N) (D) between the four sampling sites in the Swartkops River during the study period (August 2009–September 2012).





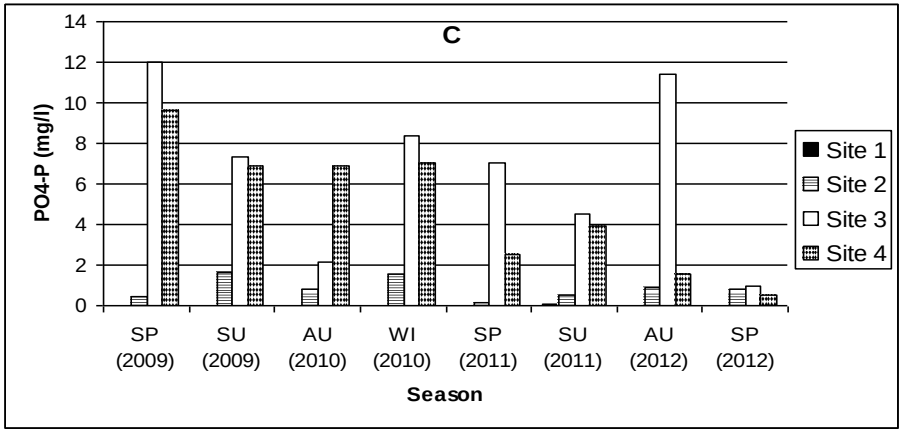


Figure 3.1c: Seasonal variability of the ammonium-nitrogen (NH_4-N) (A), total inorganic nitrogen (TIN) (B) and orthophosphate-phosphorus (PO_4-P) (C) concentrations between the four sampling sites in the Swartkops River during the study period (August 2009–September 2012).

3.3.2 Analysed metal concentrations

The concentrations of the analysed metals in the water of the Swartkops River were generally low (Table 3.2). Chromium (VI) (Cr^{+6}) and copper (Cu) did not vary significantly between the four sampling sites ($P > 0.05$) (Table 3.2), though the mean concentration of Cu was higher at Site 3 relative to the remaining three sites. Lead (Pb), Zn and Mn were statistically significantly different between the sampling sites ($P < 0.05$). The mean concentrations of Zn and Mn were statistically significantly higher at Sites 1 and 3 than at Site 2, where its concentration was lowest. The mean concentrations of Pb were also significantly lower at Site 2 than at Sites 1 and 4.

Table 3.2: Mean ± standard deviation and range (in parenthesis) of the analysed metals (n = 4) in the Swartkops River during the study period (August 2009–September 2012). P and F values are indicated by ANOVA. Different superscript letters per variable across sites indicate significant differences revealed by Tukey's HSD post-hoc test. The same superscript letter between sites per variable indicates no significant differences.

Variable	**Site 1**	**Site 2**	**Site 3**	**Site 4**	***P* value**	***F* value**
Chromium (VI) (Cr^{6+}) (mg/l)	0.035 ± 0.024 (0.02 – 0.07)	0.041 ± 0.021 (0.025– 0.07)	0.032 ± 0.01 (0.025 – 0.048)	0.032 ±0.001 (0.025-0.046)	0.243	1.475
Copper (Cu) (mg/l)	0.004 ± 0.002 (0.002– 0.006)	0.006 ± 0.002 (0.003– 0.008)	0.007 ± 0.001 (0.006 – 0.009)	0.006 ± 0.001 (0.005–0.007)	0.065	2.947
Lead (Pb) (mg/l)	0.005 ± 0.003^{b} (0.001 – 0.008)	0.002 ± 0.001^{a} (0.001 – 0.003)	0.003 ± 0.001^{ab} (0.002 – 0.004)	0.005 ± 0.005^{b} (0.001 – 0.012)	0.006	5.050
Zinc (Zn) (mg/l)	0.016 ± 0.001^{a} (0.015– 0.016)	0.003 ± 0.002^{b} (0.002 – 0.006)	0.011 ± 0.004^{c} (0.004 – 0.016)	0.004 ± 0.00^{b} (0.004– 0.004)	0.000	77.96
Manganese (Mn) (mg/l)	0.102 ± 0.08^{ab} (0.039– 0.216)	0.06 ± 0.09^{a} (0.001– 0.194)	0.144 ± 0.016^{b} (0.128 – 0.166)	0.068 ± 0.046^{a} (0.015 – 0.126)	0.007	4.906

3.3.3 Chlorophyll *a*: phytoplankton and periphyton concentrations in the Swartkops River

The seasonal variation of chlorophyll *a* used as surrogate for phytoplankton and periphyton are presented in Figure 3.2. Seasonally, the concentrations of phytoplankton and periphyton were

generally higher during spring and summer. The highest phytoplankton concentration was at Site 2 during spring (2011) sampling event, whereas the lowest concentrations consistently occurred at Site 1 throughout the sampling seasons. Periphyton concentrations were higher at Sites 3 and 4. With the exception of Site 2 during summer (2011), the lowest periphyton concentrations were observed at Site 1 in all the sampling seasons. One-way ANOVA indicated that the mean concentrations of periphyton differed significantly between the four sampling sites ($P < 0.05$; F = 12.62) and the Tukey's HSD post-hoc test indicated that the mean concentrations at Sites 2, 3 and 4 were significantly higher than at Site 1 (Figure 3.3). However, no statistical significantly difference was observed between the sampling sites based on their phytoplankton concentrations ($P > 0.05$; F = 1.69).

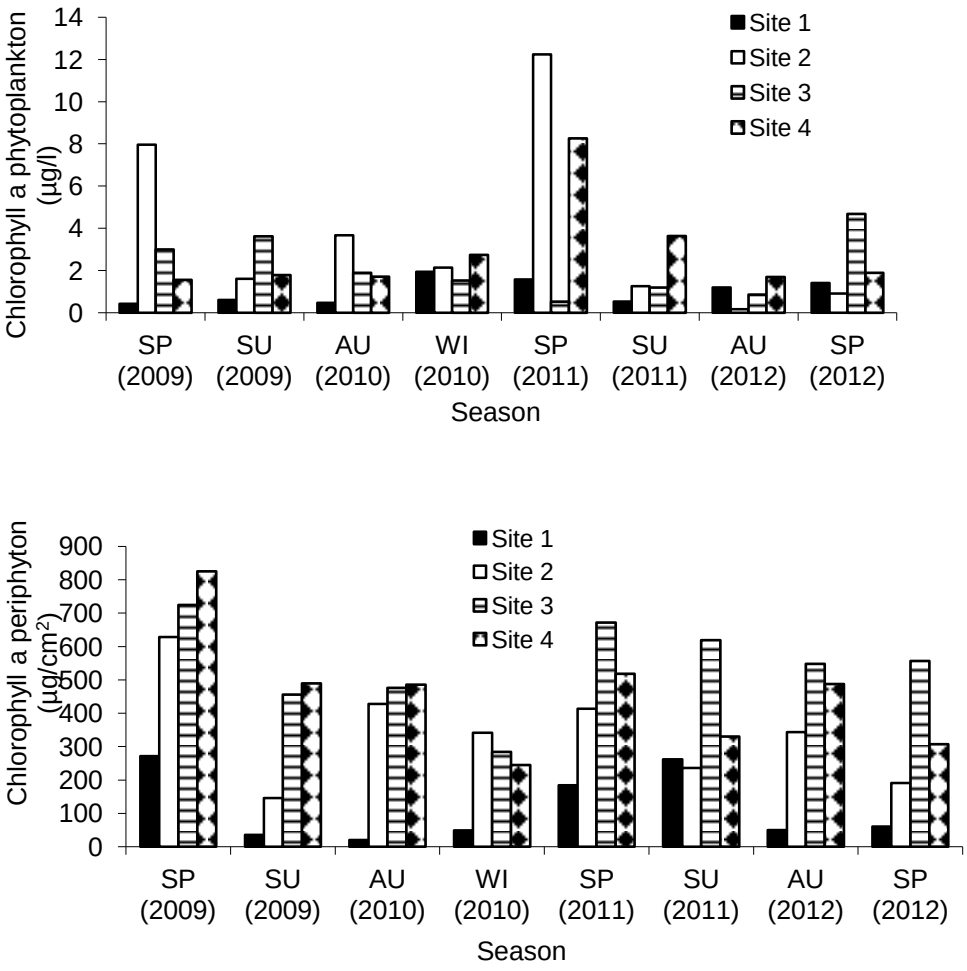


Figure 3.2: Seasonal variations in the concentrations of chlorophyll a: phytoplankton and periphyton at the four sampling sites in the Swartkops River during the study period (August 2009–September 2012).

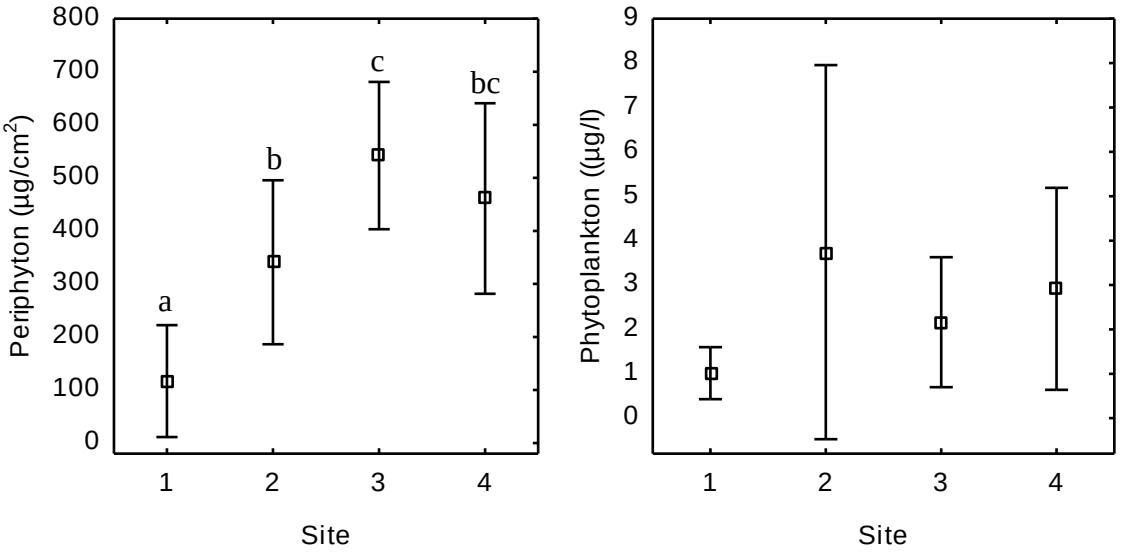


Figure 3.3: Mean (square) and standard deviation (bar) of chlorophyll *a:* periphyton and phytoplankton concentrations at the four sampling sites in the Swartkops River during the study period (August 2009–September 2012). The same alphabet letter on the bars indicates no statistically significant difference ($P > 0.05$), whereas different alphabet letters show statistical significantly difference ($P < 0.05$).

3.3.4 Assessing the Swartkops River's health using the biotic index South African Scoring System version 5 (SASS5)

The interpretation of the SASS5-based results were based on the range of SASS5 scores and ASPT values reflecting ecological categories A, B, C, D and E/F indicative of natural, good, fair, poor and very poor river water quality conditions, respectively (Table 2.2, Chapter 2, section 2.3). The physical habitat conditions assessed using the integrated habitat assessment system (IHAS) revealed that the physical habitat did not vary much between the sampling sites in all seasons (Appendix A, Figure A1). The SASS5 scores and ASPT values revealed that the river's health conditions differed between the sampling sites. Seasonally, with the exception of the autumn and spring (2012) collections, SASS5 scores at Site 1 indicated the B ecological category indicative of good water quality condition (Figure 3.4). The ASPT values on the other hand, in all the sampling seasons, indicated the C ecological category, suggesting that the water quality at Site 1 was fair (Figure 3.5). The numbers of taxa vary slightly between the sampling seasons at Site 1 with more taxa occurring in spring (2012) (Figure 3.6). Overall, the SASS5 score and the ASPT value showed good water quality (ecological category B) for Site 1, but the ASPT indicated that the water quality condition was fair (ecological category C) (Figure 3.7).

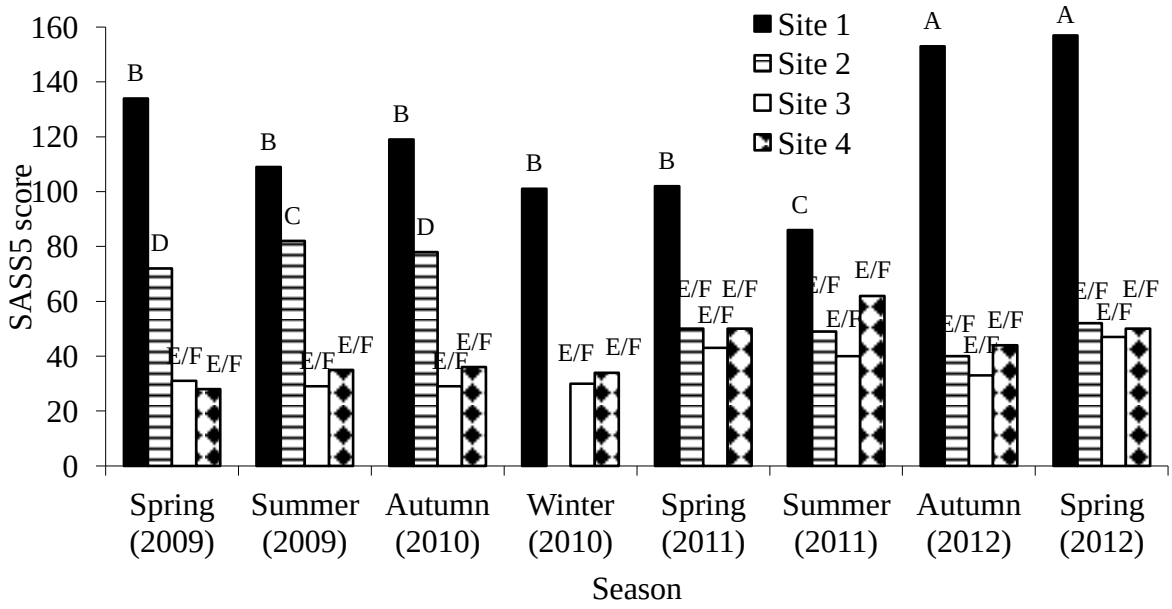


Figure 3.4: Seasonal variations for the South African Scoring System version 5 (SASS5) score at the four sampling sites in the Swartkops River during the study period (August 2009–September 2012). The ecological categories: A (natural water quality), B (good water quality), C (fair water quality), D (poor water quality) and E/F (very poor water quality) are indicated on the bars.

At Site 2, SASS5 scores indicated the D ecological category, i.e. poor water quality, in spring (2009) and in autumn (2010), while in summer (2009), it revealed the C category indicative of fair water quality (Figure 3.4). During the rest of the sampling events, SASS5 scores revealed the E/F ecological category indicating very poor water quality. Although the SASS5 scores reflected other ecological categories in addition to the E/F, the ASPT values consistently showed that Site 2 was in the E/F ecological category, indicating that the water quality at the site was very poor throughout the sampling seasons (Figure 3.5). Nevertheless, the means of the indices both indicated very poor water quality for the site. Although the number of taxa did not vary significantly between the sampling seasons, the highest number of taxa (20) was recorded during autumn (2010). Heavy rain preceded the sampling event in winter and Site 2 was severely affected, with very few organisms collected. The winter samples at Site 2 were therefore eliminated from SASS and other analyses because their biotic composition was evidently different during data exploration. At Sites 3 and 4, SASS5 scores and ASPT values revealed the E/F ecological category, (very poor water quality) throughout the sampling seasons. The overall lowest number of taxa (8) in the river was recorded at Site 3 in winter 2010.

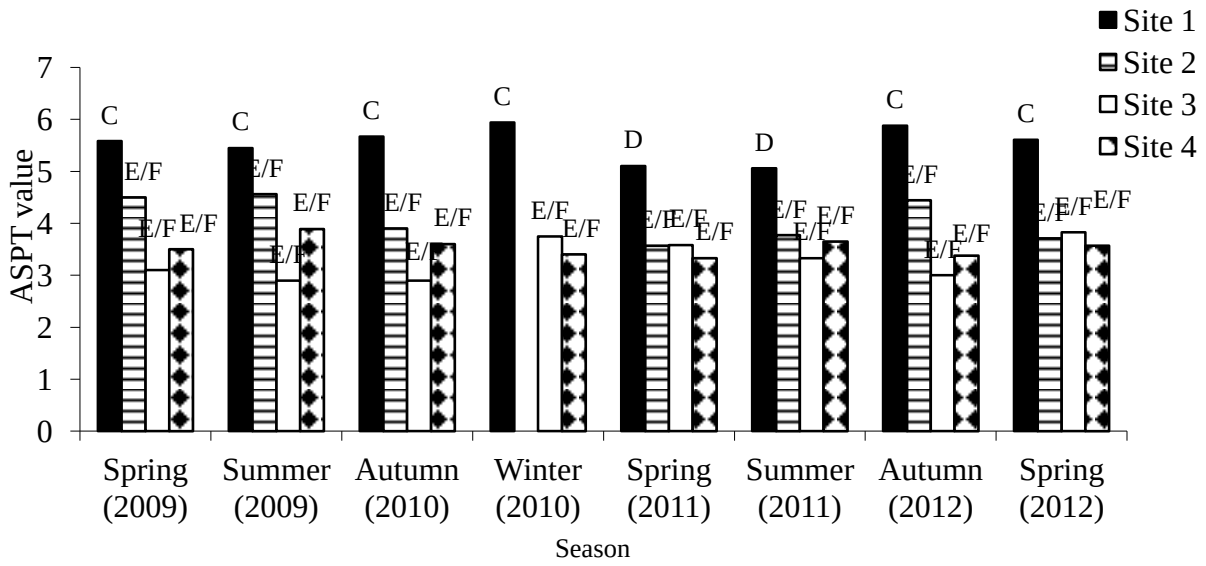


Figure 3.5: Seasonal variations for the average score per recorded taxon (ASPT) at the four sampling sites in the Swartkops River during the study period (August 2009–September 2012). The ecological categories: C (fair water quality), D (poor water quality) and E/F (very poor water quality) are indicated on the bars.

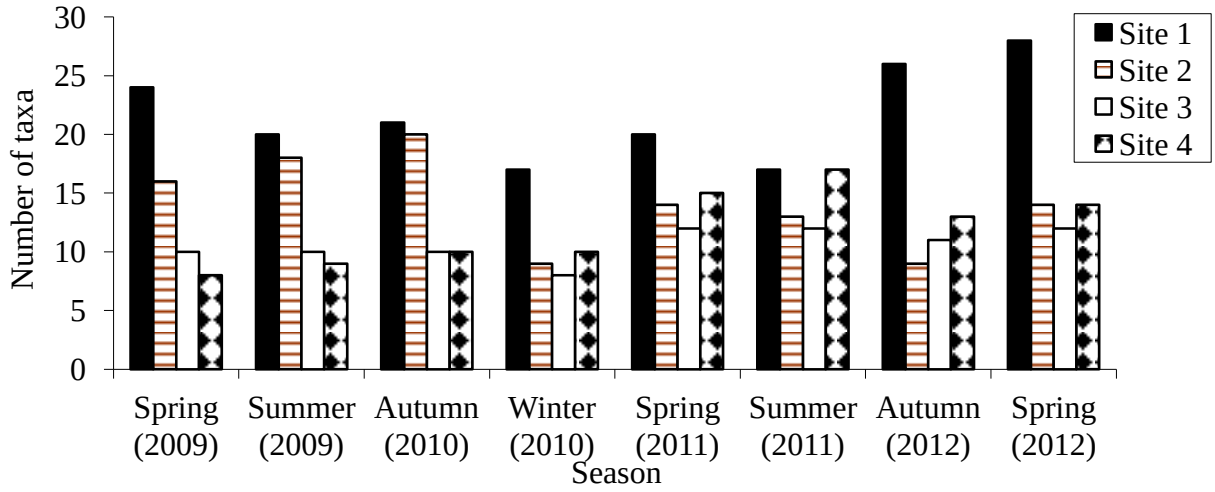


Figure 3.6: Seasonal variations for the number of taxa at the four sampling sites in the Swartkops River during the study period (August 2009–September 2012).

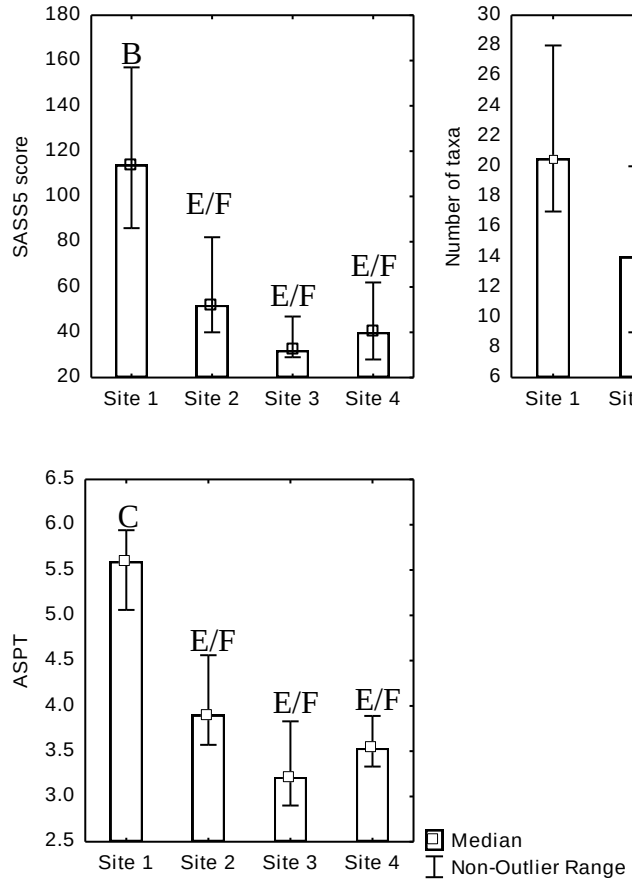


Figure 3.7: Summary of the SASS5 scores, number of taxa and ASPT values at the four sampling sites in the Swartkops River during the study period (August 2009 – September 2012). The overall ecological categories: B (good water quality), C (fair water quality), D (poor water quality) and E/F (very poor water quality) are indicated on the bars.

Comparing SASS5 scores, ASPT values and the numbers of taxa between the selected biotopes (stone, vegetation and GSM)

The vegetation and stone biotope had higher SASS5 scores, ASPT values, and numbers of taxa than the GSM biotope at Site 1 (Figure 3.8). Statistically, the Kruskal-Wallis multiple comparison test revealed that SASS5 scores were significantly higher for the vegetation than for the GSM biotope at Site 1 ($P < 0.05$; KW-H = 7.21). Similarly, at Site 2, SASS5 scores were significantly higher for the vegetation than for the GSM biotope ($P < 0.05$; KW-H = 10.13) and though the stone had higher SASS5 scores, they were not statistically higher than the scores recorded for the GSM biotope. The pattern described for Site 2 was similar to that observed for Sites 3 and 4 where the SASS5 scores were significantly higher for the vegetation biotope than the stone and GSM biotopes (Site 3: $P < 0.05$; KW-H = 40.44), (Site 4: $P < 0.05$; KW-H = 18.14).

The average score per recorded taxon (ASPT) values were close between the three biotopes at Site 1 and the Kruskal-Wallis multiple comparison test showed no significant differences between them ($P > 0.05$; KW-H = 0.75), but at Site 2, the ASPT values were significantly higher for the vegetation than the GSM biotope ($P < 0.05$; KW-H = 9.45). Although the ASPT value was also higher for the vegetation than the stone biotope at Site 2, it was not statistically significantly different ($P > 0.05$). Similarly, the vegetation had significantly higher ASPT values than the stone and GSM biotopes at Sites 3 ($P < 0.05$; KW-H = 26.9) and 4 ($P < 0.05$; KW-H = 14.25).

Stone and vegetation biotopes supported significantly higher numbers of taxa than the GSM biotope at Site 1 ($P < 0.05$; KW-H = 11.89), but at Site 2, only the vegetation supported significantly higher numbers of taxa than the GSM ($P < 0.05$; KW-H = 7.23). More taxa were recorded on the vegetation and GSM biotopes than on the stone biotopes at Site 3. The Kruskal-Wallis multiple comparison test indicated that the numbers of taxa for the stone biotope were significantly lower than the taxa recorded for the vegetation and GSM ($P < 0.05$; KW-H = 40.44). At Site 4, the stone and vegetation supported more taxa, but only the numbers of taxa supported by the vegetation biotope were significantly higher than the values recorded for the GSM ($P < 0.05$; KW-H = 16.27).

Generally, the stone and vegetation biotopes supported more taxa, and thus provided higher SASS5 scores and ASPT values, relative to the GSM biotope. However, at Site 3, which is downstream of the effluent discharge point, the stone biotope supported fewer taxa than the vegetation and GSM, and thus provided lower SASS5 scores and ASPT values than the vegetation and GSM (Figure 3.8).

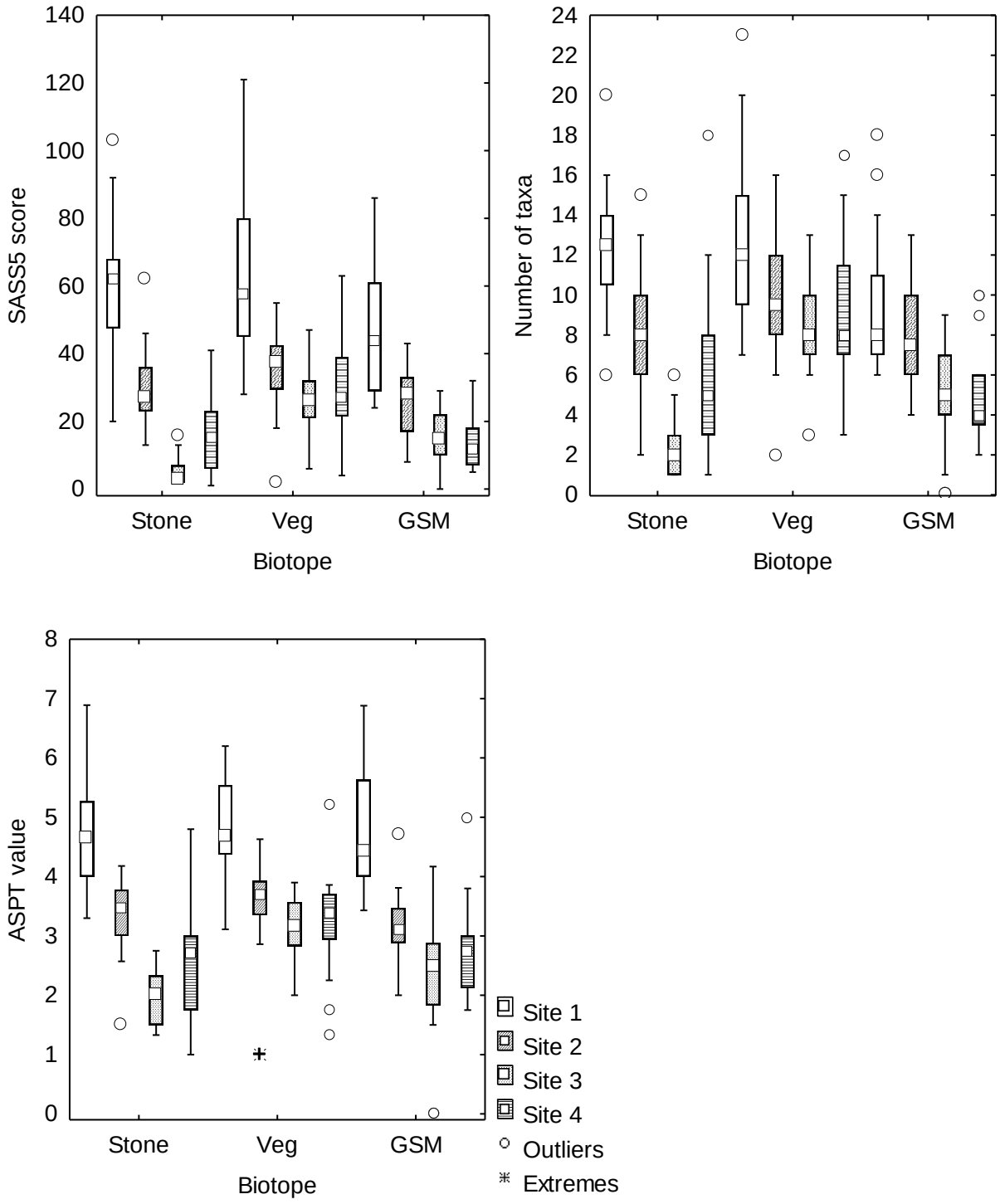


Figure 3.8: Median (small square), inter-quartile ranges (box), non-outlier ranges (bars) for SASS5 scores, numbers of taxa and ASPT values recorded per biotope at the four sampling sites in the Swartkops River during the study period (August 2009–September 2012).

3.3.5 Developing the Swartkops multimetric index (MMIswartkops) for assessing the river's health

Of the 12 family- level taxonomic metrics that enabled the discrimination of Site 1 from Sites 2, 3 and 4 (Table 2.3, Chapter 2), which were then tested for seasonal stability, only Margalef's family richness index showed significant seasonal variation between the sampling seasons ($P < 0.05$; KW-H = 8.55), so it was removed from further analysis. The Spearman's rank correlation coefficient revealed that the majority of the metrics were strongly correlated with each other (Table 3.3). Of the 11 remaining family-level taxonomic metrics, only three were weakly correlated with each other, and they were therefore retained and integrated with SASS5 scores and ASPT values to develop the Swartkops multimetric index. These were: percent Chironomidae + Oligochaeta (i.e. percent of individuals in the Chironomidae family plus order Oligochaeta relative to total number of individual in an entire sample), ET (Ephemeroptera-Trichoptera) richness (i.e. numbers of families in the ET), and Simpson family diversity index. The final five metrics including the SASS5 scores and ASPT values represented measures of composition (i.e. percent relative abundance), richness, diversity and biotic indices.

Table 3.3: Spearman's rank two-tailed correlation coefficient showing redundant macroinvertebrate metrics ($r > 0.75$, $P < 0.05$) analysed for the Swartkops River macroinvertebrate communities during the study period (August 2009–September 2012). Double asterisks revealed significant correlation at alpha < 0.01. Abbreviations: Trich abun (Trichoptera abundance), % Chiro + Olig (percent Chironomidae + Oligochaeta), % Trich (percent Trichoptera), % ETOC (percent Ephemeroptera-Trichoptera-Odonata-Coleoptera), ETOC richness (Ephemeroptera-Trichoptera-Odonata-Coleoptera richness), ET richness (Ephemeroptera-Trichoptera richness), SASS (South African Scoring System), and ASPT (Average Score Per recorded Taxon).

	Trich abun	% Chiro + Olig	% Trich	% ETOC	ETOC richness	ET richness	Pielou's evenness	Shannon	Simpson	SASS	ASPT
Trich abun	1	-.365**	.996**	.538**	.660**	.782**	.466**	.634**	.560**	.581**	.574**
% Chiro + Olig	-.365**	1	-.375**	-.704**	-.515**	-.533**	-.723**	-.752**	-.783**	-.564**	-.447**
% Trich	.996**	-.375**	1	.543**	.662**	.781**	.482**	.646**	.573**	.589**	.585**
% ETOC	.538**	-.704**	.543**	1	.788**	.732**	.750**	.832**	.816**	.812**	.679**
ETOC richness	.660**	-.515**	.662**	.788**	1	.816**	.556**	.778**	.685**	.890**	.789**
ET richness	.782**	-.533**	.781**	.732**	.816**	1	.557**	.722**	.659**	.744**	.604**
Pielou's evenness	.466**	-.723**	.482**	.750**	.556**	.557**	1	.903**	.949**	.577**	.493**
Shannon	.634**	-.752**	.646**	.832**	.778**	.722**	.903**	1	.965**	.790**	.680**
Simpsons	.560**	-.783**	.573**	.816**	.685**	.659**	.949**	.965**	1	.711**	.605**
SASS	.581**	-.564**	.589**	.812**	.890**	.744**	.577**	.790**	.711**	1	.845**
ASPT	.574**	-.447**	.585**	.679**	.789**	.604**	.493**	.680**	.605**	.845**	1

The median values of the three metrics selected in addition to SASS5 scores and ASPT values are shown in Figure 3.9. Of these metrics, percentage Chironomidae + Oligochaeta increased in numerical value with increased levels of pollution and was thus consistently higher at Sites 3 and 4 relative to Sites 1 and 2 in all sampling seasons. Conversely, the numerical value of the ET family richness and Simpson family diversity index decreased with increased levels of pollution, being lower at Sites 3 and 4 relative to Sites 1 and 2.

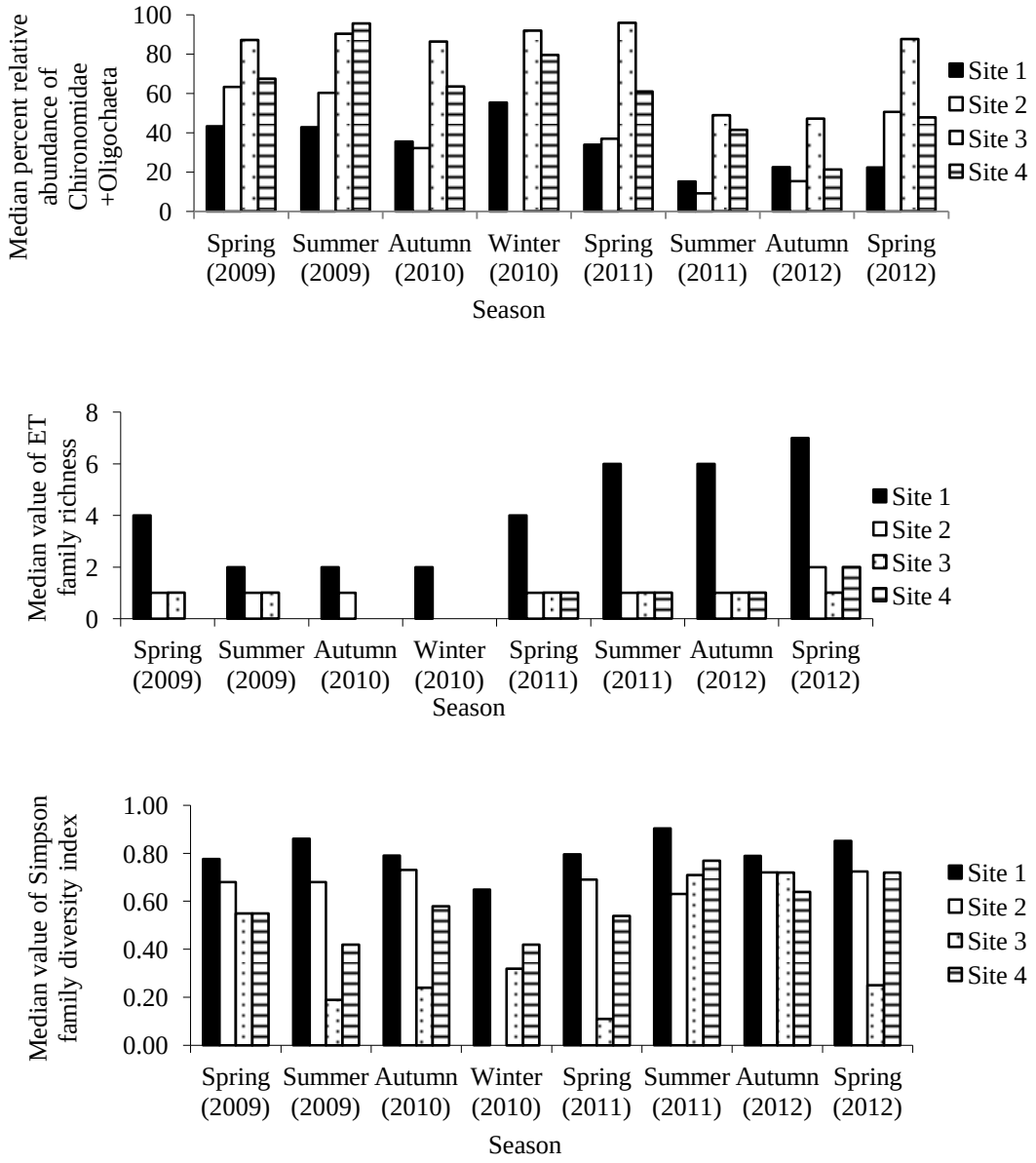


Figure 3.9: Median values of the three metrics (percent Chironomidae + Oligochaeta, ET family richness and Simpson family diversity index) selected in addition to SASS5 score and ASPT value in the Swartkops River during the study period (August 2009–September 2012).

Based on the five selected metrics, the Swartkops multimetric index (MMIswartkops) was then developed by calculating the minimum value, lower quartile (25%), mid-quartile (50%), upper quartile (75%), and maximum value of each metric for the Site 1 assemblages and these values were used as thresholds for separating the scores (Tables 3.4 and 3.5). The MMIswartkops was then computed by summing the scores of the five component metrics, and the index value range (5–25) quadrisected to obtain four ecological categories B, C, D and E/F indicative of good, fair, poor and very poor water quality conditions (Table 3.5). Ecological category A indicating pristine (i.e. natural) water quality was omitted because the macroinvertebrate assemblages used in developing the index did not come from a pristine condition, but one that was minimally impacted.

When the newly developed MMIswartkops was used to assess the Swartkops River's health at the impaired sites over the sampling seasons, it rated water quality at Site 3 as very poor indicative of ecological category E/F in all sampling seasons, and Site 4 as also very poor, except during summer (2011), and autumn (2012), for which the index indicated poor water quality i.e. the D ecological category (Figure 3.7). Water quality at Site 2 was rated as very throughout the study period (the E/F ecological category) (Figure 3.9).

Table 3.4: Calculated metric threshold based on macroinvertebrate assemblages at Site 1 for each of the five metrics integrated into the Swartkops multimetric index. These thresholds enabled the separation of the assessment scores indicative of the different ecological categories. Abbreviation: % Chi+Olig (percent Chironomidae + Oligochaeta), ET (Ephemeroptera-Trichoptera) family richness, SASS5 (South African Scoring System version 5) score, and ASPT (Average score per recorded taxon) value.

	Statistics					Score		
Metrics	Min.	25%	50%	75%	Max.	5	3	1
% Chi+Olig.	6.94	22.46	34.73	43.49	57.99	< 43.49	≥ 43.49 -58	> 58
SASS5 score	82	101.75	114	138.7	157	≥ 114	82-138.75	< 82
ASPT value	5.06	5.36	5.60	5.72	5.94	≥5.37	5.06 - 5.36	< 5.06
ET richness	3	3	4	6	9	> 3	3	<3
Simpson index	0.62	0.75	0.79	0.85	0.91	≥ 0.75	0.63- < 0.75	< 0.63

Table 3.5: Swartkops multimetric index score range indicative of different ecological and water quality categories. The range (5-25) was obtained by summing the scores of the five component metrics (Table 3.10), and then quadrisected to obtain the four ecological categories B, C, D and E/F indicative of good, fair, poor and very poor water quality.

Ecological category	E/F	D	C	B
Water quality condition	Very poor	Poor	Fair	Good
MMswartkops score range	5-10	11-16	17-21	22-25

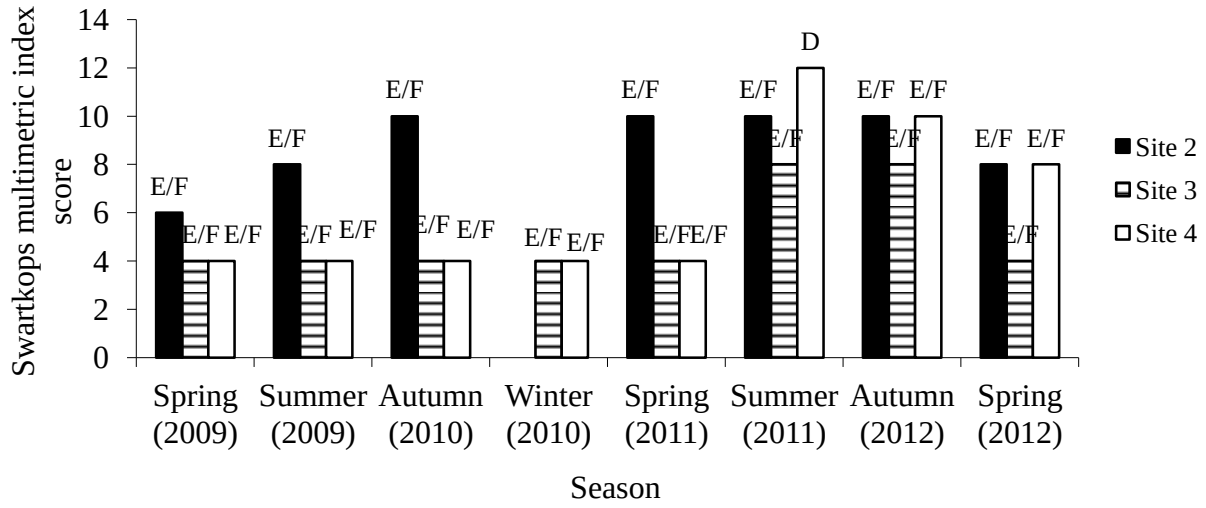


Figure 3.10: Swartkops multimetric index showing ecological categories D and E/F indicative of water quality at the three impaired sites (i.e. Sites 2, 3 and 4) in the Swartkops River during the study period (August 2009–September 2012). Ecological categories: D (poor water quality), E/F (very poor water quality).

3.3.6 Correlating the five component metrics in the Swartkops multimetric index with water physico-chemical variables

Canonical correspondence analysis (CCA) was used to correlate the five metrics with the measured water physico-chemical variables (Figure 3.11). Zinc (Zn), five-day biochemical oxygen demand (BOD_5), nitrate-nitrogen (NO_3-N), nitrite-nitrogen (NO_2-N), and ammonia-nitrogen (NH_4-N) were removed from the final CCA analysis because they displayed appreciable multi-co-linearity (Pearson's $r > 0.8$; $P < 0.05$) with other variables. Of the five metrics, only percent Chironomidae + Oligochaeta was strongly positively correlated with nutrients, electrical conductivity (EC), turbidity and copper (Cu). Although temperature, pH and manganese (Mn) were also positively correlated with percent Chironomidae + Oligochaeta, their correlations were less strong relative to nutrients, EC, turbidity and Cu. Increases in these water physico-chemical variables were associated with decreases in ET family richness, SASS5 scores, ASPT values and Simpson diversity index (Figure 3.11). Conversely, increased dissolved oxygen concentrations favoured EPT family richness, SASS5 scores, ASPT values and Simpson family diversity index.

The first CCA axis with Eigen value 0.030 explained 64.09 of the total variance and the first three axes explained a cumulative variance of 71.8%, showing that the CCA model captured

the majority of the variance within the data set used for the analysis. The Monte Carlo permutation and Person's correlation tests revealed that the first two axes of the ordination were significantly correlated with the water physico-chemical variables (Table 3.6).

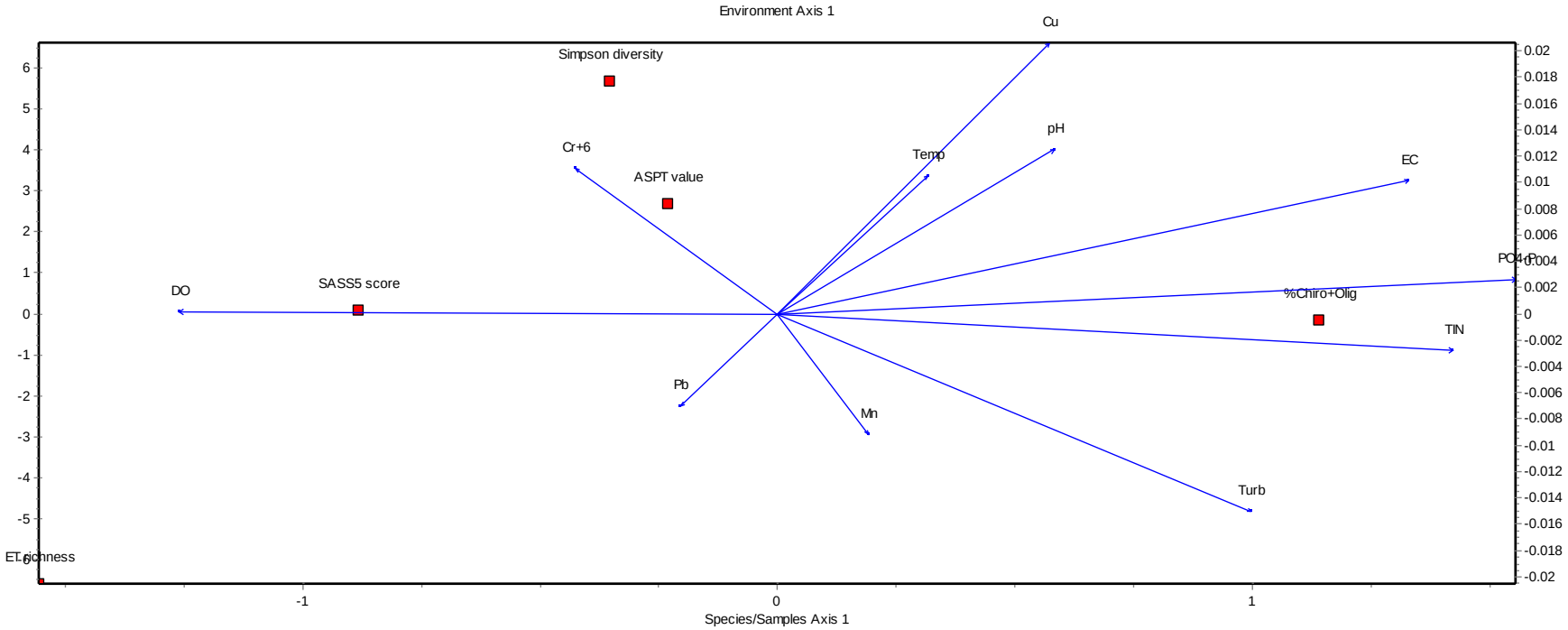


Figure 3.11 CCA ordination plot showing the correlations between the five metrics, integrated into the Swartkops multimetric index, and the selected water physico-chemical variables in the Swartkops River during the study period (August 2009–September 2012). Abbreviation of metrics: ET (Ephemeroptera-Trichoptera), SASS5 (South African Scoring System version 5), ASPT (Average score per recorded taxon), % Chiro + Olig (percent Chironomidae + Oligochaeta); water physico-chemical variables: Turb (turbidity), TIN (total inorganic nitrogen), EC (electrical conductivity), DO (dissolved oxygen) and Temp (temperature).

Table 3.6: Properties of the CCA ordination tri-plot between the water physico-chemical variables and the five metrics, percent Chironomidae + Oligochaeta, SASS5 scores, ASPT values, ET family richness and Simpson family diversity index, integrated into the Swartkops multimetric index.

Canonical properties	**Axis**		
	1	2	3
Canonical Eigen value	0.030	0.004	0.001
Percent variance explained	64.09	7.432	0.273
Percent cumulative variance explained	64.09	71.52	71.8
Monte Carlo test p – value	0.010	0.010	0.970
Pearson correlation of species and environmental Scores	0.863	0.836	0.396

3.3.7 Chironomid species-level taxonomic communities in the Swartkops River: Spatial-temporal variation

Thirty-five chironomid species belonging to three subfamilies, Chironominae, Orthocladiinae and Tanypodinae, were recorded during the study period (Appendix A). The relative abundance of species at Site 1 in all the sampling seasons were mostly dominated by *Cricotopus* sp.1, *Cricotopus trifasciata* gr., *Orthocladius* sp., *Polypedilum* sp., *Tanytarsus* sp. and *Ablabesmyia* sp. (Appendix A, Table A1). During spring and summer, species belonging to the Chironominae and Tanypodinae dominated the chironomid assemblages at Site 1 (Figure 3.12). In autumn, species of Chironominae dominated the collections at Site 1, while both Tanypodinae and Orthocladiinae had five species each. The Orthocladiinae dominated the winter collection.

During spring at Site 2, *Cricotopus* sp.1, *Cricotopus trifasciata* gr. and *Dicrotendipes* sp. dominated the chironomid relative abundance (Appendix A, Table A2). In summer and autumn, *Chironomus* sp.1, *Dicrotendipes* sp. and *Tanypus* sp. were the dominant species. However, *Chironomus* sp. contributed over 80% of the relative abundance in winter. Overall, species belonging to subfamilies Chironominae and Orthocladiinae were predominant at this site. Of the species belonging to sub-family Chironominae, the species of the tribe Chironomini were more dominant than species of the tribe Tanytarsini (Figure 3.12).

Throughout the sampling seasons, the relative abundance of chironomid species at Site 3 was dominated by *Chironomus* spp. and *Dicrotendipes* sp (Appendix A, Table A3). Apart from the autumn (2012) and spring (2012) sampling events, *Chironomus* spp. consistently contributed over 70% of species relative abundance at this site. Species of the subfamily Tanypodinae and tribe Tanytarsini did not occur at Site 3. Chironomid subfamily composition at Site 4 was similar to Site 3 with the subfamily Chironominae having the highest numbers of species in all the sampling seasons (Figure 3.12) (Appendix A, Table A4).

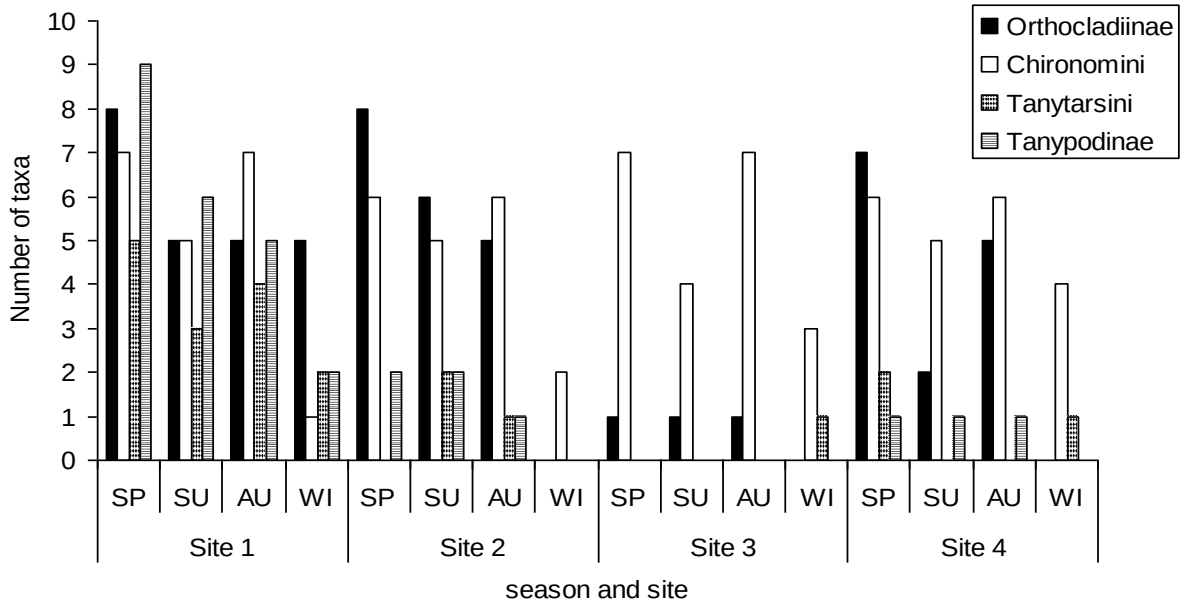


Figure 3.12: Seasonal variations in the numbers of species recorded for the subfamilies of chironomids in the Swartkops River during the study period (August 2009–September 2012). Chironomini and Tanytarsini are tribes belonging to subfamily Chironominae. Abbreviations of seasons: SP (spring), SU (summer), AU (autumn) and WI (winter).

Generally, Site 1 supported more chironomid species than Sites 2, 3 and 4. In the three downstream sites, the greatest number of species were encountered at Site 2, followed by Site 4 and then Site 3 (Figure 3.13). Seasonally, at all four of the sampling sites, more species were sampled in spring and very few species were collected in winter. The number of species recorded in autumn and summer did not show a consistent pattern across the sites, as more species were recorded in summer at Site 2, and in autumn at Sites 1, 3 and 4 (Figure 3.13)

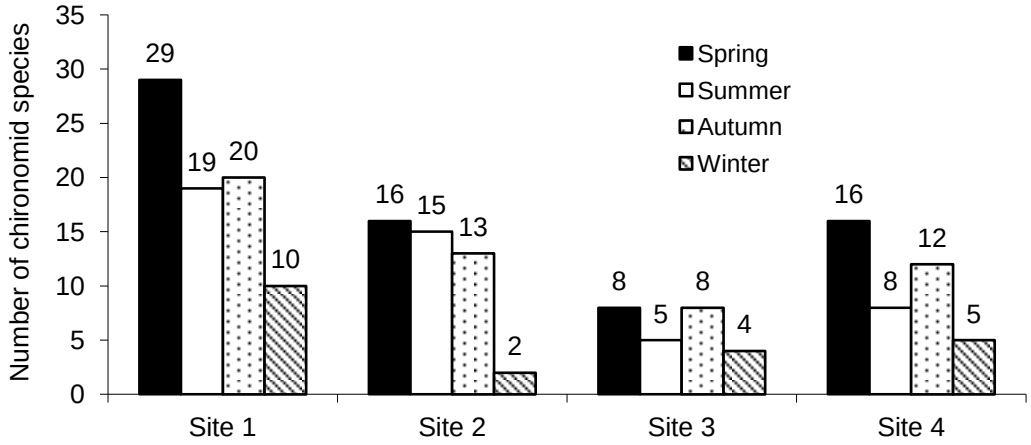


Figure 3.13: Summary of the numbers of chironomid species sampled at the four sampling sites in the Swartkops River during the sampling seasons. Numbers on top of each bar are exact numbers of species recorded for that season.

Differences between the sampling sites based on chironomid species compositions

The NMDS scaling based on Bray-Curtis similarity index undertaken to elucidate the patterns of community structure of the chironomid species, revealed that species clustered largely by sites rather than by seasons (Figure 3.13). In all the seasons, the chironomid species compositions at Site 1 were largely separated from the rest of the sampling sites. The chironomid species composition largely enabled the discrimination of Site 2 from Sites 3 and 4, with the communities at Site 2 forming a separate cluster from those at Sites 3 and 4, except in spring and autumn in which the communities at Site 4 were closely associated with Site 2 (Figure 3.13). The chironomid species communities at Site 3 were markedly different from those at Sites 1 and 2, but the differences between Site 3's assemblages and those at Site 4, were less pronounced as these two sites clustered together in summer and winter (Figure 3.13). The ANOSIM indicated a global statistically significant difference in chironomid species compositions between the sampling sites (Global $R = 0.643$; $P < 0.05$) and the highest significant difference was between Sites 1 and 3 (Table 3.7). Apart from the assemblages at Site 3 and 4, which were not significantly different, the chironomid species compositions enabled the discrimination of the sampling sites from each other. The ANOSIM results therefore agreed with the NMDS clusters described above (Table 3.7).

Furthermore, SIMPER indicated the percent dissimilarity in chironomid species composition and showed an average percent dissimilarity of 92.62% between Sites 1 and 3, which was

followed by an average dissimilarity of 77.67% between Sites 1 and 4 (Figure 3.7). Again, of all the sampling sites, the smallest percent average dissimilarity (46.03%) was between Sites 3 and 4 further suggesting that the differences between these two sites were less pronounced than between others. *Tanytarsus* sp., *Dicrotendipes* sp., *Chironomus* sp.1, *Chironomus* sp. 2, *Cricotopus trifasciata* gr. and *Ablabesmyia* sp. were the main discriminating species between the sampling sites.

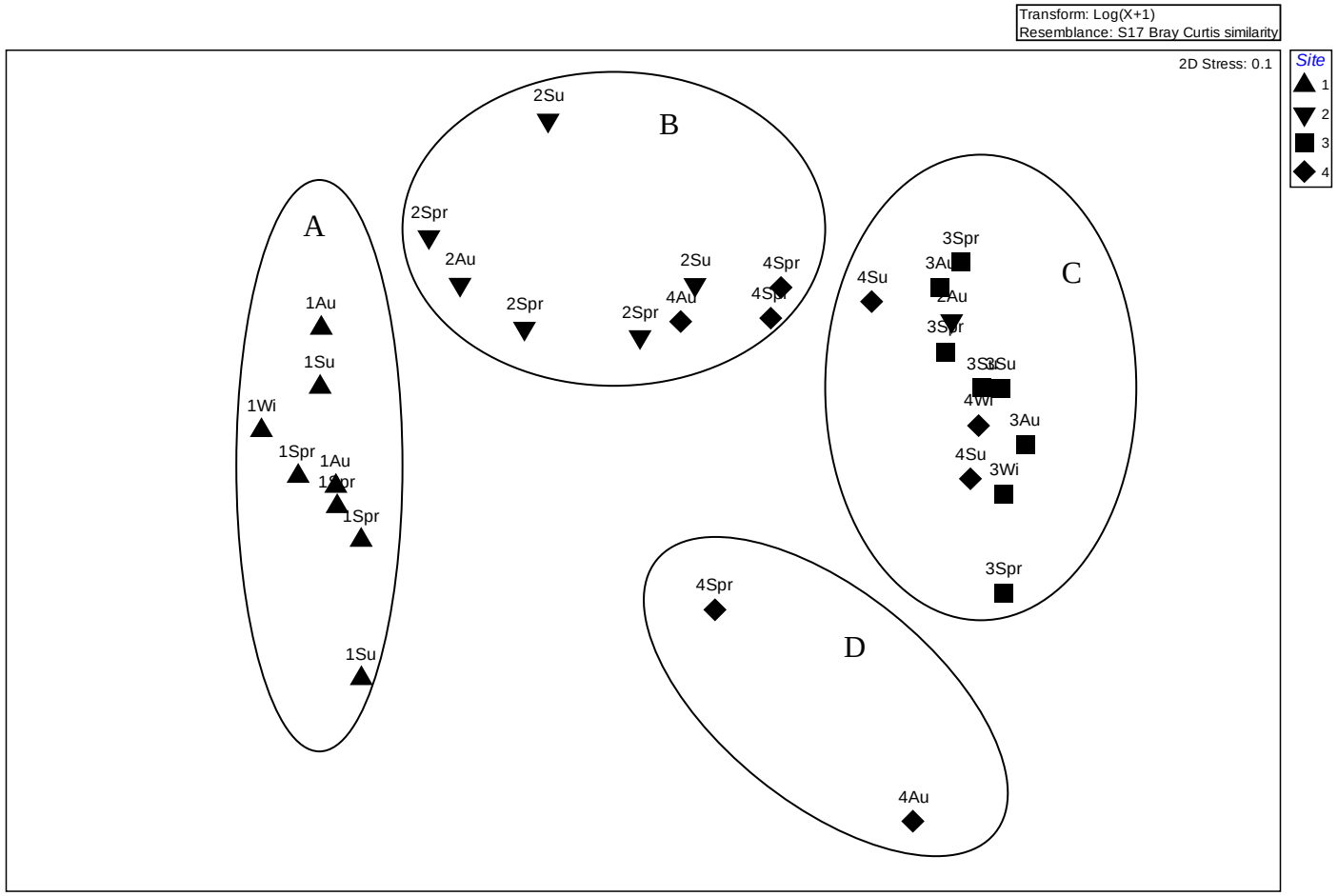


Figure 3.14: Non-metric multidimensional scaling based on Bray-Curtis similarity index showing how the sites formed clusters based on their chironomid species compositions in the Swartkops River during the study period (August 2009–September 2012). Samples collected at Site 1 formed cluster A; samples at Site 2, together with four samples at Site 4, formed cluster B; samples collected at Site 3 together with three samples at Site 4, formed cluster C. Cluster D consisted of only two samples at Site 4, which could be considered outliers. Abbreviation of seasons: Spr (spring), Su (summer), Au (autumn) and Wi (winter). Sites: 1 (Site 1), 2 (Site 2), 3 (Site 3) and 4 (Site 4).

Table 3.7: ANOSIM showing global and pair-wise tests R statistics and significance level between the sampling sites based on their chironomid species compositions in the Swartkops River over the study period (August 2009–September 2012). Boldface indicates sites that were significantly different in terms of chironomid species compositions

Sites	R statistic	Significance level %
1, 2	**0.687**	**0.1**
1, 3	**1**	**0.1**
1, 4	**0.868**	**0.1**
2, 3	**0.753**	**0.1**
2, 4	**0.241**	**2.8**
3, 4	0.164	2.5

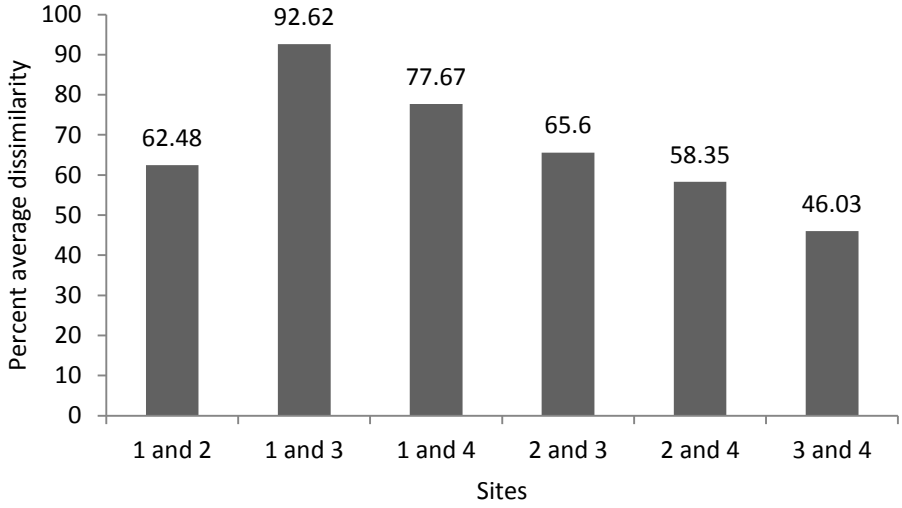


Figure 3.15: SIMPER analysis result showing average percent dissimilarities between the sampling sites based on their chironomid species compositions in the Swartkops River during the study period (August 2009–September 2012). The numbers above each bar are the actual percent average dissimilarities.

3.3.8 Relating chironomid species to water physico-chemical variables

A CCA ordination tri-plot was used to elucidate the correlation between the chironomid species and the water physico-chemical variables. Of the 16 water physico-chemical variables measured, only 11 were included in the final CCA model (Figure 3.16). Zinc (Zn), five-day biochemical oxygen demand (BOD_5), nitrate-nitrogen (NO_3-N), nitrite-nitrogen (NO_2-N) and

ammonia-nitrogen (NH_4-N) exhibited appreciable multi-co-linearity with other variables (Pearson's r > 0.8. $P < 0.05$), and were therefore eliminated from the analysis. Rare species defined in this study as species occurring in less than four replicate samples were removed from the CCA analysis to avoid their confounding effects on the final results. Consequently, *Nilotanypus* sp., *Coelotanytarsus* sp., *Virgatanytarsus* sp., *Polypedilum nubifer*, *Glyptotendipes* sp., *Clinotanypus* sp. and *Procladius* sp. were not included in the ordination (Figure 3.16).

The first axis with an Eigen value 0.525 of the CCA ordination explained 36.61% of the total variance. The first three axes explained a cumulative variance of 52.29% indicating a good ordination (Table 3.8). The Monte Carlo (1000 permutations) and the Pearson correlation tests showed significant correlations between the CCA axes and the physico-chemical variables (Table 3.8).

The CCA ordination indicated strong correlations between the chironomid species and the physicochemical variables on Axis 1. Dissolved oxygen (DO) was strongly positively correlated with species such as *Polypedilum* sp., *Ablabesmyia* sp., *Tanytarsus* sp., *Nanocladius* sp., *Macropelopia* sp., *Cricotopus trifasciata* gr, *Orthocladius* sp, *Tanytarsus* sp., *Rheotanytarsus* sp., and *Cladotanytarsus* sp. These species were negatively correlated with increased nutrients, electrical conductivity (EC) and turbidity (Figure 3.16). Conversely, electrical conductivity (EC), total inorganic nitrogen (TIN), orthophosphate-phosphorus (PO_4-P), turbidity and copper (Cu) were strongly positively correlated with *Chironomus* sp.1., *Chironomus* sp.2, *Chironomus* sp.3, *Dicrotendipes* sp. and *Kiefferulus* sp. Water physico-chemical variables including lead (Pb), chromium (VI) (Cr^{+6}), manganese (Mn), pH and temperature did not display a strong correlation with the chironomid species (Figure 3.16).

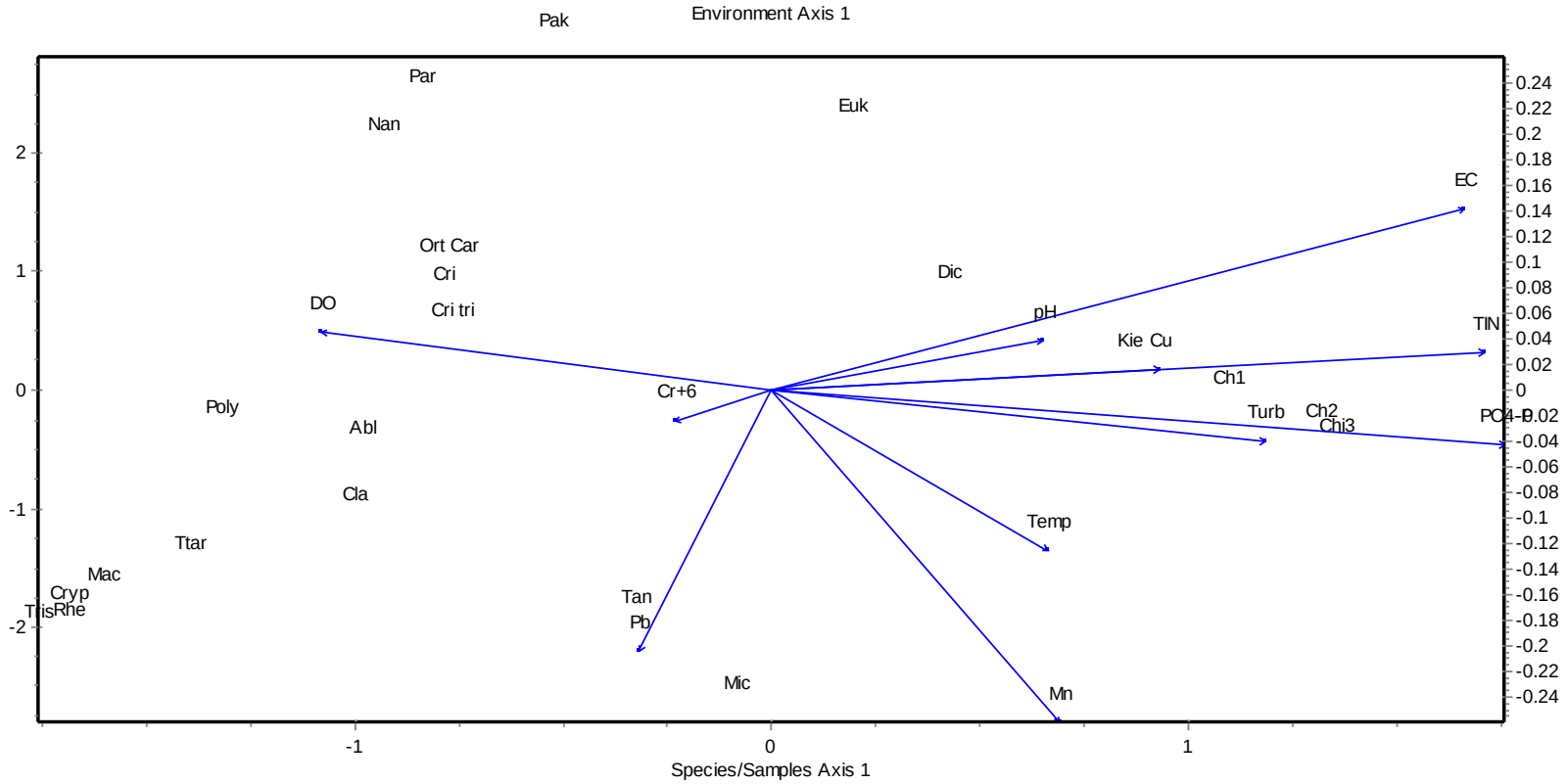


Figure 3.16. CCA ordination showing the correlations between the Swartkops River chironomid species and selected water physico-chemical variables during the study period (August 2009 – September 2012). Chironomid species abbreviations: Cri (*Cricotopus* sp.1), Cri tri (*Cricotopus trifasciata* gr), Par (*Paratrichocladius* sp.), Nan (*Nanocladius* sp.), Dic (*Dicrotendipes* sp.), Euk (*Eukiefferiella* sp.), Abl (*Ablabesmyia* sp.), Kie (*Kiefferulus* sp.), Car (*Cardiocladius* sp.), Ttar (*Tanytarsus* sp.), Poly (*Polypedilum* sp.), Pak (*Parakiefferiella* sp.), Cryp (*Cryptochironomus* sp.), Tris (*Trissopelopia* sp.), Ch1 (*Chironomus* sp.1), Ch2 (*Chironomus* sp.2), Chi3 (*Chironomus* sp.3), Ort (*Orthocladius* sp.), Rhe (*Rheotanytarsus* sp.), Mic (*Microchironomus* sp.), Tan (*Tanypus* sp.), Cla (*Cladotanytarsus* sp.), and Mac (*Macropelopia* sp.).

Table 3.8: Properties of the CCA ordination tri-plot between the chironomid species and the selected water physico-chemical variables in the Swartkops River during the study period (August 2009–September 2012).

Canonical properties	**Axis**		
	1	2	3
Canonical Eigen value	0.525	0.147	0.093
Percent variance explained	36.61	10.21	6.47
Percent cumulative variance explained	36.61	46.82	53.29
Monte Carlo test p – value	0.001	0.011	0.004
Pearson correlation of species and environmental scores	0.970	0.913	0.896

Determining chironomid species tolerances to pollution in the Swartkops River

Chironomid species pollution tolerances were determined using their relative positions along Axis 1 of the CCA ordination (i.e. species coordinate along the first axis of the CCA ordination) (Table 3.9). Based on the co-ordinates, the species tolerance scores were obtained by calculating the inter-species distances starting with the two most distant species, and the calculated distances were then converted to percent distances (see method sections). Species positions on the first axis alone were used because the axis explained over 60% of the total cumulative variance explained by the first three axes (Table 3.8). Thus, species were scored 1 to 10, with score 10 indicating the most tolerant species strongly positively associated with increased nutrients, EC, turbidity and copper, and score 1 the least tolerant, strongly negatively associated with the above-mentioned water physico-chemical variables, but positively associated with dissolved oxygen. The remaining taxa were scored in successive 10 percentile bands (Table 3.9). Thus, species such as *Trissopelopia* sp., *Cryptochironomus* sp., *Rheotanytarsus* sp., *Macropelopia* sp., *Tanytarsus* sp. and *Polypedilum* sp. were less tolerant of pollution in the Swartkops River. On the contrary, *Chironomus* sp.1, *Chironomus* sp.2, *Chironomus* sp.3, *Dicrotendipes* sp., *Kiefferulus* sp. and *Eukiefferiella* sp. were highly tolerant (Table 3.9).

Indicator species analysis corroborated the CCA-based chironomid species tolerance ranking to pollution in the Swartkops River. The indicator species analysis revealed that the least tolerant species with score 1 were significantly associated with Site 1 and they displayed absolute positive predictive power (Table 3.10). Species such as *Cricotopus trifasciata, Cricotopus* sp., *Paratrichocladius* sp. *Nanocladius* sp. having moderate pollution tolerant scores of 3 and 4 were significantly associated with both Sites 1 and 2 (Tables 3.9; 3.10). Conversely, the most pollution-tolerant species revealed by the CCA analysis, with a score of 10, were significantly associated with Sites 3 and 4 that had high nutrient and EC, and low dissolved oxygen concentrations. Thus, the Swartkops River chironomid had both pollution-sensitive and tolerant species that enabled the sites to be discriminated. The highly sensitive species were absolutely associated with Site 1 and disappeared completely at the downstream sites, particularly Sites 3 and 4.

Table 3.9: Showing scores 1–10 in order of tolerance of chironomid species to pollution in the Swartkops River. The species tolerance scores were derived from the relative position of species along the first axis of the CCA plot in Figure 3.16.

Species	Species coordinates on Axis 1	Inter-species distance	% distance	Species tolerance score
Trissopelopia sp.	-1.278	2.2603	100	1
Cryptochironomus sp.	-1.228	2.2103	98	1
Rheotantarsus sp.	-1.226	2.2083	98	1
Macropelopia sp.	-1.163	2.1453	95	1
Tanytarsus sp.	-1.015	1.9973	88	2
Polypedilum sp.	-0.9579	1.9402	86	2
Cladotanytarsus sp.	-0.7297	1.712	76	3
Ablabesmyia sp.	-0.7151	1.6974	75	3
Nanocladius sp.	-0.6783	1.6606	73	3
Paratrichocladius sp.	-0.6111	1.5934	70	4
Orthocladius sp.	-0.5962	1.5785	70	4
Cricotopus sp.1	-0.5727	1.555	69	4
Cricotopus trifasciata gr.	-0.5576	1.5399	68	4
Cardiocladius sp	-0.5395	1.5218	67	4
Parakiefferiella sp.	-0.3831	1.3654	60	5
Tanypus sp.	-0.2362	1.2185	54	5
Microchironomus sp.	-0.06198	1.04428	46	6
Eukiefferiella sp.	0.1377	0.8446	37	7
Dicrotendipes sp	0.3084	0.6739	30	8
Kiefferulus sp.	0.623	0.3593	16	9
Chironomus sp. 1	0.7945	0.1878	8	10
Chironomus sp. 2	0.9567	0.0256	1	10
Chironomus sp.3	0.9823	0	0	10

Table 3.10: Indicator species analysis revealing the association of species with the sampling sites in the Swartkops River during the study period (August 2009 – September 2012). The numbers 1 and 0 under the respective site columns indicate when a species is associated and not associated with the site. Code: 1 (species associated with Site 1), 2 (species associated with Site 2), 3 and 7 (species associated with Sites 3 and 4), 4 (species associated with Sites 1 and 2), 5, 6 and 9 (species associated with Sites 2, 3 and 4) and 8 (species indifferent to water quality differences; significantly associated with Sites 1, 2 and 4). Components: A (positive predictive power) and B (fidelity). Boldface indicates significant indicator values.

Taxa	Site 1	Site 2	Site 3	Site 4	Code	A	B	Indicator value	P-value
Ablabesmyia sp.	1	0	0	0	1	0.811	0.**597**	**0.696**	**0.001**
Tanytarsus sp.	1	0	0	0	1	0.977	**0.746**	**0.854**	**0.001**
Polypedilum sp.	1	0	0	0	1	0.952	0.**478**	**0.674**	**0.001**
Coelotanypus sp.	1	0	0	0	1	1.000	0.015	0.122	1.000
Cryptochironomus sp.	1	0	0	0	1	1.000	0.164	**0.405**	**0.001**
Trissopelopia sp.	1	0	0	0	1	1.000	0.179	**0.423**	**0.001**
Clinotanypus sp.	1	0	0	0	1	1.000	0.090	**0.299**	**0.003**
Rheotanytarsus sp.	1	0	0	0	1	1.000	0.060	**0.244**	**0.015**
Cladotanytarsus sp.	1	0	0	0	1	0.689	0.239	**0.406**	**0.001**
Nilotanypus sp.	1	0	0	0	1	1.000	0.090	**0.299**	**0.001**
Thienemannimyia sp	1	0	0	0	1	1.000	0.164	**0.405**	**0.001**
Macropelopia sp.	1	0	0	0	1	1.000	0.164	**0.405**	**0.001**
Virgatanytarsus sp.	1	0	0	0	1	1.000	0.105	**0.323**	**0.002**
Polypedilum nubifer	1	0	0	0	1	1.000	0.060	**0.244**	**0.025**
Conchapelopia sp.	1	0	0	0	1	1.000	0.015	0.122	1.000
Parakiefferiella sp.	0	1	0	0	2	0.707	0.057	0.223	0.110
Procladius sp.	0	1	0	0	2	0.874	0.283	0.223	0.053
Tanypus sp.	0	1	0	0	2	0.926	0.283	**0.512**	**0.001**

Orthocladius sp.2	0	1	0	0	2	1.000	0.019	0.137	0.197
Glyptotendipes sp.	0	0	1	0	3	0.956	0.075	**0.267**	**0.014**
Cricotopus sp.1	1	1	0	0	4	0.928	0.492	**0.676**	**0.001**
Cricotopus trifasciata gr.	1	1	0	0	4	0.879	0.558	**0.701**	**0.001**
Paratrichocladius sp.	1	1	0	0	4	0.972	0.125	**0.349**	**0.002**
Nanocladius sp.	1	1	0	0	4	1.000	0.142	**0.376**	**0.001**
Orthocladius sp.	1	1	0	0	4	0.800	0.542	**0.658**	**0.001**
Microchironomus sp.	1	1	0	0	4	0.915	0.050	0.214	0.136
Paratanytarsus sp.	1	1	0	0	4	1.000	0.033	0.183	0.234
Dicrotendipes sp.2	0	1	1	0	5	1.000	0.058	**0.242**	**0.030**
Eukiefferiella sp.	0	1	0	1	6	0.893	0.114	**0.319**	**0.023**
Chironomus sp. 1	0	0	1	1	7	0.907	0.977	**0.941**	**0.001**
Chironomus sp. 2	0	0	1	1	7	0.918	0.672	**0.785**	**0.001**
Chironomus sp .3	0	0	1	1	7	0.980	0.461	**0.672**	**0.001**
Cardiocladius sp.	1	1	0	1	8	1.000	0.166	**0.407**	**0.004**
Dicrotendipes sp.	0	1	1	1	9	0.972	0.663	**0.803**	**0.001**
Kiefferulus sp.	0	1	1	1	9	0.990	0.232	**0.479**	**0.001**

3.3.9 Identifying chironomid community types based on their preferred biotopes in the Swartkops River

Based on chironomid biotope preferences, five-chironomid community types were identified (Table 3.11). The first community type preferred the stone biotope, and consisted of eight species, but only *Cricotopus* sp.1 had a statistically significantly preference for the stone biotope. *Cricotopus* sp.1, *Cricotopus trifasciata* gr., *Paratrichocladius* sp., and *Cladotanytarsus* sp. dominated the abundance of the first community. The second community

type preferred the vegetation biotope, and consisted of only two species, i.e. *Tanytarsus* sp. and *Polypedilum* sp., with the latter showing a statistically significant preference for the vegetation biotope.

The third community type preferred the GSM biotope, and consisted of only three species: *Rheotanytarsus* sp., *Nilotanypus* sp., and *Thienemannimyia* sp., but only *Nilotanypus* sp. had a statistically significant preference for the biotope. The fourth community type consisted of three species that were weakly associated with both the stone and the vegetation biotopes, and two of these species, i.e. *Dicrotendipes* sp. and *Microchironomus* sp., belong to the subfamily Chironominae, tribe Chironomini. The last community type had five species that were weakly associated with both the stone and the GSM biotopes. This community was dominated by species of the predatory Tanypodinae i.e. *Ablabesmyia* sp., *Trissopelopia* sp., and *Macropelopia* sp.

Table 3.11 Chironomid species preferences for the selected biotope stone, vegetation (Veg.) and gravel, sand and mud (GSM) in the Swartkops River during the study period (August 2009 – September 2012). The numbers 1 and 0 under the biotope columns indicate species preferring and not preferring a particular biotope, respectively. Code (biotope preference code) indicates species preferring the stone biotope (1), the vegetation biotope (2), the GSM (3), both the stone and the vegetation biotopes (4), and both the stone and the GSM biotopes (5). Boldface indicates statistically significant preference.

Biotope						
Taxa	Stone	Veg	GSM	Code	Point-biserial coefficient	*P*-value
Cricotopus sp.1	1	0	0	1	**0.315**	**0.022**
Cricotopus trifasciata gr.	1	0	0	1	0.265	0.053
Paratrichocladius sp.	1	0	0	1	0.246	0.087
Cardiocladius sp.	1	0	0	1	0.181	0.315
Clinotanypus sp.	1	0	0	1	0.051	0.914
Orthocladius sp.	1	0	0	1	0.194	0.264
Tanypus sp.	1	0	0	1	0.066	0.869
Cladotanytarsus sp.	1	0	0	1	0.219	0.163

Tanytarsus sp.	0	1	0	2	0.128	0.562
Polypedilum sp.	0	1	0	2	**0.381**	**0.003**
Rheotanytarsus sp.	0	0	1	3	0.246	0.092
Nilotanypus sp.	0	0	1	3	**0.293**	**0.028**
Thienemannimyia sp.	0	0	1	3	0.183	0.320
Nanocladius sp.	1	1	0	4	0.133	0.587
Dicrotendipes sp.	1	1	0	4	0.173	0.386
Microchironomus sp.	1	1	0	4	0.149	0.774
Ablabesmyia sp.	1	0	1	5	0.129	0.544
Cryptochironomus sp.	1	0	1	5	0.221	0.150
Trissopelopia sp.	1	0	1	5	0.121	0.699
Macropelopia sp.	1	0	1	5	0.169	0.373
Virgatanytarsus sp.	1	0	1	5	0.107	0.723

3.4 Discussion

3.4.1 Water physico-chemical variables

Water physico-chemical variables are important factors capable of exerting influences on the species diversity and composition of freshwater ecosystems (Sundermann *et al.,* 2013). The Swartkops River drains an urbanised and industrialised catchment; the measured physico-chemical variables indicated evidence of deteriorating water quality, with elevated nutrient concentrations, turbidity and EC concentrations, and depleted dissolved oxygen concentrations recorded at the downstream sites, particularly at Sites 3 and 4 downstream of the discharge point of the Kelvin Jones Wastewater Treatment Works (WWTW) (Table 3.1; Figure 3.1a-c).

Turbidity, a measure of light scattering by suspended solids, is often used as a surrogate measure of suspended solids (Suren *et al.,* 2005). Although Davies-Colley and Smith (2001), and Bilotta and Brazier (2008) have criticised the use of turbidity as a surrogate measure of suspended solids, in the southern hemisphere it is generally considered to be strongly

correlated with some measures of suspended solids, and therefore still acceptable as a surrogate measure of suspended solids in aquatic ecosystems (DWAF, 1996b). Of the measured physico-chemical variables in this study (Table 3.1), turbidity was among the variables most strongly correlated with macroinvertebrate family-level metrics (Figure 3.11) and chironomid species (Figure 3.16). The elevated turbidity level recorded at Site 3 (Table 3.1; Figure 3.1b) could be detrimental to oxygen-sensitive biota as decomposition of solids with high organic content could lead to oxygen depletion, as was evident at Sites 3 and 4 (Table 3.1). Since most of the ET taxa use external gills for respiration, highly turbid water is likely to impact on their breathing apparatus through clogging and so affecting their species diversity and abundance (Bilotta and Braizer, 2008). This could partly explain the reduced ET species richness recorded at Sites 3 and 4 (Figure 3.9).

Given the high nutrient concentrations at Sites 3 and 4 (Table 3.1), significantly elevated concentrations of phytoplankton were expected at these sites. However, phytoplankton chlorophyll *a* concentrations were higher at Site 2 than at Sites 3 and 4 (Figure 3.2). This could be explained by the high turbidity level at Site 3, limiting light penetration and thereby affecting the phytoplankton biomass. In addition, the elevated levels of turbidity may also affect macroinvertebrate drifts and the feeding apparatus of filter-feeding invertebrates (Doeg and Milledge, 1991).

Although aquatic ecosystems require nutrients to sustain vital ecological structure, function and processes, excessive nutrient inputs could be detrimental to ecosystem health with several ecological consequences including algal blooms, excessive macrophyte growth, alteration of biological community structure, as well as morphological and histological changes in resident biota (Oberholster *et al.,* 2008; Odume, 2011; van Ginkel, 2011; Marchand *et al.,* 2012). Increased nutrients because of high organic load may lead to depletion of dissolved oxygen concentration, and this may explain why nutrients and dissolved oxygen are in opposite directions in the CCA (Figures 3.11, 3.16). Throughout the study period, considerably elevated nutrient concentrations were recorded at the three downstream sites, particularly at Sites 3 and 4 (Table 3.1). These elevated nutrient concentrations may explain the increased periphyton biomass (measured as chlorophyll *a* periphyton) and excessive macrophyte growth at Sites 3 and 4. Increased nutrient levels have previously been reported in the Swartkops River system (de Villiers and Thiart, 2007). The high organic content of the discharged effluent from the Kelvin Jones wastewater treatment works in Uitenhage (Chapter 2) is probably the main contributor to the elevated nutrient

levels, and responsible for depleting in-stream dissolved oxygen concentrations (Table 3.1; Figure 3.1a). However, run-off from the surrounding informal settlements and agricultural farmlands could also contribute to the elevated nutrient levels in the Swartkops River. Microbial activities may further release adsorbed nutrients from sediments into the water column, thereby increasing the overall nutrient concentrations available to biota (Correll, 1998). The strong negative correlation between the concentrations of nutrients and the family-level metrics (Figure 3.11) and the chironomid species (Figure 3.16) indicate that the elevated nutrient concentrations combined with other water physico-chemical variables explained the overall pattern of macroinvertebrate assemblage distribution, and general biodiversity loss in the Swartkops River system.

Electrical conductivity, which is often use as a surrogate for dissolved salts, is an important factor that can shape the distribution of aquatic macroinvertebrates (Kefford *et al.,* 2012b). In the Swartkops River, the downstream EC concentration based on recalibrated benchmarks as described in DWAF (2008c) indicated a serious deviation from the reference concentrations (Appendix B, Table B1). Thus, the elevated EC concentration may have adverse effects on the macroinvertebrate community structure of the downstream sites. Although anthropogenic sources such as discharges from WWTWs and run-off from surrounding rail and road networks could have contributed to the downstream EC concentrations, apart from such sources, the geological formations of the Swartkops River are of marine origin, which is a natural source of high EC concentrations in the Swartkops River (DWAF, 1996a). Consequently, macroinvertebrates may have adapted locally to the elevated EC concentrations.

Elevated concentrations of metals have previously been reported in the sediment of the Swartkops River (Binning and Baird, 2001), but the results in this study indicated generally low concentrations of metals in the water (apart from Cu) (Table 3.2), in ranges that could cause chronic rather than acute effects in aquatic biota (DWAF, 1996b). The South African water quality guidelines for aquatic ecosystems proposed limits above which certain metals may become either chronically or acutely toxic (DWAF, 1996b). Of the measured metals in this study, only Mn was consistently within the target water quality range (TWQR) (i.e. range in which no measurable effects were expected). Apart from Site 2, where the mean value of Zn was within the TWQR, the mean values of Cr^{+6}, Pb and Zn and Mn were in ranges that could potentially be chronically toxic to the Swartkops River biota. Although Cu was not statistically significantly different between the sampling sites (Table 3.2), in relation to the

aquatic ecosystems water quality guidelines (DWAF, 1996b), the concentrations at Sites 2, 3 and 4 may result in acute effects. Appendix B, Table B2 shows the guideline limits of the measured metals in this study.

3.4.2 Family-level taxonomic response: The South African Scoring System version 5 and the Swartkops multimetric index approach

The macroinvertebrate family-level biotic index, South African Scoring System version 5, is one of the tools that contributes information towards determining the water quality components of the Ecological Reserve for South Africa's water resources (DWAF, 2008c). This study posed the question whether integrating SASS5 with other selected metrics into a multimetric index could improve the sensitivity of the index approach to enable discrimination of differences between the three downstream sites: can any of these indices enable discrimination of biotic deterioration between these sites, considering that Site 2 is located upstream of the discharge point of the Kelvin Jones WWTW though still within an urban area, and Sites 3 and 4, downstream of the discharge point, with Site 3 being the closest? This question was asked because, in the literature, the multimetric index approach is perceived as superior to the single index, e.g. SASS5 approach in freshwater biomonitoring, because the former integrates metrics representing different aspects of macroinvertebrate taxonomic measures including diversity, composition, and richness into one single multimetric index (Baptista *et al.,* 2007; Helson and Williams, 2013; Mereta *et al.*, 2013). In this study, a Swartkops multimetric index (MMIswartkops) was developed and compared with the SASS5-based results in terms of assessing river health in the Swartkops River. The MMIswartkops consists of five metrics: SASS5 score, ASPT value, ET family richness, Simpson diversity and percent Oligochaeta + Chironomidae. These metrics represent four different metric categories: biotic index (SASS5 score and ASPT), richness (ET family richness), diversity (Simpson index) and composition (percent Chironomidae + Oligochaeta). The overall results showed that both the SASS5 and MMIswartkops index approach revealed similar ecological categories (Figures 3.4, 3.5 and 3.10) for the three downstream sites, indicating that the integration of SASS into a multimetric index did not provide additional useful insight into biotic deterioration when the multimetric index was reduced to a single score.

However, the component metrics, e.g. ET family richness and percent Chironomidae + Oligochaeta, which were masked in SASS, provided insight into the identity

of the taxonomic group of macroinvertebrate that did or did not deteriorate with increasing pollution. For example, the ET family richness decreased markedly at the three downstream sites relative to the upstream Site 1, and the percent Chironomidae + Oligochaeta increased with increasing pollution (Figure 3.9). The index approach to bioassessment as currently used in South Africa reduces biodiversity information into final scores that do not include or reflect the taxonomic identities of macroinvertebrates affected by deteriorating water quality (Dickens and Graham, 2002; Thirion, 2008). Although it is important to reduce complex biodiversity information to simple interpretable scores that can easily be conveyed to water resource managers, while retaining the benefits of simple scores, it is equally important to convey the taxonomic identities of at least some biota affected by water quality to resource managers because such information is vital for planning biodiversity conservation and devising strategies to protect vulnerable taxonomic groups. Therefore, examining the component metrics in the Swartkops multimetric index (MMIswartkops), provided additional useful insight into the taxonomic response of the ET family richness and percent Chironomidae + Oligochaeta to deteriorating water quality in the Swartkops River.

The insect orders Ephemeroptera, Plecoptera and Trichoptera taxa have been shown to exhibit strong negative responses to deteriorating water quality (Baptista *et al.,* 2007; Mereta *et al.,* 2013). The orders Ephemeroptera and Trichoptera are ecologically important groups of aquatic macroinvertebrates of vital significance in biomonitoring because of their perceived sensitivity to pollution and thus they are frequently used as indicators of water quality (Vlek *et al.,* 2004; Arimoro and Ikomi, 2009; Helson and Williams, 2013). Because they are usually among the first taxonomic groups of aquatic invertebrates to disappear from impaired sites (Mereta *et al.,* 2013), they are highly relevant for biodiversity conservation planning and assessing the success of restoration efforts. However, in this study, not a single individual of the Plecoptera order was recorded. Therefore, the metric ET (Ephemeroptera-Trichoptera) family richness was assessed instead of the conventional EPT. Their marked reduction at the downstream sites in the Swartkops River (Figure 3.9) indicates impaired water quality evident in their strong negative correlations with elevated electrical conductivity and turbidity (Figure 3.11). Organic input leading to depletion of oxygen often negatively affects the ET taxa as most of them rely on external gills for respiration, and their populations usually decrease with decreasing oxygen concentrations (de Moor *et al.,* 2003a; 2003b; Camargo *et al.,* 2004; Barber-James *et al.* 2008). Furthermore, exposure of these taxa to increased suspended solids measured as turbidity in this study could pose the risk of gill clogging and

lead to death. However, among families in the Ephemeroptera order, most species of beatids are capable of tolerating moderate organic pollution accompanied by increased periphyton and phytoplankton that serve as food because most species of baetids feed on algae by collecting-gathering and scraping exposed surfaces (Merritt *et al.,* 1996). Generally, the marked reduction of the ET taxa at the downstream sites highlights the need for management actions to improve the overall condition of the river.

The percent relative abundance of family Chironomidae increase with increasing pollution (Odume *et al.,* 2012a) and in the present study, the percent Chironomidae + Oligochaeta is the only metric that increased with increasing organic input and oxygen depletion in the Swartkops River (Figures 3.9 and 3.11). The family Chironomidae is a highly diverse group of aquatic macroinvertebrates, displaying a range of ecological sensitivities and requirements that enable them to survive in environments of varying conditions (Armitage *et al.,* 1995). Thus, some species of chironomids possess adaptive features that enable them to thrive in highly organic, oxygen-depleted environments. Furthermore, such chironomid species become opportunistic and increase in abundance in environments with low competition and hence the increased value of the percent Chironomidae + Oligochaeta metric, particularly at Sites 3 and 4. It is however important to stress that because the metric is assessed at the family level of taxonomic identification, the preponderance of a few tolerant chironomid individuals dominating the abundance counts of samples collected at the downstream sites would suggest that chironomids are all tolerant. However, studies (e.g. Adriaenssens *et al.,* 2004; Odume and Muller, 2011) have shown that chironomids, when identified to the genus or species level, have both sensitive and tolerant species (see later section).

Diversity is a reflection of aquatic ecosystem stability, which in turn reflects its integrity; the Simpson family diversity index included in the Swartkops multimetric index (MMIswartkops) has been used to assess aquatic ecosystem biotic diversity (Ravera, 2001; Carmago *et al*., 2004). The reason for using the index is that a decrease in its numerical value relative to value at an established reference site indicates a reduction in the diversity of biota and is thus an indication of environmental stress (Washington, 1984; Carmago *et al*., 2004). The limitation of the index is that it has no taxonomic meaning and thus does not provide any insight into the taxonomic identities of biota and hence it is of limited relevance with reference to biodiversity conservation. Nevertheless, it was included in the final MMIswartkops because it displayed a strong negative response to deteriorating water quality, decreasing markedly at Sites 3 and 4. Furthermore, since the Simpson diversity is a

dominance index that is highly influenced by the abundance of the commonest families (Ogbeibu, 2005; Gray and Delaney, 2008), its numerical value is a reflection of ecological factors influencing family abundance and diversity. The Pielou's evenness and Shannon diversity index correlated strongly with the Simpson diversity index. The Simpson index was chosen because it has been demonstrated to be a highly sensitive index, enabling the discrimination of impaired and unimpaired sites, as well as between sites with mild ecological differences (Ravera, 2001).

South African Scoring System version 5 and selected biotopes

The number of families (taxa), SASS5 scores and ASPT values were highest mostly in the stone and vegetation biotopes, and differed significantly between the three biotopes (Figure 3.8). These differences could be attributed to differences in hydraulic, substrate and thermal conditions between the three biotopes. The stone and vegetation biotopes are morphologically complex and more stable than the GSM biotope, and are therefore more likely to support more food and space resources, and thus more macroinvertebrate families leading to increased SASS5 scores and ASPT values. These results agree with those of Dallas (2007a) who reported that the stone and vegetation biotopes supported more macroinvertebrate families and higher SASS5 score and ASPT values than the GSM biotope. It is therefore important to sample all available biotopes to capture a wider range of biodiversity when undertaken aquatic biomonitoring.

3.4.3 Chironomid species level taxonomic response to deteriorating water quality in the Swartkops River

Although it is important to develop simple, low-cost tools that require minimal resources for sampling, sorting and identifying taxa in freshwater biomonitoring, it is equally important to conserve biodiversity, which requires understanding species ecology, and evaluating potential threats to biodiversity. Routinely used tools for freshwater biomonitoring in South Africa, which are developed at the family taxonomic resolution, are inadequate for investigating effects of deteriorating water quality on biodiversity and may not engender the desired level of biodiversity conservation envisaged in the National Water Act, Act No. 36 of 1998. Investigating pollution effects on a species-rich group of aquatic macroinvertebrates, such as chironomids, at the species taxonomic level was deemed a good compromise between the onerous task of identifying all species and a family-level taxonomic assessment. For example, the family level taxonomic metric percent Chironomidae + Oligochaeta increased at the

downstream sites (Figure 3.9), indicating that deteriorating water quality at these sites had a minimal effect on the chironomids and oligochaetes. However, the species-level taxonomic assessment indicated that the majority of the species occurring at the upstream Site 1 disappeared from the three downstream sites, particularly at Sites 3 and 4 (Figure 3.13; Appendix A). These results therefore indicated that pollution at downstream sites had an adverse effect on chironomid species diversity, richness and composition, which was undetected with the family-level assessment tools. Thus, the information based on species-level assessment is necessary to assess the true state of biodiversity in order to inform conservation planning.

Prioritising restoration efforts and assigning limited resources wisely is critically important to water resource managers and requires biomonitoring at a taxonomic level sufficiently sensitive to enable the discrimination of sites with mild differences in biotic deterioration. Although the family-level rapid SASS5 assessed the overall water quality at the three downstream sites as very poor, the observed differences in the chironomid species composition between the three sites, with more species, including the less pollution-tolerant species recorded at Site 2, indicated that Site 2 was less impacted than Sites 3 and 4. This is corroborated by the improved concentrations of the water physico-chemical variables (Table 3.1) measured at Site 2 relative to Sites 3 and 4. Species identification was needed in addition to SASS5 to investigate further the level of biotic deterioration in the Swartkops River. The importance of species-level assessment in freshwater biomonitoring has been stressed by Lenat and Resh (2001), and Schmidt-kloiber and Nijboer (2004) who demonstrated that it provides a more accurate assessment of water quality relative to the family-level assessment, as was evident by the clear separation of Site 2 from Sites 3 and 4 in the present study (Figure 3.14).

The relatively high number of chironomid species (35 species) recorded in the Swartkops River, particularly at Site 1, could be attributed to their ecological diversity (reflected in their ability to form random patches independent of each other), habitat partitioning, and flexible diet and space resource utilisation, enabling species to co-exist with limited inter-specific competition (Armitage *et al.,* 1995; Raunio *et al.,* 2011). However, the chironomid species richness decreased markedly at the downstream sites, particularly at Sites 3 and 4 (Figure 3.13) and this could be attributed to the adverse effects of pollution at these sites.

Among the Swartkops River chironomids, species belonging to the subfamily Tanypodinae and tribe Tanytarsini were most affected by deteriorating water quality (Figure 3.12). Their species abundance and composition decreased with increasing pollution and thus, with the exception of one Tanytarsini species, no other species belonging to these subfamilies was recorded at Site 3 (Appendix A, Table A3). Species of tribe Tanytarsini are small-bodied chironomids, which may increase their surface area to volume ratio and, potentially, increase their exposure to toxicants. This could increase their vulnerability to pollution and thus their inability to tolerate deteriorating water quality. Clement *et al.* (1992) and Mousavi *et al.* (2003) also noted the pollution-intolerant nature of species belonging to the tribe Tanytarsini. The Tanypodinae were mostly associated with soft sediments, i.e. gravel, sand and mud (Table 3.11), but this biotope serves as sink for most toxicants that could be toxic to biota (Beasley and Kneale, 2002; Pettigrove and Hoffmann, 2005) and may therefore potentially increase the vulnerability of associated species to the effects of deteriorating water quality. This probably explains why few species of the Tanypodinae occurred at Sites 2 and 4, and none at Site 3 (Figure 3.12). Ochieng *et al.* (2008) reported similar results in which species of the Tanypodinae were less frequent at polluted sites.

In this study, a CCA-based empirical approach was used to derive chironomid-tolerant scores based on their correlation with the water physico-chemical variables. The same approach has previously been used to derive acid-tolerance scores for macroinvertebrates in selected British streams, and the resultant index proved sensitive in diagnosing effects of acidification on macroinvertebrate communities (Murphy *et al.*, 2013). The data used to derive chironomid tolerance scores in the present study spans a period of three years, and were considered adequate. Species such *Chironomus* sp.1, *Chironomus* sp.2, *Chironomus* sp.3, *Kiefferulus* sp. and *Dicrotendipes* sp. could be considered pollution-tolerant species and good indicators of organically polluted and nutrient-enriched condition that characterised Sites 3 and 4 (Tables 3.10). These species are large-bodied scrapers that feed mostly on organic matter, and have a high concentration of haemoglobin, a combination of adaptations that enable them to cope under oxygen-depleted and organically enriched environments (Wielderholm, 1983; Adriaenssens *et al.*, 2004).

An important limitation of the chironomid-water quality CCA correlation is that the samples were collected at four sampling sites, which were not very far from each other and because of system connectivity in lotic ecosystems, they could be regarded not independent samples, but pseudo-replicates. However, the clear point source impact e.g. wastewater effluent discharges

influencing the water quality of the river served to distinguished between the sites. In addition, the study was undertaken in a relatively small river system, and the size of the river make it impossible to select sites very far from each other that lies within the same ecoregion. Nevertheless, the extended period over which the data were collected (August 2009 – September 2012), and the number of replicates collected per site during each sampling event enable robust statistical analysis of the data.

The majority of the Orthocladiinae species recorded in the Swartkops River had moderate pollution-tolerance scores ranging between 3 and 4, and were associated with water quality conditions at Sites 1 and 2 (Table 3.10). The Orthocladiinae species reportedly inhabit environments of varying water quality status (Simiao-Ferreira *et al.* 2009) and this could be attributed to their functional diversity, enabling them to survive in diverse environmental conditions (Armitage *et al.,* 1995; Cranston *et al.,* 1996). Overall, the results in this study support the argument that the highly tolerant score of 2 assigned to chironomids in the SASS5 sheet does not reflect their range of sensitivity, given their huge species diversity in South African freshwater ecosystems (Dickens and Graham, 2002; Odume and Muller, 2011). The approach presented here could be a starting point for developing a chironomid-based SASS-type system that could provide a more accurate assessment of environmental impacts as evident in their clear separation of the three downstream sites, indicating varying levels of biotic deterioration (Figure 3.14).

Seasonally, high chironomid species abundance, richness and diversity have been reported during summer and spring (Rossaro, 1991; Adriaenssens *et al.* 2004; Raunio *et al.,* 2007). For example, some authors, e.g. Adriaenssens *et al.* (2004), restricted chironomid sampling to summer only, but in the present study, seasonality did not strongly influence the overall clustering of the Swartkops River chironomid communities; rather, spatial distribution exerted the overriding influence on the clustering (Figure 3.14). Thus, seasonal differences were less important for the Swartkops River chironomid assemblages.

3.4.4 Chironomid species-biotope association in the Swartkops River

The stone biotope group supported more chironomid species (Table 3.15). Stones are morphologically and structurally complex and could support more zoobenthic communities than structurally simpler biotopes such as sand and mud (Wright *et al*., 1983; Principe *et al.,* 2008). Structurally complex biotopes are more likely to support diverse food and space

resources for species, and to provide refugia for benthic fauna and are therefore likely to support more species (Rosa *et al*., 2011; Epele *et al.,* 2012).

Chironomid species recorded in this study were associated with specific biotopes, but the majority of these associations (preferences) were not statistically significant as indicated by the Pearson's point-biserial correlation coefficient (Table 3.11). Species' traits, and general ecology, including thermal and food requirements, feeding habits, hydraulic and substratum characteristics, as well as predator-prey interaction could define chironomid preferences for specific biotopes (Armitage *et al.*, 1995; Syrovátka *et al.*, 2009). For example, in this study, *Cricotopus* sp.1, *Cricotopus trifasciata* gr., *Paratrichocladius* sp., *Cladotanytarsus* sp., *Orthocladius* sp., *Tanytarsus* sp., *Polypedilum* sp., and *Nanocladius* sp. preferred either the stone biotope, or the vegetation biotope or both (Table 3.11). These species are mostly collector-gatherers and scraper-grazers that feed on particulate organic matter deposited on stone surfaces in pools, or graze on periphyton materials. The predatory Tanypodinae, including *Nilotanypus* sp. *Thienemannimyia* sp., *Ablabesmyia* sp., *Trissopelopia* sp., and *Macropelopia* sp. were mostly associated with either the GSM biotope, or the stone biotope, or both. Since the GSM biotope group and the stone-out-of-current biotope occurred mostly in pools, the predatory Tanypodinae avoid the danger of being swept away by currents while searching for food. According to Wielderholm (1983) and Rosa *et al*. (2011), most species of the subfamily Tanypodinae are adapted to living in pools with a large amount of soft sediment. Syrovátka *et al.* (2009) also found a large proportion of predatory chironomids in pools. Species such as *Dicrotendipes* sp., *Cryptochironomus* sp., and *Virgatanytarsus* sp., which were associated with more than one biotope, have flexible diets, enabling them to occupy several biotopes under varying environmental conditions (Armitage *et al.,* 1995). Overall, knowledge of the Swartkops River chironomid-biotope association could be incorporated into biomonitoring methods requiring information on ecological traits, with the overarching goal of improving the diagnosis of freshwater impacts (see Chapter 5).

3.5 Conclusion

The results for the water physico-chemical variables and selected metals presented in this chapter revealed water quality in the Swartkops River deteriorating as it flowed downstream, reinforcing the urgent need for management intervention. Although the frequency of metal analysis in the present study did not permit a detailed interpretation of metal contaminants in the Swartkops River, the concentrations reported were mostly in ranges that could potentially

cause chronic effects. More comprehensive metal monitoring is needed to guide necessary management actions.

Both the SASS5 and MMIswartkops indices provided similar summaries of the river health assessment of the Swartkops River. However, the component metrics e.g. ET family richness and percent Chironomidae + Oligochaeta, which were masked in SASS, provided additional information on the identity of at least some of the taxonomic groups of macroinvertebrates that did, or did not, deteriorate with increasing pollution. Although it is important to reduce complex biodiversity information to simple interpretable scores, it is equally important to convey the taxonomic identities of at least some biota affected by water quality to resource managers because such information is vital for planning biodiversity conservation. Thus, the MMIswartkops was only superior to SASS5 in retaining the taxonomic identities of some macroinvertebrate groups, but not in assessing the ecological state of the river as both approaches provided similar results.

The chironomid species-level responses shed light on the importance of identifying species in freshwater biomonitoring, as the species-level assessment proved sensitive, enabling the discrimination of sites, which were grouped together in the E/F ecological categories by family-level assessment tools. Although the family level taxonomic assessment indicated that the family Chironomidae increased with pollution in the Swartkops River, this can be misleading considering that species-level taxonomic assessment indicated that most of the species occurring at the upstream Site 1 disappeared as pollution increased. Therefore, the species-level taxonomic responses provided a more refined assessment of biotic deterioration in the Swartkops River, and should be prioritised over the family-level tools for assessing the ecological state of the river and in planning biodiversity conservation. However, a decision to identify biota beyond the family level must be considered in light of the assessment goals and available resources. Nevertheless, SASS5-based family-level assessment could be improved if scores are assigned at the subfamily level for the family Chironomidae because the different subfamilies encountered in this study showed varying sensitivity to deteriorating water quality and subfamily level identification is relatively easy for the chironomids.

The functional and biological diversity among the chironomids make them a suitable group of macroinvertebrates for developing a species-level bio-assessment tool in South Africa, and the results presented showed that they have species of varying sensitivities and tolerances to pollution (Tables 3.9 and 3.10). The empirical approach for deriving the tolerance score

presented here, which has previously been used in the United Kingdom (Murphy *et al.*, 2013), can be applied widely as chironomid data become available from other South African rivers, with a view to developing a chironomid-based SASS-type system for bio-assessment of lotic ecosystems. The chironomid-biotope information obtained here forms an important contribution to chironomid ecological traits, which can be incorporated into a traits-based assessment approach (see Chapter 5).

CHAPTER 4: MACROINVERTEBRATE FAMILY-LEVEL TRAITS-BASED APPROACH TO BIOMONITORING IN THE SWARTKOPS RIVER

In Chapter 3, taxonomically-based biomonitoring approaches involving the biotic index, the South African Scoring System (SASS), and the multimetric index, the Swartkops multimetric at the family level of taxonomic resolution, and chironomid species responses at the species taxonomic resolution were used to evaluate the impact of deteriorating water quality on the Swartkops River macroinvertebrate communities. The rationale and contributions of each of the approaches to understanding macroinvertebrate in-stream responses and assessing river health were critically discussed. However, the taxonomically-based approaches as used in the previous chapter did not enable the assessment of water quality impact on ecological function of the macroinvertebrate communities. The approaches failed to provide insight into why certain species or families of macroinvertebrates persisted in the polluted sites in the Swartkops River. Empirical evidence suggests that biological and ecological traits together make it possible to investigate impact of deteriorating water quality on ecological function (Statzner et al., 2001; de Bello et al., 2010). Because the habitat template concept provides a mechanistic basis for linking traits to prevailing environmental (water quality) conditions (Townsend and Hildrew, 1994), the questions of whether applying a trait-based approach at a cost-effective family-level taxonomic resolution could provide additional insight into the Swartkops River macroinvertebrate community response was addressed in this chapter. The chapter begins with the rationale for the trait-based approach at the family-level taxonomic identification, the selection of traits and provides evidence of the potential of the selected traits in diagnosing the main water quality issues in the Swartkops River. Evidence of an association between traits clusters and the sampling sites was provided in this chapter.

4.1 Introduction

Biomonitoring in South Africa relies on taxonomic identifications, usually to family level such as in the South African Scoring System version 5 (SASS5) and occasionally to the species level (Dickens and Graham 2002; Thirion, 2008). However, taxonomically-based approaches do not reveal functional diversity within aquatic ecosystems, nor can pollution impacts on ecological function of biological communities necessarily be sensitively

elucidated (Bonada *et al.,* 2006). In addition, empirical evidence indicates that approaches aimed at protecting species composition alone, may not necessarily protect ecological function (Kefford *et al.,* 2012a). Therefore, to manage aquatic ecosystems sustainably, it is important to develop approaches and methods that indicate and enable rigorous assessment of ecological function, and which could complement existing taxonomically-based approaches currently being used for managing South Africa's water resources.

A growing body of empirical evidence suggests that *species traits* make it possible to investigate the impact of pollution on the ecological function of biological communities (Statzner *et al.,* 2001; de Bello *et al.,* 2010; Statzner and Beche, 2010). The traits-based approach (TBA) is based on the premise that habitat constraints (or "filters") select for specific species traits and eliminate others so that there is a correspondence between the habitat and the traits (Southwood, 1977; 1988; Townsend and Hildrew, 1994; Poff, 1997). Consequently, species can only survive and thrive in environments that meet their biological and ecological requirements. That is, only species having traits that enable them to adapt to prevailing abiotic conditions in an environment can survive and thrive. Therefore, the TBA provides insights into why certain species persist in a given environment, and offers a basis for predicting biological communities once the prevailing abiotic factors are known (van Kleef *et al.,* 2006). The TBA has been reviewed in Chapter 1, section 1.5 of this thesis.

As discussed in Chapter 1, section 1.5, the habitat template concept is the fundamental theoretical underpinning of the TBA in freshwater biomonitoring and it forms the basis for predicting species trends in an environment (Southwood, 1977; 1988; Townsend and Hildrew, 1994). For example, Townsend and Hildrew (1994) who adapted the habitat template concept for freshwater ecosystems predicted that in a perturbed habitat, traits that confer resilience or resistance on the organism such many offspring per reproductive event, enables the organism to survive under disturbance and dominate in such environment. The TBA has not previously been used in South Africa in a manner consistent with the river habitat template concept, and this study is the first to use the TBA in the biomonitoring of a South African river.

The Swartkops River receives discharges of wastewater effluents, which has led to elevated concentrations of nutrients, suspended solids (measured as turbidity in this study), dissolved solids (measured as electrical conductivity) and depletion of dissolved oxygen (DO) concentration (Chapter 3). Traits that enable macroinvertebrate families to adapt to these

stressors were expected to dominate the impacted sites in line with the river-habitat template concept (RHTC). For example, active locomotion enables organisms to move into refugia, which could be less polluted patches within polluted reaches in the river. Similarly, aerial respiration may enable organisms to survive in oxygen-deficient sites.

The TBA has been used both at the family- and species-level taxonomic resolutions (Liess and Von der Ohe, 2005; Tomanova *et al.,* 2008; Beketov *et al.,* 2009) and although there were conflicting views in the literature regarding the taxonomic resolution at which traits can be reliably applied, the bulk of evidence suggested that family-level taxonomic identification was sufficient for biomonitoring (Dolédec *et al.,* 1998; 2000; Gayraud *et al.,* 2003; Beketov *et al.,* 2009; Chapter 1). Since identifying all macroinvertebrates to species is onerous, particularly in Afro-tropical regions where taxonomic knowledge is sparse, the question of whether family-level traits can be reliably correlated with water quality in the Swartkops River is addressed in this chapter. Investigating the responses of family-level traits is important because: i) family-level traits could enable the development of a simple, low-cost tool that could be used easily by water resource managers; ii) the limited taxonomic knowledge of most Afro-tropical macroinvertebrate species is sparse, which could limit applying tools developed at the genus or species-level; iii) unlike in Europe and North America, very little is known about the biology and autecology of Afro-tropical species, and the information in the literature is sparse. Therefore, family-level traits were used to investigate the impacts of deteriorating water quality on the macroinvertebrate communities in the Swartkops River.

4.2 Materials and methods

4.2.1 Derivation, and fuzzy coding, of macroinvertebrate trait information

Seven macroinvertebrate traits, four biological (body size, respiration, body shape and mobility) and three ecological (biotope preference, preferred food and feeding habits) that can be easily describe at the family-level were selected. The seven selected macroinvertebrate traits were resolved into 32 trait classes (Table 4.1) and a fuzzy coding system (Chevenet *et al.,* 1994) applied to describe the link between a taxon and each trait class (Appendix C, Table C1). The fuzzy coding system describes the link between a taxon and each trait class with affinity scores that account for potential functional variations between members of the

same family and different life stages within a taxon and is then applied comparatively on a proportional basis (Chevenet *et al.,* 1994).

Table 4.1: Macroinvertebrate selected biological and ecological traits and trait class (with code) applied to biomonitoring in the Swartkops River over the study period (August 2009 – September 2012). The rationale for selecting each trait category is given in Chapter 2, section 2.6.1. The "+"indicates predicted increase in the relative abundance of the macroinvertebrate associated with that trait and "-" indicates decrease in the relative abundance of macroinvertebrate associated with that trait in relation to pollution increase.

Biological and ecological trait/class		Predicted response
Maximal body size (mm)	Code	
Very small (≤5)	A1	-
Small (>5-10)	A2	-
Medium (>10-20)	A3	±
Large (>20-40)	A4	+
Very large(>40-80)	A5	+
Respiration		
Gills	B1	-
Tegument	B2	+
Aerial: spiracles	B3	+
Aerial/vegetation: breathing tube, straps/other apparatus e.g. elytra etc	B4	+
Aerial: lung	B5	-
Mobility		
Climber	C1	+
Crawler	C2	-
Sprawler	C3	
Swimmer	C4	-
Skater	C5	-
Burrower	C6	±
Body shape		
Streamlined	D1	
Flattened	D2	
Spherical	D3	
Cylindrical	D4	
Preferred food		
FPOM(fine detritus)	E1	-
CPOM (coarse detritus)	E2	-
Plant materials/algae	E3	+
Animal materials	E4	-
Feeding habit		
Shredder	F1	-
Collector-gatherer	F2	+
Collector-filterer	F3	-
Scraper (grazer, brushers)	F4	+
Predator	F5	-

Preferred biotope		
Sediment (gravel, sand and mud)	G1	+
Stone	G2	±
Vegetation	G3	-

Affinity score, as used in this study, refers to the proportional representation of a trait class in a taxon derived from empirical analysis or the degree of reliance of a taxon on a trait class derived from the literature (for the literature derived traits, see the section below) (Chevenet *et al.,* 1994; Tomanova *et al.,* 2008). In this study, affinity scores ranging from 0 to 5 were assigned to each taxon for each trait class as follows: 0 indicates no affinity of taxon to a given trait class i.e. no individual of a taxon exhibits that trait class; 1 indicates low affinity of a taxon to a trait class; 3 indicates moderate affinity of a taxon to a trait class, while 5 indicates high affinity of a taxon to a trait class. By using these affinity scores, the fuzzy coding system avoids the obligate assignment of a taxon to a single trait class because, in reality in most cases, all individuals within a taxon rarely belong entirely to one trait class within a trait category. The coding system also helps translate qualitative trait information into quantitative data that can be subjected to further statistical analysis aimed at elucidating ecological patterns (Chevenet *et al.,* 1994; Usseglio-Polatera *et al.,* 2000).

The remaining part of this sub-section describes how trait information was obtained.

Empirically derived trait information

Information about body size (length), biotope preference, food and feeding habits, and body shape was empirically derived for macroinvertebrates families in this study and are marked X in Table 4.2 to distinguish them from literature-derived trait information marked Y in the table.

Body size

The body lengths of between 10 and 30 individuals of each macroinvertebrate taxon at each sampling site during each sampling season were measured from the tip of the head to the abdominal tip, exclusive of terminal appendages as described in Chapter 2, section 2.6.1. Body length measurements were restricted only to late juvenile stages to obtain maximum size (where the adult was non-aquatic). For the Potamonautidae, the carapace length was used as a measure of body length. All late juvenile stages of the less abundant families were

measured for each site during the study period. All measured individuals within a family were pooled and categorised into five body size classes (Table 4.1) and then expressed in percentages. A fuzzy coding system was then used to represent this information following a majority rule as follows: a body size class was scored 0 for a given family if no individual fell within that class; 1 if less than 40% of the individuals within a family fell within that body size class, 3 if between >40% and 60% of individuals fell within that class and a score of 5 was assigned if >60% of individuals within a family fell within the class. The body size classes employed in this study have been widely used to group macroinvertebrates into body size classes (e.g. Tomanova *et al.,* 2008).

Body length was not measured for the ancylids, physids, planorbids, lymnaeids and oligochaetes because length is considered not an appropriate representation of body size for these macroinvertebrates (Robson *et al.,* 2005). In addition, an alternative measure such as body mass, which could then be converted to length using published information was not used because such conversions come with series of errors and have been criticized (Robson *et al.,* 2005). Therefore, body size information was not coded for these groups of macroinvertebrates (i.e. Physidae, Planorbidae, Lymnaeidae and Oligochaeta) and are indicated as blank spaces in Table 4.2

Biotope preference

As described in Chapter 2, macroinvertebrates were collected from three distinct biotopes: GSM, stone and vegetation. Macroinvertebrate biotope preference was determined using the Pearson's point-biserial correlation (De Cáceres *et al.*, 2008; De Cáceres and Legendre, 2009). The Pearson's point-biserial correlation has already been described in Chapter 3 section 3.2.4. Macroinvertebrate biotope preference was undertaken only for the Site 1 assemblages to avoid the confounding effects of pollution. The significance of the association was tested using the Monte Carlo permutation test with 999 permutation at *alpha* = 0.05. When a family was associated with a particular biotope it was coded 3 if the association was not significant, but 5 if it was significant (Appendix C, Table C1). A code of 0 was assigned if no association was observed. During each of the sampling occasions at each site, three replicate samples were collected per biotope making nine samples per site in each season (Chapter 2), so that over the entire study period, 72 samples were collected per site, except in cases where not all replicate samples could be collected from all biotopes because of biotope inadequacies. Macroinvertebrate families occurring in less than five samples at Site 1 over

the study period were eliminated from the analysis and information regarding their biotopes preference was obtained from Thirion (2008) marked as Y in Table 4.2.

Preferred food and feeding habits

Macroinvertebrate food and feeding habits have been studied extensively and macroinvertebrates have been assigned functional feeding groups (FFG) based on their feeding habits, gut content analysis and mouth part morphology (Cummins 1974; Cummins and Klug, 1979; Palmer, 1991; Palmer and O'Keeffe, 1992; Palmer *et al.*,1993a; 1993b; Uwadiae, 2010). It has been argued that it is inappropriate to assign macroinvertebrates to FFG based on studies conducted elsewhere, particularly in different eco-regions (King *et al.*, 1988; Palmer, 1991; Tomanova *et al.*, 2006). Therefore, in this study, macroinvertebrate families were assigned feeding habits based on observed feeding behaviour, and the preferred food was determined based on gut content analysis. To determine the feeding habit, macroinvertebrates were collected at Site 1 on three different occasions corresponding to three seasons: spring, summer and autumn (2011-2012), and transported to the laboratory in a sealed plastic bag containing river water and oxygen tablets. The sealed plastic bag was kept in a cooler box filled with river water and an aerator. Concurrently, food sources including sediments, stone with attached algae and leaf litter were collected from the river. In the laboratory, on each of the sampling occasions, five individuals of different sizes within a family were selected and kept in different aquaria containing different food sources and the feeding habits were observed and recorded as described in Palmer (1991) and Palmer *et al.* (1993b). The feeding habits described in Palmer (1991) and Palmer *et al*. (1993b) complemented the information obtained during feeding habits observation.

To determine the preferred food type, beginning in September 2011 (spring), five individuals representing different sizes of the dominant macroinvertebrate families (excluding the non-insect taxa) were dissected and their gut content examined as described in Palmer (1991) and Palmer and O'Keeffe (1992). Briefly, the gut was removed from each dissected individual, and then washed with distilled water in a petri-dish. The distilled water containing the gut content was emptied into a test tube and then mixed using a voltex, and the content filtered through a GF/F filter paper 0.45 ɥm. The residues were mounted and viewed under an oil immersion lens (X 100) in an Olympus compound microscope (B X 51) equipped with an Altra 20 soft imaging system digital camera. Food materials were broadly categorised as fine particulate organic matter (FPOM) (< 1 mm), coarse particulate organic matter (CPOM)

(> 1 mm), plant materials including algae, and animal materials (Table 4.1) (Cummins and Klug, 1979). Because several food types were found in the gut of individuals belonging to the same families, the fuzzy coding was also applied to show the affinity of each family to a preferred food type, based on the proportion of food type observed in the gut of examined individuals.

Body shape

Body shape was resolved into four trait classes: streamlined, flattened, spherical and cylindrical (Table 4.1). Macroinvertebrates were assigned a body shape class based on observation. The fuzzy coding system was used to describe cases where members of the same families belong to different body shape classes.

Literature derived trait information

Respiration

All literature-derived traits information for the macroinvertebrate families are marked Y in Table 4.2.

Information about respiratory traits of macroinvertebrate families was obtained from several published and unpublished literature sources as described in Chapter 2, section 2.6.1. Macroinvertebrates were resolved into five respiratory trait classes: gills, tegument, lung (the pulmonate snails), spiracle, aerial respiration involving breathing tubes, straps and other mechanisms, e.g. elytra (Table 4.1). A fuzzy coding system was used to code taxa according to their affinity to a given respiratory trait class, where a taxon uses more than one of the respiratory trait classes (Appendix C, Table C1).

Mobility

Information about mobility of macroinvertebrate families was obtained through observation and literature sources (e.g. Merritt *et al.,* 1996; Gerber and Gabriel, 2002; Tomanova *et al.,* 2008). Mobility was categorised into six trait classes including climbers, crawlers, sprawlers, swimmers, skaters and burrowers (Table 4.1).

Table 4.2: Macroinvertebrate taxa in the Swartkops River and the analysed trait categories showing information that was empirically derived (X) and that derived from literature sources (Y). For body size, macroinvertebrate families for which individual measurements were less than 10 counts are marked Z and blank spaces indicates that body size measurements were not undertaken for the taxon.

Taxon	Body size	Respiration	Mobility	Body shape	Preferred food	Feeding habit	Preferred biotope
Oligochaeta		Y	Y	X	Y	Y	X
Hirudinea	X	Y	Y	X	Y	Y	X
Turbellaria	X	Y	Y	X	Y	Y	X
Potamonautida	X	Y	Y	X	Y	X	X
Leptophlebiidae	X	Y	Y	X	X	X	X
Baetidae	X	Y	Y	X	X	X	X
Caenidae	X	Y	Y	X	X	X	X
Heptageniidae	Z	Y	Y	X	X	X	Y
Coenagrionidae	X	Y	Y	X	Y	X	X
Lestidae	Z	Y	Y	X	X	Y	Y
Aeshnidae	X	Y	Y	X	X	X	X
Corduliidae	X	Y	Y	X	X	X	X
Gomphidae	X	Y	Y	X	X	X	X
Libellulidae	X	Y	Y	X	X	X	X
Synlestidae	X	Y	Y	X	Y	X	X
Platycnemidae	Z	Y	Y	X	Y	Y	Y
Protoneuridae	Z	Y	Y	X	Y	X	Y
Crambidae	Z	Y	Y	X	X	Y	Y
Belostomatidae	X	Y	Y	X	X	X	X
Corixidae	X	Y	Y	X	X	X	X
Naucoridae	X	Y	Y	X	Y	Y	Y
Nepidae	Z	Y	Y	X	Y	Y	Y
Notonectidae	Z	Y	Y	X	X	X	Y
Pleidae	X	Y	Y	X	X	X	X
Veliidae	X	Y	Y	X	X	X	X
Gerridae	X	Y	Y	X	X	X	X
Ecnomidae	X	Y	Y	X	X	X	X
Hydropsychidae	X	Y	Y	X	X	X	X
Leptoceridae	X	Y	Y	X	X	X	X
Hydroptilidae	X	Y	Y	X	X	X	X
Dytiscidae	X	Y	Y	X	X	X	X
Gyrinidae	X	Y	Y	X	Y	X	X
Hydraenidae	Z	Y	Y	X	X	Y	Y
Hydrophilidae	X	Y	Y	X	X	X	X
Elmidae	X	Y	Y	X	X	X	X
Helodidae	Z	Y	Y	X	X	Y	Y
Ceratopogonidae	X	Y	Y	X	X	X	X

Chironomidae	X	Y	Y	X	X	X	X
Culicidae	X	Y	Y	X	X	X	X
Muscidae	X	Y	Y	X	X	X	X
Simuliidae	X	Y	Y	X	X	X	X
Tabanidae	Z	Y	Y	X	X	Y	Y
Tipulidae	X	Y	Y	X	X	Y	Y
Athericidae	Z	Y	Y	X	X	Y	Y
Ephydridae	X	Y	Y	X	X	Y	Y
Psychodidae	X	Y	Y	X	X	Y	Y
Syrphidae	X	Y	Y	X	X	Y	Y
Ancylidae		Y	Y	X	X	X	X
Lymnaeidae		Y	Y	X	X	X	X
Physidae		Y	Y	X	X	X	X
Planorbinae		Y	Y	X	X	Y	Y
Hydrachnellae	Z	Y	Y	X	X	Y	Y

4.2.2 Statistical and data analysis

To analyse the traits-based community responses at the four sampling sites, over the study period, macroinvertebrate taxa at each site were pooled seasonally resulting in taxon-site information for each season (Appendix C, Table C2 a - d). A second table was then created containing trait-taxon with trait information coded for each taxon (Appendix, C Table C1). The trait-taxon matrix was then multiplied by the abundance of each macroinvertebrate family per season in each site to create a third table (Appendix C Table C3 a - b) containing trait-site information as described in Tamanova *et al.* (2008). The abundance of each trait category within each site was then transformed into relative abundance (Tamanova *et al.*, 2008) and analysed statistically.

To analyse traits-based community responses between the four sampling sites, the non-parametric Kruskal-Wallis multiple comparison test was used to test for significant differences between the sites for each trait class. A cluster analysis based on Bray-Curtis similarity was used to elucidate the pattern of traits association (combination) among the macroinvertebrate taxa, and box plot was then used to visualise the variation of the relative abundances of macroinvertebrates in each identified trait cluster across the four sampling sites. Cluster analysis was selected to elucidate traits association because is a multidimensional representation compared with the 2-dimensional representation of ordination methods (Poff *et al.*, 2006). The Kruskal-Wallis multiple comparison test was also

used to ascertain whether the relative abundances of macroinvertebrates in each trait cluster showing trait combinations differed significantly different between the sampling sites *(P* < 0.05). Cluster analysis and the Kruskal-Wallis test were undertaken using Primer 5 version 5.2.9 (Clarke and Warwick, 1994) and Statistica software package version 9, respectively.

4.3 Results

4.3.1 Traits-based community responses to deteriorating water quality in the Swartkops River

The response of each trait class to deteriorating water quality in the Swartkops River was analysed. For the purpose of clarity, only the median values expressed in percent relative abundances are presented in this section. The percent relative abundance of macroinvertebrate in each trait class plotted using box plots showing the medians, 25-75 percentile distributions and the non-outlier ranges at each sampling site are presented in Appendix C, Figures C1 – C4.

Macroinvertebrates belonging to body-size classes A1 (≤ 5 mm) and A2 (> 5 - 10 mm) dominated the relative abundance of macroinvertebrate communities at the four sampling sites in the Swartkops River (Figure 4.1). Of the five body-size trait classes, the relative abundance of macroinvertebrates belonging to A4 (large: >20-40 mm) increased significantly more at Site 3 than at Site 1, 2 and 4 ($P < 0.05$). The Kruskal-Wallis multiple comparison test showed that the relative abundances of macroinvertebrates belonging to the majority of the body-size trait classes including A1 (≤ 5 mm), A3 (> 10 - 20 mm), and A5 (>40 - 80 mm) did not change significantly across the four sites ($P > 0.05$) (Figure 4.1). However, the relative abundance of the body size class A2 (>5 – 10 mm) increased significantly more at Sites 2 and 4 than at Sites 1 and 3 ($P < 0.05$). The results generally indicated that the majority of the body-size classes did not respond markedly to deteriorating water quality, as there were no significant differences in macroinvertebrate relative abundances between the upstream reference Site 1 and the downstream sites.

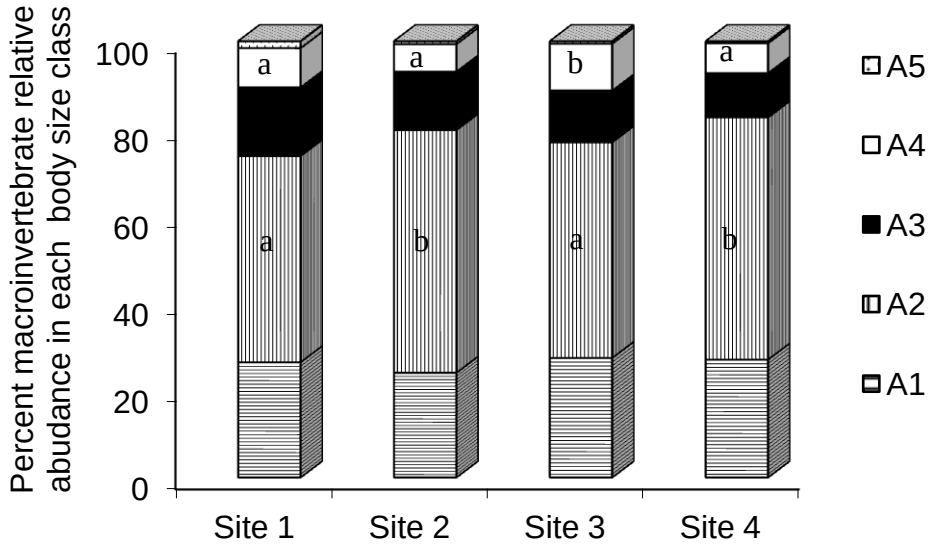


Figure 4.1: Percentage macroinvertebrate relative abundance (median value) in each of the analysed body-size trait classes at the four sampling sites in the Swartkops River during the study period (August 2009 – September 2012) showing the increase in macroinvertebrate relative abundance belonging to Class A 2 (>5 – 10) at Sites 2 and 4, and Class A4 (> 20 – 40) at Site 3, with the remaining body-size classes showing no significant change between the four sampling sites. Body-size classes having completely different alphabet letters across the sites indicate significant difference ($P < 0.05$), whereas body size classes having the same alphabet letter in common, or no alphabet letter across the site indicate no significant difference ($P > 0.05$). Body size classes (mm): A1 (≤ 5), A2 (> 5-10), A3 (>10-20), A4 (> 20-40), A5 (> 40-80).

In terms of respiration, the relative abundance of macroinvertebrates relying on spiracles for in-take of atmospheric oxygen increased significantly at Sites 3 and 4 than at Site 1 ($P > 0.05$) (Figure 4.2). Similarly, the relative abundance of macroinvertebrate using tegument for dissolved oxygen in-take also increased significantly at Sites 3 and 4 than at Site 1. The Kruskal-Wallis multiple comparison tests did not reveal a statistically significant difference ($P > 0.05$) between Sites 1 and 2 in the relative abundance of macroinvertebrates using tegument. Conversely, the relative abundance of macroinvertebrates relying mostly on gills for respiration decreased markedly at the downstream sites, particularly at Sites 3 and 4 relative to Site 1 (Figure 4.2). Although the lung of the pulmonate snails facilitates in-take of atmospheric oxygen, the relative abundance of macroinvertebrates relying on lungs as a means of respiration decreased significantly at Sites 3 and 4. Generally, the respiratory traits responded predictably to deteriorating water quality in the Swartkops River, with the relative abundance of macroinvertebrates using gills and lungs being significantly reduced at the

downstream sites, particularly at Sites 3 and 4 compared with Site 1, and relative abundance of those relying mostly tegument, and aerial respiration increasing at the three downstream sites relative to Site 1.

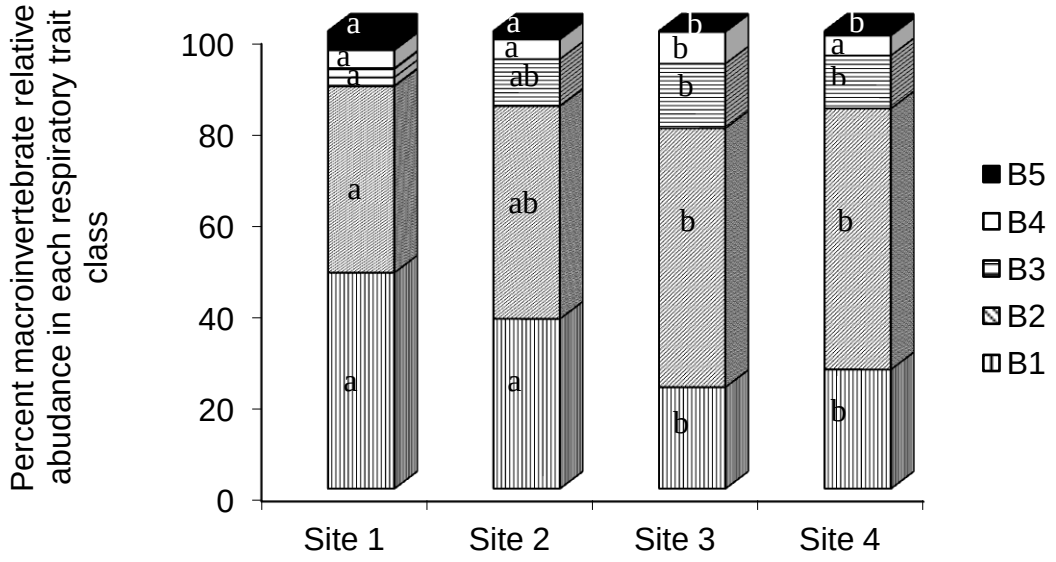


Figure 4.2: Percent macroinvertebrate relative abundance (median value) in each of the analysed respiratory trait classes at the four sampling sites in the Swartkops River during the study period (August 2009 – September 2012) showing the responses of respiratory traits to deteriorating water quality in the river. The relative abundance of macroinvertebrates using gills and lungs decreased markedly at Sites 3 and 4, and the relative abundance of those relying mostly on tegument and aerial respiration increased at Sites 3 and 4 relative to Site 1. Respiratory trait classes having completely different alphabet letters across the sites indicate significant difference ($P < 0.05$), whereas classes having the same alphabet letter in common across the site indicate no significant difference ($P > 0.05$). Trait classes: B1 (gills), B2 (tegument), B3 (spiracle), B4 (breathing tube), B5 (lung).

Six trait classes related to mobility were analysed and the relative abundance of macroinvertebrates that are crawlers, swimmers and burrowers dominated the macroinvertebrate communities at the four sampling sites (Figure 4.3). The relative abundance of the burrowers increased significantly at Site 3 than at Sites 1 and 2 (Figure 4.3). Similarly, the relative abundance of sprawlers also increased significantly at Site 3 than at Sites 1 and 2. However, the relative abundance of macroinvertebrates exhibiting the swimming and crawling habits decreased at Site 3. No significant change was observed between Sites 1, 2 and 4 in terms of the analysed traits related to mobility. Only a few families, including the veliids and gerrids, displayed the skating habit, thus macroinvertebrates displaying the skating habit contributed a very small fraction to the total

relative abundance at the four sampling sites, and no change was observed between the sampling sites. Overall, the relative abundance of the burrowers and sprawlers increased at the downstream sites, particularly at Site 3, whereas the relative abundance of the swimmers decreased with deteriorating water quality particularly at Sites 3 and 4 compared with Site 1 (Figure 4.3).

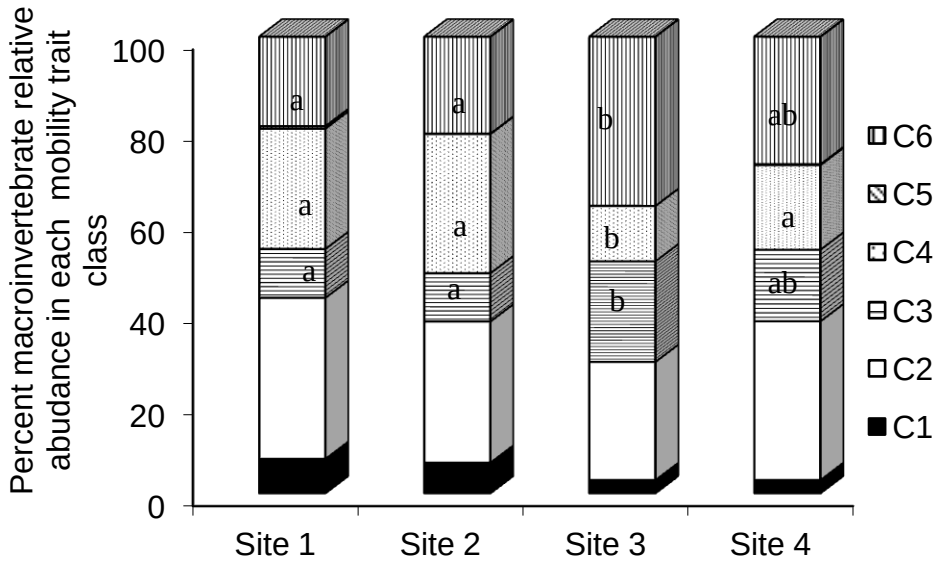


Figure 4.3: Percent macroinvertebrate relative abundance (median value) in each of the analysed mobility trait classes at the four sampling sites in the Swartkops River during the study period (August 2009 – September 2012) showing that the burrowers and sprawlers increased at the downstream sites relative to Site 1 and the swimmers decreased more at Sites 3 and 4 than at Sites 1 and 2. Mobility trait classes having completely different alphabet letters across the sites indicate statistically significant difference ($P < 0.05$), whereas classes having the same alphabet letter in common, or no alphabet letter across the sites indicate no statistically significant difference ($P > 0.05$) Trait classes: C1 (climbers), C2 (crawlers), C3 (sprawlers), C4 (swimmers), C5 (skaters) and C6 (burrowers).

The relative abundance of macroinvertebrates that are cylindrically shaped dominated the assemblages at the four sampling sites, while spherically shaped taxa contributed the least to the relative abundances at the sampling sites (Figure 4.4). For all the analysed traits related to body shape, no significant change was observed in macroinvertebrate relative abundance between Sites 1, 2 and 3. However, the relative abundances of flat-shaped taxa were significantly higher at Site 4 than at the upstream Site 1 (Figure 4.4) and the relative abundance of spherically shaped taxa decreased more (though not statistically significantly) at Sites 3 and 4 than at Sites 1 and 2.

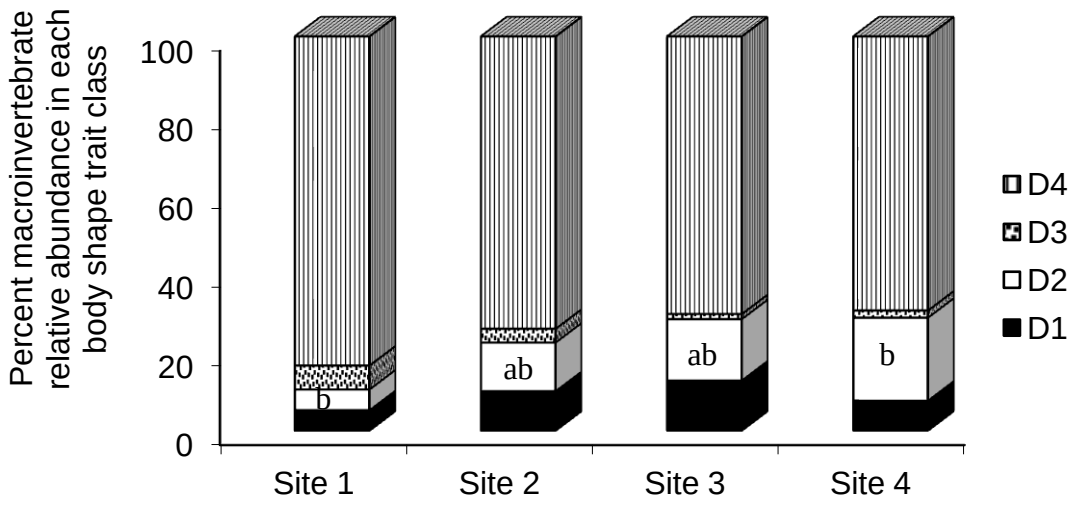


Figure 4.4: Percent macroinvertebrate relative abundance (median value) in each of the analysed body-shape trait classes at the four sampling sites in the Swartkops River during the study period (August 2009 – September 2012) showing that cylindrically shaped taxa dominated the assemblages at all four sites, and flat-shaped taxa increased markedly at the downstream sites, particularly at Site 4, relative to Site 1. Body shape trait classes having completely different alphabet letters across the sites indicate statistically significant difference ($P < 0.05$), whereas classes having the same alphabet letter in common, or no alphabet letter across the sites indicate no statistically significant difference ($P > 0.05$). Trait classes: D1 (streamlined), D2 (flattened), D3 (spherical) and D4 (cylindrical).

In terms of the preferred food and feeding habits, macroinvertebrates feeding on fine detritus (FPOM) and the collector-gatherers dominated the relative abundance of macroinvertebrate communities at the four sampling sites in the Swartkops River (Figures 4.5 and 4.6).The relative abundance the collector-filterers reduced significantly at Site 3, with no significant change observed between the remaining three sampling sites. On the other hand, the relative abundances of the predators feeding on animal materials increased markedly at Sites 3 and 4 compared with Sites 1 and 2 ($P < 0.05$) (Figures 4.6). Although the relative abundance of macroinvertebrates preferring animal materials increased significantly at Site 4 compared with Sites 1 and 2, the proportion of predators did not change significantly between Sites 1, 2 and 4 ($P > 0.05$). The relative abundance of macroinvertebrates displaying the scraping, grazing and brushing feeding habits did not change significantly between the sampling sites during the study period. The shredders were more abundant at the upstream Site 1 than at the three downstream sites (Figure 4.6).

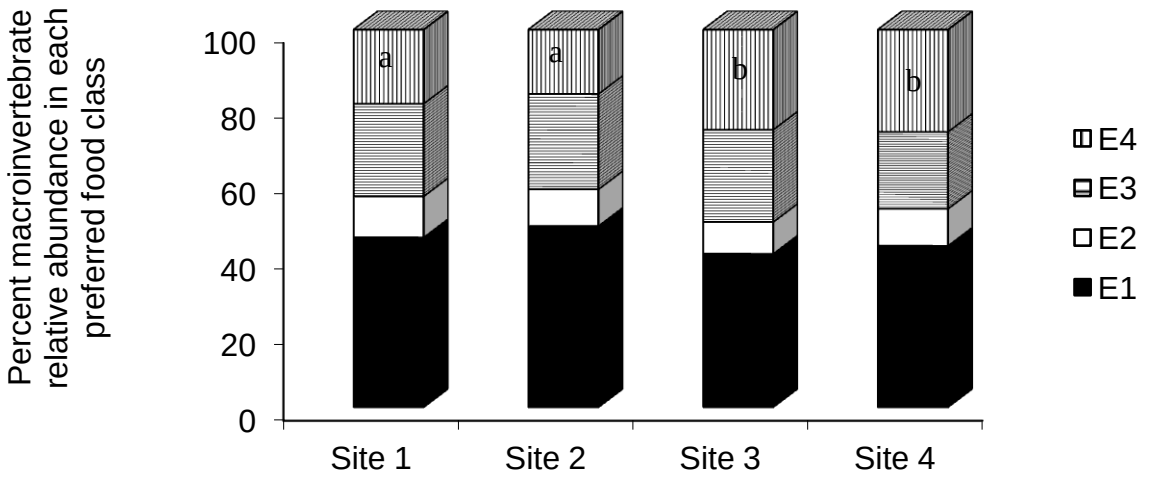


Figure 4.5 Percent macroinvertebrate relative abundance (median value) in each of the analysed preferred food trait classes at the four sampling sites in the Swartkops River during the study period (August 2009 – September 2012) showing that macroinvertebrate feeding on fine particulate organic matter dominated the relative abundances of taxa at the four sites. Preferred food classes having completely different alphabet letters across the sites indicate statistically significant difference ($P < 0.05$), whereas classes having the same alphabet letter in common, or no alphabet letter across the sites indicate no statistically significant difference ($P > 0.05$). Trait classes: E1 (FPOM), E2 (CPOM), E3 (plant materials including algae) and E4 (animal materials).

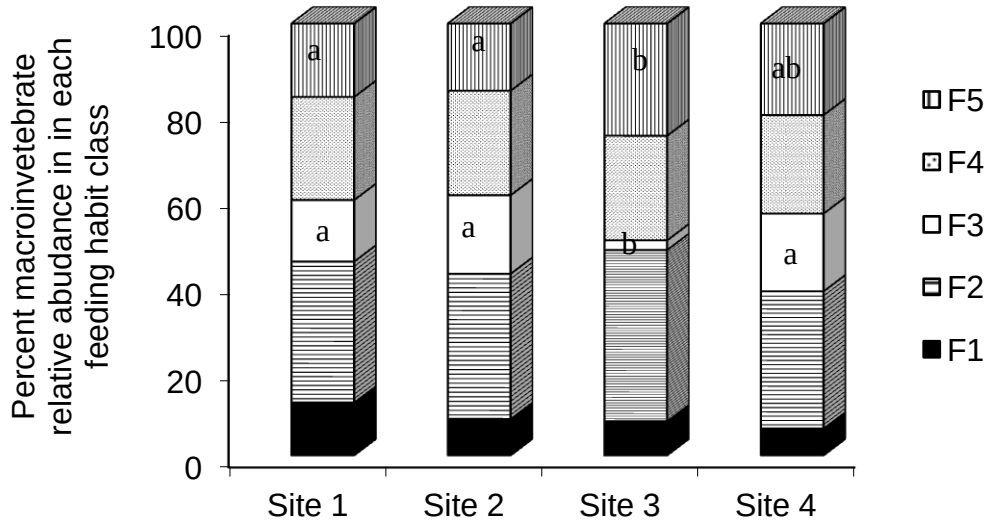


Figure 4.6: Percent macroinvertebrate relative abundance (median value) in each of the analysed feeding habit trait classes at the four sampling sites in the Swartkops River during the study period (August 2009 – September 2012) indicating that the collector-gatherers dominated the relative abundances of taxa at the four sites and the predators increased significantly at Site 3 than at Sites 1 and 2, while the shredders were more abundant at Site 1. Preferred feeding habit classes having completely different alphabet letters across the sites indicate statistically significant difference ($P < 0.05$), whereas classes having the same alphabet letter in common, or no alphabet letter across the sites indicate no statistically significant difference ($P > 0.05$). Trait classes: F1 (shredder), F2 (collector-gatherer), F3 (collector-filterer) and F4 (scraper, grazer, brusher) and F5 (predator).

Of the three biotopes, macroinvertebrates preferring the vegetation and stone biotopes dominated the relative abundances of the assemblages at all four of the sampling sites (Figure 4.7). The results showed that relative abundance of macroinvertebrates preferring the sediment biotope groups increased with increased pollution (Figure 4.7) with their relative abundances being significantly higher at Site 3 than at Site 1 ($P< 0.05$). Although the relative abundances of taxa preferring the sediment biotope groups also increased at both Sites 2 and 4, these were not significantly different from Site 1 ($P> 0.05$). The results also indicated that the relative abundances of macroinvertebrates preferring the vegetation biotope were significantly reduced at Site 3 compared with Site 1.

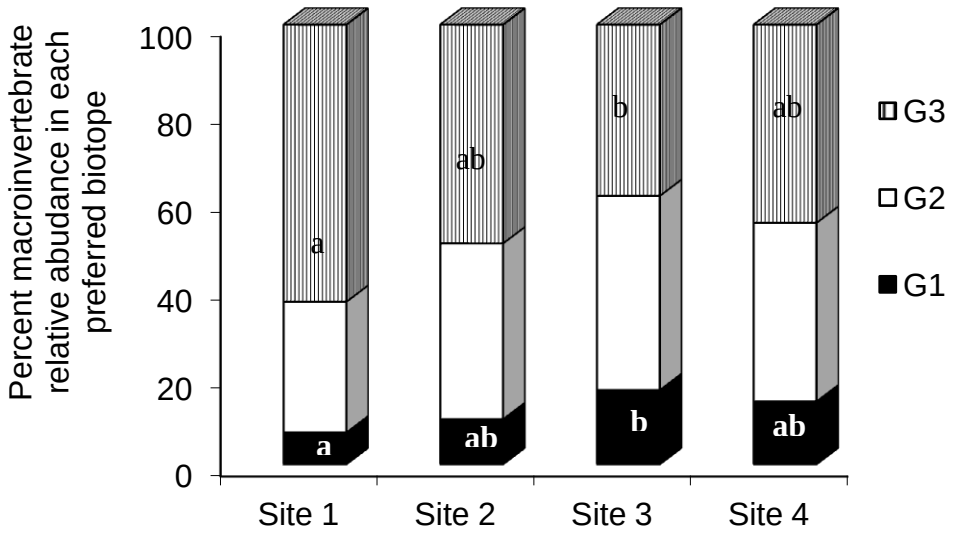


Figure 4.7: Percent macroinvertebrate relative abundance (median value) in each of the analysed preferred biotope trait classes at the four sampling sites in the Swartkops River during the study period (August 2009 – September 2012) indicating that taxa preferring the vegetation and stone biotopes dominated the relative abundances of assemblages at all four sampling site. Preferred biotopes having completely different alphabet letters across the sites indicate statistically significant difference ($P < 0.05$), whereas biotopes having the same alphabet letter in common, or no alphabet letter across the sites indicate no statistically significant difference ($P> 0.05$) Trait classes: G1 (sediment: gravel, sand and mud), G2 (stone) and G3 (vegetation).

4.3.2 Macroinvertebrate trait clusters and their associations with the four sampling sites

A cluster analysis was undertaken to elucidate the pattern of trait clustering among the Swartkops River macroinvertebrate communities. At a similarity greater than 85% subjectively defined as when traits were tightly clustered, showing trait co-occurrence, four distinct clusters were identified (Figure 4.8; Table 4.3) in order to understand how trait

combinations enable macroinvertebrate taxa to adapt to their environmental conditions at the sampling sites.

The first cluster is defined primarily by traits (spiracles and breathing tubes) used for aerial respiration linked with a climbing adaptation. The relative abundance of macroinvertebrate exhibiting traits in Cluster 1 increased more markedly at Site 3 than at the remaining three sites (Figure 4.9).

The second cluster of traits consisted of body size trait classes A 3(>10-20 mm) and A4 (>20-40 mm); preferred food E2 (CPOM), and feeding habits F1 (shredder) (Figure 4.8, Table 4.3). It is primarily a feeding association characterised by shredders feeding on coarse particulate organic matter. The relative abundance of macroinvertebrates in Cluster 2 increased at the upstream Site 1 and decreased significantly at the three downstream sites (Figure 4.9). That is, macroinvertebrates in Cluster 2 were mostly associated with Site 1 (Figure 4.9).

Cluster 3 consisted of eight traits including body size A1 (≤ 5 mm), preferred biotope G1 (sediment), preferred food E3 (plant materials) and E4 (animal materials), feeding habits F4 (scrapers, grazers and brushers) and F5 (predators), and mobility traits C4 (swimming) and C6 (burrowing). This cluster, similar to Cluster 2, is a food and feeding habit association, characterised by predators feeding on animal materials, and scrapers and grazers feeding on plant materials. Most of the macroinvertebrates in this cluster are swimmers (Figure 4.8).The relative abundance of macroinvertebrates in Cluster 3 increased markedly at the downstream sites, particularly at Site 3 (Figure 4.9). Therefore, the results indicated that macroinvertebrate families in Cluster 3 were mostly associated with Site 3 compared with Sites 1, 2 and 4 (Figure 4.9)

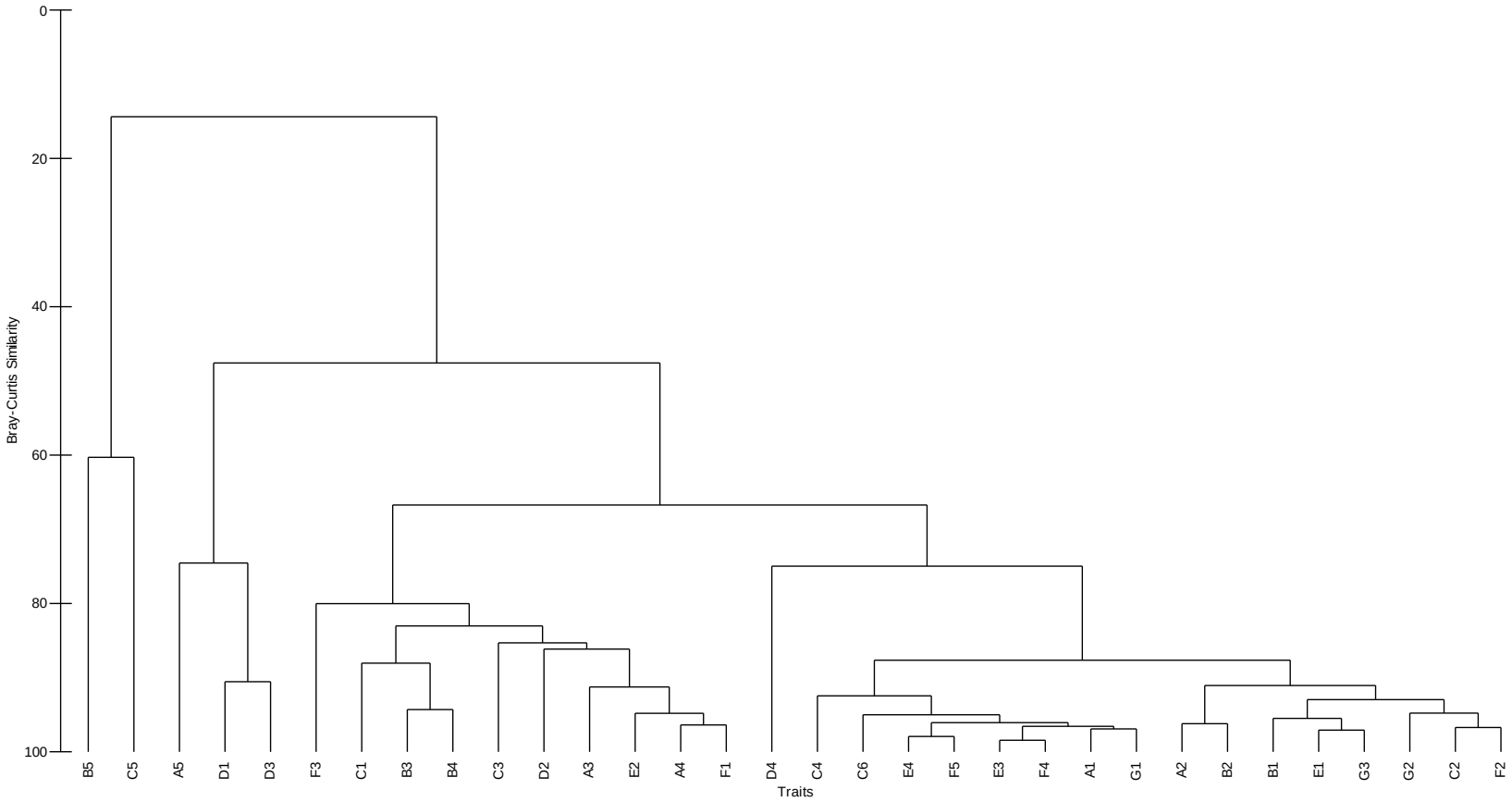


Figure 4.8 Dendrogram from cluster analysis based on Bray-Curtis similarity index showing trait combinations among the Swartkops River macroinvertebrate families over the study period (August 2009 - September 2012). Trait code: A1 (≤ 5 mm), A2 (> 5 -10 mm), A3 (> 10 – 20 mm), A4 (> 20 – 40 mm), A5 (> 40 -80 mm), B1 (gill), B2 (tegument), B3 (spiracle), B4 (aerial respiration: breathing tube, etc), B5 (aerial: lung), C1 (climber), C2 (crawler), C3 (sprawler), C4 (swimmer), C5 (skater), C6 (burrower), D1 (streamlined), D2 (flattened), D3 (spherical), D4 (cylindrical), E1 (FPOM), E2 (CPOM), E3 (plant materials/algae), E4 (animal materials), F1 (shredder), F2 (collector-gatherer), F3 (collector-filterer), F4 (scraper, grazer and brusher), F5 (predator), G1 (sediment), G2 (stone) and G3 (vegetation).

Table 4.3: Four distinct clusters of macroinvertebrate traits clustering at > 85%Bray-Curtis similarity among the Swartkops River macroinvertebrates communities based on the dendrogram in Figure 4.8.

Cluster	Trait class
1	C1 (climber)
	B3 (spiracle)
	B4 (aerial respiration: breathing tube, strap, elytra)
2	A3 (medium, > 10 – 20 mm)
	E2 (CPOM)
	A4 (large, > 20 – 40 mm)
	F1 (shredder)
3	C4 (swimmer)
	C6 (burrower)
	E4 (animal materials)
	F5 (predator)
	E3 (plant materials)
	F4 (scraper, grazer and brusher)
	A1 (very small, ≤ 5 mm)
	G1 (sediment)
4	A2 (small, > 5 -10 mm)
	B2 (tegument)
	B1 (gills)
	E1 (FPOM)
	G3 (vegetation)
	G2 (stone)
	C2 (crawler)
	F2 (collector-gatherer)

Cluster 4 consisted of eight traits, including body size A2 (> 5 – 10 mm), respiratory traits B1 (gills) and B2 (tegument), preferred food E1 (FPOM), preferred biotopes G2 (stone) and G3 (vegetation), mobility C2 (crawling) and feeding habit F2 (collecting-gathering) (Figure 4.8; Table 4.3). Macroinvertebrates exhibiting a combination of two or more of these traits were associated mostly with Sites 1, 2 and 4 and their relative abundances decreased significantly at Site 3 (Figure 4.9). Of the 33 analysed trait classes, seven i.e. lung, skater, very large body (>40-80 mm), collector-filterer, cylindrical, streamlined and spherical did not form any of the identified four clusters (Figure 4.8).

Overall, Clusters 3 and 4 consisted of most of the analysed traits, and the results indicated that macroinvertebrates in Cluster 3 were mostly associated with the downstream sites, particularly at Sites 3 and 4 than at Sites 1 and 2 (Figure 4.9), whereas the relative abundance of macroinvertebrates in Cluster 4 decreased markedly at Site 3. Cluster 1, which consisted of only three traits increased at the downstream sites, peaking at Site 3 and the relative abundance of macroinvertebrates in Cluster 2 decreased significantly at three downstream sites compared with Site 1 (Figure 4.9).

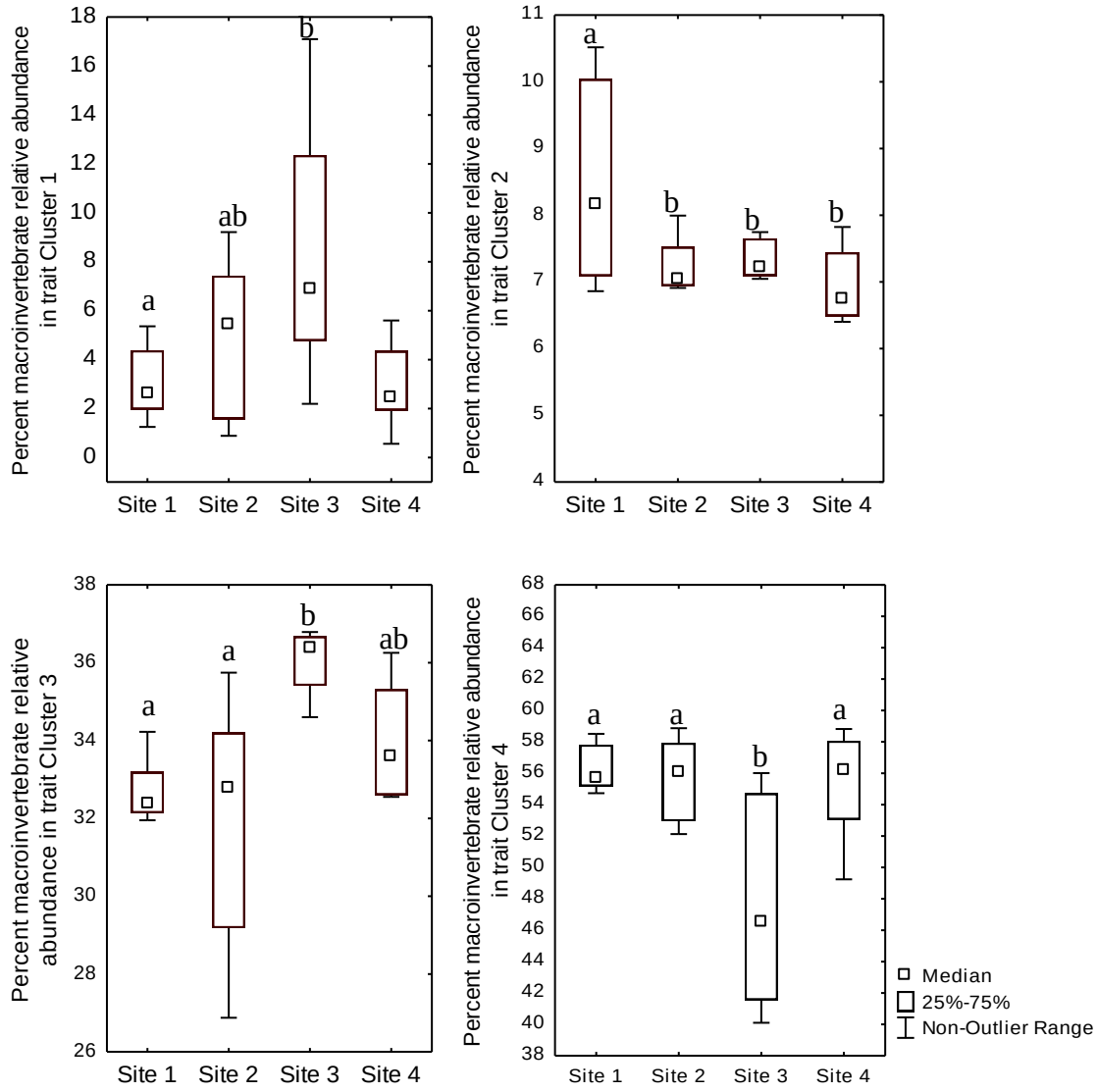


Figure 4.9: Percent macroinvertebrate relative abundance in each of the four identified trait clusters based on the dendrogram in Figure 4.8 indicating that macroinvertebrates in Cluster 2 were mostly associated with Site 1 and those in Cluster 1 and 3 were mostly associated with Site 3. Macroinvertebrates in Cluster 4 were mostly associated with Sites 1, 2 and 4 and decreased significantly at Site 3. Sites having completely different alphabet letter (s) indicate statistically significantly different ($P< 0.05$) and those having the same alphabet letter in common showed no statistically significant difference ($P > 0.05$) revealed by Kruskal-Wallis multiple comparison test.

4.4 Discussion

4.4.1 Biological and ecological traits as a tool for biomonitoring the Swartkops River

Biological and ecological traits were analysed to improve an understanding of macroinvertebrate distribution patterns, and the impact of pollution on their functional ecology in the Swartkops River. The river habitat template concept (RHTC) postulated by Townsend

and Hildrew (1994) forms the basis for applying the TBA to biomonitoring, and for a *priori* prediction of traits responses to environmental stressors because environmental conditions are matched by traits that enable the organisms to adapt to, and survive in, their environment (Culp *et al.*, 2011). Townsend and Hildrew (1994) for example, predicted that biological traits that enable rapid population growth such as short generation time, small body size, and many offspring per reproductive event, which confer resistance and resilience on biological communities were expected to dominate the traits assemblages in perturbed environments.

Predicting traits responses to environmental stressors requires an understanding of the mechanistic relationship between the traits and the given stressor (Culp *et al.*, 2011; Van den Brink *et al.*, 2011). For example, gills and small body size increase an organisms' surface area to volume ratio, thereby increasing external contact for uptake of dissolved salts and other toxicants including metals and pesticides (Liess and Von der Ohe, 2005; Statzner and Bêche, 2010). Thus, the relative abundances of gill-bearing and small-bodied macroinvertebrate taxa have been predicted to decrease as metal and dissolved salts concentrations increase (Dolédec and Statzner, 2008; Statzner and Bêche, 2010). Furthermore, in organically polluted river reaches in which the dissolved oxygen concentration is depleted, traits that enable aerial respiration may confer resilience on the communities living in such river reaches. Consequently, the relative abundances of air-breathing macroinvertebrates have been predicted to increase in an oxygen-deficient environment, relative to non-air breathers (Statzner and Bêche, 2010).

Nevertheless, predicting the response of a trait to a stressor is not straightforward because of trait syndromes that are correlated causing the response of one trait to a stressor to interfere with the predicted response of another trait (van Kleef *et al.*, 2006; Statzner and Bêche, 2010). For example, most hemipterans and coleopterans are predators and their relative abundance is expected to decrease with increasing concentrations of metals because of potential biomagnification along the food chain (Dolédec and Statzner, 2008). However, most of the families in these two orders also have specialised techniques for obtaining atmospheric oxygen in organically polluted river reaches where dissolved oxygen is depleted. Therefore, where these two stressors act simultaneously, depending on which of them influences the assemblages the most, the response of one trait (e.g. aerial respiration) may interfere with the predicted response of the other (e.g. predation). This phenomenon explains why some traits, e.g. tegument, analysed independently were positively associated with the impacted sites (i.e.

increased in relative abundance) (Figure 4.2), but analysed in combination with other traits after clustering, were negatively associated (i.e. decreased in relative abundance) (Figure 4.9), suggesting that their original response was modulated and over-ruled by another trait acting in response to a stronger stressor.

Based on separate analysis for each body-size trait class, there was no clear predictable body size response to deteriorating water quality in the Swartkops River (Figure 4.1). For example, there was no significant change in the relative abundance of the very small-bodied families across the four sites, and contrary to predictions, the relative abundance of the large-bodied individuals also did not change except at Site 3 (Figure 4.1). As already discussed above, small body size increases an organism's surface area to volume ratio, and may therefore increase exposure to, and absorption of, dissolved salts (Statzner and Bêche, 2010). Given the elevated concentrations of dissolved salts measured as electrical conductivity in this study recorded at Sites 2, 3 and 4, the relative abundance of small-bodied macroinvertebrates was expected to decrease markedly at these sites compared with Site 1.

Although body size did not respond predictably to deteriorating water quality, when analysed in combination with other traits, only the small-bodied individuals were found in a cluster (Cluster 3) that was associated mostly with the impacted sites, while the clusters (Clusters 2 and 4) containing the remaining size classes were associated with Site 1 (Figure 4.9). Several reasons could be advanced for the observed, unpredicted response of body size to deteriorating water quality at the impacted sites. Firstly, the use of predefined body size classes (though they were derived from literature) could be inappropriate for representing the macroinvertebrate body-size distribution because the natural body size distribution was not accounted for in arbitrarily defining body size classes and in assigning families to these size classes (Robson *et al.*, 2005; but see Chapter 5 of this thesis for how the Kernel curve was used to generate chironomid body size classes). Secondly, it could be that the concentrations of dissolved pollutants e.g. dissolved salts at the impacted sites, were not high enough to induce a predictable significant change in body size distribution. Thirdly, other stressor(s) acting at the impacted sites could have selected for small body size. In this case, a small body in itself becomes an adaptation for surviving at the impacted sites, as a small body, in most cases, translates into short generation time, short life span, rapid population growth, and the potential for re-colonisation and establishment, thus conferring resilience on the biological community (Southwood, 1977; 1988; Townsend and Hildrew, 1994).

The Swartkops River receives effluent discharges from the Kelvin Jones WWTW; Site 3 is immediately below the discharge point and Site 4 further downstream (Chapter 2). Consequently, the dissolved oxygen concentrations at Sites 3 and 4 were significantly lower when compared with those at Site 1 and 2 (Table 3.1; Chapter 3). Similarly, turbidity was significantly elevated at Site 3 compared to the remaining three sites. Thus, the relative abundance of air breathers using spiracles and breathing tubes was expected to increase at Sites 3 and 4, and those using gills were expected to decrease at Site 3 because of exposure to elevated concentrations of turbidity which may pose the risk of gill clogging to gill-bearing taxa (Bilotta and Braizer, 2008). The respiratory traits responded predictably to water quality impacts in the Swartkops River (Figure 4.2). The analysis of each of the traits related to respiration indicated that the relative abundance of macroinvertebrates relying on aerial respiration increased markedly at the three downstream sites, particularly at Sites 3 and 4, and those using tegument also increased at Sites 3 and 4 relative to Site 1 (Figure 4.2). The use of spiracle and specialised breathing techniques for obtaining atmospheric oxygen is an adaptation in an oxygen-deficient environment as organisms possessing such traits become less reliant on dissolved oxygen concentrations in water (Statzner and Bechê, 2010). Conversely, the relative abundance of macroinvertebrates relying more on gills and lungs for respiration increased at Site 1, and decreased at the downstream sites, particularly at Sites 3 and 4 (Figure 4.2). The significant decrease in the relative abundance of taxa relying on gills for respiration at Sites 3 was expected because as already discussed, exposure to the high turbidity concentration at this site heightened the potential risk of gill-clogging in gill-bearing taxa. Furthermore, external gills increase the overall surface area to volume ratio (Statzner and Bêche, 2010), increasing the potential up-take of dissolved salts and thereby increasing the overall vulnerability of gill-bearing organisms. These factors explain why the relative abundance of macroinvertebrates using gills for respiration decreased at the downstream sites, particularly at Site 3. The relative abundance of macroinvertebrates using lungs (the pulmonate snails) also decreased significantly at Sites 3 and 4, compared to Sites 1 and 2. Although a lung is a special adaptation for breathing atmospheric oxygen which makes lung-possessing macroinvertebrates less reliant on dissolved oxygen concentrations in water, the abundances of the lung-bearing taxa such asphysids, lymnaeids, and ancylids decreased significantly at Sites 3 and 4 (Appendix C, Table C2 c - d). Although the relative abundance of taxa using tegument increased at Sites 3 and 4 (Figure 4.2), when analysed in combination with other traits (Cluster 4), the results indicated that tegument was mostly associated with Site 1 rather than Site 3 (Figure 4.9) (Figures 4.9 and 4.10). A plausible explanation for this is that most of the macroinvertebrates relying on

tegument for respiration also use gills, and therefore, the response of tegument was probably over-ridden by the presence of gills in gill-bearing families, thus its increased association with Site 1 rather than Site 3 (Figure 4.9).

Mobility is an important trait that could enable an organism to move into refugia away from disturbed patches of rivers and streams (Ladle and Ladle, 1992). When the traits related to mobility were analysed separately, the relative abundance of the swimmers decreased significantly at Site 3, with no significant change between Sites 1, 2 and 4 (Figure 4.3).The relative abundances of the swimmers were predicted to decrease with deteriorating water quality because swimming is an important adaptation that could enable organisms to move away from polluted or perturbed river reaches (Ladle and Ladle, 1992). Thus, their reduced abundances at Site 3 in comparison with Sites 1, 2 and 4 was expected. Conversely, the burrowers were more abundant at Site 3 than at Sites 1, 2 and 4 (Figure 4.3). The increased settled river-bed sediments resulting from effluent discharges at Site 3 (personal observation) favoured the preponderance of the burrowers.

Body shape, though not directly related to deteriorating water quality, could provide insight into whether alteration in water velocity is an important stressor acting synergistically with deteriorating water quality on the Swartkops River macroinvertebrates. The increased relative abundance of flat-shaped macroinvertebrates at Site 4 (Figure 4.4), compared with the remaining three sampling sites suggests that change in the near-bottom flow was an additional stressor acting synergistically with deteriorating water quality at this site. A flat-shaped body is an important adaption for attachment, and for reducing the drag effect caused by increased water velocity (Statzner and Bechê, 2010). A small culvert constructed across the river close to this site, thereby modifying the flow, caused the alteration in water velocity at Site 4 (Chapter 2, section 2.2.2). Apart from Site 4 in which there was a significant increase in the relative abundance of flat-shaped taxa relative to Site 1, no differences existed between Sites 1, 2 and 3, suggesting that deteriorating water quality rather than flow was the main stressor influencing macroinvertebrates in the Swartkops River. Furthermore, the results of the integrated habitat assessment system (IHAS) (Appendix A, Figure A1), which revealed that habitat did not vary significantly between the sampling sites also indicated that the physical habitat was not the main influencing factor of the community structure of the macroinvertebrates in the river.

Changes in macroinvertebrate functional feeding groups (feeding habits) have been used to assess biological responses to human-induced disturbances (Moya *et al.*, 2011; Buendia *et al.*,

2013). The river continuum concept (RCC) (Vannote *et al.,* 1980), which predicts feeding group changes along the river continuum from headwaters to the mouth, forms the theoretical underpinning for a *priori* prediction of macroinvertebrate functional feeding group responses to perturbation that could impact food availability (Bonada *et al.,* 2006). For example, the relative abundances of shredders feeding on CPOM have been predicted to decrease from headwater to the mouth, with a corresponding increase in the abundance of grazers in the river middle reaches, and collectors in the lower reaches (Vannote *et al.,* 1980). Consequently, the relative abundance of shredders was predicted to decrease at the downstream sites in the Swartkops River and the results conform with the prediction, as the relative abundance of shredders feeding on CPOM were higher at Site 1, and decreased markedly at Sites 3 and 4 (Figure 4.6). The relative abundance of the predators was predicted to decrease because of toxicants e.g. metals with potential for biomagnification (Dolédec and Statzner, 2008). However, contrary to prediction, predators increased significantly at Site 3 compared with Sites 1 and 2. The concentrations of metals recorded in the water of the Swartkops River were generally low (Table 3.2) and it could be that they were not high enough to induce significant changes in the population of the predators. As explained earlier, it could also be that the predicted predatory response was over-ruled by the responses of other traits (van Kleef *et al.,* 2006).

Of the three preferred biotopes analysed, sediment serves as a physical stressor, particularly to gill-bearing taxa (Bilotta and Braizer, 2008), and as a sink for most pollutants including metals and persistent organic pollutants (POPs) (Beasley and Kneale, 2002). It is also the site for chemical interactions and speciation (Gu, *et al.,* 2013; Kalantzi *et al.,* 2013). Thus, taxa preferring sediment are likely to be more exposed to pollutants than taxa preferring the vegetation and stone biotopes that do not serve as contaminant sink. The biological effects of sediment-bound contaminants depend on both abiotic and biotic factors, including the contaminant concentrations, bioavailability, excretion and other physiological adaptations (Eggleton and Thomas, 2004). Consequently, less tolerant taxa may be more associated with the stone and vegetation than the sediment (Thirion, 2008), where tolerant taxa such as chironomids with physiological adaption become dominant (Armitage *et al.,* 1995; Syrovátka *et al*., 2009). The preponderances of tolerant taxa in the sediment at polluted sites may increase their relative abundance compared with sediment at reference site. This probably explained why the relative abundance of taxa preferring the sediment biotope increased at Sites 3 and 4 compared with Sites 1 and 2 (Figure 4.7).

In Chapter 1, section 1.5.3 of this thesis, it was argued that traits should not be seen as independent, unrelated entities, but as a complex of adaptations that enable organisms to survive in their environments (Verberk *et al.,* 2008; Menezes *et al.,* 2010). Thus, organisms faced with similar environmental stressors usually employed more than one trait, combining several traits to adapt to their environment, thereby increasing the likelihood of persisting in their environment. In this study, a cluster analysis based on the Bray-Curtis similarity index was undertaken to elucidate the patterns of traits clusters, which were then associated with the sampling sites.

The results of the cluster analysis shed light on i) the concept of traits syndrome in which the response of one traits interferes with the predicted response of other traits (Usseglio-Polatera *et al.,* 2000); ii) how group of traits were associated and their responses to deteriorating water quality. For example, the relative abundance of grazers and scrapers were expected to increase at the downstream sites compared with Site 1 because of anthropogenic nutrient input that stimulate the growth of attached algae. However, the results of the separate analysis for each trait revealed that the relative abundances of the grazers did not change significantly between the four sampling sites (Figure 4.6), but after clustering, grazing was closely associated with plant materials in Cluster 2 that was mostly associated with the three downstream sites (Figure 4.9; Table 3.1, Chapter 3) indicating that other traits overrule the predicted response of grazing, but then responded as expected after clustering.

As mentioned above, the cluster analysis also shed light on how groups of traits were associated and responded to deteriorating water quality. For example, Cluster 1 was characterised by traits that enable aerial respiration and, of the four sampling sites, the cluster was mostly associated with Site 3, where significantly low dissolved oxygen concentrations were recorded (Figure 4.9; Table 3.1, Chapter 3). Furthermore, the cluster analysis revealed that macroinvertebrates at the downstream sites, particularly at Site 3, fed on plant material and algae by grazing and scraping, and on animal material by predation (Cluster 3). The increased soft bottom sediments resulting from wastewater effluent discharges at Sites 3 and 4, favoured the preponderance of small burrowing taxa that preferred sediments (Figure 4.9, Table 4.8). On the other hand, Cluster 2, which was defined by food and feeding habit, characterised by CPOM and shredders, was mostly associated with Site 1 rather than the remaining three sites. In addition, the cluster analysis indicated that macroinvertebrates having gills were mostly associated with vegetation and stone biotopes and fed mostly on FPOM (Cluster 4). Unlike the sediment biotope, vegetation and stones do not act as a sink for toxicants, and organisms

associated with the vegetation and stone biotopes may be less exposed to pollutants. Since gills are vulnerable to increased suspended solids (Bilotta and Braizer, 2008) and also increase organism's surface areas to volume ratio (Statzner and Bechê, 2010), leading to increased exposure to sediment bound pollutants, it could be that gill-bearing taxa avoided the sediment, and thus their association with the vegetation and stone biotopes.

In summary, pollution in the Swartkops River induced several environmental constraints that local biota must resist to survive at the impacted sites. The first sets of constraints relate to altered water chemistry characteristics, including elevated dissolved solids (measured as electrical conductivity in this study) and significantly reduced dissolved oxygen concentrations (Figures 3.1a-c, Tables 3.1 and 3.2). These abiotic factors required physiological, ecological, morphological and behavioural traits for organisms to survive exposure to them. By combining two or more traits such as climbing ability, aerial respiration, feeding on a variety of food sources, including animal and plant material, and employing different feeding habits (grazing and predation) taxa at the downstream sites were able to survive impact of deteriorating water quality.

The second set of constraints relate to physical stressors such as increased suspended solids resulting in high turbidity and high levels of settled river-bed sediments particularly at Site 3 Again, these stressors also required specific traits to enable organisms survive successfully. Therefore, these stressors reduced the relative abundance of lung and gill-bearing families at Site 3 because exposure to elevated suspended solid could pose the risk of gill clogging to gill-bearing taxa.

4.4.2 Effects of taxonomic resolution on the applied trait-based approach (TBA)

As mentioned earlier in Chapter 1, an important consideration in applying the TBA is the level of taxonomic resolution at which associated traits can accurately reflect change in biological communities in relation to human-induced impacts (Gayraud *et al.,* 2003; Poff *et al.,* 2006; Tomanova *et al.,* 2008). In this chapter, traits that could potentially be applied at the family-level were used to investigate pollution effects on macroinvertebrate communities in the Swartkops River. Although the selected traits, particularly the respiratory traits, were responses associated with deteriorating water quality in the Swartkops River, the use of traits at the family level without knowledge of species or genus identity is evidently limited, because important traits, particularly those related to reproduction, growth rate and dispersal, cannot be reliably applied at the family level (Tomanova *et al.,* 2008). Reproductive traits such as

generation time, number of eggs, parental care, oviposition, and voltinism are functionally connected with both community resistance and resilience (Townsend and Hildrew, 1994). For example, short generation time, rapid growth and early maturity at reproduction and multi-voltinism are determinants of community persistence in perturbed aquatic ecosystems as these traits are likely to confer resilience on the biological community (Townsend and Hildrew, 1994). However, it was impossible to code these traits in this study at the family level without knowledge of species or at least genus identity. Therefore, the sparse taxonomic knowledge of, and limited autecological information on Afro-tropical macroinvertebrate species limit the application of the TBA. It is thus safe to argue that the strength of the TBA is in its community predictive and impact-diagnostic potential and not in deepening information provided by coarse taxonomic resolution in freshwater biomonitoring, at least in Afro-tropical and subtropical regions. Although using traits that could be applied at the family level provided insight into the Swartkops River community's functional response to pollution, it is essential to acknowledge that a family-level TBA represents a compromise between simplicity and loss of potential useful ecological and biological information. Nevertheless, the family-level TBA applied in this chapter proved useful in deepening understanding of responses to prevailing abiotic factors shaping the Swartkops River macroinvertebrate communities. It also provides insight into why certain families occurred at the impacted sites, which may therefore inform prediction of expected communities in similar rivers experiencing similar perturbations.

4.5 Conclusion

The TBA used in this study indicated that deteriorating water quality is the main driver of change on the traits composition of the macroinvertebrate communities. Furthermore, specific trait responses, particularly those related to respiration indicated that depletion of oxygen, favouring aerial respiration and increased turbidity which may affect gills of gill-bearing taxa were among the main abiotic stressors of biotic communities in the Swartkops River. In addition, increased river bed sediments that favoured the burrowers was an additional abiotic factor shaping the macroinvertebrate communities, particularly at Site 3. Furthermore, the TBA also revealed that increased near-bottom flow caused by a culvert at Site 4 was an additional physical stressor at this site. Therefore, unlike the taxonomically-based approaches applied in Chapter 3 of this thesis, the TBA enabled the diagnosis of the potential abiotic drivers shaping the biological communities. Consequently, the TBA can help direct management strategies to

focus on specific aspects of the river rehabilitation, e.g. strategies to improve dissolved oxygen concentrations, and to reduce river nutrient inputs and input of suspended solids.

The potential drawback of the TBA based on sparse taxonomic knowledge and the limited autecological information has been stated. To resolve some of these challenges, it is recommended that applied research be directed towards compiling the limited autecological information available on Afro-tropical species in a database, and also to undertake basic ecological research focusing on species biology and autecology in order to harness the full potential of the TBA in managing South Africa's water resources. However, the results in this study indicate that including a family-level TBA in freshwater biomonitoring in South Africa has the potential to improve managing freshwater resources because of the TBA's diagnostic and predictive potential, and its capacity to indicate the impact of pollution on ecological function.

CHAPTER 5: FUNCTIONAL RESPONSE OF COMMUNITIES OF THE FAMILY CHIRONOMIDAE TO DETERIORATING WATER QUALITY IN THE SWARTKOPS RIVER

In Chapter 3, the taxonomic responses of chironomid species to deteriorating water quality was analysed and in Chapter 4, the traits-based approach (TBA) was used at the family level to assess macroinvertebrate community response to deteriorating water quality in the Swartkops River. The chironomid species taxonomically-based approach offered no insight into why certain chironomid species occurred at the reference site (i.e. Site 1), but not at the impacted sites. This chapter therefore focuses on using the traits-based approach (TBA) to investigate functional responses of species from the family Chironomidae to deteriorating water quality in the Swartkops River to ascertain whether species level TBA would provide a basis for adaptive and mechanistic interpretation of chironomid species compositions in relation to deteriorating water quality. To this end, based on body size and other analysed traits (haemoglobin, preferred food and biotopes) chironomid species were grouped into "functional strategies" reflecting chironomid species with combinations of similar sets of traits. The chironomid functional strategy (FNS) approach was used as a biomonitoring tool for investigating functional responses of chironomid species to deteriorating water quality. The chapter begins with a brief introduction justifying the use of chironomid functional response in biomonitoring, followed by a methods section that describes the chironomid traits and the specific approach utilised to investigate the chironomid functional response. The results showed that the FNS developed in this chapter was sensitive to water quality differences and the approach was diagnostic of the main water quality stressors. The approach provided adaptive and mechanistic basis for interpreting chironomid species occurrence in relation to deteriorating water quality, and could to be a potentially useful tool for biomonitoring South Africa's lotic ecosystems.

5.1 Introduction

In Chapter 3 of this thesis, responses of chironomid species (based on taxonomic identification) to deteriorating water quality was analysed, but the taxonomically-based approach offered no insight into why some chironomid species occurred at the reference site (i.e. Site 1), but not at

the impacted sites. A traits-based approach (TBA) involving carefully selected traits that could provide a mechanistic and adaptive basis for explaining functional response of chironomid species to deteriorating water quality was therefore undertaken. Understanding why certain chironomid species are present in an environment requires the knowledge of the prevailing abiotic factors, and the traits that enable species to adapt to their environment (Verberk *et al.*, 2008; Van Kleef, 2010).

A biological trait such as body size is important because it constrains several other traits related to reproduction, adult life span, locomotion and metabolic rate, and has been regarded as an "integrative taxon-free" trait that can provide clues to understanding species interaction with their environments (Pianka, 1970; Armitage *et al.*, 1995; Blanckenhorn, 2000; Robson *et al.*, 2005; Siqueira *et al.*, 2008). However the very use of traits to understand species responses fundamentally includes taxonomic recognition. Therefore trait-base information may well be valuable, but is in addition to taxonomic identification. As already discussed in Chapters 2 and 4 of this thesis, body size has a mechanistic relationship with freshwater pollution because aquatic macroinvertebrates in polluted rivers, particularly with elevated concentrations of dissolved salts may seek to maintain body sizes that reduce body surface area to volume ratio. Therefore, it was hypothesised that to minimise external exposure to pollutants such as metals and increased dissolved salts (measured as electrical conductivity in this study), large-bodied chironomid species would dominate the species abundance at the downstream sites, particularly at Sites 3 and 4.

Although this chapter tests the hypothesis stated above, chironomids species combine other traits with body size to adapt to their environment (Armitage *et al.*, 1995). Consequently, a chironomid traits-based approach that combines body size with other traits that have mechanistic and adaptive values was developed to investigate the functional response of the Swartkops River chironomid communities to deteriorating water quality. Verberk *et al.* (2008), using life-history theory, provide a context-specific basis for defining species that employ similar traits to adapt to similar environmental constraints. Therefore, in the chironomid traits-based approach utilised in this study, species were grouped into "functional strategies" to provide an integrative insight into how chironomid species responded to the prevailing abiotic stressors in their environments. The term functional strategy (FNS) as used here refers to a context-specific combination of traits that contribute to the adaptation of the Swartkops River chironomids to the prevailing abiotic stressors in their environment. The term FNS was coined

in contrast to life-history tactics (LHT), which was used by Van Kleef (2010), because life-history refers mostly to biological traits characteristics of biological aspects of organisms including recruitment, mortality, growth, development, dormancy and synchronisation, reproduction, dispersal, voltinism, and phenology (Resh and Rosenberg, 2010), but usually does not include ecological traits. Therefore the term FNS was coined in this study to emphasise the functional response combining both biological and ecological traits.

5.2 Materials and methods

5.2.1 Biological and ecological traits

Four traits were selected for analysis: two biological (body size and haemoglobin) and two ecological (preferred food and biotope-preference), which potentially have both a mechanistic and adaptive relationship with deteriorating water quality in the Swartkops River. As already stated, large body size is a morphological adaptation that could enable organisms to minimise external contact with pollutants due to reduced surface area to volume ratio (Statzner and Bêche, 2010). Small-bodied chironomids were expected to be more vulnerable to effects of increased pollutants particularly elevated dissolved salt concentrations (measured as electrical conductivity in this study) due to their increased surface area to volume ratio. Chironomid body-size measurements were undertaken as described in Chapter 2, section 2.5.1. All chironomid body-size measurements for the recorded species were used to produce a kernel curve fitted unto a histogram. Body-size classes were obtained based on the modes and troughs of the kernel curve (Chapter 2, section 2.8.5).

Haemoglobin protein is a physiological adaptation that enables chironomid species to survive in an oxygen-deficient environment because of its high affinity for oxygen (Armitage *et al.*, 1995). Chironomid species living with a limited supply of oxygen, saturate their haemoglobin with oxygen, and release it rapidly when needed. Haemoglobin-possessing species were therefore expected to thrive in low oxygen concentrations and their high abundance could be indicative of an environment with low oxygen concentration (Armitage *et al.*, 1995). Information on haemoglobin presence or absence in chironomids was obtained from the literature (Armitage *et al.*, 1995; Cranston, 1996; Van Kleef, 2010).

The Swartkops River receives pollutants from both non-point and point sources of pollution, including wastewater effluent discharges (Chapter 2) and the third selected trait, which is preferred food, could also be related to effects of increase in nutrient inputs, and toxicants with

potential for bio-magnification along the food chain. For example, nutrients stimulate the growth of algae and may therefore favour the preponderance of chironomids that are detritivore-algivores, i.e. chironomid species, feeding on detritus and grazing on algae because of an increased supply of food resources from sewage-based nutrient. Elevated concentrations of metals were expected at the downstream sites due to surrounding industries and wastewater effluent discharges (Chapter 2), and because of the biomagnification potential of metals across trophic levels (Beasley and Kneale, 2002; Croteau *et al.,* 2005), the abundance of the predatory chironomids preferring animal materials were expected to decrease at the downstream sites. The abundances of macroinvertebrates feeding on animal materials (i.e. predators) have previously been predicted to decrease in relation to toxicants with biomagnification potentials (Dolédec and Statzner, 2008).

Chironomid food preference was investigated using gut content analysis (Sanseverino and Nessimian, 2008). Gut contents of mounted chironomids (Chapter 2, section 2.4) were examined directly from slides (Sanseverino and Nessimian, 2008), under an oil immersion lens (X 100) in an Olympus compound microscope (B X 51) equipped with an Altra 20 soft imaging system digital camera. Chironomid gut contents were identified broadly as detritus, algae, macrophytes and animal parts.

The fourth trait is biotope preference, and it included stone, vegetation and sediment. Of these three biotopes, sediment act as a sink for a range of pollutants including metals and persistent organic pollutants (POPs) and it is also the site of chemical speciation, interactions and toxicant releases during disturbances (Beasley and Kneale, 2002; Gu, *et al.,* 2013; Kalantzi *et al.,* 2013). These factors may lead to increase exposure of sediment-associated species to contaminants. On the other hand, stones and vegetation do not usually act as contaminant sink, and therefore free-flowing water over these biotopes (stones and vegetation) may offer a water quality refuge to chironomid species compared with the sediment. Chironomid-biotope preference was undertaken as described in Chapter 3, section 3.2.1 using the Pearson's point-biserial correlation coefficient (De Cáceres *et al.,* 2008; De Cáceres and Legendre, 2009). Where chironomid biotope association could not be determined for any species because of insufficient data for that species, the information for a generic level was obtained from the literature (Armitage *et al.,* 1995; Principe *et al.,* 2008; Syrovátka *et al*., 2009; Rosa *et al.,* 2011; Epele *et al.,* 2012).

Overall, using these four traits, a functional strategy (FNS) classification system was developed for used in chironomid traits based analysis. Based on the FNS classification chironomids were assigned functional strategies that reflect specific combinations of these traits. Other useful traits, such as voltinism and synchronisation, were not used in this study for defining chironomid functional strategies because the lack of information in the literature makes it too uncertain to use them.

Describing the chironomid functional strategies (FNS) classification system applied for the traits-based analysis

Based on the analysed trait combinations by chironomid species, seven functional strategies (A-G) were identified and described for the Swartkops River chironomid assemblages. Functional strategy A (FNS A: small body size with no haemoglobin) consisted of small-bodied chironomid species with the third and fourth instars larvae ranging mostly between 3-7 mm, with some species in this FNS growing as much as 12 mm in length. No species in this FNS invest in haemoglobin formation, with the majority of them preferring the stone biotope. They are mostly detritivore-algivorous, feeding on detritus and algae with few species feeding on animal parts (predators). The defining characteristics of species in this FNS are their small body size with no investment in haemoglobin formation. The relatively small body sizes of species in this FNS, coupled with the lack of haemoglobin would make them vulnerable to effects of oxygen depletion and elevated dissolved salt concentrations. Thus, their relative abundance was expected to decrease with pollution in the Swartkops River.

FNS B (very large body size with haemoglobin): Species belonging to this functional strategy are very large-bodied (> 12 mm), growing to as much as >16 mm in length. The very large body would enable them to minimise external contact with pollutants through reduced body surface area to volume ratio. Species in this FNS are able to tolerate harsh environmental conditions (e.g. significantly reduced dissolved oxygen concentrations) by investing in haemoglobin formation. They are mostly burrowers, preferring the soft sediments of streams and the interstitial spaces between stones where they feed on detritus and graze attached algae. Species in this FNS are all detritivore-algivorous. The defining characteristics of this species are their large body sizes with investment in haemoglobin formation. The large body, coupled with the investment in haemoglobin and a preference for detritus and algae as the main diet enable these species to thrive in organically enriched sites. Their relative abundance is expected to increase with increase pollution in the Swartkops River.

Species in FNS C (medium to large body size with haemoglobin) are medium to large bodied (> 9 – 16 mm) chironomid species similar to those in FNS B except that they do not grow as much as > 16 mm and are mostly associated with the vegetation and the stone biotope groups (Table 5.1). They invest in haemoglobin formation and are therefore able to thrive in organically enriched sites. These medium to large bodied chironomid have a size advantage compared with the small species that investment in haemoglobin formation. Because of their body size advantage and investment in haemoglobin, their relative abundance is expected to increase with increased pollution in the Swartkops River.

FNS D (small to medium body size with haemoglobin): These are relatively small to medium-bodied chironomid species whose body size ranges between < 3 – 9 mm. In terms of body size, species in FNS D are similar to those in FNS A, but unlike species in FNS A, species in FNS D invest in haemoglobin formation and consist of both predators and herbivores, preferring macrophytes instead of algae. However, their diets also included detritus. In addition, the majority of species in this FNS prefer the vegetation biotope group. Because they invest in haemoglobin formation, they are likely to tolerate decreased oxygen concentrations, but their often-predatory lifestyle and small body may limit their ability to inhabit heavily polluted sites, and therefore, their relative abundance is expected to decrease with pollution, particularly at Sites 3 and 4 in the Swartkops River, with elevated concentration of EC. The defining characteristics of species in this FNS are their small to medium body sizes with investment haemoglobin formation.

FNS E (very small body size with haemoglobin): Species in FNS E are similar to those in FNS D, but are smaller with their third and fourth instars larvae ranging between < 3 – 7 mm and are entirely detritivorous. They do not feed on macrophytes and do not use vegetation as their preferred biotope; instead, they are mostly associated with sediment and stones. The investment in haemoglobin may allow them to tolerate decreased dissolved oxygen concentrations, but their very small body-sizes may render them vulnerable to effects of elevated electrical conductivity because of increase in surface area to volume ratio. Their relative abundance is therefore expected to decrease with pollution in the Swartkops River.

FNS F (medium sized predators with no haemoglobin): Species of FNS F are all predators, and do not invest in haemoglobin formation. They are of medium sized (> 7 – 12 mm) body and prefer the sediment where they can easily search for their prey. Their lack of haemoglobin, coupled with their predatory lifestyle render species of FNS F vulnerable to harsh water quality

conditions. Their relative abundance is therefore expected to decrease with pollution in the Swartkops River.

FNS G: Species in FNS G could not be confidently classified because not all the traits could be analysed for them, as they were not frequently encountered in the Swartkops River. However, they are all small-bodied, and do not invest in haemoglobin formation.

5.2.2 Statistical analysis

Instead of using arbitrarily predefined body-size classes, all chironomid body-size measurements for the recorded species were used to produce a kernel curve fitted unto a histogram. Body-size classes were obtained based on the modes and troughs of the kernel. A detailed description of the kernel curve is provided in Chapter 2, section 2.8.5. The chironomid communities were analysed in terms of body size by calculating the relative abundance of individuals belonging to each of the obtained body-size classes and the Kruskal-Wallis multiple comparison test was used to ascertain whether there were significant differences between the sites in terms of chironomid body size classes. The Kruskal-Wallis test was undertaken using the Statistica software package version 9.

To assess whether the described chironomid FNSs could enable the discrimination of the sites, box plots were used to assess the discriminatory potential of each of the defined chironomid FNS in terms of association with Site 1 (the reference site) compared with the three downstream sites (i.e. Sites 2, 3 and 4). Chironomid functional strategies (FNSs) enabled discrimination of Site 1 from Sites 2, 3 and 4, indicating that the FNS were affected by deteriorating water quality. The degree of overlap of the medians and the inter-quartile ranges (IQRs) between Site 1 and the downstream sites was considered an indicator of the discriminatory potential of each of the FNSs. Two levels of discrimination between Site 1, and Sites 2, 3 and 4 were considered satisfactory. The first level of discrimination was in cases where the inter-quartile ranges of the FNSs between Site 1 and Sites 2, 3 and 4 did not overlap, whereas in the second level of discrimination, the inter-quartile ranges overlap, but the medians were outside of the inter-quartile ranges. Similarly, to test whether the chironomid FNSs could be used to detect subtle differences between the three downstream sites, the same criteria were used to judge whether FNSs that enable discrimination of Site 1 from Sites 2, 3 and 4, could also enable discrimination between the three downstream sites (i.e. Sites 2, 3 and 4).

The correlations between the chironomid FNSs and the measured water physicochemical variables were elucidated using canonical correspondence analysis (CCA) (ter Braak and Verdonschot, 1995). Water physicochemical variables exhibiting high multi-colinearity (r > 0.8) were eliminated from the CCA model (ter Braak and Verdonschot, 1995; Zuur *et al.*, 2007). The CCA was undertaken using Environment Community Analysis 1.33 package (ECOM) (Pisces conservation Ltd, 2000). The CCA method has been described in details in Chapter 2, section 2.8.7.

5.3 Results

5.3.1 Patterns of chironomid body-size distribution in the Swartkops River

The Kernel density curve showed that the overall body-size distribution of the Swartkops River chironomids was multimodal (Figures 5.1). Based on the kernel curve, the chironomids were divided into the following five classes corresponding to modes and troughs: A (<3 – 7 mm), B (>7 – 9 mm), C (> 9 – 12 mm), D (>12 – 16 mm), and E (>16 mm). The relative abundance of species at Sites 1 was dominated by small-bodied chironomids A (<3 – 7 mm) (Figure 5.2) and the Kruskal-Wallis multiple comparison test showed that the abundance of these species was significantly higher at Sites 1 than at Sites 2, 3 and 4 ($P < 0.05$). The small-bodied species (Class A) were absent at Site 3 and were higher at Site 2 than at Site 4 (Figure 5.2). The relative abundance of species belonging to Class B ((>7 – 9 mm) were also higher at Site 1 and declined markedly at Site 3. The large- and very large-bodied chironomid species Class D and E dominated the relative abundance of species at Sites 3 and 4, and the Kruskal-Wallis multiple comparison test revealed that their abundances were significantly higher at Sites 3 and 4 than at Site 1 ($P < 0.05$). The abundances of these species i.e. the large and very large-bodied species contributed the least to total relative abundance of species at Site 1. The chironomids species belonging to Class C (> 9 – 12 mm) were the dominant species at Site 2 and their abundance was significantly higher at this site than at Sites 1 and 3. Species belonging to all the identified classes were represented at Site 2.

Generally, the chironomid body size responded predictably to deteriorating water quality, with the abundance of small bodied-species dominating the chironomid communities at Site 1, and declining significantly at the downstream sites particularly at Sites 3 and 4. Conversely, the large and very large-bodied species dominated the relative abundance of the assemblages at the downstream sites, and declined significantly at the upstream Site 1 (Figure 5.2).

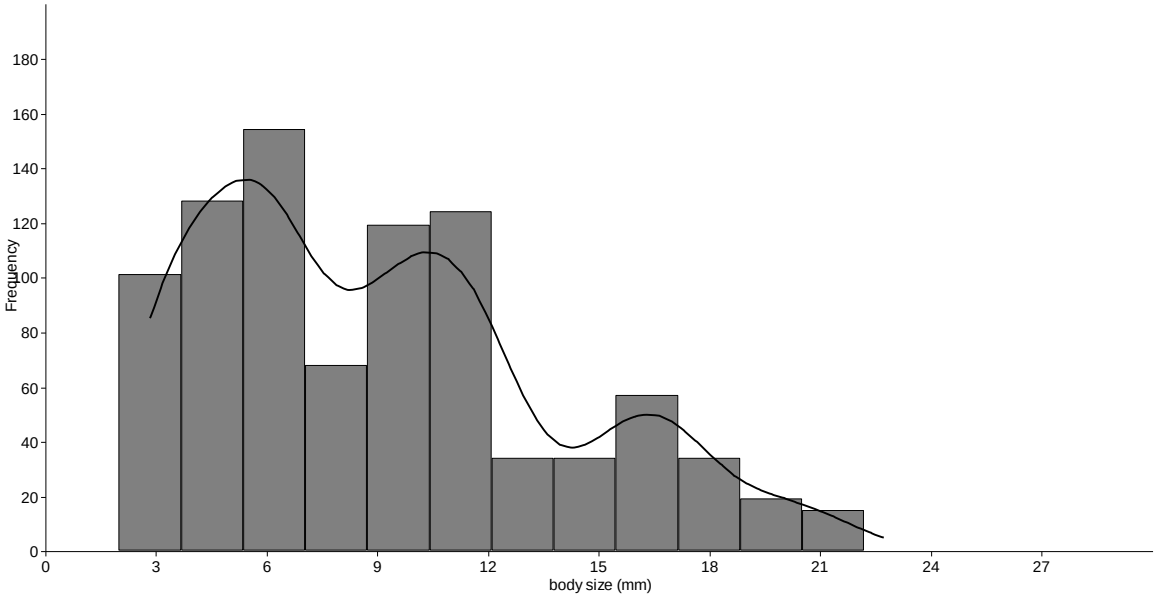


Figure 5.1: Kernel curve fitted onto a histogram showing that the overall body-size distribution of the Swartkops River chironomids was multimodal (three modes) during the study period (August 2009 – September 2012) and based on the Kernel curve, the following body size classes corresponding to modes and troughs were estimated: <3 – 7 mm, >7 – 9 mm, > 9 – 12 mm, >12 – 16 mm, and >16 mm.

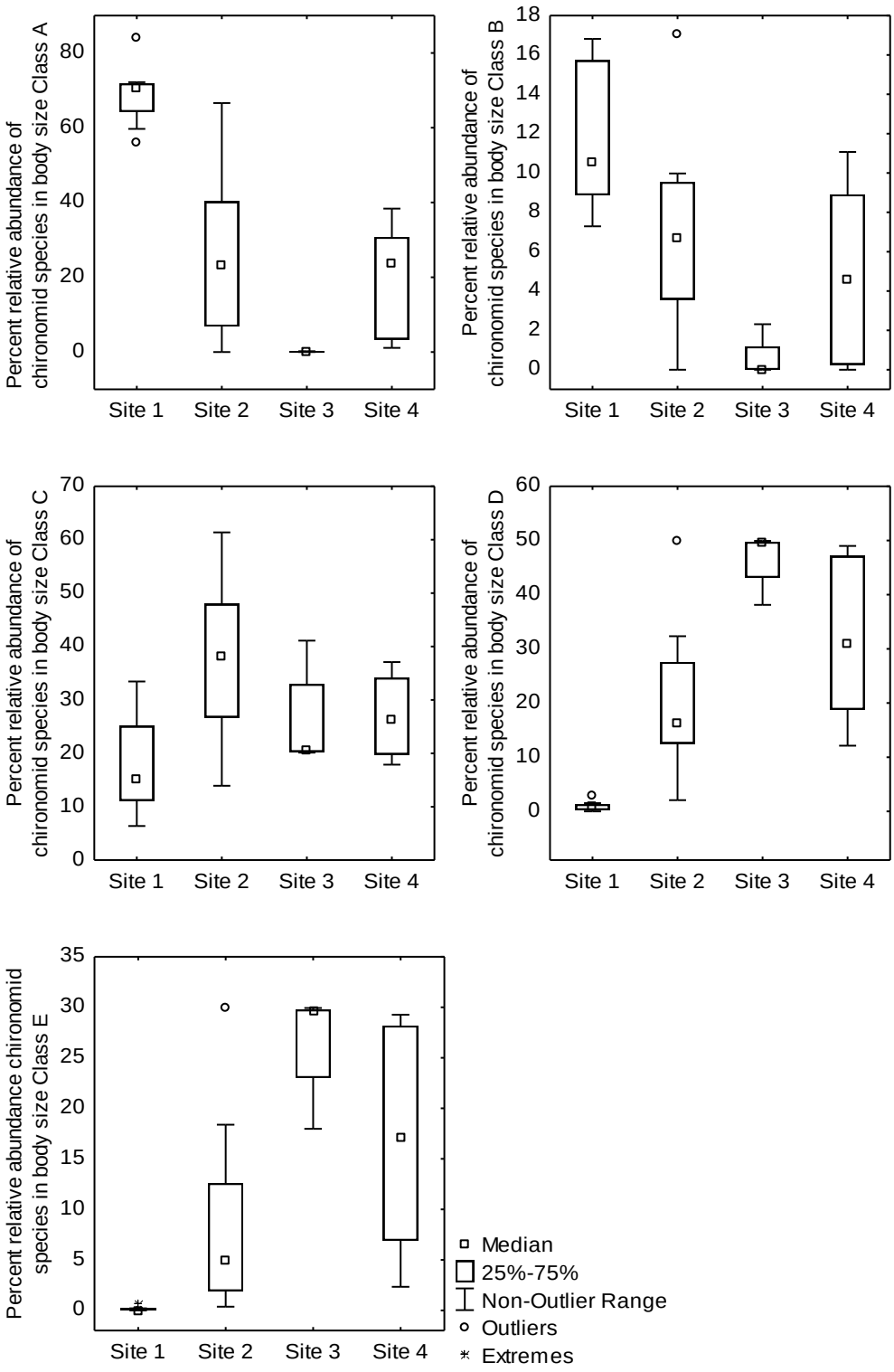


Figure 5.2: Percent relative abundance of chironomid species in the five body size classes identified for the chironomid assemblages in the Swartkops River showing the small-bodied species were more abundant at Site 1 than at Sites 2, 3 and 4, whereas the medium, large and very large bodied species (C, D and E) dominated the relative abundances of species at three downstream sites during the study period (August 2009 – September 2012). Body-size classes: A (<3 – 7 mm), B (>7 – 9 mm), C (>9 – 12 mm), D (>12 – 16 mm), and E (>16 mm).

5.3.2 Spatial and temporal distribution of chironomid functional strategies (FNS) in the Swartkops River

Of the 35 species recorded in this study, seven were grouped in FNS A (small body size with no haemoglobin), four each in FNS B (very large body size with haemoglobin) and C (medium to large body size with haemoglobin), nine in FNS D (small to medium body size with haemoglobin), three each in FNS E (very small body size with haemoglobin) and G (species in this group could not be confidently assigned because of lack of information) and five in FNS F (medium sized predators with no haemoglobin) (Table 5.1). Overall, most species belong to FNS A and D.

Tables 5.1: Chironomid species in the Swartkops River collected over the study period (August 2009 – September 2012) and their defined functional strategies (FNSs). Note that the descriptive name written by the side of each FNS is to enable easy differentiation of the FNS; full description of each FNS has been provided in the method section.

Taxon	Chironomid functional strategy
Orthocladiinae	
Cricotopus sp.1	A (small body size with no haemoglobin)
Cricotopus trifasciata gr.	A (small body size with no haemoglobin)
Paratrichocladius sp.	A (small body size with no haemoglobin)
Nanocladius sp.	G (species in this group could not be confidently assigned because of lack of information)
Eukiefferiella sp.	G (species in this group could not be confidently assigned because of lack of information)
Cardiocladius sp.	A (small body size with no haemoglobin)
Parakiefferiella sp.	G (species in this group could not be confidently assigned because of lack of information)
Orthocladius sp.	A (small body size with no haemoglobin)
Orthocladius sp.2	A (small body size with no haemoglobin)
Chironominae	
Tribe Chironomini	
Dicrotendipes sp.	C (medium to large body size with haemoglobin)
Kiefferulus sp.	C (medium to large body size with haemoglobin)
Polypedilum sp	D (small to medium body size with haemoglobin)
Cryptochironomus sp.	C (medium to large body size with haemoglobin)
Chironomus sp. 1	B (very large body size with haemoglobin)
Chironomus sp. 2	B (very large body size with haemoglobin)
Chironomus sp. 3	B (very large body size with haemoglobin)
Microchironomus sp.	D (small to medium body size with haemoglobin)
Polypedilum nubifer	D (small to medium body size with haemoglobin)
Glyptotendipes sp.	B (very large body size with haemoglobin)
Dicrotendipes sp.2	C (medium to large body size with haemoglobin)
Tribe Tanytarsini	

Tanytarsus sp.	D (small to medium body size with haemoglobin)
Rheotanytarsus sp.	E (very small body size with haemoglobin)
Cladotanytarsus sp.	E (very small body size with haemoglobin)
Virgatanytarsus sp.	E (very small body size with haemoglobin)
Paratanytarsus sp.	D (small to medium body size with haemoglobin)
Tanypodinae	
Ablabesmyia sp.	A (large body size with no haemoglobin, predator)
Coelotanypus sp.	D (small to medium body size with haemoglobin)
Procladius sp.	F (medium sized predators with no haemoglobin)
Trissopelopia sp.	F (small to medium body size with haemoglobin)
Clinotanypus sp.	D (small to medium body size with haemoglobin)
Tanypus sp	D (small to medium body size with haemoglobin)
Nilotanypus sp.	F (medium sized predators with no haemoglobin)
Thienemannimyia sp.	F (medium sized predators with no haemoglobin)
Macropelopia	D (small to medium body size with haemoglobin)
Conchapelopia sp.	F (medium sized predators with no haemoglobin)

The results for the spatial and temporal distribution of chironomid functional strategies (FNSs) during the four sampling seasons at the four sampling sites indicated that at Site 1 functional strategy A (FNS A: small body size with no haemoglobin) dominated the relative abundance of chironomid species in all the sampling seasons (Figure 5.4) except in winter. During winter, FNS D (small to medium body size with haemoglobin) was the most dominant functional strategy, but it was closely followed by the relative abundance of species belonging to FNS A. Chironomid species belonging to FNSs B (very large body size with haemoglobin), C (medium to large body size with haemoglobin) and FNS G (species that could not be confidently assigned) were the least dominant at Site 1 throughout the sampling seasons (Figure 5.3). Thus, the results showed that the small bodied chironomid species with or without investment in haemoglobin formation, preferring the stone and vegetation biotopes, and feeding mostly on algae, detritus and animal part were the most associated species with Site 1 in all the sampling seasons. On the other hand, the medium, large and very large bodied species investing in haemoglobin formation, preferring sediment and feeding mostly on algae, were the species least associated with Site 1 in all the sampling seasons.

The chironomid species belonging to FNSs A (small body size with no haemoglobin), B (very large body size with haemoglobin) and C (medium to large body size with haemoglobin) dominated the overall relative abundances of species at Site 2 in all the sampling seasons (Figure 5.3). However, there was a noticeable increase in the relative abundance of species

belonging to FNS D (small to medium body size with haemoglobin) in summer and autumn. Conversely, species belonging to FNS F (medium body sized predators with no haemoglobin) and E (very small body size with haemoglobin) were the least abundant at Site 2. The results therefore indicated that small, large and very large bodied chironomids, that invested in haemoglobin formation, preferring mostly sediment and stones, and feeding on algae and detritus were the species most associated with Sites 2. The predators, which have no investment in haemoglobin formation were the species least associated with Site 2 throughout the sampling seasons.

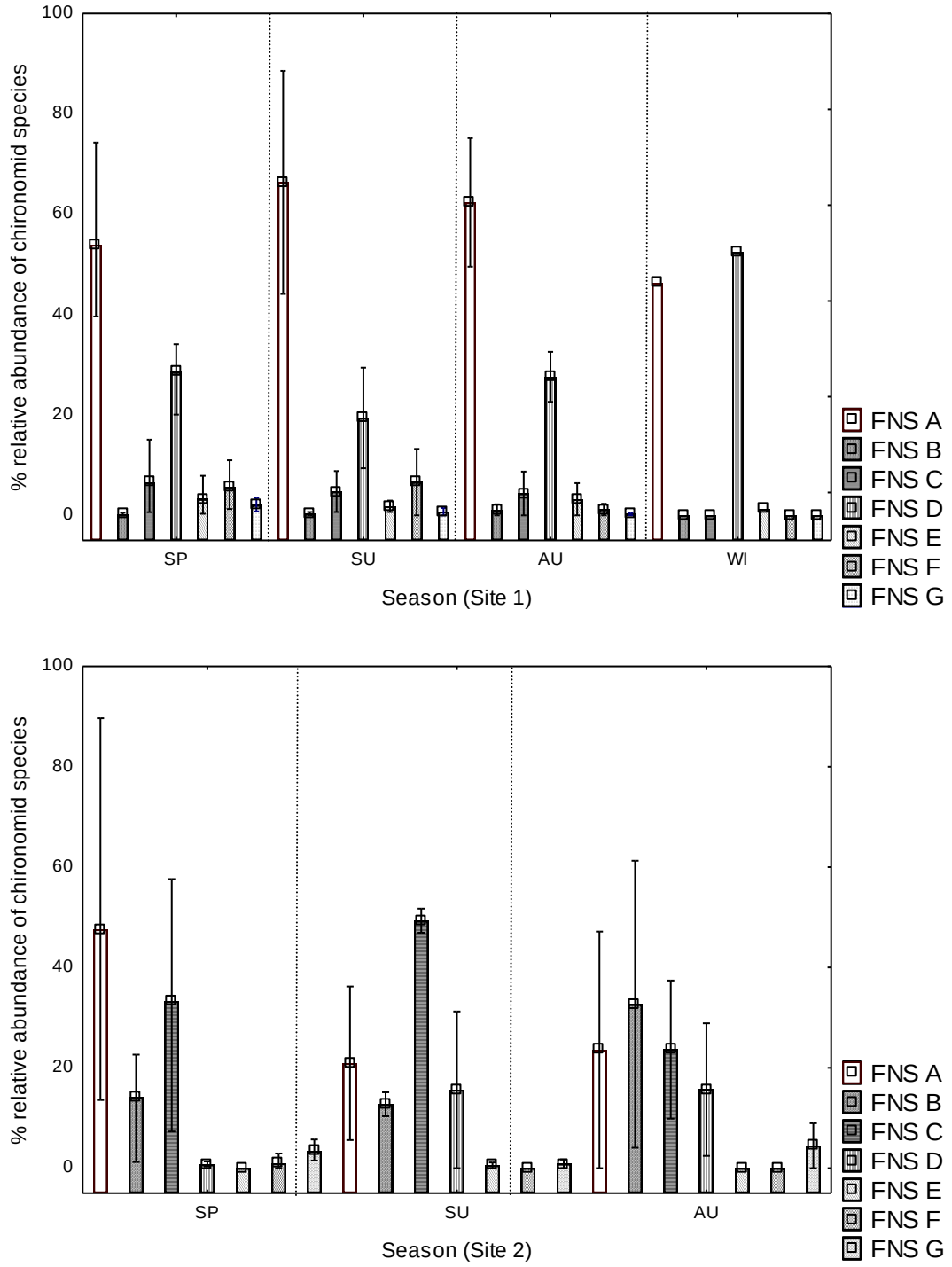


Figure 5.3: Seasonal variation in average percent relative abundance of chironomid species belonging to each of the defined seven functional strategies at Sites 1 and 2 in the Swartkops River during the study period (August 2009 – September 2012) showing that species belonging to FNS A and D were the most abundant at Site 1, whereas species in FNS B, and C were the lease abundant at Site 1 in most of the sampling seasons. The figure also indicated that species in FNS A, B and C were the most abundant at Site 2, while the least abundant species belong to FNS E and F. The vertical bars represent standard deviation from the means. Abbreviations: Seasons: SP (spring), SU (summer), AU (autumn), WI (winter).

The relative abundance of chironomid species at Sites 3 and 4 were almost entirely dominated by species belonging to FNSs B (very large-bodied species with haemoglobin) and C (medium and large-bodied species with haemoglobin), with species in the remaining FNSs contributing very little to the total relative abundance of chironomid assemblages at the two sites. There seemed to be a gradual improvement at Site 4 with the appearance of species belonging to FNS A (small-bodied species with no haemoglobin) and D (small-bodied species with haemoglobin), particularly during spring and autumn (Figure 5.4). Generally, the results indicated that the water quality condition at Sites 3 and 4 favoured the preponderances of the large- and very large-bodied chironomid species investing in haemoglobin formation that feed mostly on algae and detritus, and were mostly associated with the soft sediment and stone biotopes.

5.3.3 Do the chironomid functional strategies (FNSs) enable discrimination between the sampling sites in the Swartkops River?

Box plots were used to explore whether the defined chironomid functional strategies (FNSs) can enable the discrimination between the sampling sites in the Swartkops River (Figures 5.5a and 5.5b). Of the seven defined functional strategies (FNSs), five enabled the discrimination between Site 1, and the three downstream sites (i.e. Sites 2, 3 and 4). That is, based on the criteria explained in the methods section of this chapter; these FNSs enabled the separation of Site 1 from Sites 2, 3 and 4. The five FNSs that enabled the discrimination of Site 1 from Sites 2, 3 and 4 include FNSs A (small-bodied species with no haemoglobin), B (very large bodied-species with haemoglobin), D (small to medium sized species with haemoglobin), E (very small bodied species with haemoglobin) and F (medium sized predatory species with no haemoglobin). Two of the defined FNSs, i.e. C (medium to large bodied species with haemoglobin) and G (species that could not be confidently assigned to any of the FNS), did not enable the discrimination of Site 1 from the three downstream sites (i.e. Sites 2, 3 and 4) (Figures 5.5a and 5.5b). Of the seven chironomid FNSs, three (i.e. A, B and G) enabled discrimination between the three downstream sites. The relative abundances of chironomid species belonging to FNSs A (small-bodied species with no haemoglobin) and G (species that could not be confidently assigned to any of the FNS) were higher at Sites 2 than at Sites 3 and 4, whereas the species belonging to FNS B (very large bodied species with haemoglobin) were more associated with Sites 3 and 4 than Site 2, and thus, species in these FNS enabled the discrimination of Site 2 from Sites 3 and 4 (Figures 5.5a and 5.5b).

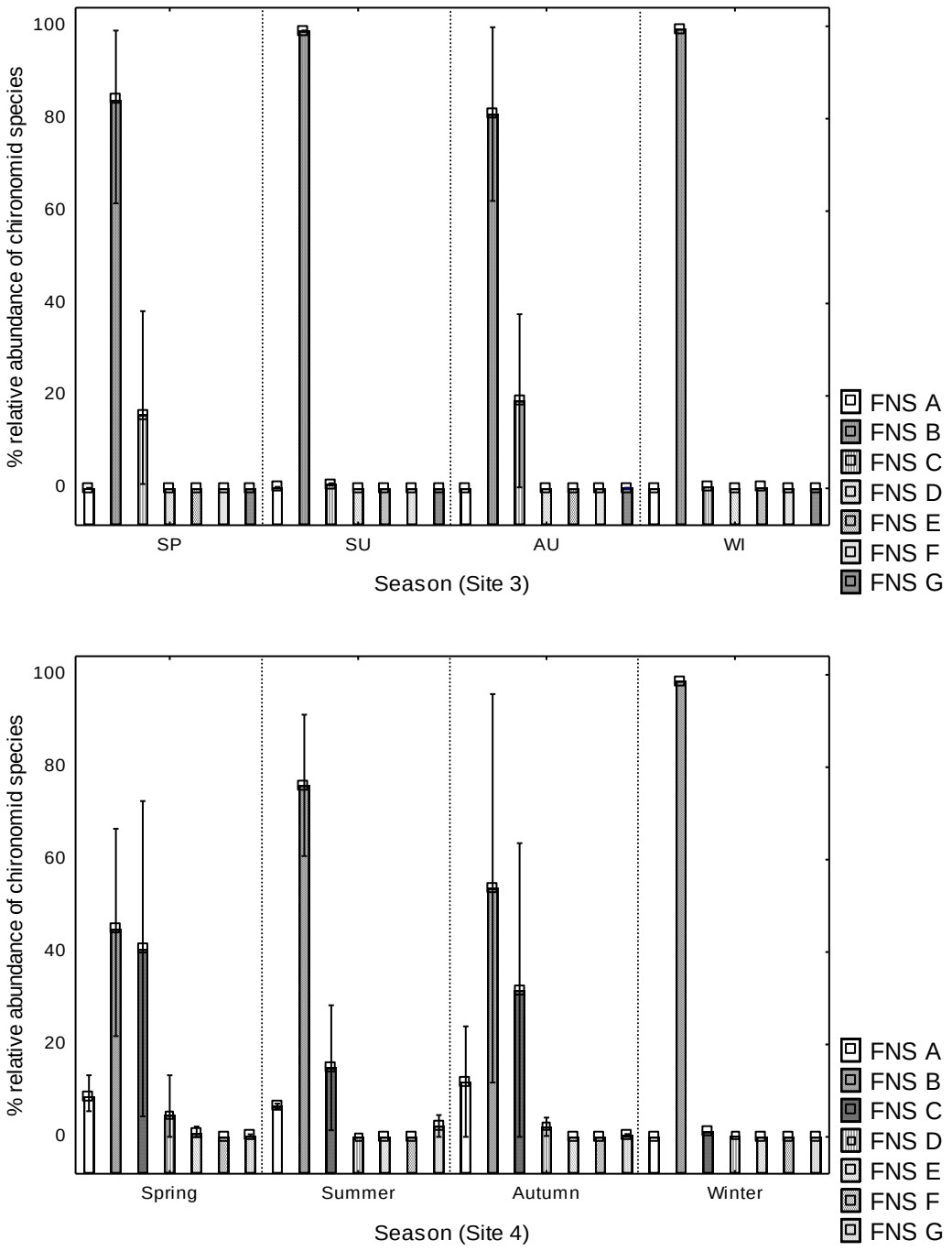


Figure 5.4: Seasonal variation in average percent relative abundance of chironomid species belonging to each of the defined seven functional strategies at Sites 3 and 4 in the Swartkops River during the study period (August 2009 – September 2012) showing that in all the sampling seasons, species in FNS B and C dominated the relative abundance of the assemblages at Sites 3 and 4, with slight increases in the relative abundance of species in FNS A and D at Site 4 compared with Site 3. The vertical bars represent standard deviation from the means. Abbreviations: Seasons: SP (spring), SU (summer), AU (autumn), WI (winter).

In summary, the results showed that the small-bodied species with or without haemoglobin, preferring the stone and sediment biotopes and feeding on algae and detritus grouped in FNS A and E were mostly associated with Site 1 than the remaining three sites. Similarly, the small- to medium-bodied species with haemoglobin that were mostly predators and herbivores, feeding on animals and macrophyte parts, preferring the vegetation biotopes (FNS D) were also mostly associated with Sites 1 than the three remaining sites. In addition, the medium-bodied predatory species with no investment in haemoglobin grouped in FNS F were also mostly associated with Site 1 than the three downstream sites and thus, species belonging to these FNSs enabled the discrimination of Sites 1 from Sites 2, 3 and 4. Conversely, the large bodied-species with haemoglobin, preferring the sediment biotope and feeding mostly on detritus and algae, which were grouped in FNS B, were mostly associated with Sites 2, 3 and 4 than Site 1, and thus species in this FNS also enabled the discrimination of Sites 2, 3 and 4 from Site 1. The medium to large bodied species with haemoglobin, preferring the stone and vegetation biotopes were associated with all the four sites, and thus, the four sites could not be discriminated based on species in this FNS (Figure 5.5a). In terms of discrimination between the three downstream sites (i.e. Sites 2, 3 and 4), the results revealed that the small bodied chironomid species with no investment in haemoglobin, preferring the stone biotope and feeding on detritus and algae (FNS A), were mostly associated with Site 2 than Sites 3 and 4, while the very large-bodied species with haemoglobin, preferring the sediment biotope and feeding mostly on algae and detritus (detritivore-algivorous), were mostly associated with Sites 3 and 4 than Site 2 (Figure 5.5 a and b). Thus, species in these FNSs enabled the discrimination of Site 2 from Sites 3 and 4.

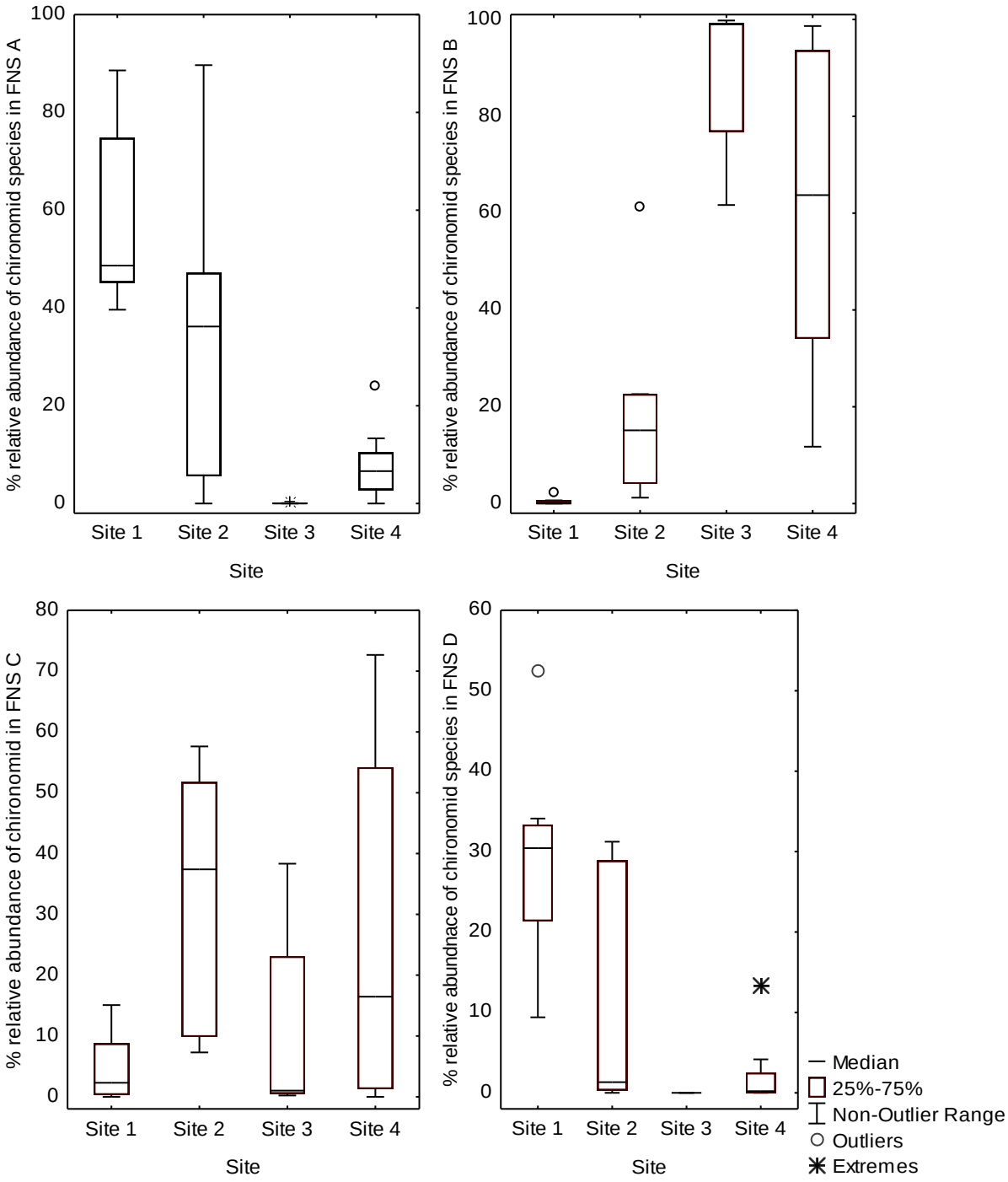


Figure 5.5a: Box plots showing the medians and inter-quartile ranges (25%-75%) indicating the discriminatory potential of the chironomid functional strategies between the four sampling sites during the study period (August 2009 – September 2012) showing that species in FNS A and D were mostly associated with Site 1 than Sites 2, 3 and 4 and those in FNS B were mostly associated with Sites 2, 3 and 4 than Site 1, whereas those in FNS C were associated Sites 3 and 4 than Sites 1 and 2.

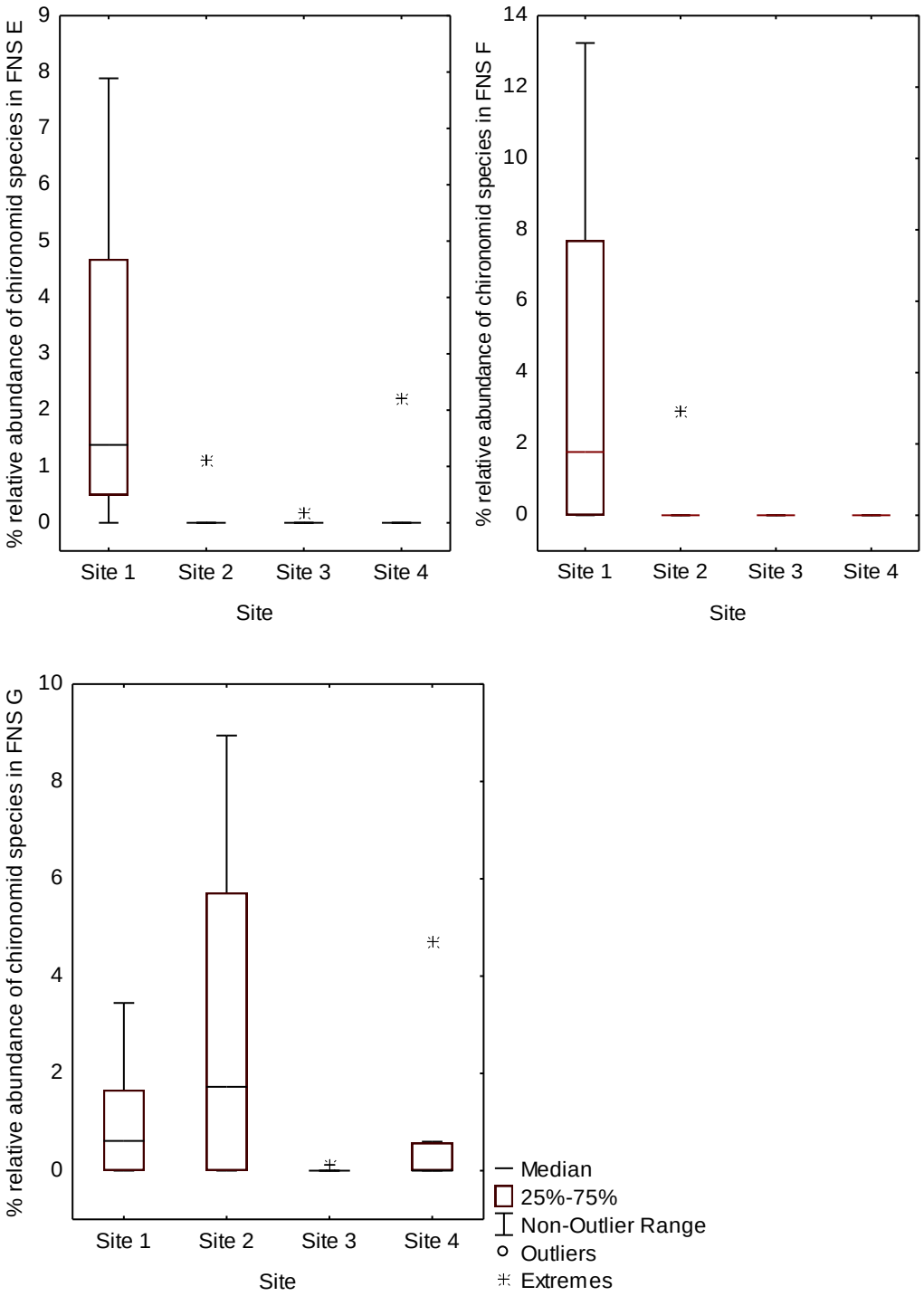


Figure 5.5b: Box plots showing the medians and inter-quartile ranges (25%-75%) indicating the discriminatory potential of the chironomid functional strategies between the four sampling sites during the study period (August 2009 – September 2012) showing that species in FNS E and F were mostly associated with Site 1 than Sites 2, 3 and 4, while species belonging to FNS G were mostly associated with Site 2 than Sites 1, 3 and 4.

5.3.4 Relating chironomid functional strategies (FNSs) to water physico-chemical variables in the Swartkops River

To elucidate the chironomid functional distribution between the sites in relation to water physico-chemical variables, the multivariate CCA analysis was used. The ordination of the sampling sites based on the chironomid FNSs using the CCA analysis (CCA) revealed distinct separation of Site 1 from Sites 2, 3 and 4, thereby supporting the box plot results (Figure 5.6). With the exception of winter, in which Site 4 clustered closely with Site 3, the three downstream sites did not show close clustering, indicating that the chironomid FNSs enabled discrimination between these sites.

The CCA ordination triplot revealed that the concentration of dissolved oxygen (DO) was the main water physico-chemical variable that favoured chironomid species belonging to FNSs A (small bodied species with no haemoglobin), D (small to medium bodied species with haemoglobin), E (very small bodied species with haemoglobin) and F (medium sized predatory species with no haemoglobin). On the other hand, the dominance of chironomid species belonging to FNSs B (very large bodied species with haemoglobin) could be attributed to their ability to tolerate depleted concentration of dissolved oxygen (DO) and electrical conductivity (EC). Chironomid species belonging to FNS C (medium to large bodied species with haemoglobin) were more closely associated with Site 4 and were positively correlated with temperature on Axis 1 of the CCA ordination triplot (Figure 5.6).

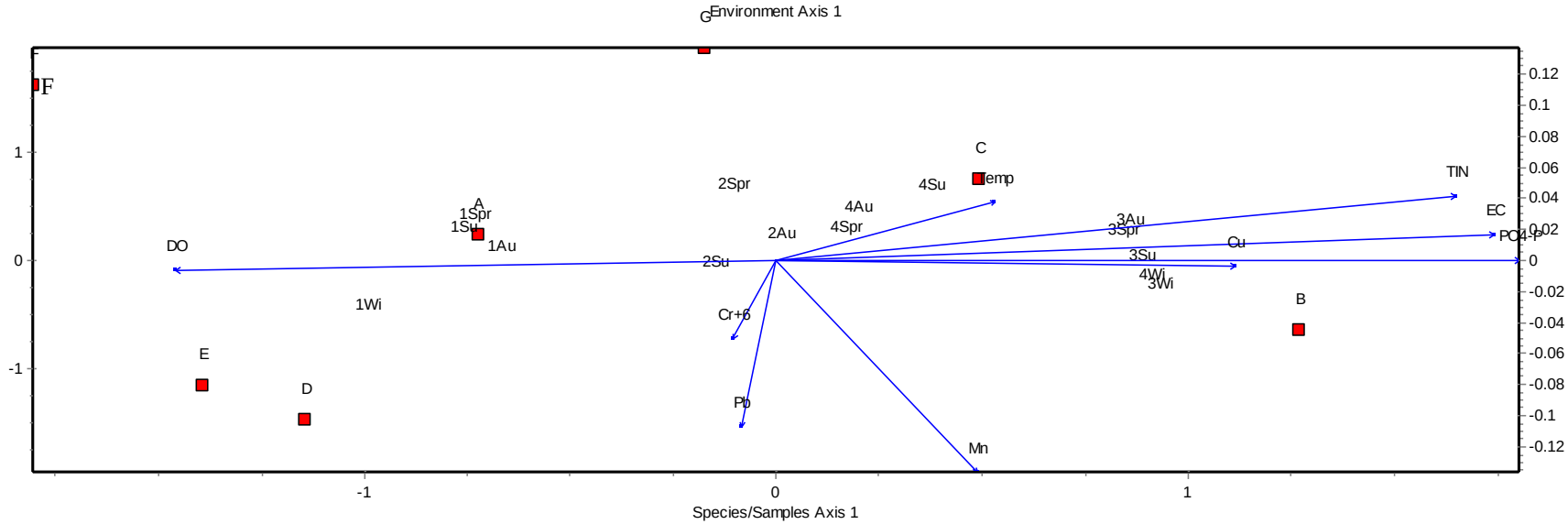


Figure 5.6: CCA ordination triplots showing the correlation between the chironomid functional strategies and the selected water physicochemical variables in the Swartkops River during the study period (August 2009 – September 2012). Abbreviations: functional strategies (FNS), A (FNS A: small bodied species with no haemoglobin)), B (FNS B: very large bodied species with haemoglobin), C (FNS C: medium to large bodied species with haemoglobin), D (FNS D: small to medium bodied species with haemoglobin), E (FNS E: very small bodied species with haemoglobin), F (FNS F: medium sized predatory species with no haemoglobin), G (FNS G: species that could not be confidently assigned to any of the FNSs); site: 1 (Site 1), 2 (Site 2), 3 (Site 3), 4 (Site 4); season: Spr (spring), Su (summer), Au (autumn) and Wi (winter).

The canonical properties of the CCA indicated a good ordination between the chironomid FNSs and the selected water physicochemical variables (Table 5.2). The first three axes of the ordination explained a cumulative variance of 81.88%, with the first axis alone explaining a total variance of 62.74% (Table 5.2). However, the Monte Carlo test, which produced the probability that the observed Eigen value could have been generated by chance, indicated that only the Eigen value of Axis 1 was statistically significant ($P < 0.05$), whereas those of Axes 2 and 3 were not ($P > 0.05$) (Table 5.1). Overall, the CCA ordination revealed strong correlation between the chironomid FNSs and the selected water physico-chemical variables.

Table 5.2: Properties of the Canonical correspondence triplot of the correlations between the chironomid functional strategies (FNS) and the selected water physicochemical variables in the Swartkops River during the study period (August 2009 – September 2012), showing that only the Eigen value of Axis 1 was statistically significant.

Canonical properties	Axis		
	1	2	3
Eigen value	0.342	0.059	0.046
% variance explained	62.74	10.78	8.36
Cumulative % variance explained	62.74	73.52	81.88
Monte Carlo test (p-value)	0.002	0.522	0.089
Pearson correlation of FNSs and environment score	0.987	0.876	0.905

5.4 Discussion

5.4.1 Chironomid body-size distribution

The chironomid body-size responded predictably to the deteriorating water quality in the Swartkops River, with the body-size distribution showing a clear pattern between the sampling sites, particularly at Sites 1 and 3, as the small and medium sized chironomids dominated the species abundance at Site 1, and large and very large bodied-species

dominated the species abundance at Site 3 (Figure 5.2). Although theoretically the habitat template concept predicts small body size to be favoured by disturbances because it is a trait perceived to confer resilience on biological communities (Townsend and Hildrew, 1994), in the present study, at polluted sites, medium- size to very large-bodied chironomids dominated the species abundance. The nature of disturbance (i.e. changes in water chemistry occasioned by pollution) investigated in this study is critical to small-bodied individuals because of their increased surface area to volume ratio, which may lead to increased exposure to pollutants, particularly the recorded elevated dissolved salts measured as electrical conductivity in this study (Chapter 3, Table 3.1). Statzner and Béche (2010) postulated that elevated dissolved salts were likely to favour large-bodied invertebrates because of their reduced surface area to volume ratio. The results presented in this chapter were consistent with this postulation.

In Chapter 4, when all macroinvertebrate body sizes were analysed at the family level of taxonomic resolution, no predictable body-size response was observed between the sampling sites and several possible reasons were advanced to explain the non-predicted body-size responses. One of these reasons was that body-size classes were arbitrarily demarcated for macroinvertebrate families based on the literature. An arbitrary demarcation of body sizes which does not account for natural animal body-size distribution has been criticized by Robson *et al.* (2005). The kernel curve is used to produce modes and troughs corresponding to body-size classes, produced better results because it avoided arbitrary demarcation of size classes in contrast with the literature-based approach used in Chapter 4. It is therefore recommended that studies using animal body sizes in biomonitoring should use the kernel curve approach.

A multimodal chironomid body-size distribution was observed for the chironomid species data sets in the Swartkops River (Figures 5.1). The multimodal distribution observed in this study is consistent with the finding of Havlicek and Carpenter (2001) who found multiple gaps (troughs) and lumps (modes) within each of phytoplankton, zooplankton and fish community functional groups. The existence of multiple modes and troughs in body-size distribution may be attributed to organisms' adaptation to the habitat (Holling, 1992). The multimodal distribution observed in this study could be explained by environmental factors (including physicochemical conditions) at the different sites favouring specific body-size classes corresponding to the modes. Several hypotheses including the energetic, phylogenetic, bio-geographical, community interaction and textural discontinuity have been

postulated concerning patterns of animal body-size distribution in their environment (Allen *et al.*, 2006). Of these five hypotheses, the textural discontinuity, which emphasises the importance of environmental conditions in constraining body size distribution, and the community interaction that places emphasis on biological interactions among species as the determinants of body-size distributions, are the most relevant hypotheses for explaining spatial variations at the scale of the present study (Allen *et al.*, 2006). The textural discontinuity hypothesis viewed the habitat conditions (including water physico-chemical conditions) as the main drivers of animal body-size distributions. Many studies have given empirical evidence to support the claim that habitat conditions, including water chemistry, as emphasised by the textural discontinuity hypothesis, are primarily responsible for shaping animal body-size distribution (e.g. de Bruyn *et al*., 2002; Stead *et al*., 2005). For example, when de Bruyn *et al*. (2002) related fish body-size distribution to organic input (sewage enrichment), they found that large-bodied fish species increased with increased levels of organic pollution. Stead *et al*. (2005) also found that changes in water physico-chemical conditions could influence body-size patterns of stream assemblages. Consequently, the clear patterns in chironomid body-size classes between the sampling sites seemed to suggest that differences in water physico-chemical conditions between the sites influenced the body-size distributions of the Swartkops River chironomids.

Although the results presented in this chapter suggest that differences in water physico-chemical conditions seemed to be influencing the patterns of chironomid body-size distributions, it is important to note that body-size distributions are dynamic and may not be explained entirely by any single factor (Allen *et al*., 2006; Battle *et al*., 2007). Consequently, unravelling the cause of body-size distribution in any stream system is a complex exercise. For instance, the community interaction hypothesis views biological interactions such as predator-prey relationship as the primary driver of animal body-size distribution and predation has been found to exert influence on the body-size distribution of aquatic macroinvertebrates (Whale, 1992). However, by combining body size with other traits could shed light on important environmental factors influencing the Swartkops River chironomid assemblages.

5.4.2 The application of chironomid functional strategies as a biomonitoring tool

Developing a simple but efficient and robust biomonitoring tool that indicates not only biotic change, but also provides insight into the main drivers of the biotic change is important for

sustainable freshwater resource management, evaluating the successes of environmental policies, and protecting biodiversity. In this chapter, a novel approach that takes account of species adaptation to specific environmental conditions was developed as a tool for biomonitoring. In the chironomid functional strategy approach, because species were grouped based on the combination of traits functionally geared towards enabling the organisms to adapt to specific environmental conditions, the approach provides a mechanistic and adaptive basis for explaining the reasons for biotic change, a feat yet to be achieved by taxonomically-based biomonitoring approaches. For example, elevated concentrations of nutrients, electrical conductivity (EC) and turbidity corresponded with increased abundance of species belonging to functional strategy B (FNS B) (Figure 5.6). Species in FNS B are large-bodied and they invest in haemoglobin formation, and thus could thrive in a organically-enriched environment with low concentrations of dissolve oxygen. In addition, these species are mainly detritivore-algivorous, feeding mostly on detritus and algae, and therefore the increased nutrient concentrations accompanied by algal growth (periphyton) could favour their dominance in organically impacted river reaches (e.g. Sites 3 and 4). In contrast, species belonging FNSs A (small bodied species with no haemoglobin), E (very small bodied species with haemoglobin), D (small to medium bodied species with haemoglobin) and F (medium sized predatory species with no haemoglobin), which did not have adaptive features needed to survive under all of the above-mentioned environmental conditions were almost entirely absent at Sites 3 and 4 where these environmental conditions prevailed (Figure 5.4). This mechanistic and adaptive basis for explaining biotic change is therefore insightful as it could potentially enable resource managers to focus on specific environmental conditions preventing species survival at a site of interest.

The FNS approach proved sensitive in that it enabled discrimination between the four sampling sites (Figures 5.5a and 5.5b) as does the chironomid species taxonomically-based assessment. Of the seven chironomid FNSs defined in this study, five FNSs A (small-bodied species with no haemoglobin), B (very large-bodied species with haemoglobin), D (small- to medium-bodied species with haemoglobin), E (very small bodied species with haemoglobin) and F(medium-bodied predatory species with no haemoglobin) enabled the discrimination of Site 1 from Sites 2, 3 and 4. Three FNSs A, B and G (species that could not be confidently assigned to any of the FNSs) enabled discrimination between the three downstream sites, i.e. Sites 2, 3 and 4. This, then, follows the Poff (1997) habitat filtering concept in which the habitat is seen as acting as a traits filter, whereby increased environmental stress reduces the

mesh size of the filter and consequently reduces the number of traits that pass through the filter, which then results in decreased trait diversity. Based on this concept, of the three downstream sites, Sites 3 and 4 could be seen as having the harshest water quality conditions resulting in the preponderances of species belonging only to FNS B (very large-bodied species with haemoglobin) and C (medium- and large-bodied species with haemoglobin). Water quality at Site 2 was less harsh than at Sites 3 and 4, resulting in the appearances of species belonging to FNS A (small-bodied species with no haemoglobin) and D (small- to medium-bodied species with haemoglobin) in addition to species belonging to FNS B and C (Figures 5.3 and 5.5a). Species in these two FNSs are small-bodied, some are predators (large bodied), and most of them lack haemoglobin (Table 5.1). Thus, the chironomid FNS approach revealed distinct differences between the three downstream sites, with the occurrence of species belonging to FNSs A and D at Site 2, suggesting that Site 2 is less impacted than Sites 3 and 4.

The river habitat template of Townsend and Hildrew (1994) provides the basis for predicting expected species under known or hypothesised environmental conditions. The predicted responses of the chironomid functional strategies (FNSs) were all confirmed except for species belonging to FNS G, which could not be confidently defined due to lack of adequate information on their biological and ecological traits. Of particular interest is the almost complete absence of species belonging to FNS F (medium sized predatory species with no haemoglobin) at the three downstream sites (Figures 5.5b). Species belonging to FNS F are all predatory chironomids that do not invest in haemoglobin formation and therefore occupy a higher trophic level. Predatory lifestyle could be negatively impacted by aquatic toxicants, e.g. some persistent organic pollutants and metals, because of effects of trophic transfer that could result in bio-magnification (Goodyear and McNeill, 1999; Fisk *et al.*, 2001; Croteau *et al.*, 2005). Persistent organic pollutants (POPs), though not measured in this study, have been reported in wastewater treatment works and in receiving freshwater ecosystems (Sánchez-Bayo, 2011; Sibley and Hanson, 2011). Because of the industrialised and urbanised catchment of the Swartkops River, the potential presence of POPs in the Swartkops River, particularly at the downstream sites, cannot be completely ruled out. It seemed therefore that the predatory lifestyle, coupled with the lack of haemoglobin of species belonging to FNS F, made it difficult for them to survive and thrive under the prevailing environmental conditions at the downstream sites. Overall, based on the predictable response of the chironomid FNSs (Figures 5.5a and 5.5b), and the strong theoretical basis for applying the FNS approach

(Southwood 1977; Townsend and Hildrew, 1994; Verberk, 2008), it could be argued that in reaches of rivers and streams impacted with similar conditions as with Sites 3 and 4, chironomid species belonging to FNS B (very large-bodied species with haemoglobin) and C (medium- and large-bodied species with haemoglobin), which were mostly associated with the sediment and feeding mostly on algae and detritus, would be likely to dominate the species abundance.

Chapter 1, section 1.5.3 of this thesis, highlighted a major constraint with the use of the traits-based approach (TBA) in freshwater biomonitoring which is the effect of trait trade-offs in which the response of one trait may override the expected response of another trait. This problem was encountered in Chapter 4 in which the expected response of predatory lifestyle was overruled by other traits. However, the chironomid FNS approach helped overcome this problem because, instead of analysing the responses of individual trait, species were grouped based on their possession of a combination of traits having an adaptive and mechanistic relationship with the prevailing water quality conditions in the Swartkops River. In this way, the effects of trait trade-offs were avoided with the recognition that the adaptive value of one trait might depend on other traits possessed by the organism. It is, however, important to stress that species belonging to the same FNS are not ecologically equivalent, but they could be regarded as adapting to their environment in a more similar way than species belonging to another FNS. Thus, they are ecologically closer than species belonging to another FNS. Statzner and Béche (2010) emphasised the need to avoid indiscriminate use of species traits, but to focus on traits for which a mechanistic a *priori* prediction was possible. Although a limited number of traits were used to define the FNSs, the approach proved very useful as a potential biomonitoring tool. Consequently, this study has demonstrated the adaptive and mechanistic values of the chironomid traits utilised for the biomonitoring of the Swartkops River, and the approach not only provides a statement of biotic change, but also provides insight into major abiotic factors driving the change. However, despite the usefulness of the chironomid TBA, the results also highlight the inextricable link between functional analyses and taxonomic identification, clearly showing that a functional approach is always additional to, and not an alternative to, taxonomic recognition because traits cannot be assigned without taxonomic recognition.

5.5 Conclusion

The chironomid body-size classes responded predictably to the deteriorating water quality in the Swartkops River, with large-bodied species dominating the species relative abundances at the downstream sites, particularly at Sites 3 and 4. Although body size may have some mechanistic and adaptive values in relation to the water quality conditions in the Swartkops River, analysing chironomid body size alone is inadequate in order to provide a critical insight into why certain species of chironomids occurred at Site 1, but not at other sites. The functional strategy approach developed and used in this chapter proved both robust and sensitive in diagnosing the potential main abiotic driver of biotic change, and in enabling discrimination between the sampling sites. The sensitive, diagnostic and predictive potential of the chironomid FNS approach and the adaptive basis for interpreting species occurrence makes the chironomid FNS approach a potentially useful biomonitoring tool for sustainable freshwater resource management and it can be incorporated into existing water quality methods and approaches in South Africa for managing water resources within the context of the ecological Reserve. A simple framework for incorporating the traits-based approach including the chironomid FNS approach into existing methods for biomonitoring is provided in Chapter 8. A comprehensive discussion on the ecological Reserve as used in South Africa has already been provided in Chapter 1.

CHAPTER 6: MORPHOLOGICAL DEFORMITIES IN THE MENTUM OF CHIRONOMIDAE LARVAE AS SUB-LETHAL RESPONSE TO ANTHROPOGENIC IMPACTS

In Chapters 3, 4, and 5, the taxonomically-based community structure and traits-based biomonitoring approaches were used to explore the Swartkops River health and macroinvertebrate responses to deteriorating water quality. The interpretative power of each of the approaches and their shortcomings were discussed. However, none of the approaches used in previous chapters accounted for sub-lethal in-stream responses to deteriorating water quality. Since sub-lethal effects would be likely to precede the manifestation of lethality in the biological community, this Chapter asks whether screening morphological deformities in chironomids could provide insight into in-stream sub-lethal effects of deteriorating water quality, and whether the sites could be separated based on sub-lethal effects. In developing a practical biomonitoring tool for comparing sites based on sub-lethal effects, the Lenat (1993) toxic score index (TSI), which is a Chironomus spp. *mentum deformity-based index used for assessing sub-lethality and comparing sites, was developed further. Therefore, this chapter begins with a rationale for screening morphological deformities, continues with a method section justifying the approach used, and provides evidence of sub-lethal effects.*

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6.1 Introduction

A taxonomically-based assemblage structure approach that focuses primarily on absence-presence and relative abundance of species could be regarded as an approach not enabling measuring sub-lethal in-stream response to deteriorating water quality, because evidence of the effects of deteriorating water quality can be detected only after the disappearance or reduced abundance and diversity of pollution-sensitive species. Approaches that do not enable the measurement of sub-lethal response are not suitable as early warning

biomonitoring of deteriorating water quality in freshwater ecosystems (Faria *et al.,* 2006; Odume *et al.,* 2012b). Furthermore, at polluted sites, usually dominated by pollution-tolerant species such as *Chironomus* spp. and *Dicrotendipes* spp., *Kiefferulus* sp. (Chapter 3), the question remains whether such species, though present, are sub-lethally impacted, which may affect their ability to perform ecological function optimally. Morphological deformity in chironomid larval mouthparts, particularly the mentum, represents a sub-lethal in-stream biological response to deteriorating water quality (Ochieng *et al.,* 2008). It is also an ecologically relevant endpoint directly related to traits such as feeding, development and emergence (Janssens de Bisthoven *et al.,* 1998).

The term "deformity", already defined in Chapter 1 at the end of section 1.5.3, refers to morphological features that depart from the normal configuration of the mentum of chironomid larvae. Mechanical wear, breakage or abrasion are distinguished from deformities by their "chipped" or "rough" edges and are not included in deformity screening (Vermeulen, 1995; Nazarova *et al*., 2004).

Although several structures, including the mentum, mandible, ligula, paraligula, pecten epipheryngis and antennae, in Chironomidae larvae have been examined for deformities, and it has been observed that expression of deformities varies between different structures in the same taxon (Warwick, 1985; 1990; Bisthoven and Gerhardt, 2003; Bhattacharyay *et al.,* 2005; Di Veroli *et al*., 2008; Odume, 2011), only the mentum was screened for deformities in this study. The screening of deformities was rescrited to the mentum because it has imparted the greatest amount of information on chironomid deformities; it is easily prepared for examination, and the deformities are rapidly quantified (Odume, 2011; Lewis *et al.,* 2012; Odume *et al.,* 2012b; Planello *et al.,* 2013). The ligula (analogous to the mentum in other chironomid subfamilies) was screened for deformities in the Tanypodinae subfamily because the mentum is not well developed in this subfamily (Wielderholm, 1983; Cranston, 1996; Harrison, 2003). Therefore, the term 'mentum' as used in this study refers to both the mentum in Chironominae and Orthocladiinae and the ligula in Tanypodinae.

The chironomid deformity-based index, the community incidences of deformities (CID), expressed as the percentage of deformed larvae to the total number of screened larvae is widely used for monitoring the sub-lethal in-stream effects of pollutants on chironomid communities (Vermeulen, 1995; Ochieng *et al.,* 2008). The rationale is that percent CID greater than 8% is considered a departure from the reference level (estimated to range

between 0 - 8%) and thus is an indication of toxic in-stream contamination (Nazarova *et al.*, 2004; Ochieng *et al.*, 2008; Odume *et al.*, 2012b). However, Odume (2011) argued that ecological conditions vary between aquatic ecosystems and between regions, and questioned such an arbitrarily designated reference value as a biological criterion for every study. Odume (2011) furthermore argued that an 8% incidence of deformities in different chironomid communities consisting of differentially sensitive species might not necessarily indicate the same levels of contamination. For these reasons, the author suggested developing a simple numeric index, similar to the toxic score index TSI (Lenat, 1993) and suggested that such an index should take account of the differential sensitivity of chironomid species to deteriorating water quality. The Lenat (1993) TSI has been reviewed in Chapter 1, section 1.6. The index is based on deformities in *Chironomus* spp. mentum and weighting factors are assigned to classes of deformities according to their apparent severity. The deformities were classified into three classes, Class I, II and III, with weighting factors 1, 2, and 3, respectively. However, the limitations of the TSI include: i) its development was based on *Chironomus* genus only, thereby ignoring deformities occurring in other genera, and as such it cannot be use to compare sites if the genus does not occur in sufficient numbers (at least 25 larvae) at sites being compared (Odume 2011); ii) the definition of the Class I deformities is ambiguous and has been criticised because deformities belonging to Class I are difficult to distinguish from breakage or abrasion (Hämäläinen, 1999); iii) it does not account for differential sensitivity of species to pollution (Warwick, 1985; Burt, 1998).

The screening of mentum deformities in chironomid larvae was employed in this study as an additional biomonitoring tool to assess i) whether it could provide insight into sub-lethal in-stream biological response of chironomids to deteriorating water quality, and ii) whether the sites could be differentiated based on sub-lethal effects in addition to the taxonomic and trait compositions used in previous chapters. In addition, the TSI was further developed by taking account of the differential sensitivity of chironomid species to deteriorating water quality, which resulted in an extended toxic score index (ETSI) that included other chironomid genera. Nevetheless, apart from sensitivity of a taxon, which may influence its expression of deformities, other factors such niche inhabited that could influence level of exposure may also influence expression of deformities in chironomid larvae. A concise review of chironomid deformities has already been provided in Chapter 1, section 1.6.

6.2 Materials and methods

6.2.1 Screening Chironomidae larval for mentum deformities

Chironomid larvae collected during the study period at the four sampling sites were screened for deformities in the mentum as described in Chapter 2, section 2.4. Larvae instar stage was not considered when screening deformities. The community incidences of deformities (CID) were calculated as the percentage of the numbers of all deformed larvae, irrespective of the species, to the total larvae screened for each site per season (Janssens de Bisthoven and Gerhardt, 2003; Ochieng *et al.,* 2008). The percent CID was also calculated seasonally per site for each chironomid subfamily as the percentage of deformed individuals belonging to a subfamily to the total number of larvae screened in that subfamily.

The incidences of deformities for selected dominant chironomid species collected from the same site (i.e. assumed to have been exposed to the same environmental conditions over time) were calculated as the percentages (i.e. proportions) of deformed individuals to the total numbers of larvae screened for that species in a particular site. Selected species had at least 20 larvae within a site per season, and occurred in more than one sampling season. This was necessary to avoid a seasonal bias and to provide a large enough sample size for deformity screening. A sample size of at least 20 larvae per taxa has been reported in the literature for comparison (MacDonald and Taylor, 2006) and was considered appropriate in this study.

6.2.2 Further development of the toxic score index (TSI)

The Lenat (1993) TSI was further developed in this study by taking account of the differential sensitivity of chironomid species to deteriorating water quality and the new index called the extended toxic score index (ETSI) was used to assess chironomid sub-lethal response to pollution. The ETSI was developed based on the five most dominant chironomid genera: *Chironomus* spp., *Cricotopus* spp., *Tanytarsus* sp. *Polypedilum* sp. and *Dicrotendipes* sp. A five-step approach summarised in Figure 6.1 was followed in developing the index. Step one involved using a canonical correspondence analysis (CCA) to score the chironomid species response to deteriorating water quality in order to obtain the species pollution-tolerance scores. The CCA ordination and the calculation of the species tolerance scores have been described in Chapter 3, section 3.2.4. The tolerance scores for the five selected genera in increasing order of tolerance to deteriorating water quality in the Swartkops River were: 2, 2, 4, 8 and 10 for *Tanytarsus* sp., *Polypedilum* sp., *Cricotopus* spp., *Dicrotendipes* sp., and

Chironomus spp., respectively (Table 3.9, Chapter 3). In step two, the observed deformities were classified into Class 1 and 2 according to their apparent severity. Class 1 deformities corresponded to Lenat's Class II deformities and included unambiguous deformity types such as extra teeth, fused teeth, missing teeth, slit teeth and distinct asymmetry (Lenat, 1993; Servia *et al*., 1998; 2000). Class 2 of the ETSI corresponded to Lenat's Class III deformities and included larvae having at least two Class 1 deformity types (i.e. a single larva having multiple deformities). In step three, weighting factors were assigned the two classes of deformities, with Class 1 a weighting factor of 1, and Class 2 a weighting factor of 2.

The weighting factors were used to distinguish the severity of the two classes of deformities, with larvae exhibiting the Class 2 deformities assumed to be more severely deformed than those exhibiting the Class 1 deformities. In step four, the calculated tolerance scores for each of the genera were used as weighting factors to account for differences in the response of the selected genera to deteriorating water quality. The last step involved calculating the index as follows:

$$\text{ETSI} = \frac{\{2[(\text{NI})+2(\text{NII})]T+2[(\text{NI})+2(\text{NII})]P+4[(\text{NI})+2(\text{NII})]C+8[(\text{NI})+2(\text{NII})]\text{D}+10[(\text{NI})+2(\text{NII})]\text{Ch}\}\text{X } 100}{\text{Total No.of larvae screened for deformities}}$$

where: NI (number of larvae with Class 1 deformities); NII (number of larvae with Class 2 deformities); T (*Tanytarsus* sp.); P (*Polypedilum* sp.); C (*Cricotopus* spp.); D (*Dicrotendipes* sp.); Ch (*Chironomus* spp.). The value 100 is a constant taken from the Lenat (1993) index.

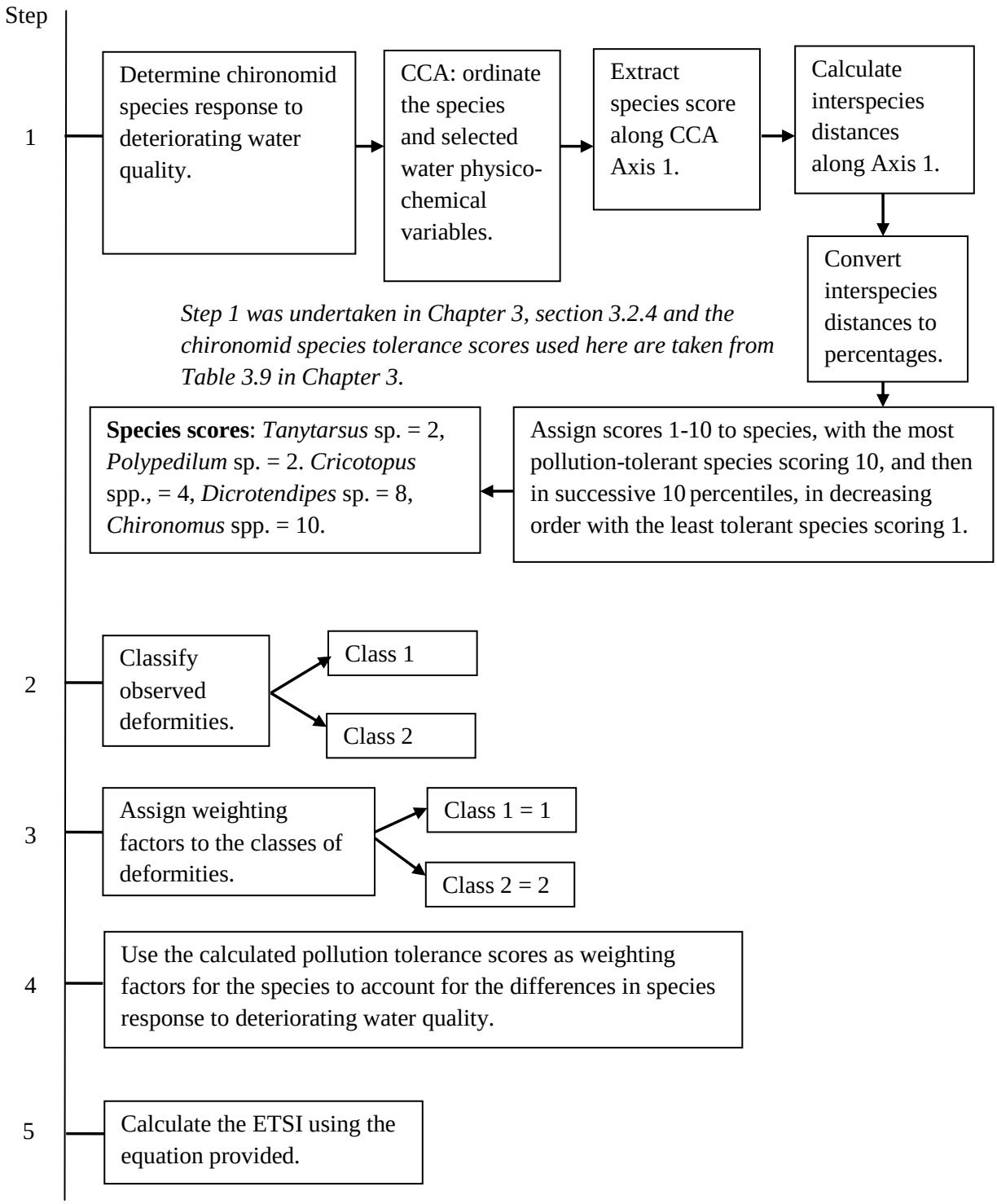


Figure 6.1: Summary of the five steps followed in developing the extended toxic score index.

6.2.3 Statistical analysis

The community incidences of deformities were arcsine transformed and one-way analysis of variance (ANOVA) was used to compare the differences between the sites, and between the seasons across the sites. Arcsine transformation is best suited for proportional data (Ogbeibu,

2005). The incidences of deformities calculated as the percentage (i.e. proportion) of deformed individuals to the total number of larvae screened within a taxon in selected Chironomidae species per site were compared using one-way ANOVA. Box plots were used to compare the calculated ETSI for the four sampling sites and the sampling seasons. Based on the ETSI, incidences of deformities at any of the three downstream sites (i.e. Sites 2, 3 and 4) were considered elevated (i.e. departed from the background levels) if the 25 percentile of the ETSI at the downstream site was higher than the 75 percentile at Site 1. The same criterion was applied to assess whether the ETSI enabled discrimination between the three downstream sites. The Kruskal-Wallis multiple comparison test was used to test statistically for significant differences between the ETSI at the four sampling sites, and during the sampling seasons across the sites.

Relating the incidences of deformities to water physico-chemical variables

Multiple-linear regression based on forward selection was used to relate incidences of deformities in chironomid to the measured water physico-chemical variables. The best subset models were selected based on the *F* maximisation criterion. Detailed descriptions of all statistical analyses are provided in Chapter 2, section 2.8.

6.3 Results

6.3.1 Screening Chironomidae larval for mentum deformities

Thirty-five species in three subfamilies, Chironominae, Orthocladiinae and Tanypodinae, were screened for deformities during the study period. The community incidences of deformities calculated at the subfamily level indicated that larvae of the subfamily Chironominae, tribes: Chironomini and Tanytarsini were more deformed than larvae belonging to the subfamilies Orthocladiinae and Tanypodinae (Figure 6.2). The Orthocladiinae were less abundant at Sites 3 and 4 (Appendix A, Tables A3 and A4), and the frequency of incidences of deformities among the Orthocladiinae larvae at these sites was lower than at Sites 2 and 1. Of the three subfamilies, larvae of the predatory Tanypodinae were less vulnerable to deformity-inducing stressors with the least incidences of deformities recorded (Figure 6.2).

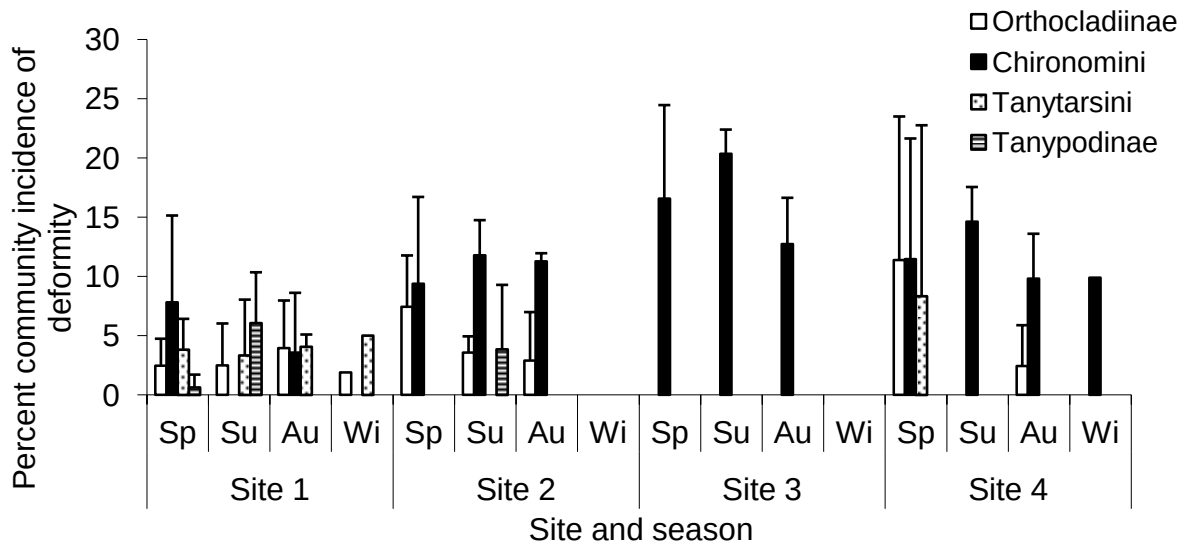


Figure 6.2: Mean percent community incidences of deformities among the chironomid subfamilies collected in the Swartkops River during the study period (August 2009-September 2012) showing that larvae belonging to subfamily Chironominae, tribes Chironomini were more frequently deformed than larvae belonging to the remaining two subfamilies. The subfamily Chironominae, the most species rich in the Swartkops River, is divided into the tribes Chironomini and Tanytarsini in the graph. The vertical bars are the standard deviations from the means. Seasons: Sp (spring), Su (summer), Au (autumn) and Wi (winter).

Chironomid larvae collected in the Swartkops River exhibited deformities during the study period (Figure 6.3). Larvae collected at Sites 3 and 4 were more frequently deformed as indicated by the community incidences of deformities (Figure 6.3). With the exception of spring 2012, the community incidence of deformities (CID) was higher at Site 3 than at Sites 1, 2 and 4. In all the sampling seasons, larvae collected at Site 1 were less frequently deformed compared with those at the remaining three sampling sites.

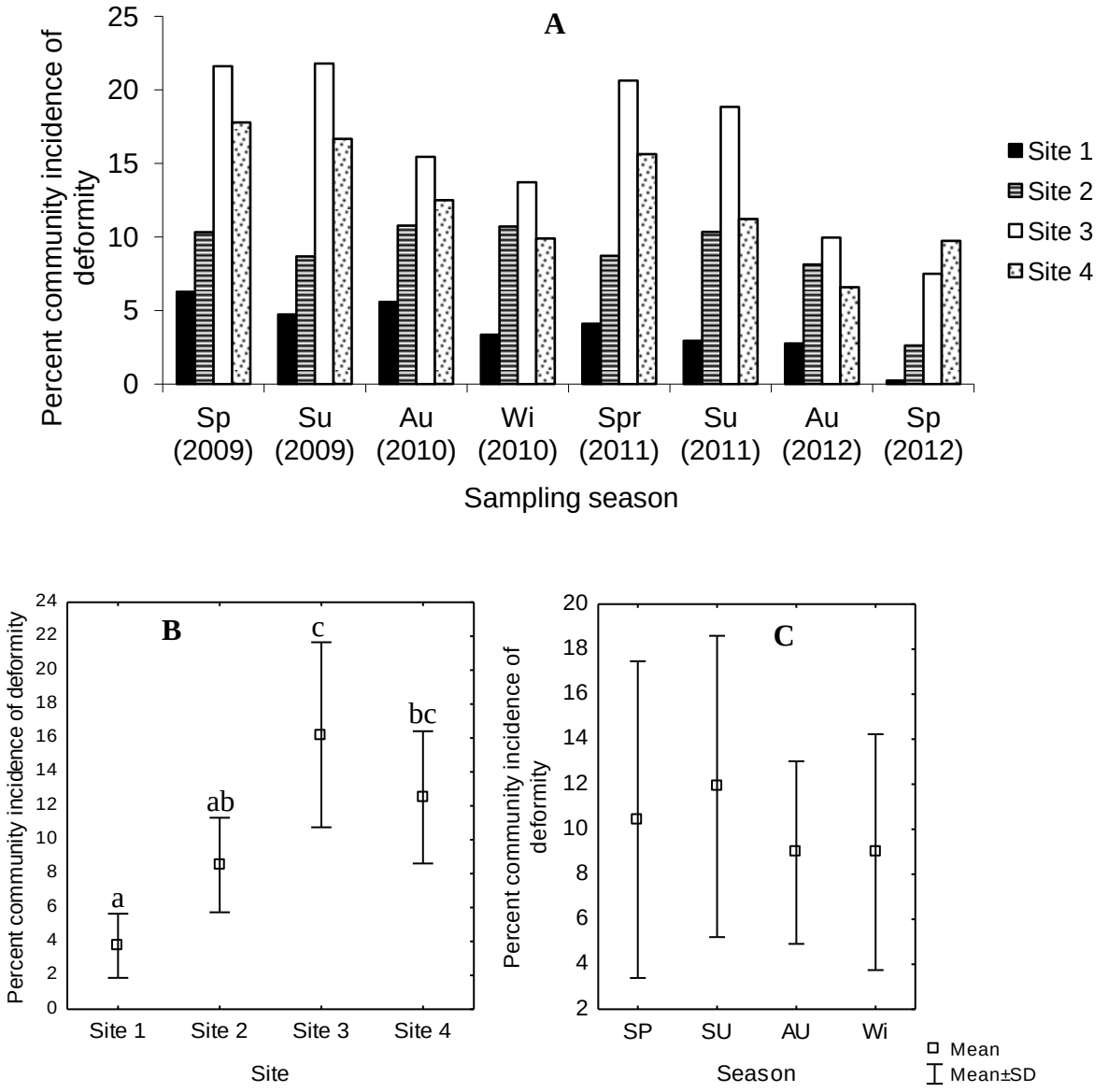


Figure 6.3: Mean percent community incidences of deformities (CID) calculated as the percentages of the numbers of all deformed larvae irrespective of the species to the total larvae screened in the Swartkops River during the study period (August 2009-September 2012). **A** (CID per site per sampling occasion) showing that larvae were more frequently deformed at Sites 3 and 4 than at Sites 1 and 2 in most of the sampling seasons. **B** (Means and standard deviations of the percent CID per site during the study period) showing that mean CID was statistically higher at Sites 3 and 4 than at Site 1, **C** (Means and standard deviations of the percent CID per seasons across the sites) indicating no statistically significant difference between the season. Vertical bars having a small alphabet letter in common are not significantly different ($P > 0.05$), whereas those having no small alphabet letter in common are significantly different ($P < 0.05$) indicated by ANOVA. Seasons: Sp (spring), Su (summer), Au (autumn) and Wi (winter).

In spring (2012), larvae at Site 4 were more frequently deformed compared to those collected at the remaining sites (Figure 6.3). In general, incidences of deformities decreased between

autumn and spring 2012 at the four sampling sites. One-way analysis of variance (ANOVA) conducted on arcsine-transformed deformity data revealed significant differences between the sampling sites ($P < 0.05$). The Tukey's honestly significant different (HSD) post-hoc test indicated that the community incidences of deformities were significantly higher at Sites 3 and 4 than at Site 1 ($P < 0.05$) (Figure 6.3). No statistically significant differences were indicated between Sites 1 and 2, and between Sites 2 and 4 ($P > 0.05$). The incidences of deformities were generally higher during summer (Figures 6.3A and B). Nevertheless, although the community incidences of mentum deformities varied between the sampling seasons, no statistically significant differences were observed between the sampling seasons ($P > 0.05$) (Figure 6.3).

6.3.2 Spatial and temporal patterns of Chironomidae larval mentum deformity types

The spatial and temporal patterns of chironomid larval mentum deformity types are shown in Figure 6.4 and illustrated for *Chironomus* spp. in Figure 6.5. The detailed descriptions of the observed deformity types are provided in Appendix D, Tables D1 – D8. Eight deformity types were observed in the mentum of the chironomid species: missing teeth, fused teeth, extra teeth, Köhn gaps (mentum gap), asymmetry, lateral gaps, split teeth and multiple deformities (i.e. a single larva having more than one deformity type, classified as Class 2). Of the eight observed deformity types, missing teeth and fused teeth were the commonest types of deformities at all four sampling sites (Figure 6.4). Larvae exhibiting multiple deformities (Class 2 deformities) were mostly associated with Sites 3 and 4, and with the spring and summer (2011) sampling occasions. Deformity types including asymmetry, Köhn gaps and extra teeth were less frequent and did not occur in the chironomid larvae collected at Site 1 (Figure 6.4). Seasonally, more types of deformities were recorded during spring and summer.

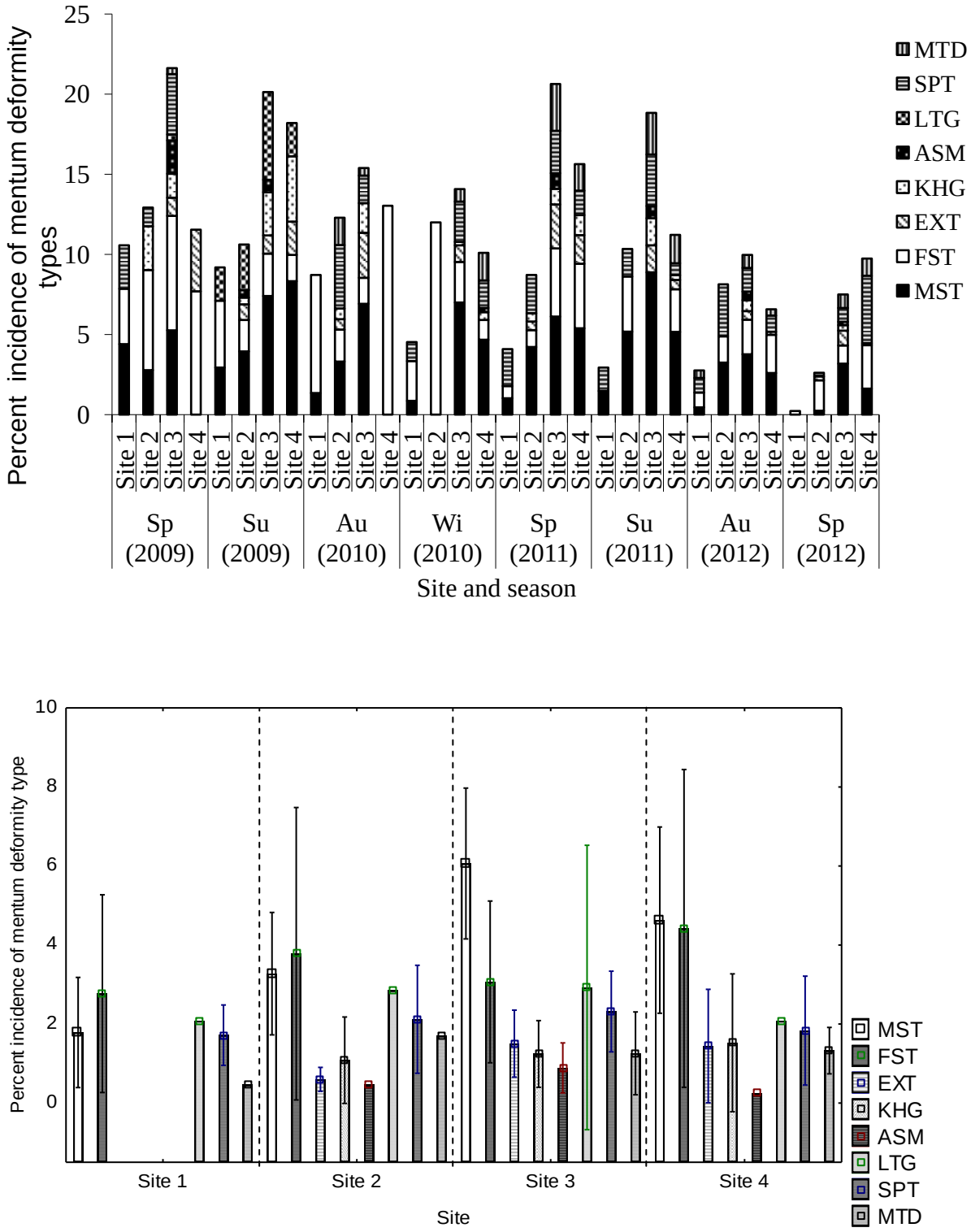


Figure 6.4: Mean percent incidences of deformity types recorded in the mentum of chironomid species during the sampling occasion at the four sampling sites (first graph, top) showing that missing teeth and fused teeth were the commonest types of deformities and percent incidence of deformity types summarised for the four sampling sites in the Swartkops River (second graph). Vertical bars are standard deviations. Deformity types: MST (missing teeth), FST (fused teeth), EXT (extra teeth), KHG (Köhn gap), ASM (asymmetry), LTG (lateral gap), SPT (split teeth), MTD (multiple deformities). Seasons; Sp (spring), Su (summer), Au (autumn) and Wi (winter).

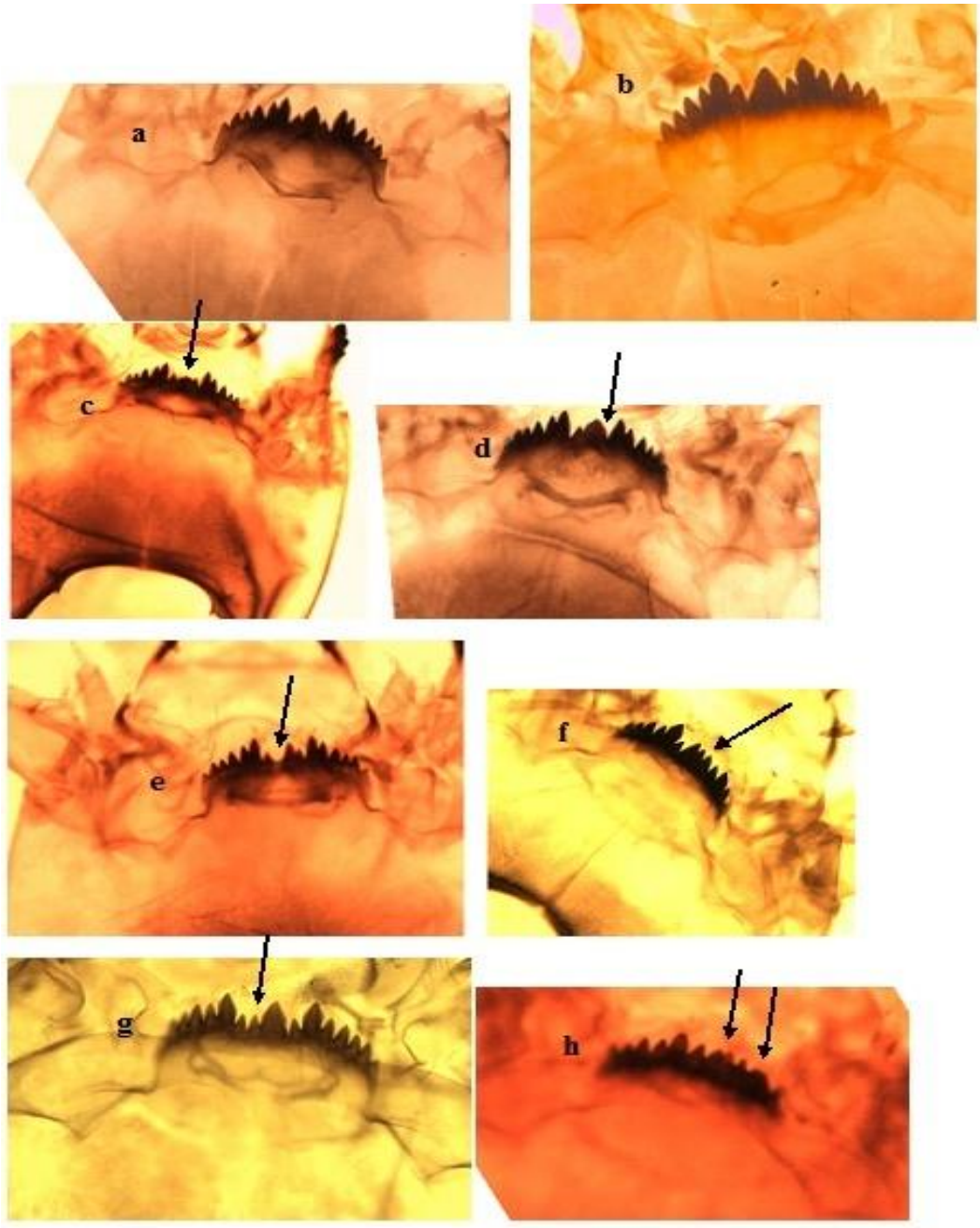


Figure 6.5: Illustration of deformity types in the mentum of *Chironomus* spp: (a) normal mentum of *Chironomus* sp.1; (b) normal mentum of *Chironomus* sp. 2; (c) split median teeth of *Chironomus* sp.1.; (d) missing right median tooth of *Chironomus* sp.1.; (e) Köhn gap in *Chironomus* sp.1 (f) fused median teeth of *Chironomus* sp1. (g) Extra left median tooth in *Chironomus* sp. 2. (h) multiple deformities in *Chironomus* sp.1

6.3.3 Incidences of mentum deformities in selected Chironomidae species at the four sampling sites

To investigate whether the expression of deformities varied between selected chironomid species collected from the same sites, the incidences of deformities calculated for the dominant species at each sites were compared. At Site 1, the incidences of deformities between *Cricotopus* sp.1, *Cricotopus trifasciata* gr., *Polypedilum* sp. *Tanytarsus* sp. and *Ablabesmyia* sp. were compared (Figure 6.6). Of the five species, the mean deformity incidence was highest in *Cricotopus trifasciata* gr., and lowest in *Tanytarsus* sp. The *Cricotopus trifasciata* gr. had the highest within-species variation in incidences of deformities. One-way analysis of variance (ANOVA) conducted on the arcsine transformed deformity data revealed no statistically significant differences between the mean incidences of deformities of the five species ($P > 0.05$).

At Site 2, incidences of deformities for five species: *Chironomus* sp.1, *Cricotopus* sp.1 *Cricotopus trifasciata* gr., *Dicrotendipes* sp. and *Tanypus* sp. were compared (Figure 6.6). The *Dicrotendipes* sp. larvae were more frequently deformed than the larvae of the remaining four species, but the differences in the incidences of deformities between the species were not statistically significant ($P > 0.05$).

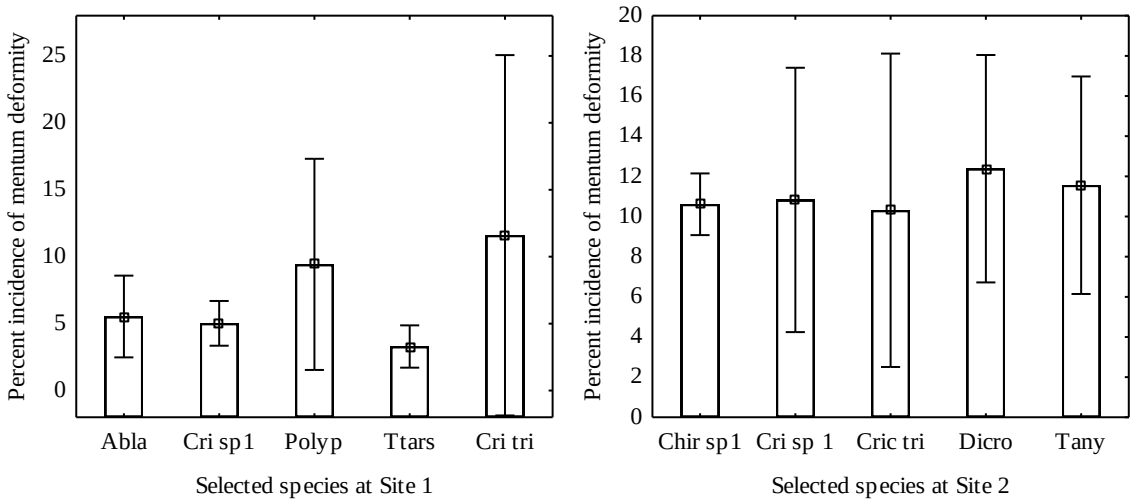


Figure 6.6: Mean and standard deviation (vertical bar) of percent incidences of deformities in selected chironomid species at Sites 1 and 2 in the Swartkops River (August 2009– September 2012) indicating that although there were variations in deformities between the species, they were not statistically significant (P > 0.05. Abbreviations: Abla (Ablabesmyia sp.) Cri sp1 (Cricotopus sp.1), Polyp (Polypedilum sp.) Ttars (Tanytarsus sp.), Cri tri (Cricotopus trifasciata gr.), Chir sp1 (Chironomus sp.1), Dicro (Dicrotendipes sp.), Tany (Tanypus sp.).

At Site 3, the larvae of *Chironomus* sp.1 and *Chironomus* sp.2 were more frequently deformed than the larvae of *Dicrotendipes* sp. (Figure 6.7), whereas at Site 4, *Cricotopus trifasciata* gr. larvae were the most deformed (Figure 6.7). No statistically significant differences were observed between the incidences of mentum deformities for the dominant species collected at Site 3 ($P > 0.05$) and Site 4 ($P > 0.05$; $F = 2.058$). Overall, although the incidences of deformities varied between species within a site, in all cases, the one-way ANOVA indicated that they were not statistically significantly different ($P > 0.05$).

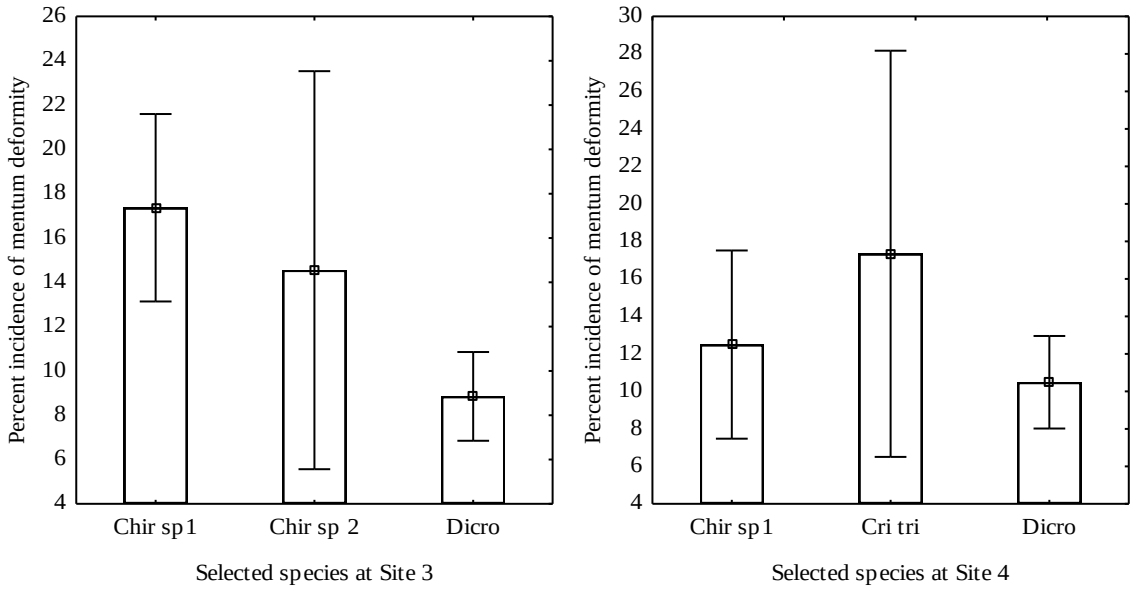


Figure 6.7: Mean and standard deviation (vertical bars) of percent incidences of deformities in selected chironomid species at Sites 3 and 4 in the Swartkops River during the study period (August 2009–September 2012) showing that although there were variations in the incidences of deformities between the species, they were not statistically significant ($P > 0.05$). Abbreviations: Chir sp1 (*Chironomus* sp.1), Chir sp 2 (*Chironomus* sp. 2), Dicro (*Dicrotendipes* sp.), Cri tri (*Cricotopus trifasciata* gr.).

6.3.4 Using the extended toxic score index (ETSI) to enable discrimination between the sampling sites

The extended toxic score index (ETSI) used to investigate whether the sites could be distinguished based on the sub-lethal effects indicated that all the three downstream sites, i.e. Sites 2, 3 and 4, were discriminated from Site 1 (Figure 6.8); that is, the 25 percentile of the ETSI at Sites 2, 3 and 4 were higher than the 75 percentile at Site 1, indicating that deformities at the three downstream sites were elevated above the reference levels at Site 1. This was further supported by the Kruskal-Wallis multiple comparison test which showed statistically significant differences between Site 1, and Sites 3 and 4 (Figure 6.6) ($P < 0.05$).

Furthermore, between the three downstream sites, the ETSI enabled discrimination of Site 2 from Sites 3 and 4, indicating that the sub-lethal effect-based index was very sensitive. However, comparing the ETSI between the sampling seasons across the sites, the box plot and the Kruskal-Wallis test showed that the ETSI between the seasons across the sites did not differ significantly ($P > 0.05$) (Figure 6.8).

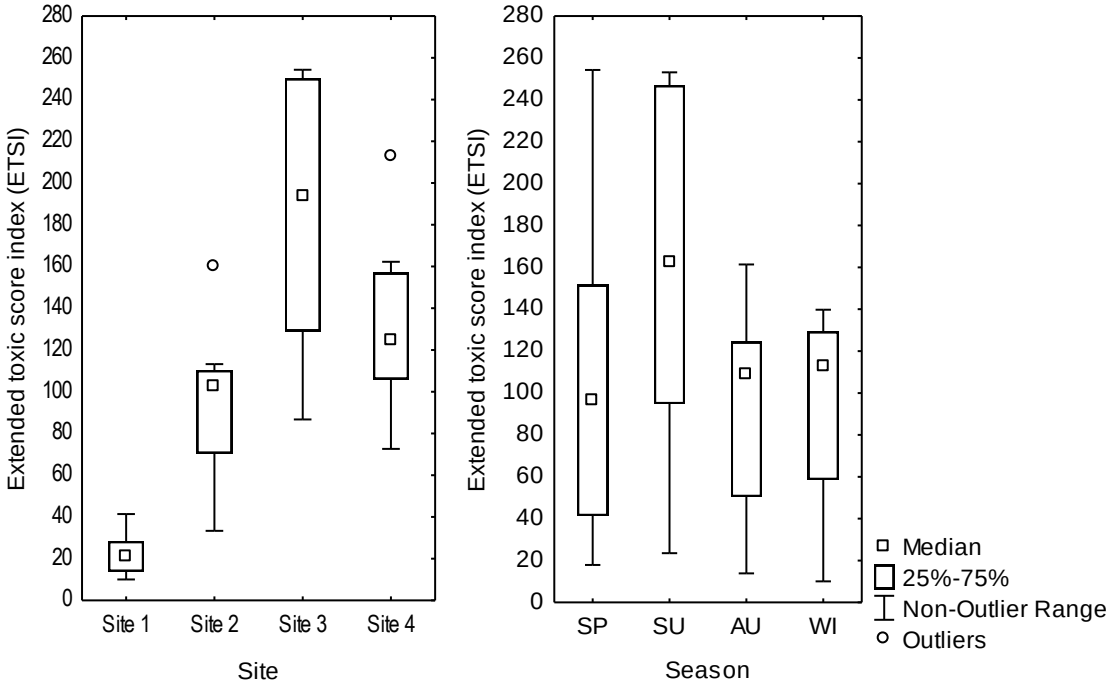


Figure 6.8: Box plots showing the extended toxic score index at the four sampling sites (left) in the Swartkops River and during the four sampling seasons (right) over the study period (August 2009–September 2012) indicating that deformities at Sites 2, 3 and 4 were elevated above the reference levels at Site 1 (left) and that there were no significant variations in the deformities between the seasons (right). Abbreviations: seasons: SP (spring), SU (summer), AU (autumn), WI (winter).

6.3.5 Relating incidences of deformities to the measured water physico-chemical variables

The percent community incidences of deformities (CID) and the extended toxic score index were related to the measured water physico-chemical variables using multiple-linear regression. The results revealed that the best subset model between the percent CID and the physico-chemical variables was obtained with seven variables: orthophosphate-phosphorus (PO_4-P), turbidity, ammonium-nitrogen (NH_4-H), nitrite-nitrogen (NO_2-N), temperature, lead (Pb), and dissolved oxygen (DO) (Table 6.1). The adjusted R^2 value showed that the subset model explained 78.3% variability between the percent CID and the selected physico-

chemical variables. Of the seven selected variables in the subset model, three variables: PO_4-P, turbidity and NO_2-N were significantly positively correlated with the percent CID. Increased DO and Pb were negatively correlated with the percent CID, but these were not significant (Table 6.1). Orthophosphate-phosphorus (PO_4-P) and nitrite-nitrogen (NO_2-N) were the variables with the highest correlations with the percent CID (Table 6.1).

The multiple-linear regression between the extended toxic score index (ETSI) and the physico-chemical variables indicated that five variables yielded the best subset model: total inorganic nitrogen (TIN), electrical conductivity (EC), manganese (Mn), lead (Pb) and chromium (VI) (Cr^{+6}). The adjusted R^2 value revealed that the subset model explained 59.6% variability between the ETSI and the physico-chemical variables (Table 6.1). Of the five variables, TIN, EC and Mn were significantly positively correlated with the extended toxic score index. The slope of the model showed that the relationship between the ETSI and the variables was greatest for TIN and EC.

Table 6.1: Properties of the best subset model of the multiple linear regressions between the extended toxic score index and the selected water physico-chemical variables, and between the percent community incidences of deformities (CID) and the selected water physico-chemical variables in the Swartkops River during the study period (August 2009–September 2012). Variables that were significantly correlated with the ETSI and CID are indicated in bold face.

ETSI: F = 8.136; P = 0.000; Adjusted R^2 =59.6%				
	Slope	Standard error of slope	t-value	p-value
Intercept			-1.343	0.192
Total inorganic nitrogen (TIN)	**0.563**	**0.211**	**2.661**	**0.014**
Electrical conductivity (EC)	**0.468**	**0.217**	**2.162**	**0.041**
Manganese (Mn)	**0.350**	**0.132**	**2.653**	**0.014**
Lead (Pb)	-0.207	0.141	-1.466	0.156
Chromium (VI) (Cr^{+6})	-0.147	0.143	-1.025	0.316
Percent CID: F = 14.564; P = 0.000; Adjusted R^2 = 78.3%				
	Slope	Standard error of slope	t-value	p-value
Intercept			-0.422	0.677
Orthophosphate-phosphorus (PO_4-P)	**0.518**	**0.071**	**3.109**	**0.005**
Turbidity	**0.314**	**0.040**	**2.856**	**0.009**
Ammonium-nitrogen (NH_4-N)	0.088	0.130	0.276	0.785
Nitrite-nitrogen (NO_2-N)	**0.518**	**0.503**	**3.334**	**0.003**
Temperature	0.178	0.112	1.981	0.060
Lead (Pb)	-0.165	14.925	-1.798	0.086
Dissolved oxygen (DO)	-0.205	0.110	-1.506	0.146

6.4 Discussion

6.4.1 Chironomidae larval mentum deformities as a biomonitoring tool

Morphological deformities in the mentum of chironomid species screened in this study indicated the sub-lethal effects of pollutants in the Swartkops River (Figures 6.3- 6.8). Several authors (e.g. Janssens de Bisthoven and Gerhardt, 2003; Nazarova *et al.*, 2004; Ochieng *et al.*, 2008) have implicated metals as the main cause of deformities in chironomid species. However, chironomids under field conditions in this study were exposed to a wide array of in-stream chemical pollutants originating from wastewater discharges and other diffuse sources including run-off from surrounding rail and road networks. The in-stream chemical pollutants may undergo transformation and degradation through several processes

including hydrolysis, photolysis, oxidation and reduction, producing by-products that could also induce deformities (Vermeulen, 1995). Exposure to contaminated sediment may also influence deformities in chironomid larvae (Vermeulen, 1995). Therefore, it is inappropriate to implicate metals as the sole causative agents of deformities observed in chironomid communities in the Swartkops River: the elevated incidences of deformities observed in this study (Figures 6.3 and 6.8) could be interpreted as sub-lethal biological effects of exposure to a wide array of chemical pollutants. Nevertheless, the significant positive relationship indicated by the multiple linear regressions between the percent community incidences of deformities and nutrients, turbidity, as well as between the ETSI and EC, nutrients and Mn (Table 6.1) seemed to suggest that elevated concentrations of these water physico-chemical variables are likely to directly or indirectly contribute to inducing deformities in the mentum of chironomids. It is however important to note that correlation is not causal evidence and experiments would be required to confirm whether elevated levels of these variables could cause deformities in chironomid larvae. Although nutrients may not directly induce deformities in chironomids, their elevated concentrations, provided other growth limiting factors are satisfied, can stimulate the growth of harmful micro-algae and bacteria that produce bio-toxins that can induce deformities in chironomid species (Lewis *et al.,* 2012; Carey *et al.,* 2013). Lewis *et al.* (2012) reported that bio-toxin extracted from an actinobacteria induced deformities in *Chironomus riparius* after 96 hours exposure period. Results from Lewis *et al.* (2012) therefore suggest that factors such as elevated nutrients that can stimulate the growth of bio-toxin-producing bacteria and micro-algae are indirect causative agents of deformities in chironomids. Given the algal cover observed in the Swartkops River between 2009 and 2010 (personal observation), the possibility of bio-toxin-producing species that can induce deformities, cannot be completely ruled out. Furthermore, the organic input into the Swartkops River, and the generally low concentrations of metals recorded in the water of the river in this study (Chapter 3), seemed to suggest that organic pollutants not measured in this study were acting synergistically with metals to induce significantly elevated levels of deformities among the Swartkops River chironomid assemblages at the downstream sites. Organic pollution has been reported to induce deformities in chironomid larvae (Servia *et al.*, 1998; MacDonald *et al*., 2006).

Based on the newly developed extended toxic score index (ETSI), it could be argued that the observed incidences of deformities at the downstream sites i.e. Sites 2, 3 and 4, were truly elevated above the reference values at Site 1 because the index relied on an objective

criterion. The index proved sensitive because it not only allowed the discrimination of Site 1 from the downstream sites, but it also enabled the discrimination of Site 2, from Sites 3 and 4 (Figure 6.8). This indicates that the index could be used as a reliable tool for assessing sub-lethal in-stream biological response. The ETSI is scientifically defensible because i) it is based on determining an appropriate reference study site, and using a statistical criterion to determine whether incidences of deformities at impacted sites were elevated above the reference levels; ii) it is based on only one structure, thereby avoiding the confounding effects of pooling together the incidences of deformities in multiple structures that differ in expressing morphological deformities; iii) it accounted for the potential pollution-tolerance differences between the chironomid species; iv) it accounted for the potential severity of observed deformities. Therefore, it is recommended that studies utilising chironomid deformities as a biological screening tool could use the ETSI instead of relying solely on 8% community incidences of deformities as a criterion for judging whether deformities were elevated above the reference levels. It should be noted, however, that, depending on the dominant species in such studies, the constituent species in the ETSI would change, but the approach used for deriving the sensitivity scores could be applied for deriving the scores of such dominant chironomid species.

The observed incidences of deformities in the Swartkops River, apart from indicating sub-lethal biological effects, could also be considered as a relevant ecological endpoint related to feeding, growth and development. Although these biological parameters were not directly related to deformities in this study, Janssens de Bisthoven *et al.* (1998) reported that in chironomid larvae collected from a wastewater- and copper-polluted site in the River Ijese, Belgium, normal larvae were larger than deformed larvae, and that the normal larvae survived better and developed faster than the deformed larvae. These authors attributed these differences to the fact that deformed individuals were probably less fit than normal larvae suggesting that deformities could affect the fitness, growth and survival of chironomid larvae. Furthermore, since chironomids rely partly on the mentum for feeding (Armitage *et al.*, 1995), deformities in the mentum could affect feeding with possible effects on ecological function such as recycling of nutrients because leaf fragmentation caused by chironomid feeding habits could be hindered as a result of deformed mouthparts. Therefore, the elevated incidences of mentum deformities at Sites 2, 3 and 4, particularly at Site 3, is a cause for concern because it suggests a community whose performance could have been affected as a result of deformed individuals, thereby potentially impacting on important ecological

function. However, the link between chironomid deformities and ecological function such as resource acquisition and nutrient recycling, which were not considered in this study, remains hypothetical and deserves further research.

In the Vermeulen (1995) review, it was noted that the diagnostic power of chironomid deformity types could be enhanced if deformities could be linked with specific kinds of pollutants and, possibly, levels of pollution. Chironomid larvae collected at Site 1 exhibited fewer deformity types than those at Sites 2, 3 and 4 (Figures 6.4 and 6.5). Of the eight observed deformity types, Köhn gap (i.e. mentum gap), extra teeth and asymmetry did not occur at Site 1, but were present in larvae collected at the downstream sites, particularly at Sites 3 and 4, which probably suggests that these types of deformity could be induced only under the severe pollution prevailing at Sites 3 and 4.

6.4.2 Seasonal variations in the incidences of mentum deformities

Servia *et al.* (2000) emphasised the importance of seasonal and inter-annual variations when using chironomid deformities as a biomonitoring tool. In this study, deformities were screened seasonally over an extended period (August 2009 - September 2012). Contrary to the observation of Servia *et al.* (2000) who reported significantly higher incidences of deformities during winter, in this study, the community incidences of deformities and the extended toxic score index were not statistically significantly different ($p > 0.05$) between the seasons across the sampling sites (Figures 6.3 and 6.8). Nevertheless, more deformities, in most cases, were found in the larvae collected during summer and spring (Figure 6.3). Elevated incidences of deformities during summer could be attributed to increased temperature, which would increase the bioavailability of deformity-inducing pollutants by altering the rate of both chemical and biological reactions (Jeyasingham and Ling, 2000; Eggleton and Thomas, 2004). The elevated incidences of deformities during spring has been attributed to spring "overwintering" larvae that had been developing at a slower rate under low temperature conditions and were therefore exposed to pollutants much longer (Jeyasingham and Ling, 2000). The elevated incidences of deformities observed in this study during summer (Figures 6.3 and 6.8) are in agreement with Jeyasingham and Ling (2000) who reported higher incidences of deformities during summer in Hamilton Lake and Lake Ngaroto in New Zealand. Although significant seasonal differences have been observed in other studies, particularly in the temperate regions, the non-significant differences observed

in the incidences of deformities in the Swartkops River between seasons showed that seasonal differences were less influential than site differences in inducing deformities.

6.4.3 Incidences of deformities in chironomid subfamilies and selected species

Of the three chironomid subfamilies recorded in this study, incidences of deformity were highest among species belonging to the Chironominae subfamily, particularly within the tribe Chironomini (Figure 6.2). Species belonging to the Chironominae live in close association with the sediments, where they feed on detritus and graze on algae (Olafasson, 1992; Berg, 1995). This close association with the sediments predisposes them more to the sediment-bound pollutants than species that are less associated with the sediments. Since the sediment acts as a sink for most pollutants including metals, the associated fauna are therefore more likely to be affected by these pollutants. This phenomenon probably explains why species of the subfamily Chironominae were more deformed than species belonging to the other two subfamilies in the present study. Bhattacharyay *et al*. (2006) and Ochieng *et al.* (2008) have also reported higher incidences of deformities in species belonging to the Chironominae subfamily in the River Damodar and Lake Victoria, respectively.

The ligula in Tanypodinae is adapted for a predatory lifestyle and has been reported to exhibit fewer incidences of deformities than the mentum in other chironomid subfamilies (Ochieng *et al.,* 2008). In this study, deformities in the subfamily Tanypodinae were less frequent than in either the Orthocladiinae or Chironominae (Figure 6.2). The configuration of the ligula, which is adapted for engulfing and piercing, and the free-living lifestyle of most species belonging to the Tanypodinae (Berg, 1995) would enable them to avoid extended contact with the sediment-bound contaminants. The Tanypodinae, being predators, presumably spend more time actively searching for prey and less time buried in sediments. The above reasons could partly explain why there were fewer deformities among the Tanypodinae species.

The observed incidences of deformities varied between species within a Site, but these variations were not statistically significantly different (Figures 6.6 and 6.7). The differences (though not statistically significant) could be attributed to species differential sensitivity to deteriorating water quality (Warwick, 1990). Although the *Chironomus* spp. were deemed more tolerant than *Dicrotendipes* sp. in this study, the *Dicrotendipes* sp. exhibited fewer incidences of deformities than *Chironomus* spp. at both Sites 3 and 4. Warwick (1988) indicated that each chironomid species would express deformities within tolerable

contaminant concentrations after which mortality would reduce the population of such species. Thus, the fewer incidences of deformities in *Dicrotendipes* sp. than *Chironomus* spp. at Sites 3 and 4 (Figure 6.7) could be due to high mortality among the deformed larvae compared to their normal counterparts such that normal larvae were more often encountered in samples than the deformed larvae. For example, in a laboratory experiment, Janssens de Bisthoven *et al.* (1998) found that deformities influenced mortality in *Chironomus* population, with a higher mortality rate recorded for the deformed larvae than the normal ones. Therefore, if deformity screening is reduced to only one species, and the pollution level is higher than its presumed response range (Warwick, 1990), resulting in the mortality of deformed larvae and frequent sampling of normal larvae than deformed ones, then the biomonitoring implication is that fewer incidences of deformities than expected would be reported and could lead to an erroneous conclusion. This was overcome in this study by screening deformities in more than one chironomid species and by developing an index that relied on more than one species. However, it could also be that the observed differences between species were influenced by different instar stages of the larvae, a potentially important factor that was not considered in this study. Differences in instar stages are a potential source of uncertainty that deserves further exploration to determine their influences on the expression of deformities in chironomid larvae.

Furthermore, although the overall community incidences of deformities and the extended toxic score index were low at Site 1 (Figures 6.3 and 6.8), there were considerably higher incidences of deformities in the mentum of *Polypedilum* sp. and *Cricotopus trifasciata* gr. (Figure 6.6). The higher incidences of deformities in these species, despite the overall good physico-chemical water conditions at the site, could be early-warning indicators of impending biological effects. On the other hand, the deformities could also be considered as inherent in the population and are being transferred through generations. In this case, the deformities may not have been induced by environmental conditions. However, it could be argued with some degree of certainty that the elevated incidences of deformities observed in the selected species at Site 1 are potentially early warning signals of exposure to environmental stressors, which may or may not be related to water quality. This is because several deformity-studies (e.g. Frank and Köhn, 1982; Dickman and Rygiel, 1996; Groenendijk *et al*., 1998; Martinez *et al.*, 2004) have indicated that chironomid deformities are probably teratogenic, with incidences of deformity dropping in offspring populations reared in clean substrates

compared to their parent populations collected in contaminated sediments. The results of the studies cited above suggested that deformities were not passed from parents to offspring.

6.5 Conclusion

The screening of morphological deformities in chironomids provided evidence of a sub-lethal in-stream biological response to deteriorating water quality. The newly developed extended toxic score index proved sensitive, and allowed the discrimination of Sites 1 from Sites 2, 3 and 4, and Site 2, from Sites 3 and 4. The results, therefore, indicated that a biomonitoring tool based on sub-lethal effects could distinguish the sampling sites before the manifestation of lethality in the biological community. The elevated incidences of deformities in *Polypedilum* sp. and *Cricotopus trifasciata* gr. at Site 1 were considered early warning indicators of biological effects of exposure to environmental stressor that only a sub-lethal assessment would provide. Overall, the study of chironomid deformities added value to the lines of evidence gathered in the multi-criteria biomonitoring investigation of pollution undertaken in this study.

CHAPTER 7: MACROINVERTEBRATE COMMUNITY RESPONSE TO TREATED WASTEWATER EFFLUENT IN MODEL STREAM ECOSYSTEMS

Although there are several non-point sources of pollution in the Swartkops River (Chapter 2), a major contributor to the deteriorating water quality in the river is the discharge of wastewater effluent from the Kelvin Jones wastewater treatment works (WWTW) in Uitenhage. Sites 1 and 2 in the Swartkops River are upstream of the discharge point, whereas Site 3 is just downstream of the discharge point and Site 4 about 2.5 km further downstream. The previous chapters recorded observations that the greatest water quality impact on the Swartkops River macroinvertebrate communities (taxonomic-, traits-based and sub-lethal) was at Sites 3 and 4, and this was mostly attributed to the effects of wastewater effluent discharges. However, using biomonitoring data alone it is difficult to establish whether the wastewater effluent was the main cause of the observed in-stream macroinvertebrate community response. This chapter describes nine outdoor multispecies model-stream ecosystems that were developed and used to isolate and investigate the effects of the treated wastewater effluent on macroinvertebrate communities. The chapter also presents the results of the effluent physico-chemical quality compliance of the Kelvin Jones WWTW, provides evidence of the effects of the wastewater effluent on macroinvertebrate communities and presents interpretations of the results in the context of the observed field-based patterns presented in previous chapters.

7.1 Introduction

Wastewater effluent discharges constitute approximately 50% of the downstream flow of the Swartkops River (i.e. below Uitenhage) during low-flow periods (Taljaard *et al.*, 1998) and are thus a major contributor to deteriorating water quality in the river. The Kelvin Jones, Despatch and KwaNobuhle wastewater treatment works (WWTWs) are all within the river catchment (Chapter 2) but, of these three WWTWs, only the Kelvin Jones WWTW in Uitenhage is located within the sampling sites in this study and thus it is the focus of this chapter. Daily flow data from 1995 to 2013 in the Swartkops River, upstream of the Kelvin Jones effluent discharge point, and data on the daily operating capacity of the Kelvin Jones

wastewater treatment works, revealed that the Kelvin Jones WWTW contribute an average of 60% to the Swartkops River flow at the discharge point (Slaughter, *pers. comm.*).

Chapters 3, 4, 5 and 6 described the taxonomic-, traits-based and sub-lethal biomonitoring approaches that were used to assess macroinvertebrate community response to deteriorating water quality, and the communities at Sites 3 and 4 had the greatest deviation from the reference assemblages at Site 1. The evidence presented in the previous chapters suggested that the changes in macroinvertebrate community structure were caused chiefly by the discharges of wastewater effluents. However, because biomonitoring data rely on correlative inferences, it is difficult to establish a direct causal relationship or to ascertain whether the wastewater effluent discharges were the main cause of the observed in-stream macroinvertebrate response. This gap was filled by experimentally investigating the effects of the treated wastewater effluent on macroinvertebrate communities and thus eliminating effects of diffuse sources of pollution.

In South Africa, the potential effects of wastewater effluent on biological communities are assessed through physico-chemical measurements and laboratory-based single-species toxicity tests (Slabbert, 2004; DWAF, 2009; DWA, 2011c). However, the green-drop programme of the South African Department of Water Affairs (DWA) relies on the physico-chemical measurements alone for ascertaining effluent quality, and compliance with legislated standards and license conditions. The green-drop programme (discussed in Chapter 1, section 1.2.2) is an incentive-based regulatory framework for managing all operational aspects of wastewater treatment works to ensure compliance of the final effluent before discharging into a receiving water resource (DWA 2009; 2011c). Reliance on physico-chemical measurements alone as measures for ascertaining effluent quality compliance aimed at protecting in-stream biological resources has several limitations. First, physico-chemical measurements alone cannot fully determine the biological effects of the effluent because these measurements cannot assess the effects of the effluent-constituent chemicals, their by-products, or their antagonistic and synergistic interactions on the biotic communities. Second, the number of dissolved and suspended chemicals in the effluent and the analytical expense and expertise required to measure them, make exhaustive chemical characterisation uneconomical and practically impossible. Thus, only the few physico-chemical variables that are considered important are measured in order to assess effluent quality. For these reasons, laboratory-based single-species toxicity tests have been used as complementary methods to

assess the effects of effluent on biological communities in South Africa (Palmer *et al.,* 2004; Slabbert, 2004).

Although the laboratory-based, single-species toxicity tests are widely used and recommended for assessing wastewater effluent effects (EPA, 2002), they are unable to provide information about long-term population, community or ecosystem level effects, or adequate environmental realism (Ledger *et al.,* 2009). Therefore, the laboratory-based single-species tests rely on applying safety assessment factors or the species sensitivity distribution (SSD) approach for ecological interpretation of toxicity data (Schmitt-Jansen *et al.,* 2008; Jesenská *et al.,* 2011). To reduce the uncertainty associated with laboratory to field extrapolation, and to provide a more ecologically relevant and realistic assessment of long-term effects of the Kelvin Jones WWTW effluent on macroinvertebrate communities, nine outdoor multispecies model-stream ecosystems were constructed and used in this study. The direct effects of the effluent on the macroinvertebrate community structure were related to the observed in-stream effects to complement the biomonitoring results in order to understand the influence of the wastewater effluent on the in-stream macroinvertebrate communities in the Swartkops River.

The outdoor multispecies model-stream ecosystem approach used in this study occupies an intermediate space between the field-based biomonitoring undertaken in the Swartkops River (Chapters 3, 4, 5 and 6), and the laboratory-based single-species toxicity test approach. This intermediate position enables the multispecies model-stream ecosystems to provide a high degree of environmental realism, enabling complex biophysical interaction and the evaluation of direct effluent effects at higher biological organisations (Ledger *et al.,* 2009; Grantham *et al.,* 2012). However, unlike the single-species laboratory-based approach that offers a high degree of precision, repeatability and simplicity, the model-stream ecosystem approach used in this study represents a trade-off between these factors and their offered advantages outlined above. Nevertheless, the need to investigate the effects of the effluent on higher biological organisations, similar to the field-based biomonitoring, informed the approach used in this study because such results could give a direct insight into the effects of the wastewater effluent in the Swartkops River when interpreted in the context of the field-based macroinvertebrate response.

7.2 Materials and methods

7.2.1 Study site

The experiment was conducted at the Kelvin Jones WWTW in Uitenhage (S33^{o}46.997', E025^{o}25.615'), which is about 31.1 km north of Port Elizabeth, Eastern Cape, South Africa. The WWTW serves a population of approximately 82 275. It also receives influent from several sources, including automobile and allied industries, a tannery, wool and textile factories. The treated effluent is therefore both domestic and industrial. The WWTW has a design capacity of 24-ML/ day, but currently operates at 18.40-ML/ day (Nelson Mandela Bay Municipality, 2008).

The WWTW is an activated sludge system equipped with two bioreactors. Both reactors have been designed to operate either in the anoxic/ aerobic mode for biological nitrogen removal with chemical phosphorus removal, or in the conventional UCT (University of Cape Town) mode for biological removal of nitrogen and phosphorus. The inlet is equipped with screens and grit removal facilities that remove solids from the influent before undergoing further treatment processes. The final treated effluent in the clarifiers (settling tanks) is chlorinated before discharging into the Swartkops River at sampling Site 3 (Figure 7.1)

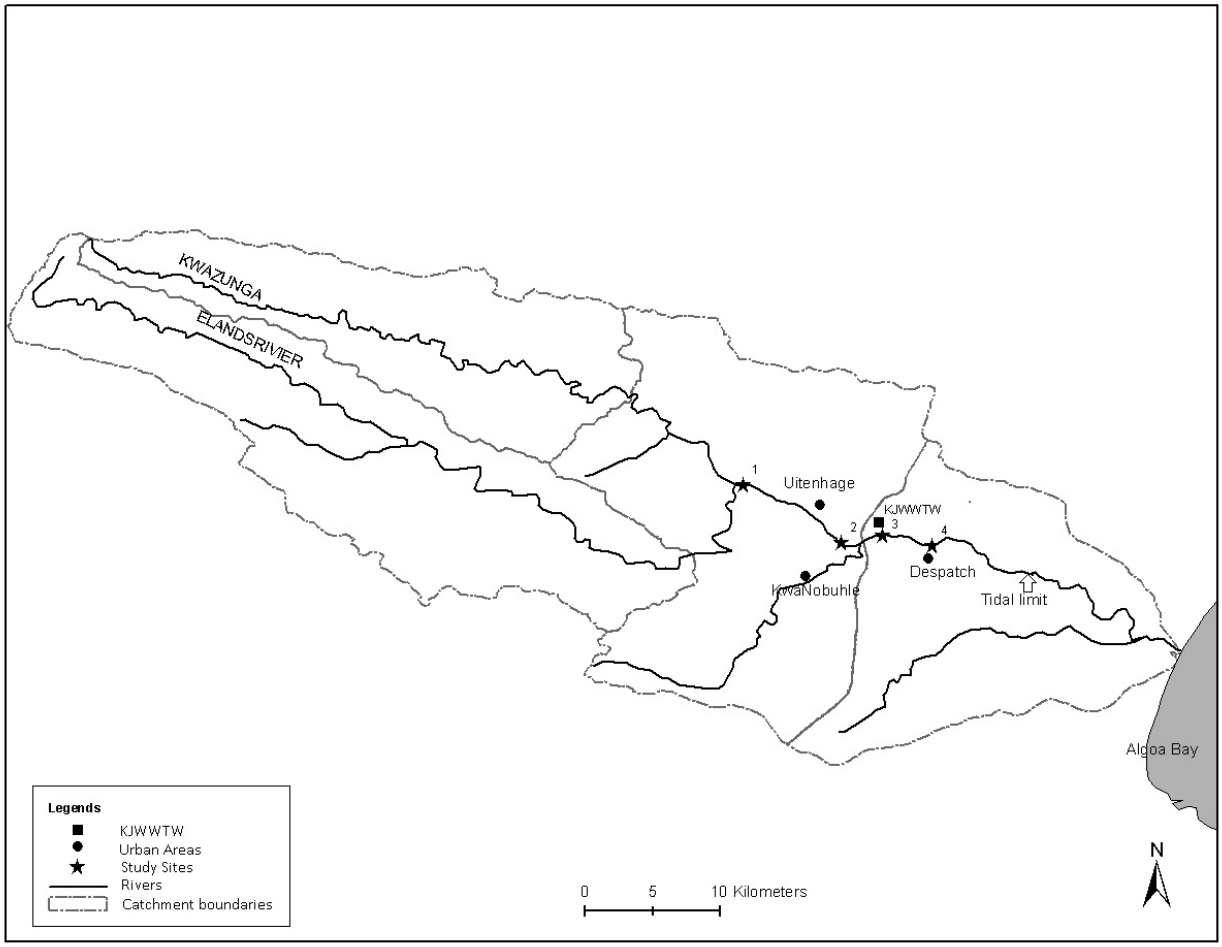


Figure 7.1: Swartkops River showing the sampling sites and the relative position of the Kelvin Jones Wastewater Treatment Works (shaded square)

7.2.2 Description of the model-stream ecosystems

Nine outdoor model-stream ecosystems were constructed at the Rhodes University Engineering Workshop, and then transported to the Kelvin Jones WWTW, where the models were assembled at the experimental site (Figure 7.2), about 400 metres away from the Swartkops River. Each model-stream channel was made of 4.5 m long white PVC guttering, with a 91 mm wide base, and side height of 70 mm. Three stream channels were fitted onto a single steel frame made of angle iron, so that the nine streams were on three separate frames i.e. three streams per frame (Figure 7.3). Each frame was 6 m long and 1.2 m wide, with adjustable legs made of threaded rods. The adjustable legs made it possible to achieve the desired slopes.

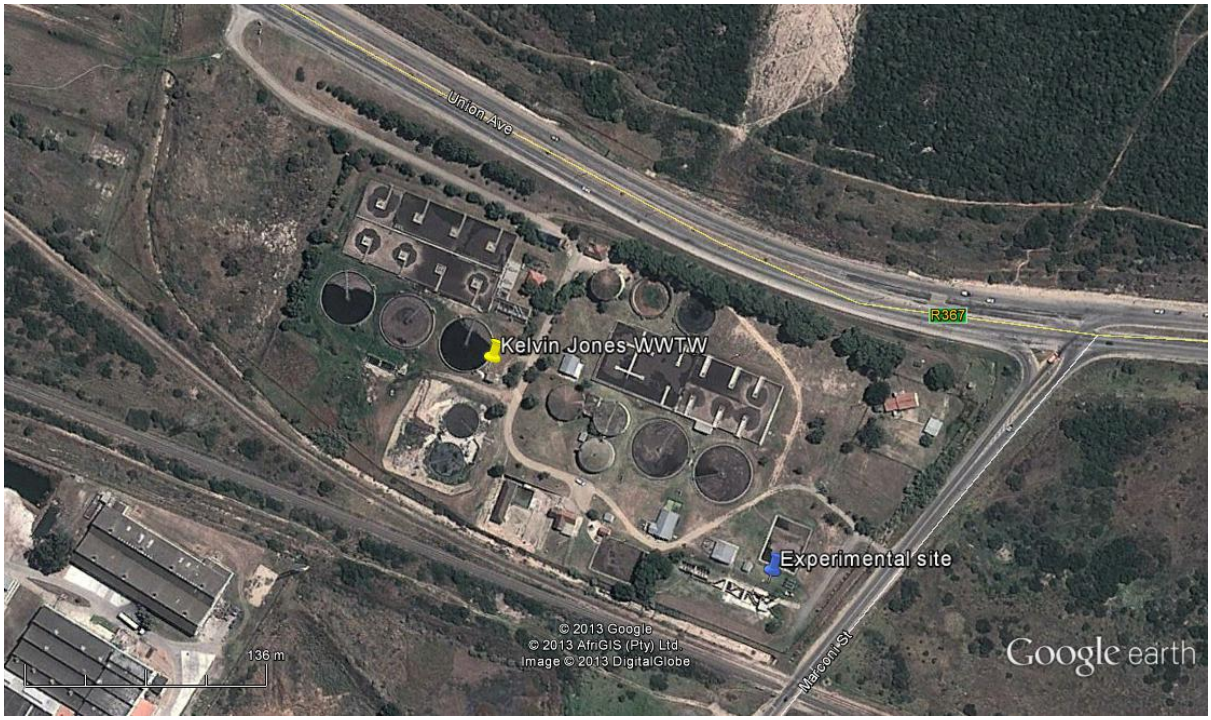


Figure 7.2: Google map showing the Kelvin Jones wastewater treatment works and the location of the experimental site.

Figure 7.3: The full experimental set-up at the beginning of the experiment (top); the experimental view from behind the upstream of the stream channel (bottom left), and the control streams after 90 days (bottom right). Tanks: A (de-chlorinated tap water), B (100% treated effluent), C (50% treated effluent) and D (50-litre tank with a Pedrollo top 2 pump for recirculation of dechlorinated tap water).

The three streams on each frame served as replicates: de-chlorinated tap water (control), 50% (diluted) and 100% (undiluted) effluent concentrations were made to flow through three replicate streams on each frame (Figure 7.3). A 20 mm diameter PVC tube was used to connect tap water from a nearby tap (freshwater source) to the control streams. The tap water flowed into a 210-litre tank (Tank A) through a 1.5 m long, perforated drainage pipe of diameter 110 mm containing activated charcoal. The purpose of the activated charcoal was to remove the chlorine in the tap water and the presence of residual chlorine that could remain

in the systems was analysed using a Merck chloride test kit. The de-chlorinated tap water in the 210-litre tank then flowed by gravity into the three replicate control streams through valves of 50 mm diameter that enabled the flow rate into the streams to be regulated. Downstream of the three replicate control streams, the de-chlorinated water was channelled into a 50-litre tank (Tank D) containing a Pedrollo top 2 pump, from where the de-chlorinated tap water was pumped back to the upstream of the control streams, through the activated charcoal chamber, and then into the 210-litre tank. Thus, the control streams were re-circulatory. Regular supply of water from the tap compensated for water loss through evaporation. The re-circulatory system could lead to higher temperature in the control streams, thereby creating a confounding factor of temperature. However, regular temperature measurements across the streams (controls, 50%-effluent treated and 100%-effluent treated) at the beginning of the experiment suggested that temperature was not elevated in the control streams over time.

The final treated effluent (100% concentration) en-route to the receiving Swartkops River was pumped through a PVC tube of diameter 20 mm into a 210-litre tank (Tank B) using a 2-inch trash pump. Before entering the tank, the effluent flowed through a mesh to remove the solids and rags. The 100% effluent concentration in the 210-litre tank then flowed by gravity into the three replicate 100% effluent concentration streams, but the flow rate was regulated with valves 50 mm in diameter. To avoid effluent overflow in the 210-litre tank, a 50 mm diameter PVC pipe was connected to the tank about 30 cm below the inlet pipe. This allowed the effluent to flow back into the drainage pipe en-route to the river without overflowing from the tank.

A third 210-litre tank (Tank C) was connected to both Tanks A and B containing the de-chlorinated tap water and 100% effluent concentration, respectively. The de-chlorinated tap water and the 100% effluent concentration were led into the third tank at approximately the same flow rate to achieve 50% effluent dilution. The 50% effluent concentration in the third tank then flowed by gravity into the three replicate 50% effluent streams. Valves were used to regulate the flow to achieve uniform velocity in the streams. To avoid the 50% effluent concentration in the third tank (Tank C) from contaminating the tap water (i.e. preventing effluent from flowing back into the tap water tank through the tap water inlet into the 50% effluent concentration tank), an outflow of diameter 50 mm was made in the tank containing the 50% effluent concentration. This outflow point was about 15 cm below the inflow points from both the tap water and 100% effluent concentration tanks. This design effectively

prevented any cross contamination. The 50% and 100% effluent concentrations flowing through the respective replicate streams were not re-circulated because continuous re-circulation of chlorinated effluent could transform chlorine into more toxic forms. Thus, the treatment streams were open flow-through systems, while the control streams were open re-circulatory systems. Each of the pumps used in the experiment had an automatic flow switch as a precautionary measure to avoid burning out should the water drop below an acceptable level.

Stream substrates

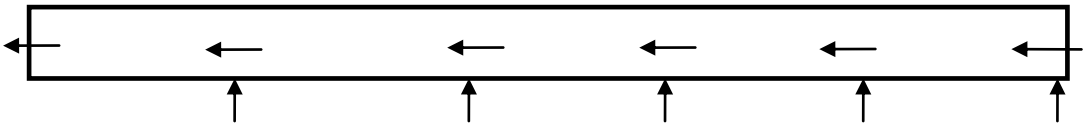
Eight baskets made of perforated drainage pipe of diameter 110 mm, and 200 mm long were cut open at the surface (Figure 7.4) and placed in each stream channel. Four were filled with stones and the other four with plastic ribbons (Figure 7.4) which served as surrogate vegetation. The baskets containing the stones and those with ribbons were arranged alternately along the length of each stream. The baskets served two purposes: first, they contained the substrate for macroinvertebrate colonisation and thus served as quantitative sampling units. The substrates were never completely submerged, thus providing exposed surfaces for insects to perch on while attempting to access the streams. Second, they help in providing flow dynamics within each channel. As the water flowed from upstream to downstream of each channel, it built up close to each basket, providing a deeper pool-like condition, and then trickled over the basket, creating shallower riffle-like condition behind each basket, so that pools and riffles alternated along the length of each stream. During a preliminary test run of the experiment, the ribbon clogged quickly in the 50% and 100% effluent concentration streams, causing overflow. For these reasons, the amount of plastic ribbon in each basket was reduced across all the streams. The baskets were fixed in position using round iron rods connected to each stream channel behind each basket. Two different substrates were used to provide an element of habitat diversity, a characteristics of natural stream systems.

Figure 7.4: Baskets containing the stone substrates on the left and the plastic ribbons on the right.

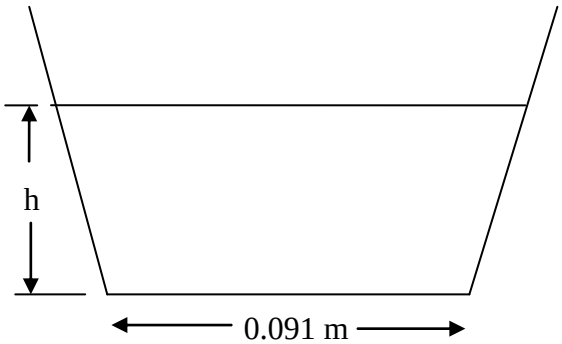
Stream hydraulics

At the beginning of the experiment, the hydraulics of each stream was established by measuring the water depth, water velocity and cross-sectional area at various points corresponding to pools and riffles. Williams' formula (1996) was used to calculate the cross-sectional area. The procedure followed to establish the hydraulic characteristics of the stream channels using one of the stream as an example is summarised in Figure 7.5 and a summary of the hydraulic characteristics of the streams is provided in Table 7.1.

Stream viewed from above:



Distance (m) from upstream	3.8	2.8	2.4	0.11	0
In-stream condition:	Pool	Riffle	Pool	Riffle	Pool
Water depth (m)	0.063	0.028	0.042	0.036	0.042
Water velocity (m/s)	0.015	0.036	0.023	0.027	0.023
Area (A) (m^2)	0.0068	0.0028	0.0043	0.0036	0.0043



Formula for calculating cross-sectional area (William, 1996)

h = water depth (m)

A (cross-sectional area) (m^2) = ($0.091h + 0.2632h^2$)

Figure 7.5: Procedures followed in establishing the model-stream hydraulic characteristics showing all the necessary measurements taken using one the stream channels as an example.

Table 7.1 Hydraulic characteristics of the model-stream ecosystems used to investigate the macroinvertebrate community response to the treated wastewater effluent at the Kelvin Jones wastewater treatment works.

	Control (De-chlorinated tap water)			50% effluent concentration			100% effluent concentration		
Replicate stream	1	2	3	1	2	3	1	2	3
Combined discharge (m^3/s)	2.9×10^{-4}			4.3×10^{-4}			4.3×10^{-4}		
Flow velocity (m/s)	0.025	0.024	0.025	0.027	0.028	0.030	0.031	0.029	0.030
Slope	01:59			01:27			01:21		

The differences in slope between the streams were important because of the differences in fluid viscosity. When all streams were at the same slope, the effluent (50% and 100% concentrations) flowed too slowly, resulting in clogging and consequently overflowing the stream channels. Although slight hydraulic variations existed between the streams, these were considered insignificant, as shown in Table 7.1. Efforts were made to maintain these hydraulic conditions constant throughout the duration of the experiment.

7.2.3 Experimental design

The experimental design involved three replicate control streams (de-chlorinated tap water), three replicate 50% diluted effluent-treated streams and three replicate 100% undiluted effluent-treated streams. The control and the treatment streams were not arranged in a stratified random design because such arrangement would make it difficult to achieve a uniform flow in the replicate streams. The experiment was conducted for a period of nine months and three weeks, from 8 August 2012 to 31 May 2013. The experiment was started just before the onset of spring, when insect emergence is greatest and the streams were colonised aerially for a period of three months before the first sampling event in November 2012. In addition, to aerial colonisation, the legs of the frames on which the stream channels rested, and the closeness of the streams to the ground could also enable non-flying macroinvertebrates such as snails and worms to colonise the streams. To provide food sources, at the beginning of the experiment, leaf-litter was placed in all the nine stream channels. No food source was added during the rest of the experimental period. Food sources was needed only for the control streams, but to provide similar conditions in all the streams, food sources were added to all the nine streams.

7.2.4 Water and effluent sampling, and physico-chemical measurements

On days 1, 2, 3, and 10, after start of the experiment and before commencing biological sampling, temperature, dissolved oxygen (DO), electrical conductivity (EC), turbidity, and pH were measured in the model streams using a mercury-in-glass thermometer, Cyberscan DO 300, Cyberscan Con 300, Orbeco-Hellige 966, and Cyberscan pH 300 metres, respectively. These variables were also measured on each biological sampling day, i.e. day 90, 165, 207, 235, 263 and 284. Water and effluent samples were collected in acid-washed, 250 ml plastic bottles from the control and the effluent-treated streams, and then transported to the laboratory in an ice-filled cooler box. In the laboratory, within 24 hours, the collected samples were analysed for nitrate-nitrogen (NO_3-N), nitrite-nitrogen (NO_2-N), ammonia-nitrogen (NH_3-N), orthophosphate-phosphorus (PO_4-P), and free chlorine. Nitrate-nitrogen was analysed according to Ondrus (1996). Nitrite-nitrogen , NH_3-N, PO_4-P were analysed as described in Chapter 2, section 2.7. A Merck chloride test kit was used to analysed free-chlorine, according to the manufacturer's instructions.

Metal analysis

On days 90, 165, 207, and 235 effluent samples were collected from the treatment streams using 250 litre plastic bottles and analysed for the zinc (Zn), manganese (Mn), copper (Cu), lead (Pb) and chromium (VI) (Cr^{+6}). Inno Venton Analytica undertook the metal analyses. Inno Venton Analytical is accredited by the South African National Accreditation System (SANAS). With the exception of Cr^{+6}, metal analyses were undertaken using inductively coupled plasma spectrometry, according to EPA (1984) (EPA method number 200.7). Chromium (VI) Cr^{+6} was analysed using a Merck chromate test kit.

Effluent quality compliance

Effluent quality compliance of the Kelvin Jones WWTW was determined over the study period. For an effluent sample to be deemed compliant, at least 90% concentrations of the measured physico-chemical variables must be within the legislated limits as contained in the general authorisation, special permit or license applicable to the WWTW (DWA, 2011c). The Kelvin Jones WWTW operates with a license and the calculation of its effluent quality compliance was based on the license conditions. However, in this study, additional variables were measured and used together with variables in the license to determine the effluent quality compliance (Table 7.2); calculating the effluent quality compliance of the Kelvin

Jones was based on 11 variables. Discharge limits of variables not required in the license were based on the general authorisation (GA) (DWA 2011c) (Table 7.2).

Table 7.2 Wastewater effluent quality limits applicable to Wastewater Treatment Works (WWTWs) discharging into a water resource in South Africa (DWA, 2011c). The discharge limits with an asterisk (*) were obtained from the Kelvin Jones WWTW license (Nelson Mandela Bay Municipality 2008). Limits without an asterisk were obtained from the general authorisation (DWA, 2011c).

Variable	General limit
Ammonia-nitrogen (NH_3-N) (mg/l)	10*
Nitrate- nitrite-nitrogen (NO_3-N + NO_2-N) (mg/l)	15*
Orthophosphate-phosphorus (PO4-P) (mg/l)	1.0*
pH	5.5- 9.5*
Electrical conductivity (EC) (mS/s)	70 -150 above the background level of the receiving water resource
Free chlorine (Cl)(mg/l)	0.25
Chromium (VI) (Cr^{6+})(mg/l)	0.05
Copper (Cu) (mg/l)	0.01
Lead (Pb) (mg/l)	0.01
Manganese (Mn) (mg/l)	0.1
Zinc (Zn) (mg/l)	0.1

On each sampling occasion, the effluent quality compliance for both the 50% diluted and 100% undiluted effluent was calculated. Compliance was calculated using variables that were monitored over the study period. The concentrations of each measured variable was compared with the limit given in Table 7.2. The daily effluent compliance was calculated by dividing numbers of variables whose concentrations were compliant for that day by the total numbers of variables measured and then multiplying by 100%. The overall compliance of the effluent over the study period was calculated by dividing the sum of the numbers of compliant samples by the sum of the total numbers of samples collected and then multiplying by 100% (see formula below). In addition, each variable compliance over the study period was calculated by dividing the number of samples whose concentrations were compliant for that variable by the total numbers of samples and multiplying by 100% (DWA, 2011c). The final calculated percentage compliance in each case was compared to the 90% criterion (DWA, 2011c).

$$\text{Variable compliance over the study period} = \frac{\text{number of compliant samples}}{\text{Total number of samples}} \text{X } 100\%$$

$$\text{Overall effluent compliance } = \frac{\text{sum of number of compliant samples}}{\text{Sum of total number of samples}} \text{X } 100\%$$

7.2.5 Chlorophyll *a:* phytoplankton and periphyton analysis

On each sampling day (90, 165, 207, 235, 263 and 284), 200 ml of water and effluent were collected from each replicate control and treatment streams and analysed for phytoplankton chlorophyll *a* as described in Chapter 2, section 2.7.1. Concurrently, three stones were randomly selected from each replicate stream, a premeasured circular ring of 6.16 cm^2 was placed over a flat surface of the collected stones, and the area within the ring scraped, using a scraper. The scraped stone surface area was rinsed with distilled water into a beaker, filtered using a Whatman GF/F filter paper, and then the filter paper placed in a 10 ml tube containing 8 ml 90% acetone and analysed for periphyton chlorophyll *a* as described in Chapter 2, section 2.7.1.

7.2.6 Macroinvertebrate sampling

On each sampling day (90, 165, 207, 235, 263 and 284), two baskets, one with stones and the other with plastic ribbons (surrogate vegetation) were randomly collected from each replicate stream. Thus, during each sampling event, six quantitative samples each were collected from the control, 50% effluent- and 100% effluent-treated streams. Prior to the sampling, the stream valves regulating the supply of water and effluent were closed for few seconds to reduce the flow so that no macroinvertebrate was washed off the sampling unit. Once the water velocity was at acceptable rate, the two randomly selected baskets were gently removed from the stream at the same time and washed thoroughly, dislodging attached organisms onto two separate white rectangular trays. The area surrounding the baskets within each stream was also washed and the content emptied onto the respective trays. Immediately after the removal of the baskets from each stream, the valves were re-opened. After emptying the contents of the baskets into the trays, the baskets and the substrates were placed back in the streams and in subsequent sampling event on later dates, these baskets were not re-sampled until all the other baskets had been sampled.

In the trays, all solids were removed from the samples collected in the treatment streams. Removing solids from the control samples was not necessary. The samples were sieved

through a net of mesh size 200 µm and then the contents from the two baskets gently emptied into separate plastic bottles containing 70% ethanol. The samples were then transported to the laboratory for sorting, identification and abundance count. The contents of the two baskets collected from each stream per sampling occasion were processed separately. Gerber and Gabriel (2002) keys were used to identify macroinvertebrate families.

7.2.7 Statistical analysis

Effects of the effluent (50% and 100% concentrations) on the macroinvertebrate communities in the model streams were analysed using principal response curve (PRC) (van den Brink and ter Braak, 1999), with Canoco version 4.5 (ter Braak and Smilauer, 1998). The PRC is a multivariate method developed specifically for analysing model-stream community response over time, by focusing on the taxa composition of the treatment streams and those of the control streams collected at the same time.

The PRC generates a simple graph with the sampling time on the horizontal axis and the treatment effect (canonical coefficient *Cdt*) on the vertical axis expressed as deviation from the control (van den Brink and ter Braak, 1999). The PRC produced taxa weights that allowed an interpretation of the treatment effect on each taxon. A taxon with positive weight was more abundant in the control relative to the treatments, whereas a taxon with negative weight was more abundant in the treatments relative to the control. That is, a taxon with negative weight was less affected by the treatment than a taxon with positive weight. A taxon having near zero weight showed either no response or a response not related to the PRC (van den Brink and ter Braak, 1999; Choung *et al.*, 2013). The PRC is therefore able to identify important taxa, enabling discrimination between the control and the treatment streams. The Monte Carlo permutation test was used to evaluate the significance of the PRC model. Prior to the PRC analysis, macroinvertebrate abundance data were log (2x + 1) transformed (Choung *et al.,* 2013). The PRC was undertaken separately for macroinvertebrate collected for the stone substrate and the surrogate vegetation, and then the data were pooled for combined analysis.

The multivariate analysis of similarity (ANOSIM) based on the Bray-Curtis similarity index was used to assess significant differences between the control and the treatment streams per each sampling day. Pair-wise tests were used to compare each treatment to the control, and between the treatments (i.e. 50% and 100% effluent-treated streams). ANOSIM was applied

on log (x +1) transformed abundance data. Similarity percentage analysis (SIMPER) was also used to ascertain the percent dissimilarities between the control and the treatments per sampling event. This analysis was undertaken to investigate whether trends similar to those of the PRC would be observed. Both ANOSIM and SIMPER were carried out using Primer 5 version 5.2.9 (Clarke and Warwick, 1994).

The univariate analysis of variance ANOVA was used to analyse the responses of the taxa enabling the discrimination of the streams (indicated by the taxa weight from the PRC) to ascertain whether significant differences existed between the treatment and the control streams over time. The ANOVA was followed by the Tukey's honestly significant different (HSD) post-hoc test. The analysis was performed using all six samples per sampling occasion per the treatment and the control. One-way ANOVA was used to compare the physico-chemical variables and the chlorophyll *a* data between the streams. ANOVAs were undertaken using the Statistica software package version 9.

7.3 Results

7.3.1 Physico-chemical variables and effluent quality compliance

There were noticeable variations in the concentrations of electrical conductivity (EC) between the streams during the experimental period (Figure 7.6). The EC concentrations were consistently lower in the control streams than in the 50% and 100% effluent-treated streams. The overall highest EC concentration was recorded in the 100% effluent on day 165. One-way ANOVA, followed by the Tukey's HSD post-hoc test indicated that the mean EC concentration was significantly lower ($P < 0.05$) in the control streams than in the 50% and 100% effluent-treated streams. The mean EC concentrations between the 50% and 100% effluent-treated streams did not differ significantly ($P > 0.05$). The concentrations of EC in the 100% effluent-treated streams were 30% compliant with the required standard, but after 50% dilution, compliance of EC concentrations increased to approximately 50% during the experimental duration (Figure 7.7).

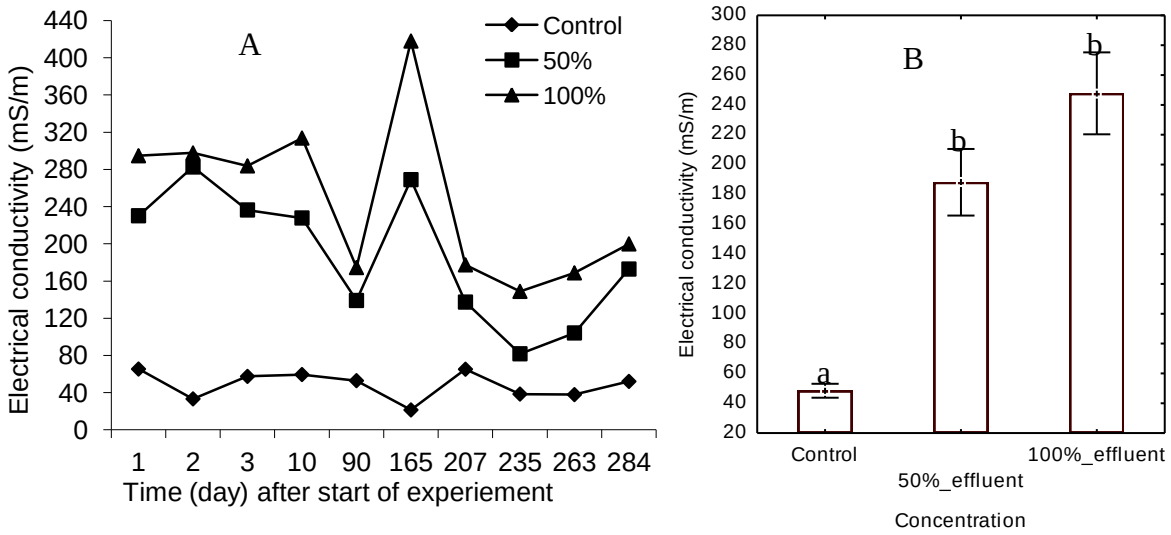


Figure 7.6: Concentrations of electrical conductivity (EC) on each sampling day in the control, 50% and 100% effluent-treated streams (A) and the means ± standard errors of EC (B) during the experimental period (August 2012 – May 2013). Different small alphabet letters on the bars indicate across the streams significant differences ($P < 0.05$), whereas the same alphabet letters across the streams indicate no significant differences by ANOVA and the Tukey's HSD test ($P > 0.05$).

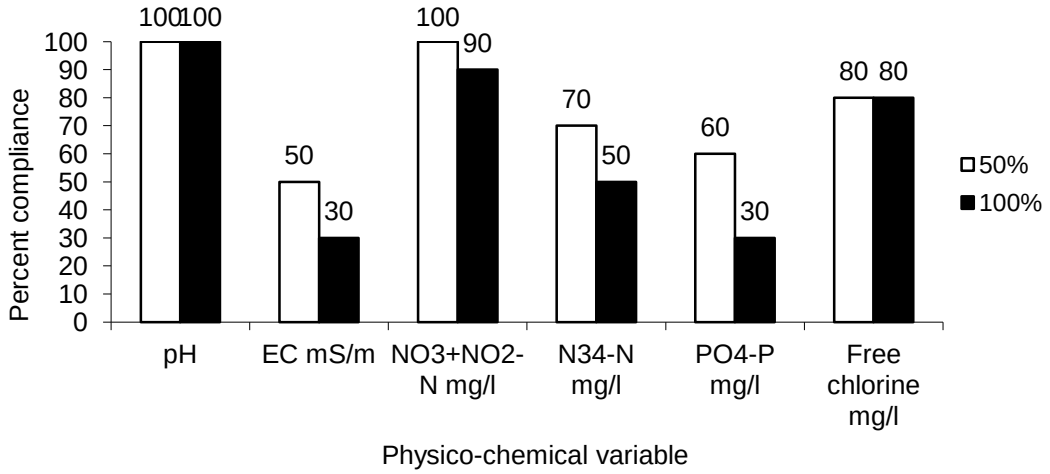


Figure 7.7: Percent compliance level of the concentrations of the measured effluent physico-chemical variables in the 50% and 100% effluent-treated streams during the experimental period (August 2013 – May 2013) showing that compliance was higher in the 50% effluent- than in the 100% effluent-treated stream.

The streams showed similar temperature and pH results (Figure 7.8). Nevertheless, pH was slightly higher in the 100% effluent-treated streams between days 1 and 90. One-way ANOVA revealed that the mean concentrations of pH and temperature were not statistically significantly different between the streams during the duration of the experiment ($P > 0.05$).

The results also showed that the concentrations of pH in the 50% and 100% effluent-treated streams were 100% within the compliance limits (Figure 7.7)

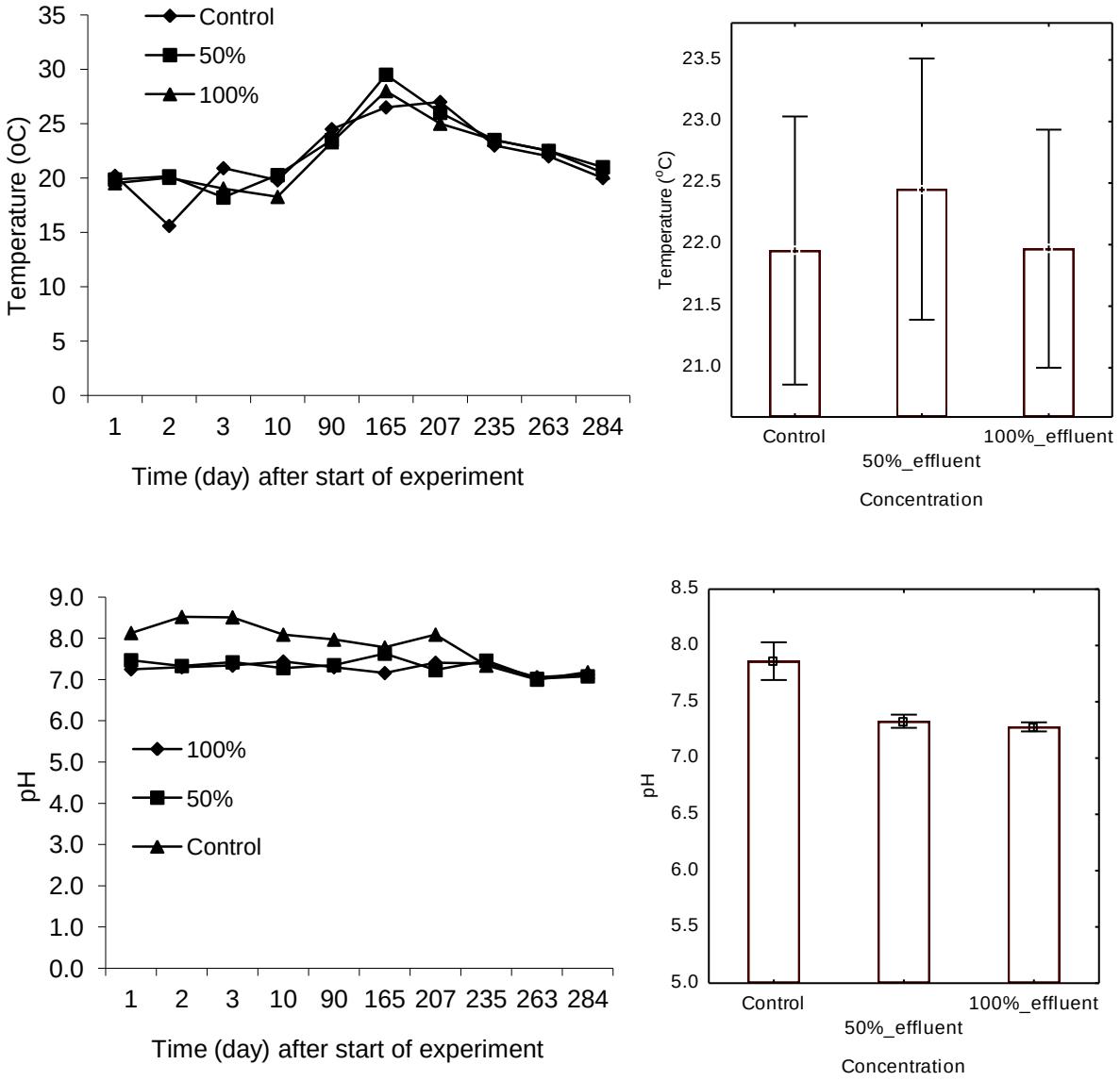


Figure 7.8: Temperature and pH on each sampling day in the control, 50% and 100% effluent-treated streams on the left and the means ± standard errors of temperature and pH on the right during the experimental period (August 2012 – May 2013).

Dissolved oxygen concentrations (DO) were consistently higher in the control streams than in the 50% and 100% effluent-treated streams throughout the experimental period (Figure 7.9). The overall highest DO concentration was recorded on sampling day 263 in the control streams. One-way ANOVA, followed by the Tukey's HSD post-hoc test showed that the mean DO concentrations differed significantly between all the three stream sets ($P < 0.05$).

The DO concentrations were significantly lower in the 100% effluent-treated streams than in the control and 50% effluent-treated streams indicating that the effluent affected dissolved oxygen level in the treatment streams.

Turbidity was relatively stable in the 50% and 100% effluent-treated streams between sampling days 1 to 10 and dropped to its lowest on day 90. A huge peak in turbidity was observed on day 263 in the treatment streams (Figure 7.9). One-way ANOVA, followed by the Tukey's HSD post-hoc test indicated that the concentrations of turbidity were significantly lower in the control streams than in the 50% and 100% effluent-treated streams ($P < 0.05$). The mean concentrations of turbidity did not differ significantly between the 50% and 100% effluent-treated streams ($P > 0.05$).

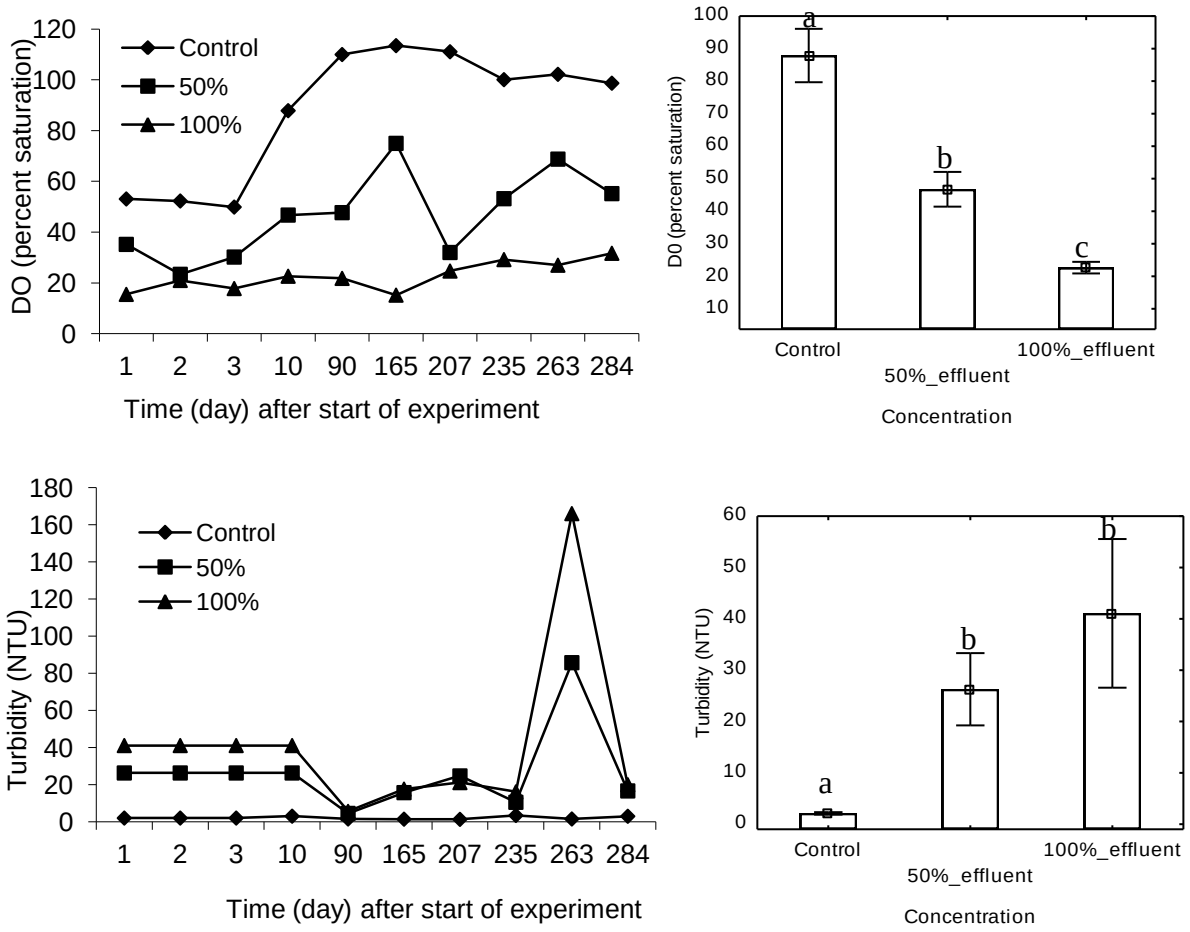


Figure 7.9: Concentrations of dissolved oxygen and turbidity on each sampling day in the control, 50% and 100% effluent-treated streams on the left and the means ± standard errors on the right, during the experimental period (August 2012 – May 2013). Different small alphabet letters on the bars across the streams indicate significant differences ($P < 0.05$), whereas the same alphabet letters across the streams indicate no significant differences by ANOVA and the Tukey's HSD test ($P > 0.05$).

The results revealed that there were noticeable variations in the concentrations of ammonia-nitrogen (NH_3-N) between the sampling days in the treatment streams, with peak concentrations recorded on days 10 and 263 in the 100% effluent- and on days 2 and 263 in the 50% effluent-treated streams (Figure 7.10). Generally, with the exception of day 3, NH_3-N was consistently highest in the 100% effluent-treated streams. One-way ANOVA, followed by the Tukey's HSD post-hoc test showed that the mean concentrations of NH_3-N were significantly lower in the control and higher in the 100% effluent-treated streams ($P < 0.05$). The overall compliance of concentrations of NH_3-N in the undiluted 100% effluent was only 50%, and after 50% dilution, 70% compliance was achieved (Figure 7.7).

Nitrate- and nitrite-nitrogen (NO_3-N + NO_2-N) concentrations showed significant variation between the streams ($P < 0.05$) (Figure 7.10) and noticeable variations between the sampling days in the 50% and 100% effluent-treated streams were observed, with concentration peaking on day 284 (Figure 7.10). With the exception of day 3, the nitrate- and nitrite-nitrogen were consistently highest in the 100% effluent-treated streams. The mean concentrations were significantly higher in the 100% effluent-treated streams than in the controls and 50% effluent-treated streams ($P < 0.05$). A comparison of the concentrations to the discharge limits (Table 7.2) revealed that they were 100% compliant in the 50% diluted effluent and 90% compliant in the undiluted effluent concentration (Figure 7.7). Generally, the NO_3-N + NO_2-N results revealed that nutrient fluctuated in the effluent-treated streams, but the concentrations were mostly below the discharged limits.

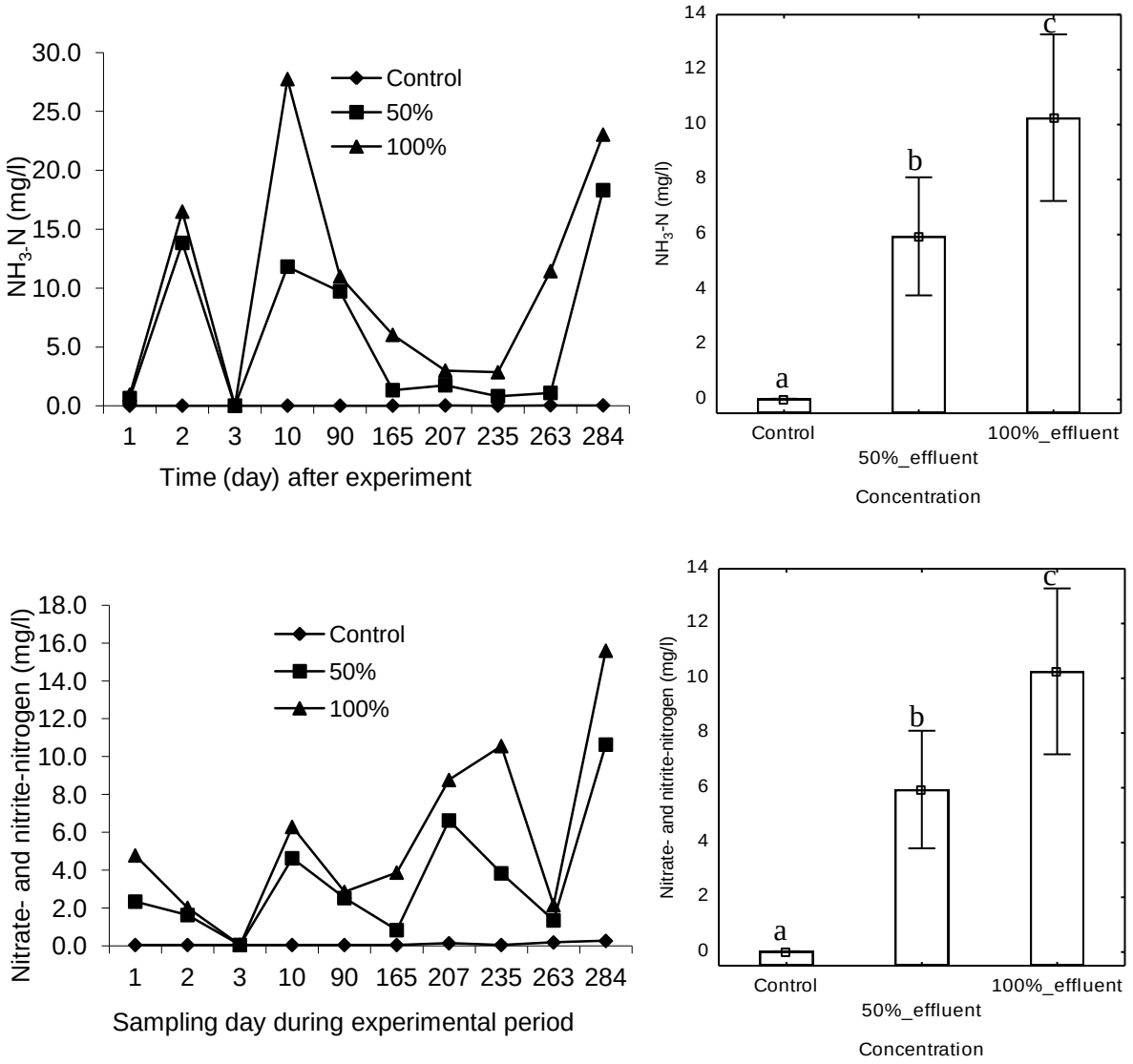


Figure 7.10: Ammonia-nitrogen and nitrate-and nitrite-nitrogen concentrations on each sampling day in the control, 50% and 100% effluent-treated streams on the left and the means ± standard errors on the right during the experimental period (August 2012 – May 2013). Different small alphabet letters on the bars across the streams indicate significant differences ($P < 0.05$), whereas the same alphabet letters across the streams indicate no significant differences by ANOVA and the Tukey's HSD test ($P > 0.05$).

Orthophosphate-phosphorus (PO_4-P) concentrations were considerably low on days 1, 3, 165, 207 and 235 during the experimental period (Figure 7.11). The concentrations of PO_4-P peaked on day 10 in the 50% diluted effluent and 100% effluent. The concentrations of PO_4-P in the control streams were low throughout the duration of the experiment. One-way ANOVA, followed by the Tukey's HSD post-hoc test showed that the mean concentrations of PO_4-P were significantly lower in the control streams than in the 50% and 100% effluent-

treated streams ($P < 0.05$). In term of compliance, the concentrations of PO_4-P in the 100% effluent was only 30% within compliance limit, but after 50% dilution, the compliance of the concentrations increased to approximately 60% (Figure 7.7) indicating that the effluent discharges were a main source of phosphorus input into the Swartkops River.

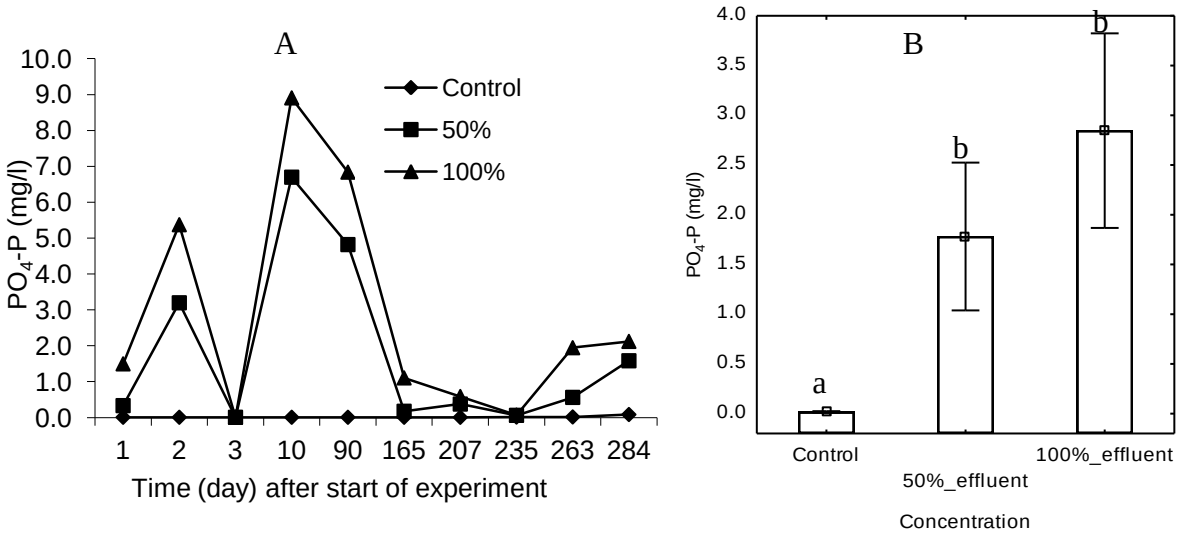


Figure 7.11: Orthophosphate-phosphorus concentrations on each sampling day in the control, 50% and 100% effluent-treated streams (A) and the means ± standard errors (B) during the experimental period (August 2012 – May 2013). Different small alphabet letters on the bars across the streams indicate significant differences ($P < 0.05$), whereas the same alphabet letters across the streams indicate no significant differences by ANOVA and the Tukey's HSD test ($P > 0.05$).

Peaks in free-chlorine concentrations corresponded to sampling days 165 and 263 during the experimental period (Figure 7.12). The first peak was recorded in the 50% diluted effluent, while the second was recorded in the 100% undiluted effluent concentration. During most of the sampling days, free-chlorine concentrations were within the compliance limits in both the 50% and 100% effluent concentrations (Figure 7.7). The free-chlorine concentrations were generally low in the control streams, below the compliance limits.

The concentrations of the five analysed metals measured on days 90, 165, 207 and 235 were generally low in the 50% and 100% effluents (Figure 7.12). Metals were not measured in the control de-chlorinated tap water. Apart from manganese (Mn), the results revealed that metal concentrations were generally below the acceptable limits contained in the general authorisation (Table 7.2). Thus, the concentrations of chromium (VI), copper, lead, and zinc in the 50% and 100% effluent-treated streams were 100% within the complaint limits.

However, the concentrations of manganese were only 25% compliant limits in the 100% effluent and 75% within the compliant limits in the 50% effluent (Figure 7.13).

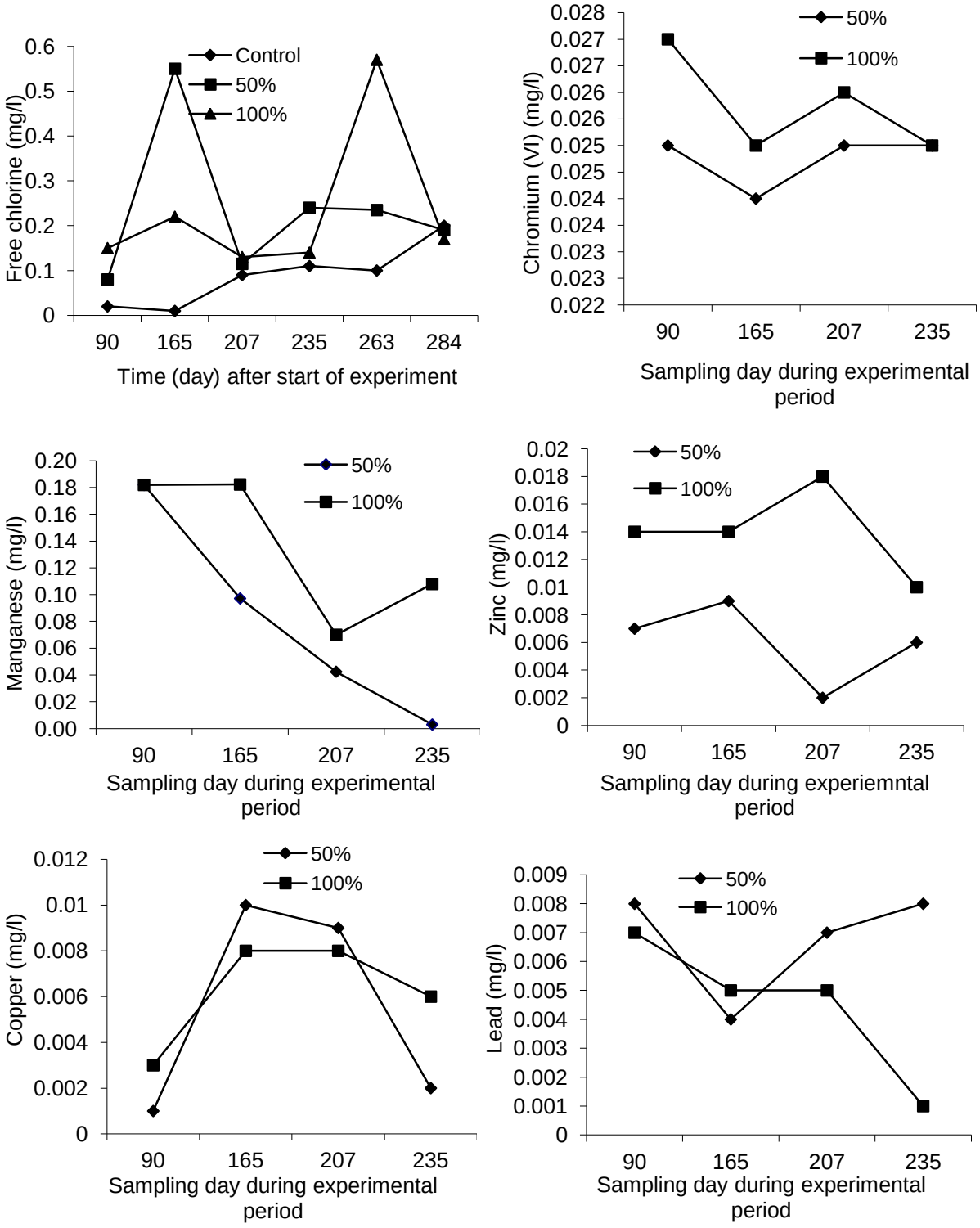


Figure 7.12: Free-chlorine concentration in the control and treatment streams, and the analysed metal concentrations on sampling day 90, 165, 207 and 235 in the 50% and 100% effluent-treated streams during the experimental period (August 2012 - May 2013).

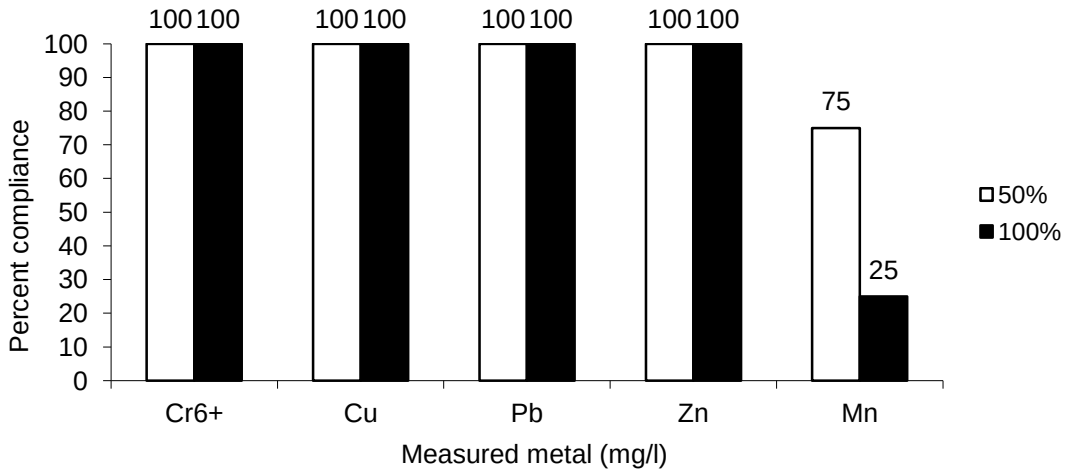


Figure 7.13: Percent compliance level of the concentrations of the analysed metals in the 50% and 100% effluent-treated streams showing that the concentrations of majority of the metals were 100% compliant during the experimental period (August 2012-May 2013).

The daily effluent quality compliance on each sampling day showed that the 100% undiluted effluent quality compliance was less than 70% on seven out of the ten sampling days (Figure 7.14). However, after 50% dilution, on six of the ten sampling occasions, the diluted effluent compliance was greater than 70% (Figure 7.14). On days 207, 263 and 284, the diluted effluent achieved 100% compliance, whereas the undiluted 100% effluent achieved 100% compliance on a single sampling event (i.e. day 207). The overall compliance of the undiluted effluent was approximately 68.4%, but after 50% dilution, the overall compliance increased to 81.6% over the study period (Figure 7.14).

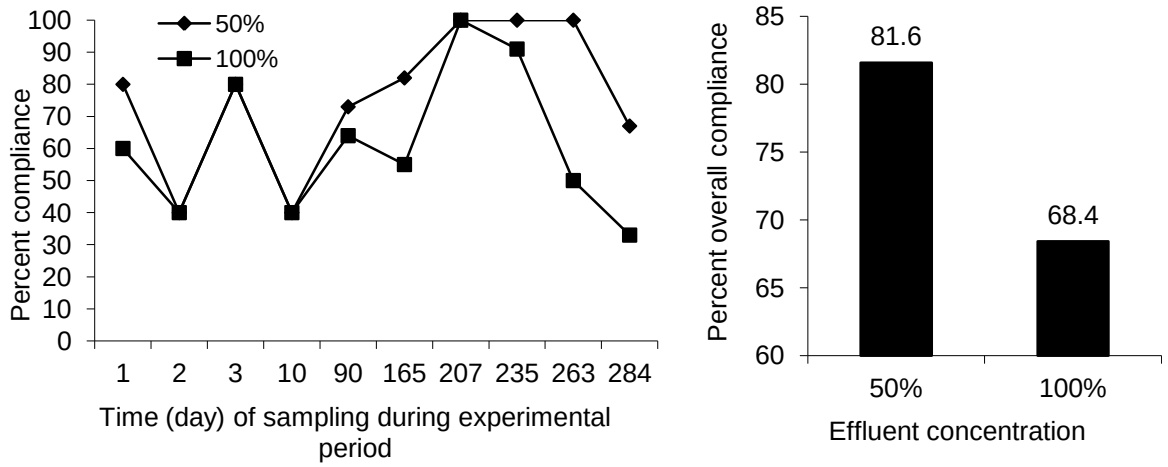


Figure 7.14: Percent effluent daily compliance levels (left) and the overall compliance levels of the effluent (right) in the 50% diluted and 100% undiluted effluent concentrations showing that the compliance increased from 68.4% to 81.6% after 50% dilution of the effluent during the experimental period (August 2012 - May 2013).

7.3.2 Macroinvertebrate community response to the treated wastewater effluent

Twenty-two macroinvertebrate taxa were recorded in the model streams during the experimental period (Appendix E). There were slight differences in the number of taxa recorded per streams (i.e. controls, 50% and 100% effluent-treated) over time (Figure 7.15). In the control streams, the highest number of taxa occurred on day 90, and the lowest number of taxa was recorded on day 207. In the 50% effluent-treated streams, the highest number of taxa was recorded on day 165 and the lowest number of taxa was recorded on days 90, 235 and 263 (Figure 7.15). In the 100% effluent streams, more macroinvertebrate taxa occurred on days 207 and 263 than the other sampling days. In terms of differences in the number of taxa between the control and treatment streams, with the exception of days 165 and 207, more macroinvertebrate taxa occurred in the control streams (Figure 7.15). On days 165 and 207, the 50% effluent-treated streams had more taxa than the control and 100% effluent-treated streams. Apart from day 263, the 100% effluent streams had fewer taxa than the remaining streams throughout the experimental duration (Figure 7.15).

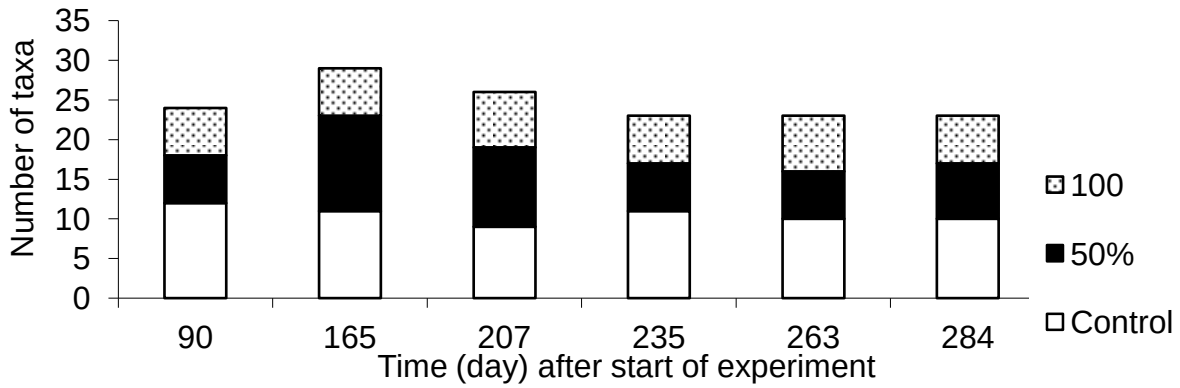


Figure 7.15 Number of macroinvertebrate taxa in the control, 50% and 100% effluent-treated streams on each of the sampling days during the experimental period (August 2012 - May 2013).

The principal response curve (PRC) for the combined data (i.e. data pooled from the stone and surrogate vegetation substrates) showed a clear deviation between the effluent-treated streams and the control (Figure 7.16). The macroinvertebrate communities in the 100% effluent streams deviated most from the control, with the time of greatest deviation being day 165 (Figure 7.16). The PRC analysis revealed that 71.60% of the total variance was caused by the effect of the effluent (i.e. treatment differences) whereas, differences between the sampling times accounted for only 9.40% of the total variance. The remaining 18.99% was attributed to the variability between the replicate streams. The first axis of the PRC with Eigen value 0.566 was statistically significant ($P < 0.05$) and it explained 79.05% of the variance captured by the treatment effect. Although the second axis with Eigen value 0.084 was also statistically significant ($P < 0.05$), it accounted for only 11.73% of the variance captured by the treatment effect. Thus, only the first PRC axis is shown in Figure 7.16.

Analysis of similarity (ANOSIM) indicated that the community compositions in the two treatments, i.e. the 50% and 100% effluents, were significantly different from the control on each of the sampling days ($P < 0.05$). However, between the treatments, the community compositions in the 100% effluent-treated streams were significantly different from those in the 50% effluent-treated stream on days 90, 165 and 207 ($P < 0.05$). No significant differences were observed between the two treatments on the remaining sampling days. Similarity percentage analysis (SIMPER) supported the overall trends seen in the PRC (Appendix E, Figure E1). The highest percent dissimilarity was consistently observed between the control and 100% effluent-treated streams, whereas the two treatments had the

lowest percent dissimilarities throughout the duration of the experiment (Appendix E, Figure E1).

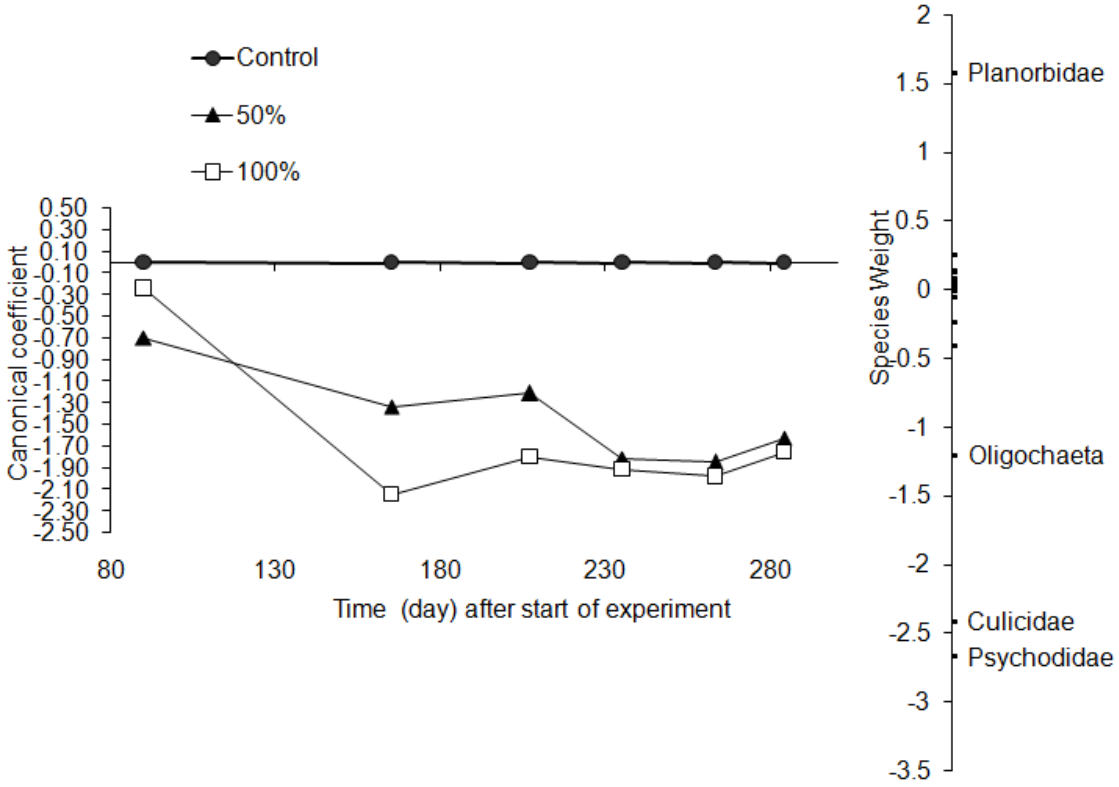


Figure 7.16: Principal response curve with taxa weights showing the macroinvertebrate community response to the treated wastewater effluent from the Kelvin Jones WWTW. Taxa with weights between 0.5 and -0.5 has been omitted for clarity. The PRC was undertaken using macroinvertebrate combined data for macroinvertebrate collected from the stone and surrogate vegetation substrates.

The PRC taxa weights showed that Planorbidae was the taxon most affected by the effluent. The abundance of Planorbidae decreased in the treatment streams. Conversely, taxa such as Psychodidae, Culicidae and Oligochaeta were less affected by the treatment, and their abundance increased in the treatment streams relative to the control streams. The remaining taxa not shown in Figure 7.16 had weights near zero and were deemed to have shown no response or a response unrelated to the PRC.

When the PRC was undertaken separately on macroinvertebrate data collected from the two substrates (stones and surrogate vegetation) over the experimental period, the individual PRCs indicated trends similar to the PRC of the combined data (i.e. pooled data from both substrates) described above. However, the differences between the macroinvertebrate community compositions in the 50% and 100% effluent-treated streams were more pronounced for the macroinvertebrate communities collected on the stone substrates (Figure

7.17). Between days 207 and 284, the PRC for the macroinvertebrate data collected on the surrogate vegetation indicated that the community compositions in the two treatments were very similar (Figure 7.18).

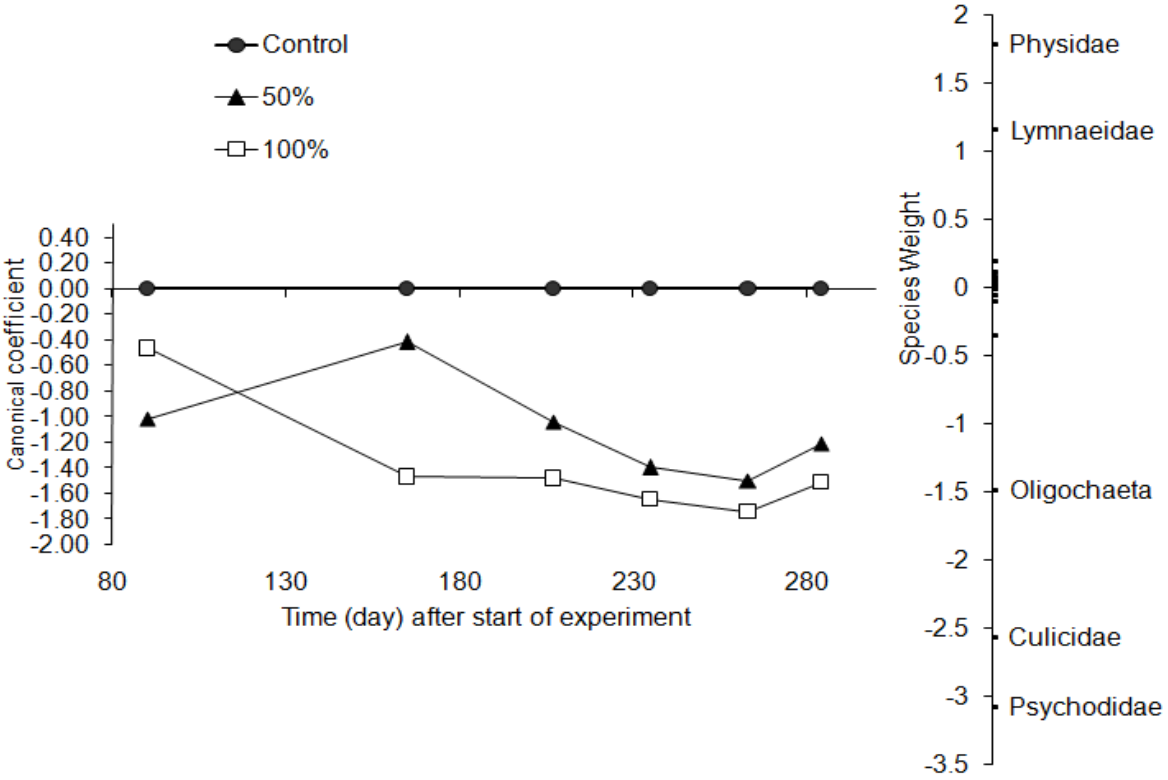


Figure 7.17: Principal response curve with taxa weights showing the response of the macroinvertebrate community collected on the stone substrates to the treated wastewater effluent from the Kelvin Jones WWTW. Taxa with weights between 0.5 and -0.5 has been omitted for clarity.

The PRC for the macroinvertebrate data collected on the stone substrate showed that the treatment effects explained 64.10% of the total variance, while 8.40% variance was explained by the differences between the sampling times. The remaining 27.5% was attributed to variability between the replicate streams. The first axis of the PRC with Eigen value 0.515 was statistically significant ($P < 0.05$) and explained 80.34% of the variance accounted for by the treatment effect. The second axis was not statistically significant ($P > 0.05$) and explained only 10.45% of the variance captured by the treatment effect.

The PRC for the macroinvertebrate data collected on the surrogate vegetation substrate showed that the treatment (effluent) effects explained 71.60% of the total variance, whereas differences between the sampling times accounted for 9.40% of the variance. The remaining 19% was attributed to variability between the stream replicates. The first axis with Eigen value 0.566 of the PRC accounted for 79.05% of the variance explained by the treatment

effect. Although the second axis with Eigen value 0.084 was statistically significant ($P < 0.05$) it accounted for only 11.73% of the variance captured by the treatment effect. Thus, it was not presented in the PRC diagram (Figure 7.18).

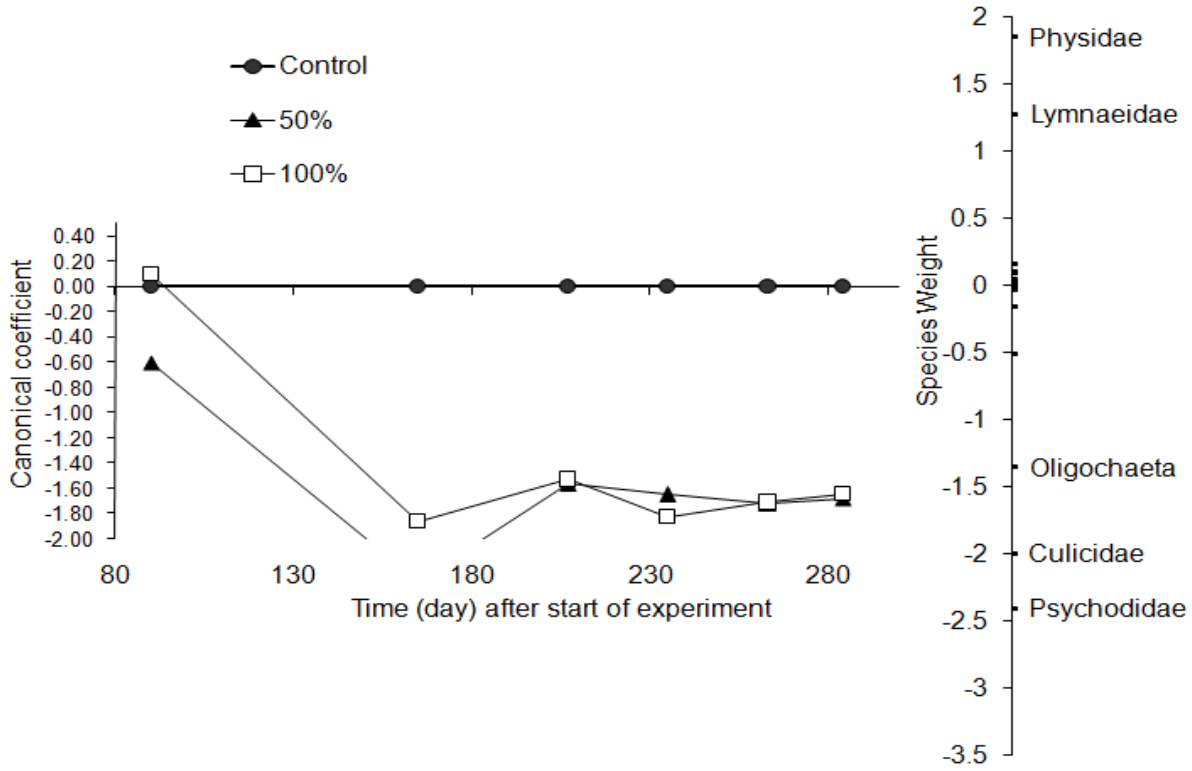


Figure 7.18: Principal response curve with taxa weights showing the response of the macroinvertebrate community collected on the surrogate vegetation substrates to the treated wastewater effluent from the Kelvin Jones WWTW. Taxa with weight between 0.5 and -0.5 has been omitted for clarity.

The taxa weights in the two PRCs (i.e. the stone and surrogate vegetation macroinvertebrate data) indicated that the effluent less affected the same taxa (Oligochaeta, Culicidae, Psychodidae). The abundance of these taxa increased in the effluent-treated streams relative to the controls. However, unlike the PRC of the combined data, taxa weights in the PRCs for the stone and surrogate vegetation macroinvertebrate data revealed that Physidae and Lymnaeidae were the taxa most affected by the effluent and declined in abundance in the effluent-treated streams relative to the controls (Figures 7.17 and 7.18).

7.3.3 Responses of the taxa that enabled the discrimination of the streams indicated by the PRC analyses

Planorbidae, Physidae and Lymnaeidae

Planorbidae did not occur in the 50% and 100% effluent concentration streams at any time during the experimental period (Figure 7.19). The highest abundance of the planorbids in the controls was recorded on day 165, decreased towards day 207 and 235, and increased slightly after 263 days. On the first sampling day, i.e. day 90, no individual of Physidae was recorded in the streams. Thereafter, the abundance of the physids was consistently highest in the control streams, with the period of highest abundance corresponding to sampling day 165 (Figure 7.19). The physids did not occur in the 100% effluent-treated streams and when they did occurred in the 50% effluent-treated streams, their mean abundances were consistently less than 10%, and significantly different from the control streams ($P < 0.05$) (Figure 7.19).

Although no individual of Lymnaeidae was caught in the 100% effluent treated streams, their abundances were similar in the control and 50% effluent-treated streams (Figure 7.19). Analysis of variance did not indicate statistical significant difference between the control and 50% effluent-treated streams in terms of the lymnaeids abundance.

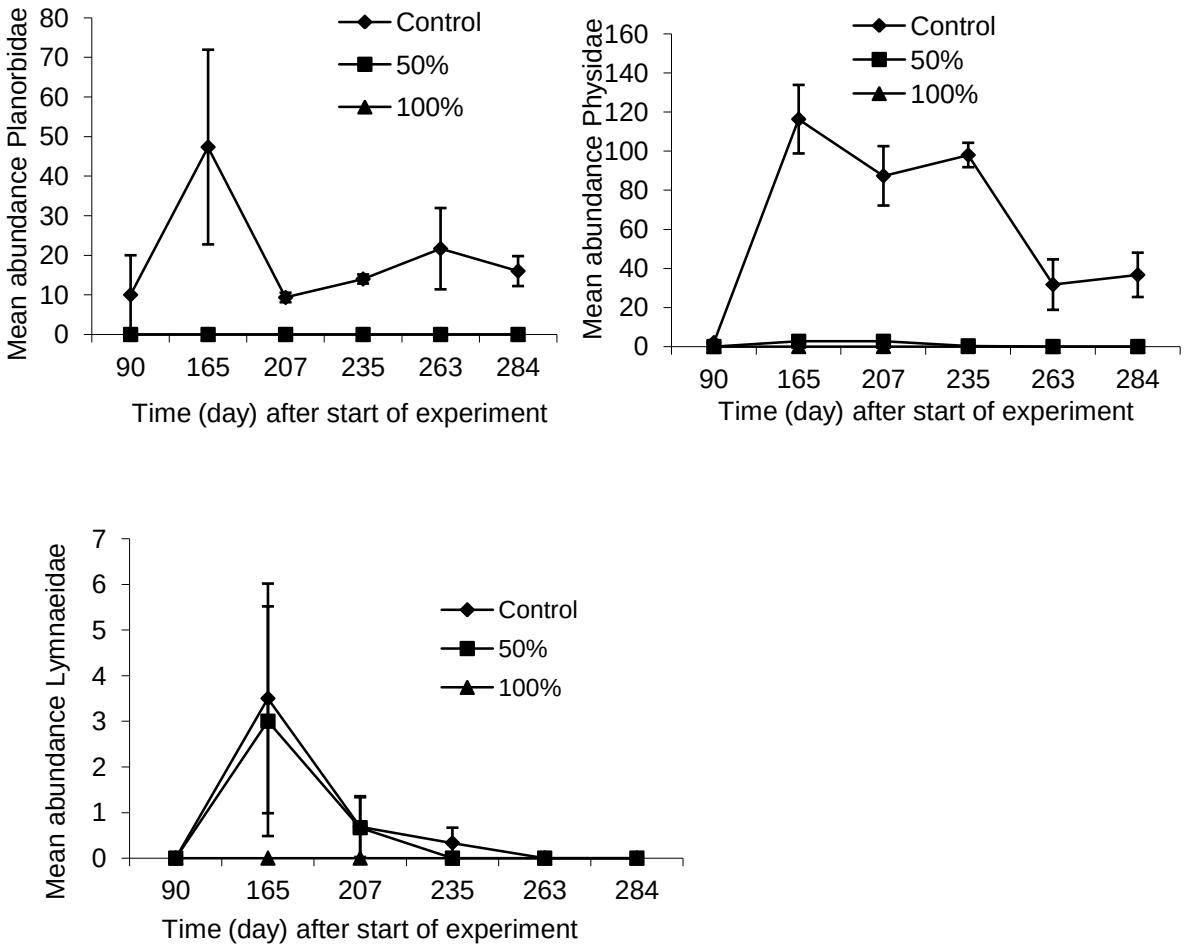


Figure 7.19 Mean abundance ± standard error of Planorbidae, Physidae and Lymnaeidae in the control, 50% and 100% effluent-treated streams on each sampling day showing that the abundance of these taxa were consistently higher in the control streams than in the effluent-treated streams during the experimental period (August 2012 - May 2013).

Oligochaeta, Culicidae and Psychodidae

The abundance of the oligochaetes was consistently lowest in the 100% effluent-treated streams, and consistently highest in the 50% effluent-treated streams (Figure 7.20). One-way ANOVA, followed by the Tukey's HSD post-hoc test revealed significant differences across the streams on sampling days 165, 235 and 263 when the oligochaetes were significantly more abundant in the 50% effluent treated streams compared to the control and 100% effluent treated streams ($P < 0.05$).

The culicids were more abundant in the 100% effluent-treated streams compared to the control and 50% effluent-treated streams (Figure 7.20). One-way ANOVA, followed by the Tukey's HSD post-hoc test indicated that on days 165 and 263, the abundance of the culicids

was significantly higher in the 100% effluent-treated streams than in the control and 50% effluent-treated streams ($P < 0.05$). However, on sampling day 235 no significant difference was indicated between the 50% and 100% effluent-treated streams ($P > 0$ .05).

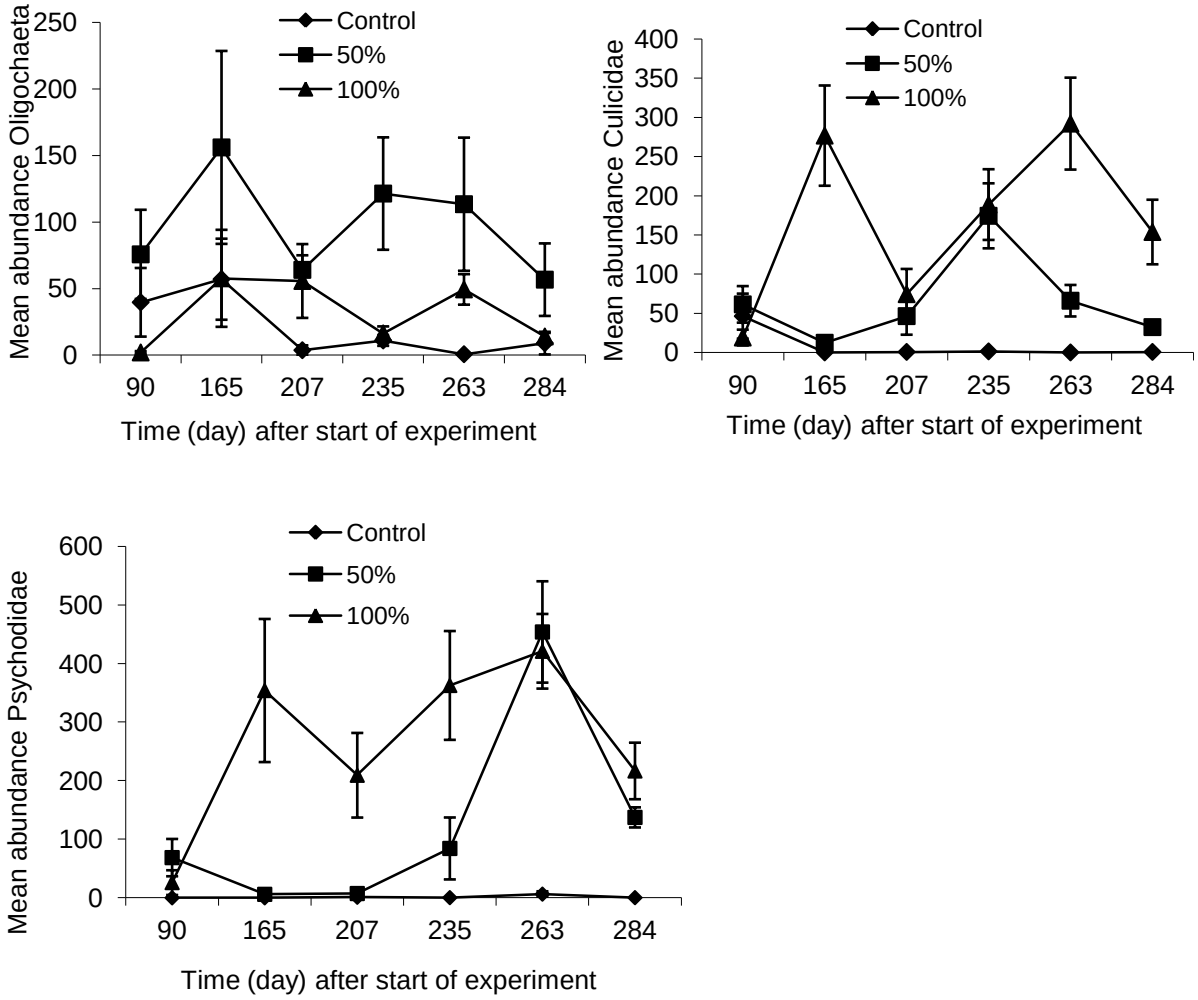


Figure 7.20 Mean abundance ± standard error of Oligochaeta, Culicidae and Psychodidae in the control, 50% and 100% effluent-treated streams on each sampling day during the experimental period (August 2012 - May 2013).

Throughout the experimental period, no individuals of the Psychodidae were recorded in the control streams (Figure 7.20). The psychodids were significantly more abundant in the 100% effluent-treated streams on sampling days 165, 207 and 235 than in the control and 50% effluent-treated streams ($P < 0.05$). The abundance of the psychodids on sampling days 263 and 284 was similar between the 100% and 50% effluent-treated streams (Figure 7.20).

7.3.4 Chlorophyll *a*: phytoplankton and periphyton

With the exception of sampling day 165, the concentration of phytoplankton chlorophyll *a* was consistently higher in the control streams than in the 50% and 100% effluent-treated streams (Figure 7.21). On sampling day 165, the concentration of phytoplankton chlorophyll *a* in the 50% effluent-treated streams was higher than in the control and 100% effluent treated streams (Figure 7.21). One-way ANOVA, followed by the Tukey's HSD post-hoc test showed that the mean concentrations of phytoplankton chlorophyll *a* was significantly higher in the control streams ($P < 0.05$) than in the 50% and 100% effluent-treated streams. However, there was no statistically significant difference in the concentration of phytoplankton chlorophyll *a* between the 50% and the 100% effluent-treated streams ($P > 0.05$).

The concentrations of periphyton chlorophyll *a* were higher in the control streams on sampling days 90, 235 and 263, but declined towards the end of the experiment (Figure 7.21). The concentrations of periphyton chlorophyll *a* were higher in the 50% effluent-treated streams on sampling days 165, 207 and 284 than in the control and 100% effluent-treated streams (Figure 7.21). Although there were variations in the periphyton concentrations across the streams over the duration of the experiment, one-way ANOVA did not indicate statistically significant differences between the streams ($P > 0.05$).

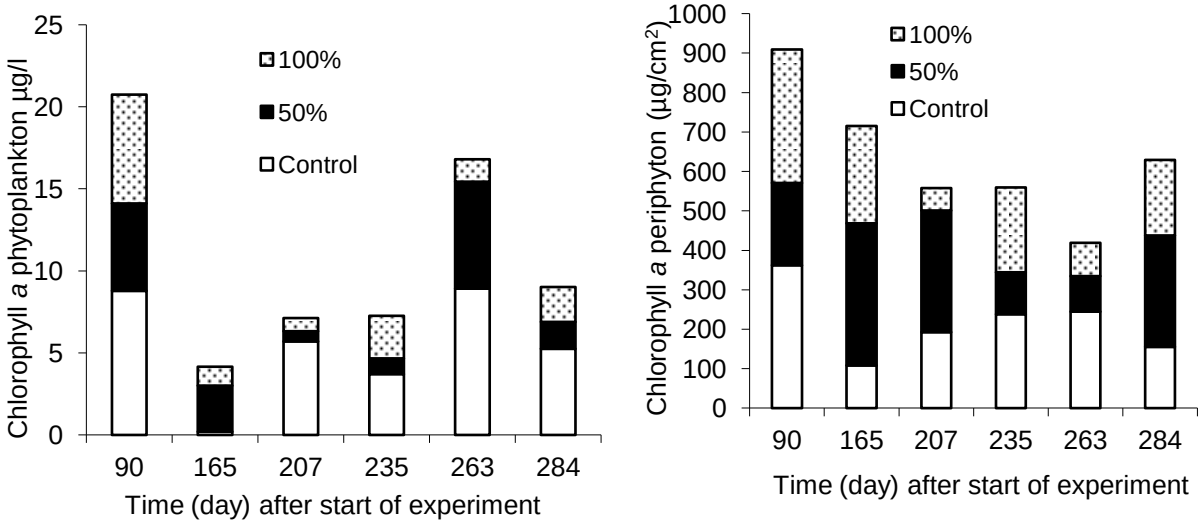


Figure 7.21: Chlorophyll a phytoplankton and periphyton concentrations in the control, 50% and 100% effluent-treated streams on each sampling day during the experimental period (August 2012-May 2013).

7.4 Discussion

7.4.1 Effluent quality compliance and physico-chemical variables

The South African Department of Water Affairs uses only physico-chemical variables as the basis for managing wastewater effluent quality (DWAF, 2009; DWA, 2011c). In this study, selected physico-chemical variables were measured to ascertain the quality and compliance of the final treated effluents from the Kelvin Jones wastewater treatment work discharging into the Swartkops River. The results revealed elevated concentrations of ammonia-nitrogen (NH_3-N), orthophosphate-phosphorus (PO_4-P), electrical conductivity (EC), manganese (Mn) and turbidity in the 50% diluted and 100% undiluted effluents, and the compliance of the concentrations of these variables were far below the 90% compliance criterion (Figures 7.7 and 7.13).

Although the overall compliance of the effluent improved (81.6%) after 50% dilution, the results revealed that, despite the improvement, the diluted effluent still significantly influenced the macroinvertebrate communities, deviating clearly from the controls (Figures 7.16, 7.17 and 7.18). The implication is that the current method of measuring effluent quality compliance, which relies on physico-chemical and microbiological variables alone, may not guarantee the protection of effluent-receiving biological communities. The current method assumes that each effluent quality variable is an independent entity, and does not consider possible interactions between chemicals (Vermeulen, 1995). However, wastewater effluents are a complex mixture of chemicals, whose synergistic interactions could aggravate their effects on the aquatic biological communities (Slabbert, 2004). Thus, the clear deviations of the macroinvertebrate communities between the effluent-treated streams and the controls (particularly in the 50% diluted effluent, despite the improvement in the compliance of the concentrations physico-chemical variables) could be attributed to chemical interactions potentially aggravating the effects of the effluent on the macroinvertebrate communities.

Elevated nutrient concentrations coupled with other factors, such as sufficient in-stream retention time and reduced capacity to dilute nutrients in a receiving water body, could have deleterious effects in the aquatic ecosystems (van Ginkel, 2011). Wastewater effluent discharges have been implicated as the main contributors to the elevated nutrient concentrations in the Swartkops River (de Villiers and Thiart, 2007; Odume and Muller, 2011; Chapter 3), and although the Department of Water Affairs has imposed stricter nutrient

criteria on the Kelvin Jones WWTW (Nelson Mandela Bay Municipality, 2008; Table 7.2), the results of the present study revealed that, of the analysed physico-chemical variables, nutrients were among the least compliant (Figure 7.7). In particular, NH_3-N and PO_4-P were far below the 90% compliant criterion, indicating that the effluent from the Kelvin Jones WWTW is still an important contributor to the Swartkops River nutrient loads. This claim is supported by the elevated nutrient concentrations recorded at sites (i.e. Sites 3 and 4) downstream of the discharge point, which were higher than concentrations recorded at the upstream Sites 1 and 2. Interestingly, even after diluting the effluent by 50%, the concentrations of both NH_3-N and PO_4-P remained far below the 90% compliant criterion. This is a cause for concern because during low flows, effluent discharges from the Kelvin Jones WWTW constituted approximately 60% of the river flow at sampling Site 3 in the Swartkops River (*Slaughter personal comm.*); and, together with other effluents from the Despatch and KwaNobuhle WWTWs, contribute about 50% of the Swartkops River downstream flow (Taljaard *et al.,* 1998). Thus, it is necessary to improve the nutrient removing efficiency of the Kelvin Jones WWTW, or to develop appropriate measures, in collaboration with the Department of Water Affairs, so that the effluent discharges constitute far less than 50% of the river flow throughout the year. This would provide for adequate in-stream dilution. Nevertheless, if this course is followed, then it needs to be balanced with the in-stream flow requirements of the river, as the discharged effluent is an important component sustaining the river's in-stream flow (Taljaard *et al.*, 1998).

The concentrations of electrical conductivity (EC) in the 50% and 100% effluent-treated streams were significantly higher than those in the controls (Figure 7.6). The elevated concentrations of EC could have contributed to the observed macroinvertebrate response in the effluent-treated streams as EC is an important variable directly related to osmotic and homeostatic regulation among aquatic organisms (Dallas and Day, 2004; Palmer *et al.*, 2005). The EC concentrations were among those with the lowest percent compliance (Figure 7.7). Although after diluting the effluent by 50%, the percent EC concentration compliance rose from 30% to 50%, the results suggested that the effluent discharges are a major contributor to the elevated in-stream EC concentrations at Sites 3 and 4 in the Swartkops River, which were significantly higher than Sites 1 and 2 upstream of the discharge point (Chapter 3).

Differences in influent daily loads from events such as storm-water run-off, and changes in the patterns of discharges from industrial facilities into public wastewater treatment works could cause major changes to the quality of the final, treated effluent. For example, during the

experimental period, high amount of solids were found in the influent on several occasions, and these corresponded to sampling events when high turbidity was recorded in the experimental streams, and in particular, on day 263. Elevated levels of turbidity can have deleterious biological effects through gill clogging, and can cause dissolved oxygen (DO) depletion through decomposing organic matter (Bilotta and Braizer, 2008). This probably explained the significantly low DO concentrations in the effluent-treated streams, which could affect the survival of organisms that are sensitive to oxygen depletion. Macroinvertebrates less reliant on dissolved oxygen concentrations were less affected in the effluent-treated streams (Figures 7.16 and 7.20).

The elevated concentrations of nutrients recorded in the effluent-treated streams, led the researcher to expect higher concentrations of phytoplankton chlorophyll *a* in these streams than in the controls. However, the results showed that the effluent significantly reduced the growth of phytoplankton in the 50% and 100% effluent-treated streams (Figure 7.21). The high turbidity, which limited light penetration and thus photosynthetic activities (DWAF, 1996b) in these streams, is a plausible explanation for the significantly reduced phytoplankton chlorophyll *a* concentrations. These results corresponded with the field-based results in Chapter 3 indicating elevated turbidity levels, and reduced phytoplankton chlorophyll *a* concentrations at Sites 3 and 4 than at Sites 1 and 2 in the Swartkops River.

The composition of final treated effluent is determined primarily by the origin of the influent and the level and quality of the treatment the effluent is subjected to (Popa *et al.*, 2012). Although some of the influent into the Kelvin Jones WWTW is from industrial sources, the concentrations of the measured metals were generally below the discharge limits in the general authorisation (GA) (Table 7.2). Apart from manganese (Mn), the concentrations of all the metals were 100% compliant (Figure 7.13). The Nelson Mandela Bay Municipality has an effluent trade unit that enforces the municipal bye laws requiring industries that discharge effluents into the treatment works to achieve approved effluent standards before such discharges (Maduma *personal comm.*). Therefore, the pre-treatment of industrial effluent before discharging into the sewer system was probably responsible for the generally low metal concentrations recorded in the 100% undiluted, and the 50% diluted effluent concentrations. However, continuously detecting metals in the effluents, though below acceptable limits, could contribute to elevated metal loads in the sediments of the receiving Swartkops River. Sediment acts as a sink for most metals and accumulates metals to levels several-fold higher than the concentrations in the water column (Beasley and Kneale, 2002).

Although metal concentrations were not measured in the Swartkops River's sediment in this study, an earlier study by Binning and Baird (2001) indicated elevated metal concentrations in the river's sediment. Furthermore, the concentrations of the metals analysed in the water of the Swartkops River, reported in Chapter 3 of this thesis, were in ranges most likely to be chronically toxic to the downstream river biota. Although, based on the results presented in this chapter, it could be argued that the effluent discharges contributed little to the metal loads of the river, there is a need to initiate a long-term metal monitoring programme for the Kelvin Jones WWTW. Such programme would provide long-term trends, which could lead to definite conclusions regarding the potential contribution of the effluents to the in-stream metal concentrations.

Overall, the results of the physico-chemical variables presented in this chapter, and their correspondence with the field-based physico-chemical results presented in Chapter 3 provided evidence that the effluents from the Kelvin Jones WWTW are a major contributor to the deteriorating water quality in the Swartkops River. These results add to the lines of evidence gathered in previous chapters that suggested that the wastewater effluent discharges were the main anthropogenic factors responsible for the deteriorating water quality in the Swartkops River.

7.4.2 Macroinvertebrate community response to the treated wastewater effluent

Isolating the potential biological effects of the Kelvin Jones wastewater effluents from other landscape activities to explore a causal relationship is critical to developing appropriate management strategies for the Swartkops River. The results presented in this chapter indicated that long-term exposure to the treated wastewater effluent from the Kelvin Jones WWTW has significant negative effects on the macroinvertebrate communities in the model-stream ecosystems (Figure 7.16). The wastewater effluent reduced the number of macroinvertebrate taxa that colonised and survived in the effluent-treated streams (Figure 7.15). The effects were greater in the 100% undiluted effluent than in the 50% diluted effluent (Figures 7.16, 7.17 and 7.18).

The PRC attributed the changes in the macroinvertebrate assemblages to the effluent effects rather than variability between the times of samplings or between the replicate streams. Thus, the results revealed that the wastewater effluent was the main factor responsible for the observed changes in the macroinvertebrate community structure in the model-stream

ecosystems. The Planorbidae, Physidae and Lymnaeidae were completely absent from the 100% effluent-treated streams throughout the experimental period and their abundances were significantly reduced in the 50% effluent-treated streams (Figure 7.19). These pulmonate snails rely on lungs for respiration (Day and de Moor 2002b) and in Chapter 4 it was shown that deteriorating water quality in the Swartkops River, particularly at Sites 3 and 4, downstream of the discharge point of the Kelvin Jones wastewater treatment works, significantly reduced the abundance of macroinvertebrates that rely on lungs for respiration. Therefore, the correspondence between the model-stream ecosystem and the biomonitoring results suggests that the discharged wastewater effluent was primarily responsible for the almost complete elimination of the pulmonate snails at Sites 3 and 4 in the Swartkops River (Appendix F). The pulmonate snails are important component of the aquatic food webs (Bronmark, 1989), and they play important ecological and ecosystem engineering roles (Hunter, 1980; Gutierrez *et al.*, 2003), and thus, their almost complete elimination may have serious ecological consequences at Sites 3 and 4. For example, the pulmonate snails are mostly herbivorous and detritivorous grazing on attached periphyton, and feeding on detritus (Bronmark, 1989). Hunter (1980) found that snails reduced the abundance of periphyton significantly. Their presence at Sites 3 and 4 would probably have contributed to keeping in check the preponderance of periphyton that characterised the sites during the study period (Chapter 3).

The PRC taxa weights showed that the treated wastewater effluent affected Oligochaeta, Culicidae and Psychodidae less, and their abundances increased in the effluent-treated streams relative to the controls (Figure 7.20). These taxa are less affected by the reduced dissolved oxygen concentrations that characterised the 50% and 100% effluent-treated streams. The macroinvertebrate taxonomic metrics percent Chironomidae + Oligochaeta was found to increase in abundance at Sites 3 and 4 relative to Sites 1 and 2 in the Swartkops River (Chapter 3), indicating that trends observed in the field-based surveys were also being detected in the model-stream ecosystems. These findings are consistent with those of Grantham *et al.* (2012) who reported increased Oligochaeta abundance in effluent-treated model streams relative to their control streams. The culicids and the phychodids have spiracles that enable them to utilised atmospheric oxygen (i.e. aerial respiration), becoming less reliant on dissolved oxygen concentrations. Aerial respiration was found to increase at Sites 3 and 4 downstream of the discharge point (Chapter 4), further supporting a correspondence between the field-based and experimental macroinvertebrate responses.

The PRC analysis undertaken separately for the substrate types (stones and surrogate vegetation) showed clear community responses for macroinvertebrates collected from each of the substrates (Figures 7.17 and 7.18). However, although the macroinvertebrate communities collected from the stones were differentiate in the 50% and 100% effluent-treated streams, this distinction was not clear for the communities collected from the vegetation, particularly between days 207 and 284 (Figure 7.17). The number of plastic ribbons, which served as surrogate for vegetation in the streams, was minimal as it caused clogging in the effluent-treated streams. Reducing the quantity of the surrogate vegetation reduced the surface area available for colonisation and probably explains why a lower number of taxa occurred in this substrate. The results indicated that the stone substrate alone supported enough macroinvertebrate taxa to reveal clear differences between the treatment streams (Figure 7.17). The model-stream results are consistent with field-based biomonitoring studies that have indicated that the stone biotope supported more taxa than other biotope groups such as sediments and vegetation (Dallas, 2007a).

The observed macroinvertebrate community response to the experimental treatment showed that the model-stream ecosystem approach developed in this study was an effective method for investigating long-term community effects of treated wastewater effluent. Although the stream channels could be considered as simplified, less dynamic systems compared to the nearby Swartkops River, important factors considered during the design of the streams improved the environmental realism of the model streams. First, the open flow-through system design and the closeness of the streams to the river may have facilitated colonisation of the streams by macroinvertebrates from the river, with more than 50% of macroinvertebrate taxa recorded in the river during the field-based surveys occurring in the model streams (Appendix E). Situating model streams close to a source river has been found to promote realism and facilitates stream invertebrate colonisation (Ledger *et al.,* 2009). Second, the streams were not artificially seeded with macroinvertebrates, thereby allowing for natural colonisation and recruitment. The non-introduction of the organisms removed a bias that would have otherwise complicated the interpretation of the data because Ledger *et al.* (2006) found that seeded model streams had macroinvertebrate communities different from those naturally colonised that could lead to attributing observed effects to treatment, when in reality such effects were caused by differences in seeding efforts. Furthermore, Ledger *et al.* (2009) argued that natural colonisation and community development in model-stream ecosystems promote ecological realism as naturally colonised systems developed

communities similar to those in the nearby river system. Third, the arrangement and design of the baskets containing the substrates created pool-like and riffle-like conditions alternating along the length of each stream channel, again re-creating the pools and riffles that are a natural characteristic of river ecosystems.

7.5 Conclusion

In this chapter the effects of the wastewater effluent were explored and the results indicated that the final treated effluent has significant effects on the macroinvertebrate communities. The significantly reduced abundance of the physid, planorbid and lymnaeid snails, as well as the increased abundance of culicids, psychodids and oligochaetes in the effluent-treated model streams, which corresponded to the field-based observations at Sites 3 and 4, provided biological effect evidence that the discharges from the Kelvin Jones WWTW have negative effects on the Swartkops River macroinvertebrate assemblages. The biological evidence is supported by the results of the effluent physico-chemical quality, showing non-compliance, and suggesting that the wastewater effluent discharges contributed to the elevated nutrients and turbidity, and to the depleted oxygen concentration recorded at sites downstream of the effluent discharge point in the river (Chapter 3). Overall, the results in this chapter added to the lines of evidence indicating that the wastewater effluent discharges were the main anthropogenic drivers of the impaired river health of the Swartkops River.

From a management perspective, isolating the effects of the effluent from other landscape activities is important because the Swartkops River is located within an urbanised catchment, and thus it is subject to multiple sources of pollution. Therefore, understanding the effects of the effluent on the biological communities would help focus management strategies to improve the quality of the effluent with a view to improving the river health. Since the effluent nutrients were among the least compliant variables, coupled with the recorded elevated turbidity, it is necessary to improve the efficiency of the WWTW in removing the nutrients as well as the solids. The results further suggested that effluent releases should be maintained at dilution rate far below 50% throughout the year to provide for adequate in-stream dilution, but the in-stream flow implications would have to be considered.

The improved physico-chemical compliance recorded in the effluent after 50% dilution did not correspond to a significant reduction in the effluent effects on the macroinvertebrate communities, as the deviations from the control streams were still significant. This then calls

into question the reliance on physico-chemical measures alone as a means for assessing effluent quality compliance, which is aimed at protecting the biological integrity of the receiving in-stream water resources. Ecologically-based methods incorporating the direct effects of effluent on biological communities are needed to adequately protect receiving water bodies' biological communities. The model-stream ecosystem method developed in this study enabled the long-term effects of effluent on the macroinvertebrate communities to be investigated and incorporated some measures of environmental realism, thus providing for better understanding of the influences of the discharged effluent on the biological communities.

CHAPTER 8: GENERAL DISCUSSION, CONCLUSION AND RECOMMENDATIONS

This chapter is a succinct discussion of the results presented in the previous chapters. The benefits of this study to freshwater biomonitoring in South Africa are discussed in a comparative analysis of the explanatory power offered by the approaches applied. The chapter concludes with a proposed framework for improving the environmental water quality of the Swartkops River, a brief conclusion and recommendations.

8.1 Introduction

Deteriorating biotic integrity and water quality occasioned by pollution is threatening freshwater ecosystem sustainability, the livelihoods of riparian communities and social-economic development (Van der Merwe-Botha, 2009; King *et al.*, 2011; UN-Water, 2011; de Groot *et al.,* 2012). Discharges of wastewater effluent into receiving water bodies is among the leading causes of deteriorating biotic integrity and water quality of South Africa's freshwater resources (de Villiers and Thiart, 2007; Van Vuuren, 2011). Wastewater effluent has led to high organic loads causing elevated nutrient concentrations and depleting dissolved oxygen in many freshwater bodies with attendant ecological consequences such as algal blooms, fish kill, changes in floristic and faunistic compositions, and excessive growth of cyanobacteria and macrophyte (de Villiers and Thiart, 2007; van Ginkel, 2011; Marchand *et al.,* 2012). Wastewater treatment effluent impacts can be exacerbated in rivers that drain urbanised and industrialised catchments, where multiple diffuse sources contribute to in-stream biotic and water quality deterioration, and this could also make it difficult to ascertain whether wastewater treatment effluent discharge is chiefly responsible for observed impacts in such rivers. Therefore, unless robust and efficient biomonitoring approaches that are diagnostic, predictive, and sufficiently sensitive to various impacts in urban landscapes are developed, the escalating water quality problems and the increasingly negative impacts on freshwater ecosystem function and biological diversity occasioned by wastewater treatment effluent discharge will be difficult to curtail.

In this study, a range of biomonitoring approaches were used to assess biotic deterioration in the Swartkops River, which receives wastewater effluent discharges and drains an urbanised and industrialised catchment in the Eastern Cape of South Africa. The purpose of this chapter is to present a concise discussion of each of the approaches applied, undertake a comparative analysis of their explanatory power and applications, and propose a simple framework for integrating the traits-based approach (TBA) into existing biomonitoring approach in South Africa.

8.2 Macroinvertebrate taxonomically based biomonitoring approaches

South African Scoring System version 5 and Swartkops multimetric index approach

Chapter 3 of this thesis described the macroinvertebrate taxonomically based assessments of biotic deterioration both at the family- and species-levels of taxonomic identification. However, in South Africa, biomonitoring is based on macroinvertebrate family-level taxonomic assessment only and the South African Scoring System version 5 (SASS5), a biotic index approach, is the primary biomonitoring tool that contributes information to managing freshwater resources (Dickens and Graham, 2002, DWAF, 2008c). The question was asked whether integrating SASS5 with other selected macroinvertebrate-based family-level metrics into a multimetric index could improve the sensitivity of the index approach to differences in biotic deterioration, particularly between the three downstream sites. Would integration of SASS5 and other metrics into a multimetric index approach enable the discrimination of these sites in-terms of their biotic deterioration, considering that Site 2 was within an urban area, but upstream of the effluent discharge point, and Sites 3 and 4 were also still within urban areas but downstream of the discharge point, with Site 3 being the closest? This is important because with Sites 2, 3 and 4 in a landscape with similar activities, further biotic deterioration at Sites 3 and 4 could then be chiefly attributed to the impact of effluent discharges, and management strategies could then be focused on improving the effluent quality. The results revealed that both approaches indicated similar ecological categories (i.e. Categories D and E/F) (Figures 3.4, 3.5, 3.7 and 3.10) for the three downstream sites indicating that the integration of SASS5 into a family-level multimetric index did not provide additional useful explanatory power on the biotic deterioration of the Swartkops River. The management implication of the results of the two family-level assessments is that biotic compositions in the downstream of the Swartkops River were already significantly impaired such that further deterioration of biotic compositions at sites receiving wastewater effluent

discharges was not detected. Thus, with these results, it would seem that the wastewater effluent discharges contributed little to further biotic deterioration of the Swartkops River. However, concentrations of dissolved oxygen (DO) were significantly lower ($P < 0.05$), and concentrations of total inorganic nitrogen (TIN), orthophosphate-phosphorus (PO_4-P), turbidity, five-day biochemical oxygen demand (BOD_5) were significantly more elevated ($P < 0.05$) at Site 3 than at Site 2, suggesting that wastewater effluent discharges contributed to further deterioration of water quality in the Swartkops River (Table 3.1). A comparative analysis of the physico-chemical and family-level taxonomic assessments implies that further water quality deterioration, as indicated by water physico-chemical variables, does not translate into further biotic deterioration as indicated by both SASS5 and MMIswartkops indices; but see the discussion on the other approaches applied.

Chironomid species-level assessment of biotic deterioration in the Swartkops River

As discussed in Chapter 3, family-level biomonitoring tools are inadequate for investigating effects of deteriorating water quality on biodiversity because many species, both sensitive and non-sensitive to deteriorating water quality, would have been aggregated into one family, thereby limiting the understanding of biotic deterioration occasioned by pollution. For instance, the family-level taxonomic metric, percent Chironomidae + Oligochaeta, increased at the downstream sites (Figure 3.9) indicating that deteriorating water quality at these sites favoured the chironomids and oligochaetes. However, the species-level taxonomic assessment indicated that most of the species occurring at the upstream Site 1 disappeared at the three downstream sites, particularly at Sites 3 and 4, which are downstream of the effluent discharge point (Figure 3.13; Appendix A). The species-level assessment therefore indicated that pollution at downstream sites had an adverse effect on chironomid species richness and composition, which was masked in a family-level assessment because species were aggregated into one family. This information, based on species-level assessment, is necessary in order to assess the realistic condition of biodiversity to inform conservation planning and rehabilitation.

Furthermore, the chironomid species-level assessment shed light on the importance of identifying species in freshwater biomonitoring, as it offers useful explanatory power, enabling the discrimination of Site 2, upstream of the wastewater effluent discharge point, from Sites 3 and 4, downstream of the discharge point (Figure 3.14). Results of the species-level assessment indicated statistically significant differences in the species compositions

between Site 2, and Sites 3 and 4 (Figure 3.14, Table 3.7), with more species recorded at Site 2, including less pollution-tolerant species. Thus, while the family-level assessment revealed that further water quality deterioration at Sites 3 and 4 did not translate into further biotic deterioration, the species-level assessment indicated further biotic deterioration at Sites 3 and 4 than at Sites 1 and 2. Therefore, the results emphasised the necessity to move beyond rapid family-level assessment tools to taxonomically refined tools sufficiently sensitive to enable the discrimination of mild differences between sites in an urban catchment receiving both diffuse and point sources of pollution in order to guide the prioritisation of management strategies in the face of limited resources.

Chironomids are functionally, taxonomically and ecologically diverse, occupying most aquatic habitat types and biotopes, providing a representation of a range of sensitivity to water quality impairment (Armitage *et al.,* 1995; Cranston *et al.,* 1996; Simiao-Ferreira *et al.* 2009). These attributes make them suitable for developing a species-level bio-assessment tool in South Africa that can offered an acceptable compromise between a biomonitoring tool that requires identifying species of all macroinvertebrates, and the current family-level assessment. If the index approach is to be followed, though the limitation of this approach has been discussed earlier in Chapter 3, then the empirical approach for deriving the tolerance score presented in Chapter 3 can be use to develop a chironomid-based SASS-type system for bio-assessment of lotic ecosystems at the species level of taxonomic resolution. It is worth noting that chironomids are inherently difficult to identify beyond the sub-family level and identifying species requires time, effort and specialist training that come with cost implications, but the investment would pay off, as they are diverse enough to provide a better biotic assessment of rivers and streams, as was evident in this study.

8.3 Macroinvertebrate traits-based biomonitoring approach

The macroinvertebrate traits-based approach (TBA) was undertaken both at the family- and species-level taxonomic identification. Altered water physico-chemical characteristics, including elevated concentrations of electrical conductivity (EC), nutrients, turbidity and significantly low dissolved oxygen (DO) concentrations occasioned by pollution are major constraints that local biota in the Swartkops River must resist to survive at the impacted sites. The TBA offered a basis for interpreting why certain macroinvertebrate families and chironomid species did not survive at the impact sites. The family-level TBA showed that macroinvertebrates that use gills and lungs were more abundant at the upstream site,

decreasing markedly at the downstream sites where the relative abundance of macroinvertebrates that rely on aerial respiration and tegument increased (Figure 4.2). These results suggest that reduced concentration of DO, and exposure to elevated turbidity, which could increase the risk of gill clogging in gill-bearing taxa, were important environmental constraints to which organisms must adapt. Thus, there were increases in the relative abundance of macroinvertebrates adapted to low oxygen concentrations and high turbidity at Sites 3 and 4 compared with Sites 1 and 2.

A novel chironomid species traits-based *functional strategies* approach developed in this study, based on species combining similar sets of traits, proved sensitive in diagnosing the main abiotic water physico-chemical stressors. Large-bodied chironomid species that have haemoglobin and feed on algae and detritus were mostly associated with Sites 3 and 4 (Figure 5.5a). Although species that do not invest in haemoglobin formation occurred at Sites 1 and 2, none of them were encountered at Site 3 that was receiving wastewater treated effluent discharges, suggesting that dissolved oxygen concentration was a major abiotic stressor at the downstream sites. The functional traits responded predictably to deteriorating water quality and provided an adaptive and mechanistic basis for interpreting chironomid species occurrences at the four sampling sites, providing insight into why certain chironomid species occurred at one site but not at the other. However, although the TBA proved useful in understanding species responses and interpreting biotic deterioration in the Swartkops River, it does not replace taxonomic identification; instead, it highlighted the inextricable link between the trait-based approach (TBA) and taxonomic identification.

8.4 Chironomids sub-lethal assessment

Biomonitoring tools based on in-stream sub-lethal effects can serve as early warning indicators of biotic deterioration because sub-lethality would normally precede the manifestation of lethality in the biological communities. In this study, in addition to taxonomically- and traits-based approaches, deformities were screened in the mentum of chironomid species to assess sub-lethality among the chironomid communities, and to assess whether, based on sub-lethal responses, additional insight could be gained in terms of biotic deterioration in the Swartkops River. The inclusion of deformities in the biomonitoring approaches used in this study offered insight into mild effects of exposure to environmental conditions in the Swartkops River. For example, at Site 1, despite the overall good water quality indicated by water physico-chemical variables and SASS5 results, there were high

incidences of deformities in the mentum of *Polypedilum* sp. and *Cricotopus trifasciata* gr. (Figure 6.6). These deformities were interpreted as chronic sub-lethal effects of exposure to unfavourable environmental conditions not severe enough to induce a lethal response. Therefore, although the water quality was good, deformities in chironomids provided an indication of biotic deterioration at this site and such information can be valuable to guide management actions in sites assigned high priority protection requiring early detection of biotic deterioration.

Furthermore, the newly developed extended toxic score index (ETSI), which was rigorously defended in Chapter 6, indicated that chironomid deformities at Sites 2, 3 and 4 were elevated above the background levels recorded at Site 1. In addition, based on the criterion developed in Chapter 6, the index enabled the discrimination of Site 2 from Sites 3 and 4 and was valuable in indicating biotic deterioration in the Swartkops River.

8.5 macroinvertebrate community response to treated wastewater effluent in the model-stream ecosystems

Apart from the family-level taxonomically based assessments, empirical evidence based on the taxonomic, traits and sub-lethal responses suggested that the biotic deterioration in the river downstream of the effluent discharge point could be chiefly attributed to the discharges of wastewater effluents. However, biomonitoring relies largely on correlative statistical analysis that allows for inferences, which do not enable exploration of cause-effect relationship. Furthermore, in-stream biological responses integrate the effects of all pollutants in the aquatic ecosystems irrespective of their sources or origin (Bonada *et al.*, 2006). Thus, in this study, nine outdoor multispecies model-stream ecosystems were developed and used to investigate experimentally the effects of treated wastewater effluent on macroinvertebrate communities, thereby eliminating the effects of other landscape activities influencing the Swartkops River water quality and biotic integrity. The interpretation of the model-stream ecosystem results and the effluent physico-chemical quality, in the context of the field-based biomonitoring results, shed light on the effluent effects in the Swartkops River.

The results indicated that long-term exposure to the treated wastewater effluent from the Kelvin Jones WWTW had adverse effects on the macroinvertebrate communities in the model-stream ecosystems (Figure 7.16). The abundance of the pulmonate snails, which were significantly reduced at the effluent impacted sites i.e. Sites 3 and 4 in the river, was also

reduced in the effluent-treated model streams, relative to the control streams. In addition, the abundances of psychodids and culicids, which increased at Sites 3 and 4, relative to Sites 1 and 2 in the river, also increased in the effluent-treated model streams relative to the controls (Figure 7.16; Appendices C, Tables C2a – d; E, Tables E1 – E3; F, Figure F1). The correspondence in biological responses between macroinvertebrates in the model streams and in the river provided further evidence that effluent discharges were the main contributor to the observed biotic deterioration in the Swartkops River at sites downstream of the treated effluent discharge point. The evidence provided by changes in macroinvertebrate community structure was supported by the results of the effluent physico-chemical quality, showing that the concentrations of most of the measured variables were not within the compliance limits. The effluent physico-chemical results further indicated that the discharges of wastewater effluent were a major contributor to the elevated concentrations of nutrients and turbidity, and to the depleted dissolved oxygen concentration recorded at Sites 3 and 4.

Overall, the model stream results indicated that improved physico-chemical effluent quality compliance even at 50% effluent dilution, did not significantly reduce the effects of the effluent on the macroinvertebrate communities, revealing that ecologically-based methods rather than physico-chemical measures alone are necessary to assess effluent quality. Thus, the experimental results add credence to the field-based assessment. These results indicated the need to develop management strategies to improve the effluent quality with a view to minimising its impacts on the Swartkops River biological resources.

8.6 Evaluating the benefit of this study to biomonitoring in South Africa

Although freshwater biomonitoring has long been used in South Africa for managing freshwater resources (Dickens and Graham, 2002; DWAF, 2008c; Thirion, 2008), this is the first study that systematically combined several biomonitoring approaches at different levels of taxonomic resolutions, with an ecotoxicological approach that incorporated some measures of environmental realism. It is therefore important to evaluate the benefits of undertaking this study and its contribution to biomonitoring in managing South Africa's water resources. For these reasons, the comparative explanatory power offered by each of the approaches was evaluated in terms of: i) providing clear evidence indicating whether wastewater effluent discharges contributed to water quality and biotic deterioration; ii) monitoring refugia and freshwater ecosystem priority areas (FEPA) for protecting biodiversity; iii) potential of impact diagnosis to inform strong inferences on causality.

Explanatory power of the applied approaches in relation to effluent impact in the Swartkops River

In addition to treated wastewater effluent discharges, the Swartkops River receives diffuse sources of pollution from surrounding urban and industrial activities (see Chapter 2); understanding whether effluent discharges significantly impact biotic integrity in a river also impacted by diffuse sources is important in focusing management strategies on improving the quality of the effluent. Thus, the explanatory power in terms of evidence provided by each of the approaches employed in this study is explored with a view to understanding whether treated wastewater effluent discharges contributed to further biotic and water quality deterioration in the Swartkops River. Sites 2, 3 and 4 are within a landscape with similar activities, with the main difference being discharges of wastewater effluent at Site 3, with Site 4 further downstream of the discharge point. Thus, further biotic deterioration at Sites 3 and 4 could be mainly attributed to the impact of the effluent.

All the approaches used in this study indicated marked biotic deterioration at the three downstream sites (i.e. Sites 2, 3 and 4) relative to the reference site (i.e. Site 1) (Table 8.1). However, the family-level taxonomically-based tools did not provide sufficient evidence to discriminate Site 2, which is upstream of the effluent discharge point from Sites 3 and 4, which are downstream of the discharges (Table 8.1). That is, no further significant biotic deterioration was noticed using the family-level taxonomically based tools. The family-level taxonomically-based results can be interpreted in two ways: either resources downstream of the river have been degraded to an extent that wastewater effluent discharges contributed no further significant deterioration, or the family-level assessments were conservative and thus assigned Site 2, upstream of the discharge point, an ecological category that was over-protective. The management implications of the first interpretation is that diffuse sources of pollution rather than the discharges of treated wastewater effluent make the over-riding impacts on the biotic integrity and water quality of the Swartkops Rive and should be prioritised in restoration efforts where resources are limited. The second interpretation could have serious socio-economic implications because when a site is assigned an over-protective ecological category that does not reflect its realistic state of deviation from the reference condition, it triggers management actions that could lead to re-assignment of resources meant for other socio-economic services (Jooste, 2001). To this end, further investigation is necessary to determine the suitability of the benchmark values for the Ecological categories

D, E/F with a view to revising them to accurately reflect ecological conditions of impacted freshwater ecosystems.

Although the family-level taxonomically-based approaches did not provide sufficient evidence to implicate the treated wastewater effluent discharges as a major contributor to further biotic deterioration in the Swartkops River, the assessments based on chironomid species identifications, family- and species-level traits-based approaches and the experimental investigation of effluent effects, provided evidence clearly indicating that wastewater effluent discharges contributed to further biotic and water quality deterioration in the river (Table 8.1). Thus, these results suggest that monitoring effluent effects in an urbanised and industrialised catchment in South Africa requires a multi-criteria approach to guide management strategies because routine biomonitoring tools proved inadequate. Furthermore, evidence obtained from the species-level taxonomically- and traits-based approaches indicated that Site 2 was less impacted than Sites 3 and 4 (Table 8.1), and was assigned an over-protective ecological category by the assessment based on family-level taxonomically-based approaches, thus further strengthening the argument for a multi-criteria approach to biomonitoring.

Table 8.1 Comparative analysis of the explanatory power offered by the applied approaches in relation to effluent impact in the Swartkops River. The explanatory power was assessed based on whether each of the approaches provided sufficient evidence to trigger management actions to improve the quality of the effluent being discharge into the Swartkops River.

Level of assessment/ approach	**Rationale for evidence necessitating management action to improve the discharge effluent quality**
Water physico-chemical analysis	Wastewater effluent discharges are often associated with high organic loads leading to increased instream nutrients and bacteria activities that reduce dissolved oxygen concentrations (Oberholster *et al.,* 2008; Odjadjare and Okoh, 2010). The physico-chemical results in this study indicated that mean dissolved oxygen was significantly lower at Site 3 than at Site 2, and improved slightly further downstream at Site 4. Mean total inorganic nitrogen (TIN) and orthophosphate-phosphorus also increased significantly at Site 3 and 4 than at Site 2 (Table 3.1). Overall, the physico-chemical results provide evidence to trigger management

	action towards improving the effluent quality.
Biomonitoring (taxonomically-based approaches)	
Family-level assessment: SASS5	SASS5 scores and ASPT values were higher at Site 2 than at Sites 3 and 4, but the overall ecological categories for the three sites were Category E/F (Figure 3.7), indicating that effluent discharges did not lead to further significant biotic deterioration at sites downstream of the effluent discharge point. The family-level SASS5 results did not indicate enough evidence suggesting that wastewater effluent discharges caused further biotic deterioration in the Swartkops River, hence management action towards improving the effluent quality may not be necessary.
Family-level assessment: Swartkops multimetric index	Similar to the SASS5 results, the multimetric index scores were higher at Site 2, relative to Sites 3 and 4 during most of the sampling events. However, in most of the sampling events, the index indicated ecological Category E/F for all three sites, revealing that effluent discharges did not lead to further significant biotic deterioration at sites downstream of the effluent discharge point. The family-level Swartkops multimetric index did not indicate enough evidence suggesting that wastewater effluent discharges caused further biotic deterioration in the Swartkops River, hence management action towards improving the effluent quality may not be necessary.
Chironomid species-level assessment	In most of the sampling events, the number of species decreased markedly at Sites 3 and 4 relative to Site 2 (Figure 3.13). Chironomid species samples collected at Site 2 formed a distinct cluster from those at Site 3, with only three samples at Site 4 clustering with Site 2 (Figure 3.14). Furthermore, chironomids species moderately intolerant of pollution in this study were recorded at Site 2, but were completely absent at Site 3, with few of such species occurring at Site 4. The chironomid species-level assessment indicated further biotic

	deterioration at sites downstream of the effluent discharge point; hence, management action towards improving the effluent quality is necessary.
Biomonitoring (trait-based approach)	
Family-level assessment	Effluent discharges are often associated with low dissolved oxygen (DO) concentration and may lead to increased turbidity if concentrations of suspended solids are elevated. The physico-chemical results indicated significantly low DO at Sites 3 and 4 than at Site 2 and significantly high turbidity at Site 3 than at Site 2. Exposure to low concentrations of DO and elevated concentrations of turbidity could lead to a decrease in the relative abundance of organisms using gills for respiration and an increased relative abundance of those using specialised techniques for aerial respiration (Statzner and Beche, 2010). The family-level traits results indicated that macroinvertebrates relying mostly on gills for respiration decreased markedly at Sites 3 and 4 relative to Site 2, and the relative abundance of those adapted for aerial respiration increased (Figure 4.2). Overall, the family level traits-based asessment provides evidence to trigger management action towards improving the effluent quality.
Chironomid species trait-assessment	The relative abundance of chironomid species not adapted to living under low oxygen concentrations investing in no haemoglobin formation decreased significantly at Sites 3 and 4 relative to Site 2, with a slight increase at Site 4 (Figure 5.5a and b). See section 5.3.1 for description of functional strategies in chironomid species. Overall, the chiironomid species traits-based assessment provides evidence to trigger management action towards improving the effluent quality.
Sub-lethal assessment	
Deformities in chironomid	The extended toxic score index was statistically significantly higher at Sites 3 and 4 than at Site 2, based on the objective criterion developed

species	in Chapter 6. That is, incidences of deformities in the mentum of chironomid species were significantly elevated at Sites 3 and 4 compared with Site 2. The sub-lethal assessment based on chiironomid deformities indicates that further biotic deterioration was evident at sites downstream of the wastewater effluent-discharge point that should necessitate management action towards improving the effluent quality.
Ecotoxicology (Model stream ecosystem approach)	
Macroinvertebrate response to treated wastewater effluent in the model stream ecosystems	Effluent discharges constitute approximately 50% of the downstream river flow at Site 3 during periods of low flow (Taljaard *et al.*, 1998; Slaughter, *pers. comm.*). The results of the model stream ecosystem indicated that after 50% dilution, the effluent still influenced the macroinvertebrate community structure significantly, showing a clear deviation from the controls (Figure 7.16). The abundance of macroinvertebrate families such as Physidae, Lymnaeidae and Planorbidae decreased significantly in the effluent-treated model streams compared with the controls, and the abundance of these families decreased at sites downstream of the effluent discharge point. The model-stream ecosystem provides evidence suggesting that effluent discharges contributed to further biotic deterioration in the Swartkops River and thus management actions are necessary to improve the quality of the discharge wastewater effluent.
General comments	
Apart from the family-level taxonomically-based tools, the remaining approaches used in this study provided sufficient evidence to indicate that the treated wastewater effluent discharges were a major contributor to deteriorating biotic and water quality in the Swartkops River . Thus, it is necessary to develop management strategies to reduce the impact of the effluent on the river, while taking account of the instream flow requirements.	

Monitoring refugia, and freshwater ecosystem priority areas (FEPA) for protecting freshwater biodiversity

An emerging water resource management practice in South Africa, particularly for highly degraded rivers in urbanised and industrialised catchment, which is already being proposed for the Olifants River catchment, for example, is to identify the least impacted sites as refugia for priority protection (DWAF, 2001). The Present Ecological States (PES) of such potential refugia determined using a range of indices, are usually in Category A and B indicating natural and good ecological conditions, respectively. The intended priority protection for the potential refugia is achieved by assigning a management Class 1 that allows for no or minimal deviation from natural conditions (DWAF, 2008b; DWA 2010; see Chapter 1, section 1.22). Since restoring or rehabilitating the ecological integrity of an entire catchment of highly degraded freshwater resources may not be economically viable, identifying refugia and assigning them priority protection is critically important for the persistence of the biodiversity in such systems (Hermoso *et al.,* 2013). Refugia could serve as biodiversity havens within the impacted systems, from where biota could re-colonise impacted sites when conditions become favourable (Magoulick *et al.,* 2003; Hermoso *et al.,* 2013; Robson *et al.,* 2013). Thus, refugia serve to reduce population losses and confer resilience on such populations (Townsend *et al.,* 1997).

To achieve the priority protection intended for refugia, sufficiently sensitive biomonitoring approaches that enable early detection of potential threats to biotic integrity in such refugia are needed. As already demonstrated in Chapter 3 of this study, family-level taxonomically-based assessments such as SASS5 are not suitable to use as early warning indicators of biotic deterioration because many species are aggregated into one family, making it impossible to detect biodiversity loss at the level of species (Chapter 3). Furthermore, biomonitoring indices based on presence or absence e.g. SASS, are essentially measures of lethality and are therefore not suitable as early indicators of biotic deterioration (see Chapters 1 and 6). Therefore, to ensure priority protection of potential refugia, it is necessary to undertake biomonitoring based on species identification. This study has demonstrated the comparative advantages of such level of assessment in freshwater biomonitoring in South Africa. Furthermore, biomonitoring based on sub-lethal assessments is also needed for monitoring refugia because they serve as early warning indicators of potential threats to biodiversity

before manifestation of lethality. The chironomid deformity-based index developed in this study can serve this purpose.

In addition, freshwater ecosystem priority areas (FEPA) deserving priority protection because of the biodiversity they support and their unique characteristics have been identified in South Africa (Nel *et al.,* 2011). The identified River FEPA are river reaches in the A and B ecological categories supporting unique biodiversity such as threatened and near-threatened fish species. To sustain the biodiversity these river ecosystems support, FEPA objectives have been set that allow no further ecological deterioration of such river ecosystems (Nel *et al.,* 2011). Therefore, similar to refugia, identified River FEPA need to be monitored using sufficiently sensitive biomonitoring approaches that would enable biotic deterioration to be detected early in order to trigger management actions. As already discussed, the routinely applied family-level biomonitoring tools are not sufficiently sensitive to be applied as early warning indicators of biotic deterioration in River FEPA. Based on the results of this study, sub-lethal- and species-level taxonomically and traits-based biomonitoring approaches are best suited to use as early warning indicators of potential biotic deterioration and should therefore be prioritised over family-level taxonomically based approaches for monitoring River FEPA.

Connectivity is an important characteristic that facilitates the propagation of threats to biological communities along the courses of rivers (Hermoso *et al.,* 2011). For example, chemical spills or effluent discharges could have adverse effects on downstream biodiversity several kilometres away. Therefore, processes operating several kilometres away could affect the biological communities protected in refugia and in River FEPA (Nel *et al.,* 2011; Hermoso *et al.,* 2012). Thus, to ensure effective protection of potential refugia and the identified River FEPA in South Africa, the surrounding areas (e.g. upstream-downstream) within the river catchment that are critical to sustaining the biological communities and ecological processes within the refugia and the River FEPA need to be prioritised for protection (if not already degraded), restoration or rehabilitation (if already degraded). These areas would also require sufficiently sensitive biomonitoring approaches to ensure adequate protection or to monitor the successes of rehabilitation or restoration efforts. Based on the results in this study, taxonomically- and trait-based species- and sub-lethal-level assessments are best suited for monitoring potential threats to biotic integrity and the success of rehabilitation efforts in areas adjacent to such refugia and River FEPA. Therefore, depending

on the context, this study provides a basis for selecting the appropriate biomonitoring approaches for use.

Impact diagnostic potential to inform strong inferences on causality

This study is the first to explicitly apply the macroinvertebrate TBA to biomonitoring in South Africa and one of the added benefits of its application is that it makes it possible to diagnose the main water quality stressors driving the observed biotic deterioration in the Swartkops River (Chapters 4 and 5). The results of the TBA approaches revealed that significantly reduced dissolved oxygen concentrations (DO) and elevated concentrations of turbidity and electrical conductivity (EC) favouring specific traits (Chapters 4 and 5) were among the main abiotic variables that explained the observed biotic deterioration in the Swartkops River. Since water resource managers are often interested in identifying causes of deteriorating water quality, the diagnostic potential of the TBA over the taxonomically based approaches, provides a strong basis for inferences on causality that can guide management strategies to improve instream DO, turbidity and EC concentrations.

8.7 Framework for integrating traits-based approach into existing taxonomically-based biomonitoring approach in South Africa

Based on the comparative advantages of the TBA approach demonstrated in this study, it is necessary to develop a framework to incorporate the TBA into existing taxonomically-based approach to biomonitoring in South Africa. The framework in Figure 8.1 shows that the integration of traits into the existing biomonitoring approach in South Africa does not require new sampling protocols because during the course of collecting macroinvertebrates for taxonomic identification, traits are also collected. In addition, macroinvertebrates housed in databases such as the National River Health database (DWAF, 2007) can also be analysed in terms of traits (Figure 8.1). Therefore, as shown in the framework, what is required is the refinement of existing biomonitoring methods to include trait information. Nevertheless, additional sample processing time would be required because some traits, e.g. body size, would have to be measured individually. Despite the added costs to biomonitoring because of additional sample processing time, the integration of traits into existing biomonitoring approach as shown on Figure 8.1 would add benefit to biomonitoring in South Africa and this study has demonstrated it (Chapters 4 and 5).

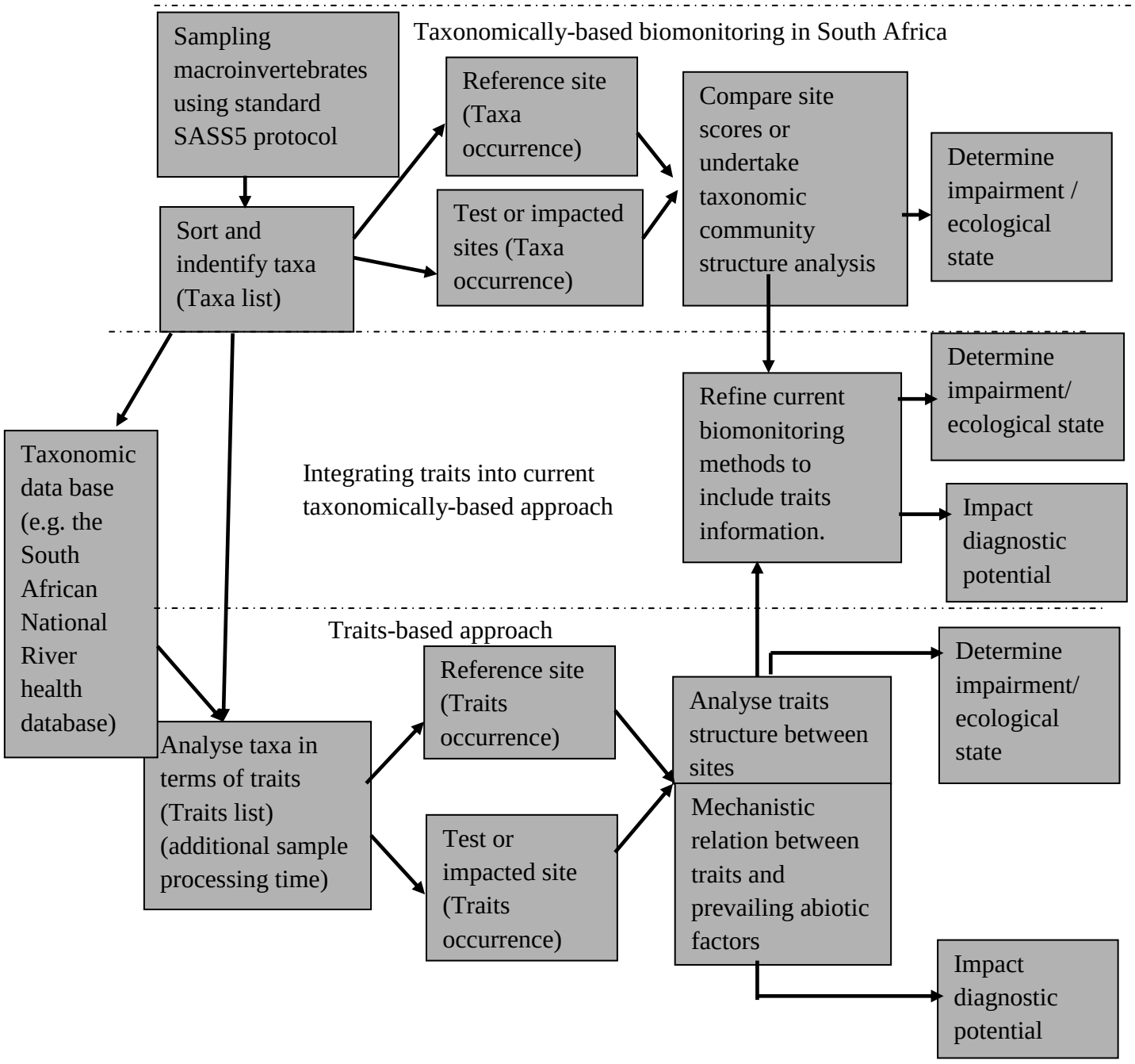


Figure 8.1 Simple schematic framework showing that integrating traits into current biomonitoring approaches in South Africa does not require new sampling protocol, but refinement of existing methods to include traits information, which would require additional sample processing time and provide the added benefit of impact diagnostic potential. (Schematic framework modified from Culp *et al.*, 2011).

8.8 Proposed framework for improving environmental water quality in the Swartkops River

Based on the results of this study, a framework for improving the biotic and water quality conditions of the Swartkops River is proposed (Figure 8.2). The framework emphasises managing the effluent quality to reduce its effects on the instream biological resources of the river. However, the biotic and water quality deterioration at Site 2, upstream of the wastewater effluent discharge point, indicated that diffuse sources of pollution are equally important contributors to deteriorating biotic integrity and water quality observed in the river (Chapters 2, 3, 4, 5, 6 and 7). Therefore, while emphasis is placed on managing the effluent quality, steps are outlined that can be taken to manage diffuse sources of pollution with the overall goal of improving the environmental water quality of the Swartkops River.

In managing the effluent quality, several indicators, including water and effluent physico-chemical variables and instream biotic conditions, are proposed as tools to assess the potential risk exposure to the discharges of wastewater effluent pose to the river's resources. The framework emphasises using the proposed indicators to monitor the effluent constantly in order to improve the effluent quality by developing effluent quality improvement plans on a regular basis, particularly improving the nutrient and solid removal efficiency of the wastewater treatment works (Figure 8.2).

The first step in the framework for managing diffuse sources of pollution is identifying the main sources through a stakeholder participatory process, and then deciding on the best management options, taking account of the socio-economic and ecological implications of such options. The importance of stakeholder engagement and participation in managing environmental quality has been emphasised (Beierle and Konisky, 2001; du Toit and Pollard, 2008). For example, du Toit and Pollard (2008) developed a comprehensive framework for stakeholder engagement within the context of integrated water resource management at the catchment level. The du Toit and Pollard (2008) framework can be applied in engaging stakeholders when managing water quality in the Swartkops River. The outcomes of implemented management strategies in the river can then be monitored using the biomonitoring approaches applied in this study with emphasis on species-level taxonomically- and traits-based assessments (Figure 8.2).

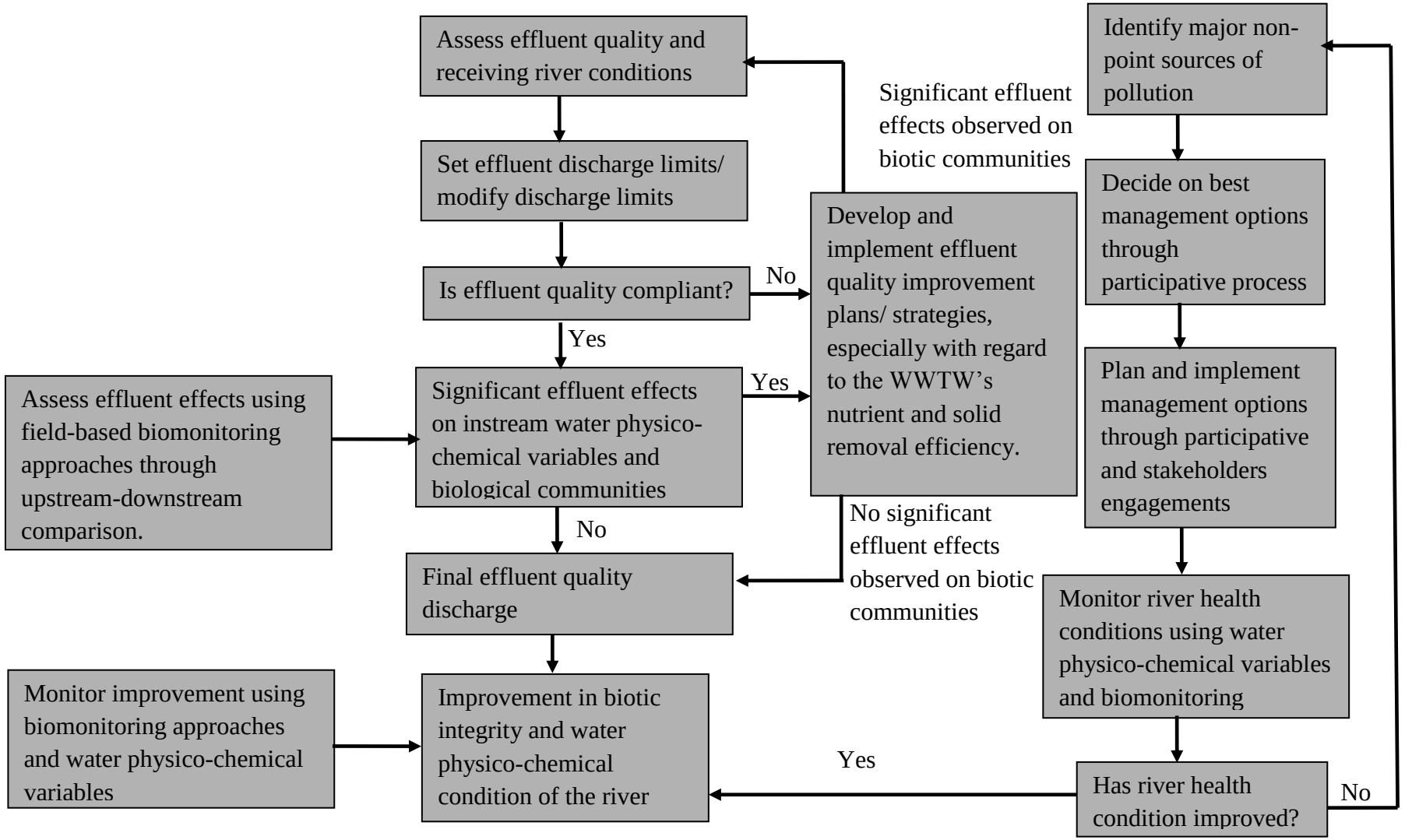


Figure 8.2 Proposed conceptual framework for improving environmental water quality of the Swartkops River taking account of management processes for both point and non-point sources of pollution.

8.9 Conclusion

This study has contributed to the field of biomonitoring in South Africa by demonstrating the importance of an integrated, multi-criteria approach to managing environmental water quality, and providing a basis for selecting the appropriate biomonitoring approaches for use. It highlighted the limitations of the current family-level taxonomically-based biomonitoring approach (Chapters 1 and 3), and then provided evidence of the comparative advantages of species-level taxonomically, sub-lethal- and traits-based approaches (Chapters 3, 4 5 and 6) in freshwater biomonitoring. The model stream ecosystem approach developed in this study shed light on the importance of ecologically-based methods for managing effluent effects, in addition to physico-chemical measures (Chapter 7), and linking field-based observations to experimental results to guide management strategies. The results indicate that effluent discharges contributed to both biotic and water quality deterioration in the Swartkops River and a framework for improving the effluent quality and biotic conditions of the river has been proposed. The results further suggested that effluent releases should be maintained at a dilution rate far below 50% throughout the year to provide for adequate instream dilution, but the instream flow implications would have to be considered.

8.10 Research limitations

The following are important limitations of this research:

- Because of the unavailability of trait information for Afro-tropical chironomid species and macroinvertebrate families, only limited numbers of traits were used to develop the chironomid traits-based approach. The traits-based approach used in this study could benefit from additional trait information e.g. reproduction and voltinism, which were not used in this study.
- Although chironomids proved useful for assessing the biotic integrity in the Swartkops River, the *inherent* difficulty associated with their identification may make the chironomid-based approaches developed in this study unattractive for biomonitoring application in a region such as South Africa where taxonomic knowledge is sparse.
- The model stream ecosystem approach, despite the offered advantages, has a number of limitations highlighted in Chapters 1 and 7, which could hinder its use as an

ecologically-based method for investigating effluent effects in relation to instream observations.

8.11 Recommendations

Based on the investigation undertaken in this study, the following are recommended for further study and in managing freshwater resources in South Africa.

- One of the potential drawbacks of the TBA as discussed in Chapter 3 is the limited autecological information on Afro-tropical macroinvertebrates. It is recommended that applied research be directed towards compiling the limited autecological information available on Afro-tropical species in a database. There is also a need to initiate basic ecological research focusing on species biology and autecology in order to harness the full potential of the TBA in managing South Africa's water resources.
- The functional and taxonomic diversity among the chironomid make them a suitable group of macroinvertebrates for developing sufficiently sensitive sub-lethal and species-level taxonomically and traits-based biomonitoring approaches. To realise the full potential of this group of macroinvertebrates, it is recommended that there should be investment in training personnel on taxonomic identification.
- To improve existing biomonitoring approaches in South Africa, it is recommended that the usefulness of the TBA be further investigated under different water quality impact conditions, with a view to refining existing methods to incorporate the TBA. As trait information becomes available in the future, other traits e.g. reproduction, voltinism, not included in the present study should be investigated.
- Improved effluent physico-chemical quality did not translate into significantly reduced effluent effects on macroinvertebrate communities. Therefore, it is necessary to incorporate ecologically-based methods in addition to physico-chemical measurements to better understand effluent effects on receiving instream resources in South Africa.

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APPENDICES

Appendix A: Number and relative abundance of chironomid species recorded at the four sampling sites in the Swartkops River during the study period (August 2009 – September 2012).

Table A 1: Number and relative abundance of chironomid species recorded at Site 1 in the Swartkops River during the study period (August 2009 – September 2012). Seasons: Sp (spring), Su (summer), Au (Autumn), Wi (winter).

	Site 1							
Taxa	Sp (2009)	Su (2009)	Au (2010)	Wi (2010)	Sp (2011)	Su (2011)	Au (2012)	Sp (2012)
Subfamily Orthocladiinae								
Cricotopus sp1	35.11	36.24	13.14	2.87	7.42	13.24	12.39	8.12
Cricotopus trifasciata gr.	10.03	32.21	54.01	34.84	3.07	1.47	16.06	10.21
Paratrichocladius sp.	2.19	0.00	0.00	0.41	0.00	0.00	0.00	0.93
Nanocladius sp.	1.25	0.00	0.00	0.00	0.77	1.47	0.46	1.86
Eukiefferiella sp.	1.88	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Cardiocladius sp	4.70	2.01	0.00	1.23	0.51	1.47	1.83	1.16
Parakiefferiella sp	0.31	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Orthocladius sp	14.11	10.74	8.03	4.92	5.88	0.00	9.63	11.60
Orthocladius sp.2	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Subfamily Chironominae								
Tribe Chironomini								
Dicrotendipes sp.	0.00	0.67	0.00	0.00	13.04	2.94	7.34	3.48
Kiefferulus sp.	0.31	0.00	0.00	0.00	0.00	2.94	0.00	0.23
Polypedilum sp.	14.42	0.00	2.92	3.28	6.65	2.94	9.17	14.15
Cryptochironomus sp.	0.31	0.00	0.00	0.00	2.05	2.94	1.38	0.23
Chironomus sp 1	0.00	0.67	1.46	0.00	0.51	0.00	0.00	0.00
Chironomus sp 2	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Chironomus sp 3	0.00	0.00	0.73	0.00	0.00	0.00	0.00	0.00
Microchironomus sp	0.00	0.00	0.73	0.00	0.51	0.00	0.00	0.00
Polypedilum nubifer	0.00	0.00	0.00	0.00	0.00	0.00	1.38	0.46
Glyptotendipes sp.	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Dicrotendipes sp.2	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Tribe Tanytarsini								
Tanytarsus sp.	4.70	9.40	15.33	47.95	17.39	19.12	21.10	10.90
Rheotanytarsus sp.	0.00	0.67	0.00	0.00	0.26	1.47	0.46	0.00
Cladotanytarsus sp.	0.31	0.00	0.00	1.23	1.28	1.47	4.13	1.86
Virgatanytarsus sp.	0.00	0.00	0.00	0.00	0.00	0.00	1.83	6.03
Paratanytarsus sp.	0.00	0.00	0.00	0.00	0.00	0.00	0.00	2.09
Subfamily Tanypodinae								

Ablabesmyia sp.	8.15	7.38	0.00	2.05	22.76	27.94	9.63	15.78
Coelotanypus sp.	0.31	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Procladius sp.	0.31	0.00	0.00	0.00	0.26	0.00	0.00	0.00
Trissopelopia sp.	0.94	0.00	0.00	0.00	3.58	4.41	0.00	0.23
Clinotanypus sp.	0.63	0.00	0.00	0.00	1.02	1.47	0.00	0.00
Tanypus sp	0.00	0.00	3.65	1.23	3.07	2.94	0.00	0.23
Nilotanypus sp.	0.00	0.00	0.00	0.00	4.60	8.82	0.00	0.00
Thienemannimyia sp.	0.00	0.00	0.00	0.00	2.56	0.00	1.83	4.18
Macropelopia sp.	0.00	0.00	0.00	0.00	2.81	2.94	0.92	6.26
Conchapelopia sp.	0.00	0.00	0.00	0.00	0.00	0.00	0.46	0.00
No. of chironomid indiv.	319.00	149.00	137.00	244.00	391.00	68.00	218.00	431.00
Number of chironomid species	18	9	9	10	21	17	17	20

Table A2: Number and relative abundance of chironomid species recorded at Site 2 in the Swartkops River during the study period (August 2009 – September 2012). Seasons: Sp (spring), Su (summer), Au (Autumn), Wi (winter).

Taxa	**Site 2**							
Subfamily Orthocladiinae	Sp (2009)	Su (2009)	Au (2010)	Wi (2010)	Sp (2011)	Su (2011)	Au (2012)	Sp (2012)
Cricotopus sp.1	25.53	0.70	0.00	0.00	21.54	8.62	11.38	3.81
Cricotopus trifasciata gr.	52.58	2.94	0.00	0.00	13.83	0.00	15.45	1.90
Paratrichocladius sp.	4.56	0.00	0.00	0.00	1.06	10.34	1.63	0.00
Nanocladius sp.	0.61	0.00	0.00	0.00	1.06	1.72	8.94	4.05
Eukiefferiella sp.	0.61	0.00	0.00	0.00	1.06	0.00	0.00	1.67
Cardiocladius sp.	0.00	0.00	0.00	0.00	0.00	0.00	0.00	4.29
Parakiefferiella sp.	0.30	0.00	0.00	0.00	0.80	0.00	0.00	0.00
Orthocladius sp.	6.99	1.82	0.00	0.00	2.39	13.79	18.70	3.57
Orthocladius sp.2	0.00	0.00	0.00	0.00	0.00	3.45	0.00	0.00
Subfamily Chironominae								
Tribe Chironomini								
Dicrotendipes sp.	7.29	32.63	7.04	0.00	34.84	51.72	37.40	57.14
Kiefferulus sp.	0.00	14.29	2.82	0.00	0.00	0.00	0.00	0.48
Polypedilum sp.	0.30	0.00	0.00	0.00	1.33	0.00	0.00	0.48
Cryptochironomus sp.	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Chironomus sp 1.	1.22	10.78	53.17	89.29	18.35	8.62	4.07	20.48
Chironomus sp 2	0.00	4.34	5.99	10.71	0.00	0.00	0.00	1.90
Chironomus sp. 3	0.00	0.00	2.11	0.00	0.00	0.00	0.00	0.24
Microchironomus sp.	0.00	0.00	2.46	0.00	0.00	0.00	0.00	0.00
Glyptotendipes sp.	0.00	0.00	0.00	0.00	0.00	1.72	0.00	0.00
Tribe Tanytarsini								
Tanytarsus sp	0.00	0.00	0.00	0.00	0.00	0.00	2.44	0.00
Cladotanytarsus sp.	0.00	1.12	0.00	0.00	0.00	0.00	0.00	0.00
Paratanytarsus sp.	0.00	0.42	0.00	0.00	0.00	0.00	0.00	0.00

Subfamily Tanypodinae								
Ablabesmyia sp.	0.00	0.14	0.00	0.00	0.80	0.00	0.00	0.00
Procladius sp.	0.00	0.00	0.00	0.00	2.93	0.00	0.00	0.00
Tanypus sp.	0.00	30.81	26.41	0.00	0.00	0.00	0.00	0.00
No. of chironomid indiv.	329.00	714.00	284.00	28.00	376.00	58.00	123.00	420.00
No. of chironomid species	10	11	7	2	12	8	8	12

Table A3: Number and relative abundance of chironomid species recorded at Site 3 in the Swartkops River during the study period (August 2009 – September 2012). Seasons: Sp (spring), Su (summer), Au (Autumn), Wi (winter).

Taxa	**Site 3**							
Subfamily Orthocladiinae	Sp (2009)	Su (2009)	Au (2010)	Wi (2010)	Sp (2011)	Su (2011)	Au (2012)	Sp (2012)
Cricotopus sp.1	0.00	0.00	0.00	0.00	0.09	0.31	0.00	0.00
Eukiefferiella sp.	0.00	0.00	0.00	0.00	0.00	0.00	0.13	0.00
Subfamily Chironominae								
Tribe Chironomini								
Dicrotendipes sp.	0.19	1.10	0.21	0.39	8.50	0.61	30.42	31.61
Kiefferulus sp.	0.74	0.00	0.00	0.00	0.00	0.00	5.79	5.16
Chironomus sp. 1	99.07	81.79	91.53	91.70	73.78	77.03	41.32	36.49
Chironomus sp. 2	0.00	11.93	6.57	7.72	9.74	13.48	12.65	14.92
Chironomus sp. 3	0.00	5.18	1.69	0.00	7.88	8.58	8.08	8.49
Glyptotendipes sp.	0.00	0.00	0.00	0.00	0.00	0.00	0.13	1.77
Dicrotendipes sp.2	0.00	0.00	0.00	0.00	0.00	0.00	1.48	1.56
Tribe Tanytarsini								
Cladotanytarsus sp.	0.00	0.00	0.00	0.19	0.00	0.00	0.00	0.00
Subfamily Tanypodinae								
No. of chironomid indiv.	537.00	637.00	472.00	518.00	1129.00	653.00	743.00	1414.00
No. of chironomid species	3	4	4	4	5	5	8	7

Table A4: Number and relative abundance of chironomid species recorded at Site 4 in the Swartkops River during the study period (August 2009 – September 2012). Seasons: Sp (spring), Su (summer), Au (Autumn), Wi (winter).

	Site 4							
Taxa	Sp (2009)	Su (2009)	Au (2010)	Wi (2010)	Sp (2011)	Su (2011)	Au (2012)	Sp (2012)
Subfamily Orthocladiinae								
Cricotopus sp. 1	2.22	0.00	0.00	0.00	1.94	0.00	1.99	1.35
Cricotopus trifasciata gr.	8.89	0.00	0.00	0.00	5.53	0.74	6.18	0.00
Paratrichocladius sp.	2.22	0.00	0.00	0.00	0.00	0.00	0.00	0.00

Eukiefferiella sp.	0.00	0.00	0.00	0.00	0.41	4.72	0.60	0.00
Cardiocladius sp.	0.00	0.00	0.00	0.00	0.00	0.00	5.98	2.17
Parakiefferiella sp.	0.00	0.00	0.00	0.00	0.14	0.00	0.00	0.00
Orthocladius sp.	0.00	0.00	0.00	0.00	0.00	0.00	9.76	2.03
Subfamily Chironominae								
Tribe Chironomini								
Dicrotendipes sp.	4.44	1.44	0.00	1.12	40.25	23.60	60.76	69.28
Kiefferulus sp.	0.00	0.00	0.00	0.00	4.56	4.87	2.79	3.38
Polypedilum sp.	6.67	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Chironomus sp. 1	57.78	69.06	95.83	91.24	41.63	44.40	8.37	18.67
Chironomus sp. 2	8.89	19.42	0.00	4.94	1.24	8.41	2.19	1.49
Chironomus sp. 3	0.00	2.88	0.00	2.47	3.46	7.96	1.20	1.62
Microchironomus sp.	0.00	0.00	4.17	0.00	0.00	0.00	0.00	0.00
Tribe Tanytarsini								
Tanytarsus sp.	6.67	0.00	0.00	0.22	0.00	0.00	0.00	0.00
Cladotanytarsus sp.	2.22	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Subfamily Tanypodinae								
Ablabesmyia sp.	0.00	7.19	0.00	0.00	0.00	5.31	0.00	0.00
Tanypus sp.	0.00	0.00	0.00	0.00	0.83	0.00	0.20	0.00
No. of chironomid indi	45.00	139.00	24.00	445.00	723.00	678.00	502.00	739.00
No. of chironomid species	9	5	2	5	10	8	11	8

Integrated Habitat Assessment Systems (IHAS)

The results of the physical habitat characteristics assessed using IHAS did not showed much difference between the sampling sites (Figure A1). Seasonally, at all four sampling sites, the IHAS score was higher during spring sampling events than the remaining three sampling seasons. The Kruskal-Wallis multiple comparison test indicated no statistically significant difference between the sites and between the seasons ($P > 0.05$). The results therefore suggest that deteriorating water quality, rather than the physical habitat differences between the sites was the main driver of the observed biotic deterioration in the Swartkops River.

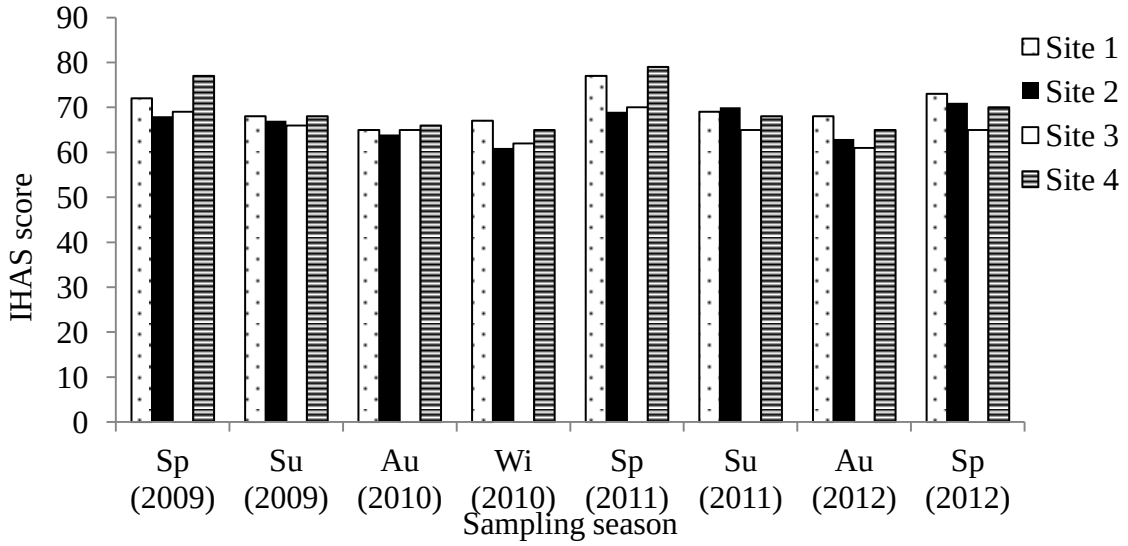

Figure A1: Integrated habitat assessment scores at the four sampling sites during the study period (August 2009 – September 2012) in the Swartkops River showing that the physical habitat condition did no vary much between the sampling sites and seasons

Appendix B: Guideline concentrations used for the interpretations of electrical conductivity and the analysed metals.

Table B1: Recalibrated EC benchmark value used for interpreting concentrations of electrical conductivity in the Swartkops River during the study period (August 2009 - September 2012). EC was recalibrated as described in DWAF (2008c).

Ecological category	Description	Rating	Recalibrated bench mark (ms/m)
A	Natural	0	57
B	Good	1	84.1 - 109
C	Upper fair	2	109.1 - 193
D	Lower fair	3	193.1 - 301
E/F	Poor	4	> 301

Table B2: Guideline (DWAF 1996b) used for the interpretation of the analysed metal concentrations in the Swartkops River during the study period (August 2009 - September 2012).

	Concentrations (µg/l)				
	Chromium (VI)	Copper	Lead	Manganese	Zinc
Target water quality range (TWQR)	7	0.8	0.5	180	2
Chronic effect value (CEV)	14	1.5	1	370	3.6
Acute effect value (AEV)	200	4.6	7	1 300	36

Appendix C: Fuzzy coding of selected macroinvertebrate traits, taxon-site matrix and traits-site matrix for the four sampling sites per sampling occasion in the Swartkops River during the study period (August 2009 – September 2012).

Table C1: Fuzzy coding of macroinvertebrate traits in the Swartkops River. Code: Body size (mm) A 1 (≤5), A2 (>5-10), A3 (>10-20), A4 (>20-40), A5 (>40-80); respiration B1 (gills), B2 (tegument), B3 (aerial: spiracle), B4 (Aerial/vegetation: breathing tube, straps/other apparatus e.g. elytra), B5 (aerial: lung); mobility C1 (climbers), C2 (crawlers), C3 (sprawlers), C4 (swimmers), C5 (skaters), C6 (burrowers); Body shape D1 (streamlined), D2 (flattened), D3 (spherical), D4 (cylindrical); preferred food E1 (fine particulate organic matter, FPOM), E2 (coarse particulate organic matter, CPOM), E3 (plant material/ algae), E4 (animal materials); feeding habit F1 (shredder), F2 (collector-gatherer), F3 (collector-filterer), F4 (scrapers, grazers and brushers), F5 (predators); preferred biotope G1 (sediment), G2 (stone) and G3 (vegetation).

Traits	Max. body size (mm)					Respiration					Mobility						Body shape				Preferred food				Feeding habit					Preferred biotope		
Code	A 1	A 2	A 3	A 4	A 5	B 1	B 2	B 3	B 4	B 5	C 1	C 2	C 3	C 4	C 5	C 6	D 1	D 2	D 3	D 4	E 1	E 2	E 3	E 4	F 1	F 2	F 3	F 4	F 6	G 1	G 2	G 3
Taxon																																
ANNELIDA																																
Oligochaeta	0	0	0	0	0	0	5	0	0	0	0	0	0	0	0	5	0	0	0	5	5	1	0	0	0	3	3	1	0	5	5	0
Hirudinea	0	3	1	0	0	0	5	0	0	0	0	3	0	3	0	0	0	5	0	3	0	0	0	5	0	0	0	0	1	0	3	0
CRUSTACEA																																
Potamonautidae	0	1	1	3	1	5	0	0	0	3	0	5	0	1	0	5	0	1	3	0	0	1	3	3	1	1	0	3	0	0	3	3
EPHEMEROPTERA																																
Leptophlebiidae	0	5	3	0	0	5	1	0	0	0	0	3	0	5	0	0	1	1	0	5	5	1	1	0	2	3	1	3	0	0	3	0
Baetidae	3	5	0	0	0	5	1	0	0	0	0	3	0	5	0	0	1	0	0	5	5	1	3	0	1	5	1	3	0	0	0	3
Caenidae	3	5	0	0	0	5	1	0	0	0	0	3	1	3	0	1	0	3	0	5	5	0	5	0	0	5	0	3	0	0	3	3
Heptageniidae	0	1	5	0	0	5	1	0	0	0	0	3	3	1	0	0	0	5	0	0	5	0	5	0	0	5	0	3	0	0	0	0
ODONATA																																
Coenagrionidae	0	1	5	5	1	5	1	0	0	0	5	5	0	3	0	0	0	0	0	5	1	0	0	5	0	0	0	0	5	0	0	5
Lestidae	0	0	5	5	1	5	1	0	0	0	5	5	0	3	0	0	0	0	0	5	1	0	1	5	0	0	0	0	5	0	0	0
Aeshnidae	0	0	5	5	3	5	1	0	0	0	5	5	0	3	0	0	0	0	0	5	1	0	1	5	0	0	0	1	5	0	3	3

Corduliidae	0	1	5	3	1	5	1	0	0	0	5	5	0	5	0	0	0	0	5	0	1	0	1	5	0	0	1	1	5	0	3	3
Gomphidae	0	1	5	3	1	5	1	0	0	0	1	5	0	3	0	0	0	3	0	5	1	0	1	5	0	0	1	1	5	0	0	5
Libellulidae	0	3	5	1	1	5	1	0	0	0	1	5	0	3	0	0	0	3	0	5	1	0	1	5	0	0	1	1	5	0	0	5
Synlestidae	0	1	5	5	3	5	1	0	0	0	3	5	0	3	0	0	0	0	0	5	1	0	1	5	0	0	1	1	5	0	0	5
Protoneuridae	0	0	5	0	0	5	1	0	0	0	5	5	0	1	0	0	0	0	0	5	1	0	1	5	0	0	1	1	5	0	0	0
Platycnemidae	0	0	5	0	0	5	1	0	0	0	5	5	0	1	0	0	0	0	0	5	1	0	1	5	0	0	1	1	5	0	0	0
LEPIDOPTERA																																
Pyralidae	0	0	5	5	0	5	1	1	0	0	1	5	0	1	0	1	0	3	0	5	3	1	3	0	3	0	0	3	0	0	0	0
HEMIPTERA																																
Belostomatidae	0	1	3	5	1	0	0	5	5	0	3	3	0	5	0	0	0	3	5	0	0	0	0	5	0	0	0	0	5	0	0	3
Naucoridae	0	5	3	0	0	0	0	5	5	0	3	3	0	5	0	0	0	3	5	1	0	0	0	5	0	0	0	0	5	0	0	0
Nepidae	0	0	5	3	1	0	0	5	5	0	3	5	0	5	0	0	0	5	0	3	0	0	0	5	0	0	0	0	5	0	0	0
Pleidae	5	0	0	0	0	0	0	5	5	0	3	0	0	5	0	0	0	0	5	0	0	0	0	5	0	0	0	0	5	0	0	3
Veliidae	5	1	0	0	0	0	0	5	5	0	0	0	0	0	5	0	0	0	5	5	0	0	0	5	0	0	0	0	5	3	0	3
Gerridae	0	5	3	0	0	0	0	5	5	0	0	0	0	0	5	0	0	5	0	5	0	0	0	5	0	0	0	0	5	0	3	3
Corixidae	0	5	1	0	0	0	0	5	5	0	3	0	0	5	0	0	0	0	0	5	1	0	5	0	0	0	1	0	0	3	0	0
Notonectidae	1	5	1	0	0	0	0	5	5	0	3	0	0	5	0	0	0	0	0	5	0	0	5	0	0	0	0	0	5	0	0	0
TRICHOPTERA																																
Ecnomidae	1	5	3	0	0	1	5	0	0	0	0	5	0	1	0	0	0	0	0	5	3	3	1	3	0	0	3	0	3	0	0	3
Hydropsychidae	0	1	5	0	0	5	1	0	0	0	0	5	0	1	0	0	0	0	0	5	5	3	3	1	1	0	5	0	1	3	0	0
Leptoceridae	0	0	5	0	0	5	1	0	0	0	3	0	3	5	0	0	0	0	0	5	3	3	1	0	5	3	0	3	0	0	0	3
Hydroptilidae	5	0	0	0	0	1	5	0	0	0	3	0	3	0	0	0	1	3	5	5	1	0	5	0	0	0	0	5	0	0	3	0
Lepidostomatidae	0	5	3	0	0	1	5	0	0	0	1	0	5	0	0		0	0	0	5	1	5	3	0	5	0	0	1	0			
COLEOPTERA																																
Dytiscidae	1	5	3	1	1	3	3	5	5	0	5	5	0	5	0	0	3	3	3	3	0	0	0	5	0	0	0	0	5	0	3	0
Gyrinidae	1	5	3	1	0	5	3	0	5	0	5	5	0	5	0	1	0	1	3	3	0	1	0	5	0	0	0	0	5	0	3	3
Hydraenidae	5	0	0	0	0	3	3	5	5	0	3	3	0	5	0	0	0	1	0	3	1	3	1	5	0	5	0	3	5	0	0	0
Hydrophilidae	1	5	3	1	1	3	1	5	5	0	5	5	0	5	0	0	0	0	5	5	0	3	3	5	3	5	0	0	5	3	0	3

Elmidae	5	1	0	0	0	5	1	5	0	0	1	5	0	0	0	0	0	3	3	3	5	3	1	0	0	5	1	0	0	0	0	3
Helodidae	1	5	0	0	0	5	3	1	0	0	5	5	0	1	0	0	0	0	0	5	1	3	0	3	0	0	0	0	5	0	0	0
DIPTERA																																
Ceratopogonidae	1	1	5	0	0	5	1	0	0	0	0	3	1	1	0	3	0	1	0	5	1	0	1	5	0	1	0	1	3	0	3	0
Chironomidae	3	5	1	1	0	3	5	0	0	0	0	3	3	1	0	5	0	0	0	5	5	1	3	3	1	5	0	3	3	0	3	3
Culicidae	1	5	0	0	0	0	0	3	3	0	0	0	0	5	0	0	0	0	0	5	5	1	1	0	0	0	5	1	0	0	3	0
Syrphidae	0	3	3	0	0	0	1	5	5	0	1	3	5	0	0	0	0	0	0	5	3	1	1	0	0	3	3	1	0	0	0	0
Muscidae	1	5	3	0	0	1	1	3	0	0	0	3	0	0	0	3	0	0	0	5	0	0	0	3	0	0	0	0	3	0	0	3
Simuliidae	3	5	0	0	0	3	5	1	0	0	0	3	0	1	0	0	0	0	0	5	5	1	1	0	0	0	5	1	0	0	0	3
Tabanidae	0	1	5	1	0	0	3	5	5	0	1	3	1	1	0	3	0	0	0	5	0	1	3	5	3	0	0	1	5	0	0	0
Tipulidae	0	3	1	1	0	3	3	5	0	0	0	1	1	0	0	5	0	0	0	5	5	1	1	3	3	1	0	1	5	0	0	0
Athericidae	0	3	3	0	0	3	3	0	0	0	1	5	3	0	0	5	0	0	0	5	1	3	1	3	1	0	0	3	3	0	0	0
Ephydridae	0	3	3	0	0	0	1	5	5	0	0	3	3	0	0	5	0	0	0	5	3	3	3	1	3	5	0	1	1	0	0	0
Psychodidae	3	3	0	0	0	0	0	5	0	0	0	5	0	1	0	0	0	3	0	3	5	3	1	0	3	5	0	3	0	0	0	0
GASTROPODA																																
Ancylidae	0	0	0	0	0	5	1	0	0	0	1	3	0	1	0	1	0	0	3	0	5	1	3	0	0	0	3	0	0	0	3	0
Lymnaeidae	0	0	0	0	0	0	5	0	0	5	1	3	0	1	0	0	0	0	3	0	3	1	3	0	0	0	0	3	0	0	0	5
Physidae	0	0	0	0	0	0	5	0	0	5	1	3	0	1	0	0	0	0	3	0	3	1	3	0	0	0	0	3	0	0	0	5
Planorbidae	0	0	0	0	0	0	5	0	0	5	1	3	0	1	0	0	0	0	3	0	3	1	3	0	0	0	0	3	0	0	0	0
HYDRACARINA																																
Hydrachnellae	5	0	0	0	0	0	5	0	0	0	1	5	0	1	0	0	0	0	5	0	1	0	0	3	0	0	0	0	0	0	0	0
Turbellaria	1	5	1	0	0	0	5	0	0	0	0	5	0	0	0	0	0	5	0	0	0	0	0	5	0	0	0	0	5	0	0	0

Table C2 a: Abundance of macronverbrate collected seasonally at Site 1 in the Swartkops River during the study period (August 2009 - September 2012).

	Site 1							
Taxon	Sp (2009)	Su (2009)	Au (2010)	Wi (2010)	Sp (2011)	Su (2011)	Au (2012)	Sp (2012)
Oligochaeta	35	27	21	18	43	38	6	83
Hirudinea	2	2	6	1	0	7	8	13
CRUSTACEA	0	0	0	0	0	0	0	0
Potamonautida	0	0	6	1	0	0	9	4
EPHEMEROPTERA	0	0	0	0	0	0	0	0
Leptophlebiidae	1	0	0	0	22	7	25	83
Baetidae	28	28	22	85	330	94	497	922
Caenidae	0	0	0	0	6	0	11	132
Heptageniidae	0	0	0	0	3	0	2	0
ODONATA	0	0	0	0	0	0	0	0
Coenagrionidae	41	38	12	9	29	81	125	163
Lestidae	0	0	0	0	0	2	0	0
Aeshnidae	1	1	3	2	2	2	7	2
Corduliidae	0	0	6	4	0	0	0	1
Gomphidae	1	2	1	0	0	4	11	3
Libellulidae	50	14	28	19	13	24	50	26
Synlestidae	14	0	6	0	0	8	11	0
Platycnemidae	0	0	0	0	0	0	3	0
Protoneuridae	0	0	0	0	0	0	2	0
LEPIDOPTERA	0	0	0	0	0	0	0	0
Pyralidae	0	0	1	1	0	0	0	0
HEMIPTERA								
Belostomatidae	0	3	0	0	3	6	3	0
Corixidae	0	0	2	0	2	16	2	0
Naucoridae	0	7	0	0	0	0	0	8
Nepidae	0	1	0	0	0	0	0	0
Pleidae	0	4	0	0	0	4	3	26
Veliidae	0	26	10	1	0	1	2	1
Gerridae	0	2	0	3	0	1	1	0
TRICHOPTERA	0	0	0	0	0	0	0	0
Ecnomidae	6	0	0	0	8	5	24	4
Hydropsychidae	158	23	10	6	3	34	10q	46
Leptoceridae	2	0	0	0	0	12	0	29
Hydroptilidae	0	0	0	0	0	0	3	42
COLEOPTERA	0	0	0	0	0	0	0	0
Dytiscidae	1	7	0	3	16	7	2	6
Gyrinidae	0	2	7	4	126	14	27	35
Hydraenidae	5	0	0	0	0	0	0	0
Hydrophilidae	0	9	1	0	1	0	1	1

Elmidae	2	0	1	0	30	0	1	10
DIPTERA								
Ceratopogonidae	5	5	18	3	12	19	1	7
Chironomidae	379	199	158	316	413	65	291	630
Culicidae	11	7	1	0	24	1	4	82
Muscidae	2	8	0	1	0	2	1	1
Simuliidae	124	39	140	124	0	96	20	263
Tabanidae	0	1	0	0	0	0	0	0
Athericidae	0	0	0	2	0	0	0	0
GASTROPODA	0	0	0	0	0	0	0	0
Ancylidae	4	1	0	0	0	0	6	17
Lymnaeidae	34	51	18	6	11	31	2	3
Physidae	8	12	13	2	112	111	8	77
Planorbidae	0	0	0	0	0	0	0	2
HYDRACARINA								
Hydrachnellae	0	0	0	0	0	0	1	0

Table C2 b: Abundance of macronverbrate collected seasonally at Site 2 in the Swartkops River during the study period (August 2009 - September 2012).

	Site 2							
Taxon	Sp (2009)	Su (2009)	Au (2010)	Wi (2010)	Sp (2011)	Su (2011)	Au (2012)	Sp (2012_
Oligochaeta	113	130	55	60	88	58	93	71
Hirudinea	16	6	10	1	31	109	306	109
CRUSTACEA	0	0	0	0	0	0	0	0
Potamonautida	4	4	18	9	1	0	0	0
EPHEMEROPTERA	0	0	0	0	0	0	0	0
Baetidae	4	160	554	0	365	8	139	374
Caenidae	0	0	0	0	0	0	0	16
ODONATA								
Coenagrionidae	84	42	38	1	5	120	40	12
Lestidae	0	3	0	0	0	0	0	0
Aeshnidae	1	4	6	0	1	5	0	0
Corduliidae	6	0	0	0	0	0	1	0
Gomphidae	3	0	1	0	0	0	0	0
Libellulidae	16	3	3	0	0	2	2	0
Platycnemidae	0	0	0	0	2	0	0	0
Protoneuridae	0	0	0	0	0	0	0	0
LEPIDOPTERA								
Belostomatidae	3	32	15	2	0	2	3	0
Corixidae	0	386	296	3	0	28	161	0
Naucoridae	0	2	0	0	0	0	0	0
Nepidae	0	0	3	0	0	0	0	0
Notonectidae	0	0	20	0	0	90	1	1

Veliidae	0	0	15	0	0	0	2	0
Gerridae	0	0	2	0	0	0	0	0
TRICHOPTERA								
Hydroptilidae	0	0	0	0	0	0	3	2
COLEOPTERA								
Dytiscidae	13	67	33	2	1	2	2	1
Gyrinidae	0	0	0	0	3	0	65	5
Hydrophilidae	0	2	0	0	0	2	0	0
Elmidae	1	0	0	0	2	0	0	0
DIPTERA								
Ceratopogonidae	2	3	42	12	0	2	2	2
Chironomidae	497	1432	460	28	453	45	147	658
Culicidae	4	47	1	19	1	3	1	45
Muscidae	1	7	0	0	8	0	3	9
Simuliidae	105	84	1	0	576	523	757	97
Tabanidae	0	0	5	0	0	0	1	0
Tipulidae	0	3	0	0	0	0	0	0
Psychodidae	0	0	0	4	0	1	1	0
GASTROPODA								
Ancylidae	6	29	9	0	0	12	16	61
Lymnaeidae	3	0	0	0	0	0	0	0
Physidae	78	252	1	2	3	0	0	0

Table C2 c: Abundance of macronverbrate collected seasonally at Site 3 in the Swartkops River during the study period (August 2009 - September 2012).

	Site 3							
Taxon	Sp (2009)	Su (2009)	Au (2010)	Wi (2010)	Sp (2011)	Su (2011)	Au (2012)	Sp (2012_
Oligochaeta	451	4	30	114	18	1	34	28
Hirudinea	42	8	0	69	3	56	202	211
EPHEMEROPTERA	0	0	0	0	0	0	0	0
Baetidae	0	2	0	0	8	204	95	0
Caenidae	0	0	0	0	0	0	0	2
ODONATA	0	0	0	0	0	0	0	0
Coenagrionidae	22	5	1	8	6	118	243	40
Aeshnidae	1	0	0	3	0	11	3	0
Libellulidae	3	0	0	0	2	2	0	1
Synlestidae	0	0	0	0	0	0	3	0
HEMIPTERA	0	0	0	0	0	0	0	0
Belostomatidae	0	30	43	0	0	0	38	6
Corixidae	0	15	0	3	8	48	204	5
Notonectidae	47	76	70	1	16	175	0	10
Pleidae	0	0	0	0	0	0	0	4
Veliidae	0	0	2	0	0	0	6	0

TRICHOPTERA								
Ecnomidae	2	0	0	0	0	0	0	0
Hydropsychidae	7	0	0	0	0	0	0	0
COLEOPTERA								
Dytiscidae	0	4	16	2	17	125	2	8
Gyrinidae	0	0	1	0	0	0	1	9
Hydrophilidae	0	6	0	0	0	12	3	3
Elmidae	4	0	0	0	0	0	0	0
Helodidae	0	0	0	0	0	0	0	2
DIPTERA								
Ceratopogonidae	0	0	0	0	0	0	1	0
Chironomidae	1052	2557	1210	1753	1857	893	901	2572
Culicidae	51	19	82	19	15	163	0	80
Muscidae	0	2	0	0	3	9	11	1
Simuliidae	0	0	0	0	7	1	36	0
Tipulidae	0	0	0	0	0	0	4	0
Ephydridae	0	0	0	0	1	0	0	0
Psychodidae	4	0	2	0	0	1	0	0
Syrphidae	0	0	10	0	0	0	8	0
GASTROPODA								
Ancylidae	0	0	0	0	0	0	1	0
Physidae	39	0	0	1	0	0	0	0
Planorbidae	1	0	0	0	0	0	0	0

Table C2 d: Abundance of macronverbrate collected seasonally at Site 4 in the Swartkops River during the study period (August 2009 - September 2012).

	Site 4							
Taxon	Sp (2009)	Su (2009)	Au (2010)	Wi (2010)	Sp (2011)	Su (2011)	Au (2012)	Sp (2012)
Oligochaeta	328	379	259	47	1	8	88	280
Hirudinea	130	1	0	192	6	76	640	1093
Turbellaria	0	0	0	0	0	0	0	16
EPHEMEROPTERA	0	0	0	0	0	0	0	0
Baetidae	1	0	0	1	87	309	65	170
Caenidae	0	0	0	0	0	0	0	1
ODONATA								
Coenagrionidae	2	0	0	7	16	58	30	35
Aeshnidae	0	1	0	3	6	6	0	0
Libellulidae	0	0	0	0	3	0	0	1
LEPIDOPTERA								
HEMIPTERA								
Belostomatidae	1	0	2	9	11	29	36	3
Corixidae	0	12	0	0	3	211	0	7
Naucoridae	0	0	0	0	0	0	0	1

Notonectidae	0	0	0	1	2	4	0	3
Pleidae	3	0	0	0	0	4	0	0
Veliidae	1	0	0	0	0	0	14	16
Gerridae	0	0	0	0	0	3	0	2
TRICHOPTERA								
Hydropsychidae	0	0	0	0	0	0	1	0
Leptoceridae	0	0	0	0	0	0	0	4
COLEOPTERA	0	0	0	0	0	0	0	0
Dytiscidae	6	1	0	4	48	38	2	0
Gyrinidae	0	0	0	0	0	0	1	3
Hydrophilidae	1	4	1	0	0	15	1	25
Elmidae	0	4	0	0	0	0	0	8
Helodidae	0	0	1	0	0	0	0	0
DIPTERA								
Ceratopogonidae	0	0	0	0	2	3	1	30
Chironomidae	36	121	42	857	1070	833	527	1520
Culicidae	0	12	172	11	40	10	5	190
Muscidae	0	2	1	3	25	33	22	32
Simuliidae	5	1	0	0	533	233	1511	218
Tabanidae	0	0	0	0	0	0	0	0
Tipulidae	0	0	0	0	0	3	0	0
Ephydridae	0	1	0	0	0	9	0	0
Syrphidae	0	0	2	0	0	0	1	0
GASTROPODA								
Physidae	41	0	0	5	17	106	0	2
Planorbidae	0	9	0	0	0	0	0	0

Table C3 a: Abundance of trait at Sites 1 and 2 per sampling occasion in the Swartkops River during the study period (August 2009 – September 2012). Trait-site matrix was obtained by multiplying the fuzzy score of each trait class per taxon by the abundance of that macroinvertebrate taxon per season as described in Tamanova *et al.* (2008).

		Site 1								**Site 2**							
Trait/ class	Code	Au (2011)	Au (2011)	Sp (2009)	Sp (2011)	Sp (2012)	Su (2009)	Su (2011)	Wi (2010)	Au (2012)	Au (2010)	Sp (2009)	Sp (2011)	Sp (2012)	Su (2009)	Su (2011)	Wi (2010)
Max. body size (mm)																	
	A1	2567	1042	1653	2584	6401	986	838	1591	3235	3311	1843	4205	3512	5154	2190	129
	A2	4926	1827	3137	4835	10688	1686	1786	2770	9067	9698	3339	9001	7472	11927	3885	314
	A3	2127	617	1771	1253	2330	740	1144	573	1700	3087	1140	1654	1536	2934	1007	121
	A4	1161	339	713	739	1576	451	617	413	435	853	1005	490	724	1924	686	72
	A5	255	81	138	68	239	77	154	42	48	126	132	10	13	165	143	14
Respiration																	
	B1	5355	1474	3054	4178	9947	1336	2016	1998	4695	7571	2477	6808	5465	6695	2453	200
	B2	2766	1916	3267	3812	7155	1809	2117	2493	7079	3956	4227	6513	5393	10143	3840	474
	B3	107	220	203	320	805	372	256	164	2644	1949	205	658	294	2781	1397	127
	B4	222	105	63	808	660	295	248	56	2198	1948	92	63	195	2661	869	107
	B5	77	173	210	615	451	315	710	43	0	59	417	18	0	1272	0	37
Mobility																	
	C1	1007	237	377	1051	1439	411	838	141	1688	1366	636	79	173	2179	983	41
	C2	4464	1445	3120	3628	7824	1519	1959	1851	5109	5446	2641	5462	4716	6625	2787	211
	C3	909	498	1148	1266	2188	603	250	958	453	1427	1493	1359	1998	4302	137	96
	C4	3665	691	1175	3262	7329	766	1372	1014	5547	8010	1225	4859	4460	6333	1886	206
	C5	26	74	0	11	41	191	41	27	10	85	3	0	0	0	0	0
	C6	1544	963	2095	2448	3806	1175	592	1698	1299	2815	3085	2737	3760	7904	533	521
Body shape																	
	D1	540	28	32	400	1098	49	122	95	595	1963	544	1186	1253	2354	95	37

	D2	360	154	183	379	904	131	203	97	2077	1586	695	986	1483	2342	649	81
	D3	214	194	131	681	732	440	322	69	455	642	204	24	211	1133	90	48
	D4	6118	2214	4320	5056	12688	2188	2644	2971	9608	10314	4260	9461	7881	12626	4686	651
Preferred food																	
	E1	5091	1913	3901	4501	11325	1813	2082	2808	6817	8521	3845	9267	7467	10817	3447	576
	E2	1276	423	1143	1207	2413	463	610	581	1361	1658	821	1861	1527	2307	666	130
	E3	2978	875	2039	2552	6332	1016	1043	1401	3656	5247	1762	3445	3755	7445	1215	178
	E4	2364	992	1919	2276	3452	1264	1150	1211	2585	2299	2226	1591	2646	5158	1355	201
Feeding habit																	
	F1	926	202	576	771	1717	280	260	413	570	2155	513	1549	1464	1931	78	49
	F2	4118	997	2189	4122	9013	1266	985	2063	2133	6957	2867	5460	6103	8850	480	361
	F3	1344	890	1696	685	3345	473	937	813	4766	1610	936	3889	1701	1856	2934	281
	F4	2649	852	1596	2561	6111	942	930	1405	2153	4873	1864	4221	4017	5794	779	216
	F5	2309	920	1901	2252	3378	1241	1084	1199	1346	2081	2134	1464	2207	4994	997	138
Preferred biotope																	
	G1	357	180	649	233	592	309	343	112	1566	1208	565	464	370	1859	524	318
	G2	1199	708	1383	2078	3580	809	545	1099	2511	3680	2224	3008	3700	5906	848	513
	G3	3729	1452	2372	3582	7485	1548	2201	1803	3561	3437	2786	4267	3537	6660	2365	132

Table C3 b: Abundance of trait at Sites 3 and 4 per sampling occasion in the Swartkops River during the study period (August 2009 – September 2012). Trait-site matrix was obtained by multiplying the fuzzy score of each trait class per taxon by the abundance of that macroinvertebrate taxon per season as described in Tamanova *et al.* (2008).

		Site 3								**Site 4**							
Trait/ class	Code	Au (2012)	Au (2010)	Sp (2009)	Sp (2011)	Sp (2012)	Su (2009)	Su (2011)	Wi (2010)	Au (2012)	Au (2010)	Sp (2009)	Sp (2011)	Sp (2012)	Su (2009)	Su (2011)	Wi (2010)
Max. body size (mm)																	
	A1	3144	3815	3288	5667	7853	7784	3781	5281	6411	300	153	5187	6146	405	4248	2593
	A2	7198	6697	5752	9615	14084	13160	7745	9101	12674	1088	639	9088	14298	775	8783	4973
	A3	2750	1495	1312	1987	3084	2807	2265	1887	1511	60	200	1468	3183	163	1837	1148
	A4	2346	1447	1170	1906	2823	2742	1677	1810	861	53	58	1286	1739	131	1354	956
	A5	304	60	28	25	58	45	290	19	69	3	10	96	64	8	158	29
Respiration																	
	B1	4580	3688	3343	5726	8010	7738	4777	5320	6630	130	159	5548	6581	408	5279	2641
	B2	6118	6142	6168	9424	14041	12856	5482	9246	13607	473	1410	8414	14840	1054	6804	5343
	B3	1394	681	240	207	383	414	1622	83	1862	544	65	1040	1197	153	1926	108
	B4	1310	676	200	191	425	408	1589	83	290	541	60	432	858	126	1579	99
	B5	0	0	200	0	0	0	0	5	0	0	205	85	10	45	530	25
Mobility																	
	C1	2004	299	209	157	356	286	1651	76	279	13	98	414	374	79	1427	103
	C2	5158	3889	3539	5762	8681	7872	4834	5532	8579	146	605	5584	9646	455	5269	3261
	C3	2748	3680	3156	5574	7718	7671	2679	5259	1587	136	108	3212	4603	366	2532	2571
	C4	3111	1993	1483	2135	3413	3026	4043	1961	3259	917	274	2618	4920	280	4411	1196
	C5	30	10	0	0	0	0	0	0	70	0	5	0	90	0	15	0
	C6	4597	6081	5711	9317	12902	12795	4493	8879	2793	472	508	5432	8070	995	4341	4341
Body shape																	
	D1	1002	1258	1052	1916	2596	2586	1472	1759	598	42	55	1301	1690	124	1256	870

	D2	2033	1394	1295	1929	3687	2714	1557	2104	3843	48	707	1288	7150	141	1432	1856
	D3	247	276	54	51	116	207	435	7	264	15	89	216	260	62	460	62
	D4	8239	6682	6338	9629	14242	13146	8027	9258	12902	1349	966	9090	14851	1167	8984	5046
Preferred food																	
	E1	5693	6531	6115	9472	13344	12914	6490	8989	10662	1335	581	8700	10905	1112	7363	4408
	E2	1089	1339	1645	1908	2698	2600	1301	1887	2201	478	414	1750	2497	549	1578	922
	E3	4075	3794	3331	5678	7851	7865	3920	5298	3297	303	160	4089	5641	483	4918	2594
	E4	5258	3945	3509	5721	9129	7942	4326	5669	5272	144	828	3745	10781	405	3776	3655
Feeding habit																	
	F1	1017	1216	1071	1868	2581	2577	1136	1753	595	45	40	1157	1785	136	1223	858
	F2	5058	6120	5751	9348	12913	12829	5551	8879	3057	480	518	5788	8942	1029	5844	4337
	F3	543	470	754	146	434	116	1075	212	7736	1125	354	2959	2506	460	1743	103
	F4	3077	3758	3716	5638	7831	7700	3472	5396	3382	559	485	4073	5806	784	3804	2640
	F5	4456	4015	3388	5725	8295	7986	4277	5394	2710	144	308	3719	6352	401	3476	2888
Preferred biotope																	
	G1	673	36	472	42	52	67	181	123	133	262	334	10	424	427	686	47
	G2	3367	3957	3889	5694	8674	7768	3745	5652	3616	901	844	3517	8797	787	2915	3248
	G3	4512	3773	3497	5665	7996	7816	3990	5313	6681	138	359	5376	6196	399	5215	2679

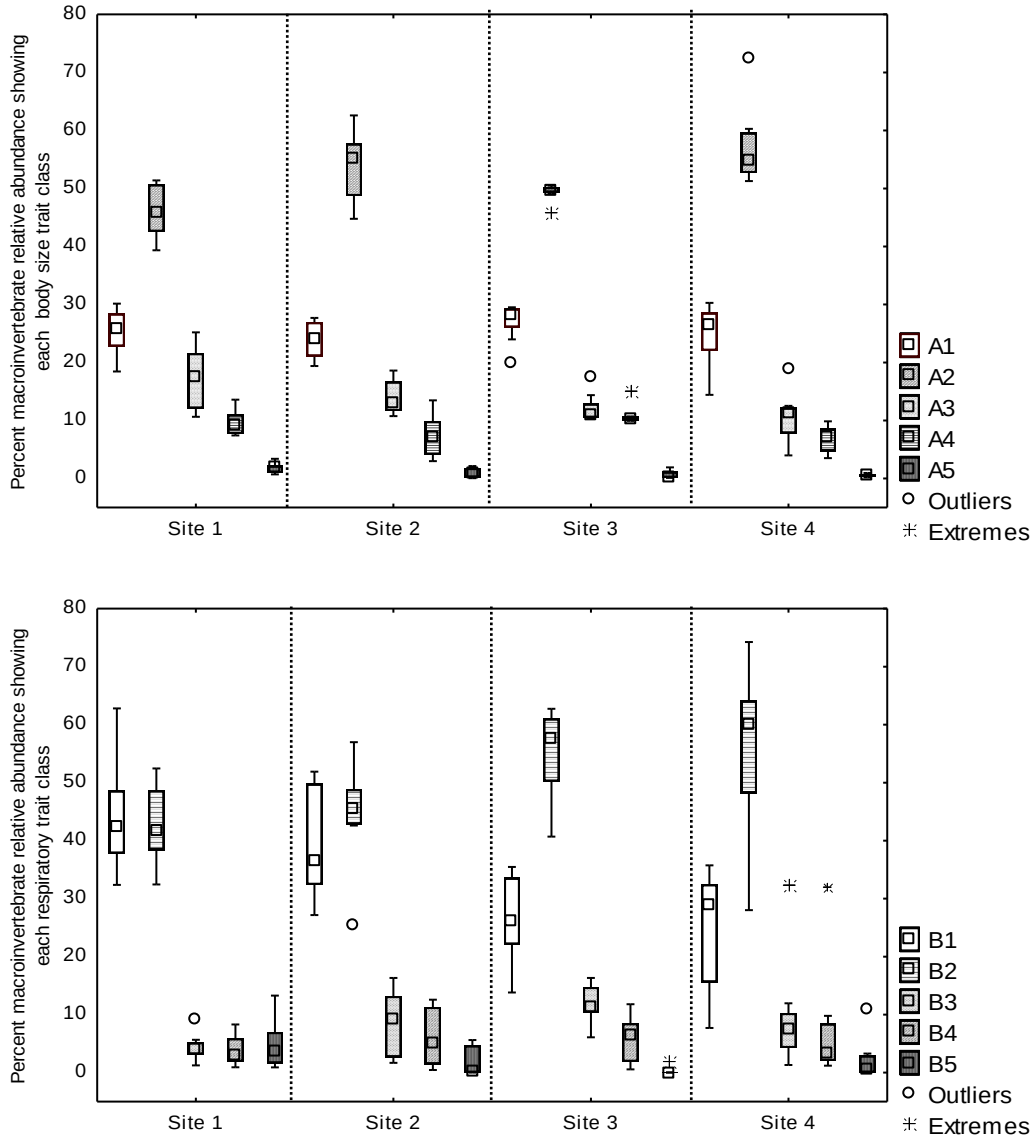

Figure C1: Percent macroinvertebrate relative abundance in each body size trait class and respiratory trait class showing the median value (small square), 25 – 75 percentile values (big box) and the non-outlier ranges (vertical bars above and below each box) at the four sampling sites in the Swartkops River during the study period (August 2009 – September 2012). Trait codes: Body Size (mm) A1 (≤ 5), A2 (> 5-10), A3 (>10-20), A4 (> 20-40), A5 (> 40-80). Respiration: B1 (gills), B2 (tegument), B3 (spiracle), B4 (breathing tube), B5 (lung)

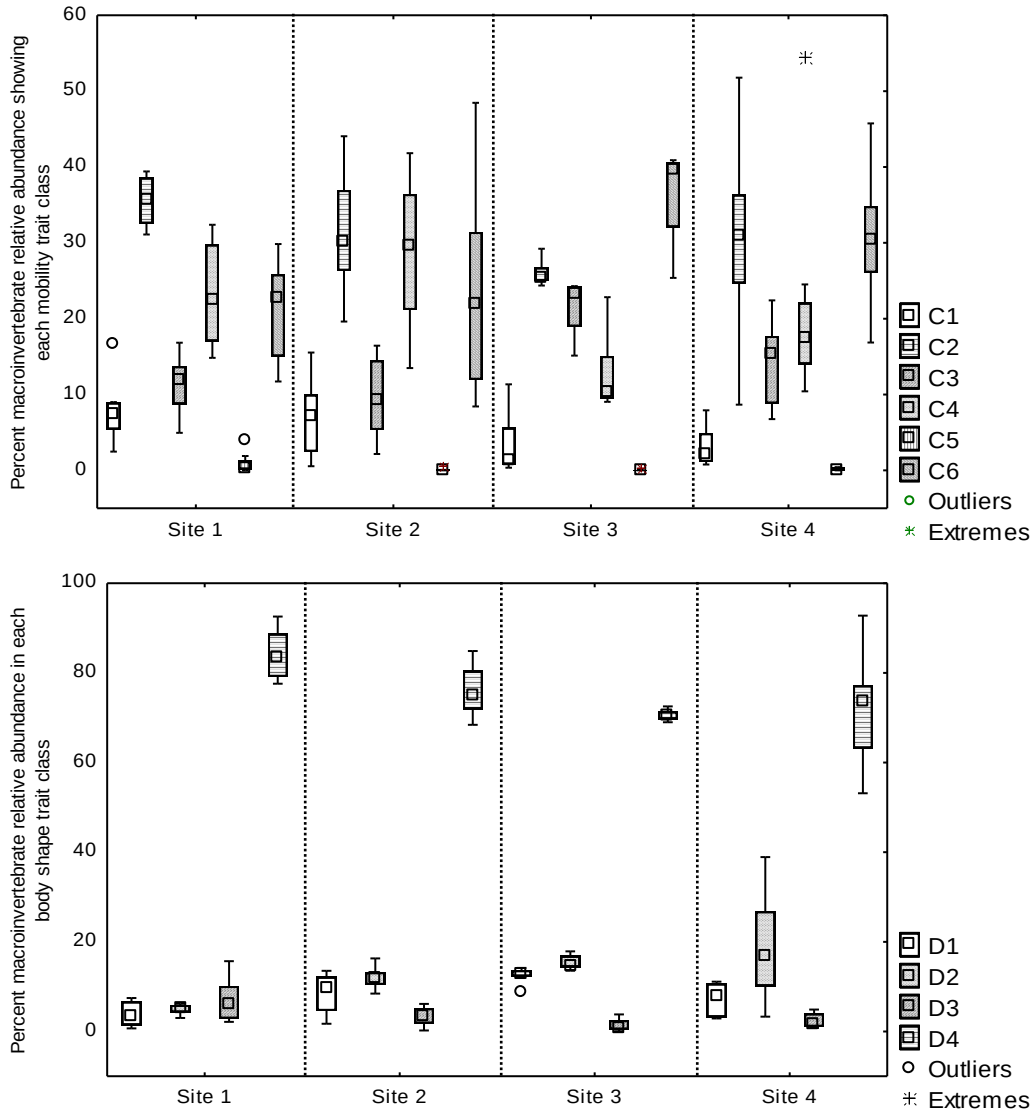

Figure C2: Percent macroinvertebrate relative abundance in each mobility trait class and body shape trait class showing the median value (small square), 25 – 75 percentile values (big box) and the non-outlier ranges (vertical bars above and below each box) at the four sampling sites in the Swartkops River during the study period (August 2009 – September 2012). Trait codes: Mobility C1 (climbers), C2 (crawlers), C3 (sprawlers), C4 (swimmers), C5 (skaters) and C6 (burrowers). Body shape D1 (streamlined), D2 (flattened), D3 (spherical) and D4 (cylindrical).

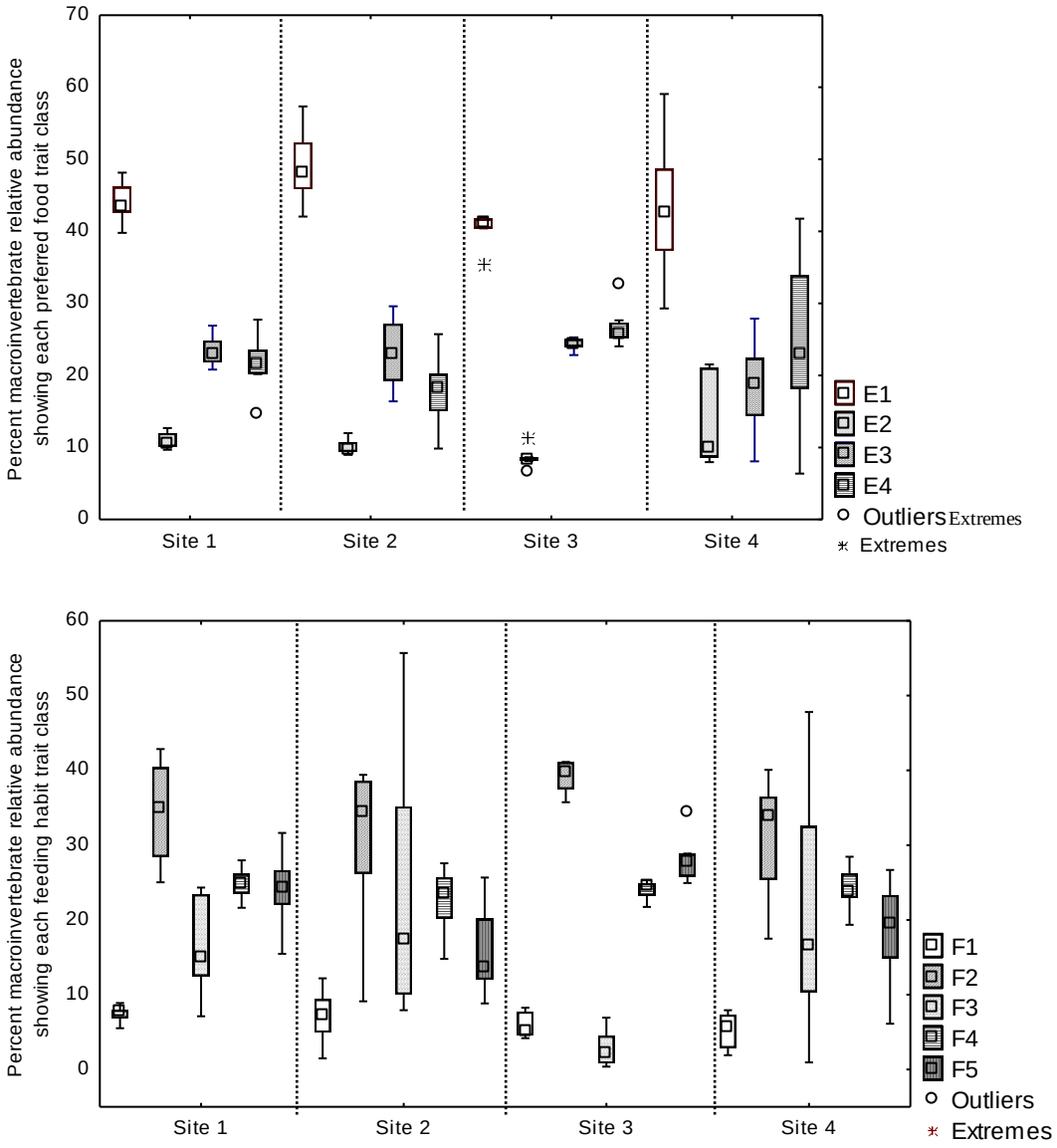

Figure C3: Percent macroinvertebrate relative abundance in each preferred food trait class and feeding habit trait class showing the median value (small square), 25 – 75 percentile values (big box) and the non-outlier ranges (vertical bars above and below each box) at the four sampling sites in the Swartkops River during the study period (August 2009 – September 2012). Trait codes: Preferred food E1 (FPOM), E2 (CPOM), E3 (plant materials including algae) and E4 (animal materials). Feeding habit F1 (shredder), F2 (collector-gatherer), F3 (collector-filterer) and F4 (scraper, grazer, brusher) and F5 (predator).

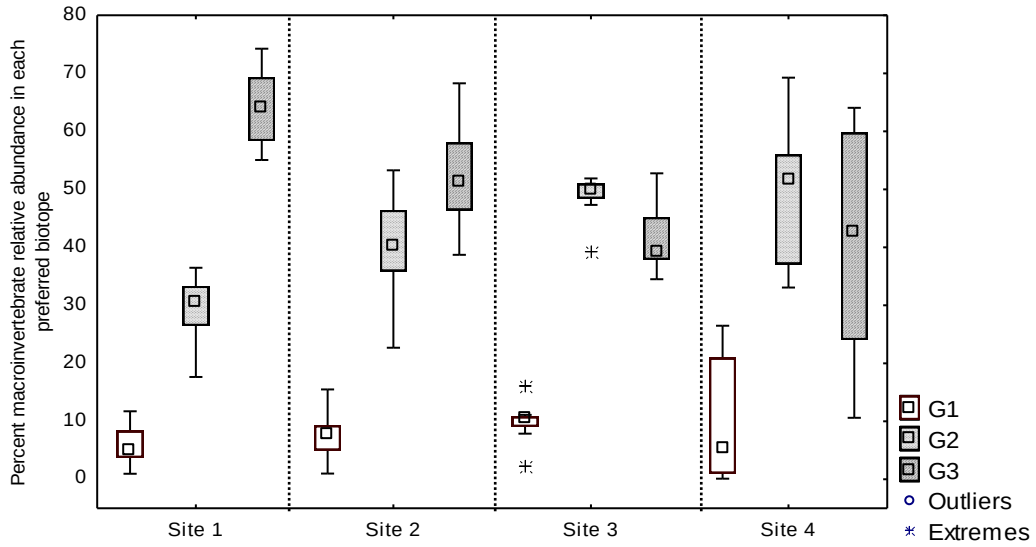

Figure C4: Percent macroinvertebrate relative abundance in each preferred biotope showing the median value (small square), 25 – 75 percentile values (big box) and the non-outlier ranges (vertical bars above and below each box) at the four sampling sites in the Swartkops River during the study period (August 2009 – September 2012). Trait classes: G1 (sediment: gravel, sand and mud), G2 (stone) and G3 (vegetation).

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Appendix D: Full description of observed mentum deformities among the Swartkops River chironomid collected at the four sampling sites during the study period (August 2009 - September 2012).

Table D1: Number and description of mentum deformities recorded among chironomid taxa at the four sampling sites during spring 2009. Biotopes replicates are also shown. Biotopes: stone (stone-in-and-out-of-current), Veg (marginal and aquatic vegetation), GSM (grave, sand and mud). Number written in fron of each biotope indicate the replicate i.e. 1 (first replicate sample), 2 (second replicate sample) and 3 (third replicate sammple).

Site	Biotope replicate	Species	Type of deformity	Description	No. of individual showing deformity
	Stone 1	*Cricotopus* sp.1	fused teeth	fused fifth and sixth lateral teeth	1
Site 1	Stone 2	*Tanytarsus* sp.	missing teeth	missing first right lateral teeth	1
		Orthocladius sp.	fused teeth	fused fourth, fifth and sixth right lateral teeth	1
		Cricotopus sp.1	split tooth	split median tooth	1
		Cardiocladius sp.	worn tooth	worn median tooth	2
	Stone 3	*Cricotopus* sp.1	fused teeth	fused fourth, fifth and sixth lateral teeth	1
		Ablabesmyia sp.	split tooth	split left inner tooth	1
	Veg. 1		split tooth	split median tooth	1
		Cricotopus sp.1	fused teeth	fused left lateral teeth	1
		Polypedilum sp.	split tooth	split left outer median tooth	1
		Ablabesmyia sp.	split tooth	split right outer tooth	1
	Veg. 3	*Polypedilum* sp.	fused teeth	fused second to sixth left lateral teeth	1
				all left lateral teeth fused with first lateral teeth	1
				fused fifth and sixth right lateral teeth	1
			missing teeth	missing outer median tooth	2
				missing sixth right lateral teeth and third to fifth left lateral teeth	1
		Orthocladius sp.	fused teeth	fused fifth and sixth left lateral teeth	1
			missing teeth	missing sixth left lateral teeth	1

Site 2			missing teeth	missing second left lateral teeth	1
				missing third lateral teeth	1
			Kohn gap	gaps on the median teeth	3
			fused teeth	fused fifth and sixth left lateral teeth	3
		Cricotopus sp.1		fused first and second left lateral teeth	1
			split tooth	split first lateral teeth	1
			lateral gap	lateral gap on left lateral teeth	1
				first and second right lateral teeth fused	1
	Stone 2	*Cricotopus trifasciata* gr.	fused teeth	fused fifth and sixth right lateral teeth	1
		Cricotopus sp.1	fused teeth	fused third, fourth, fifth and sixth right lateral teeth	1
		Cricotopus trifasciata gr.	Kohn gap	gaps on the median teeth	2
			fused teeth	fused fourth, fifth and sixth left lateral teeth	4
		Orthocladius sp.	fused teeth	fused fifth and sixth left lateral teeth	1
	Stone 1	*Chironomus* sp. 1	split tooth	split median tooth	1
	Veg. 2	*Dicrotendipes* sp.	fused teeth	fused first and second left lateral teeth	3
		Cricotopus trifasciata gr.	split tooth	split first lateral teeth	1
			lateral gap	gaps in left lateral teeth	1
				fused fifth and sixth right lateral teeth	1
		Cricotopus sp.1	fused teeth	fused first and second right lateral teeth	1
		Orthocladius sp.	fused teeth	fused fifth and sixth right lateral teeth	1
	Veg. 3	*Eukiefferiella* sp.	fused teeth	fused fourth and fifth lateral teeth	1
		Cricotopus triafasciata gr	split tooth	split left median tooth	1
	Stone 2	*Dicrotendipes* sp.	missing teeth	fifth and sixth left lateral teeth missing	1
		Cricotopus triafasciata gr	fused teeth	fused fifth and sixth left lateral teeth	1
	GSM 3				.

Site 3			Asymmetry	asymmetry between first lateral teeth	3
			fused teeth	fused fifth and sixth left lateral teeth	4
				fused lateral teeth	1
				fused third and fourth right lateral teeth	6
				fused fourth, fifth and sixth lateral teeth	1
				fused third and fourth left lateral teeth	1
			missing teeth	missing sixth right lateral teeth and third to fifth left lateral teeth	1
				missing first right lateral teeth	1
			split tooth	split first left lateral teeth	1
				split median tooth	1
				split first right lateral teeth	1
	Stone1	*Chironomus* sp. 1	Others	distortion in the basic configuration of mentum	1
	Veg. 1	*Chironomus* sp. 1	Extra teeth	extra teeth on left lateral teeth	1
			fused teeth	fused outer median and first lateral teeth	3
			split tooth	split median tooth	1
	Veg. 2	*Chironomus* sp. 1	fused teeth	fused fourth, fifth and sixth left and right lateral teeth	1
			missing teeth	missing sixth left lateral teeth	3
			split tooth	split median tooth	1
	GSM1	*Chironomus* sp. 1	missing teeth	missing outer median tooth	1
				missing fifth left lateral tooth	1
				missing sixth left lateral teeth	1
				missing first right lateral teeth	1
			Extra teeth	extra tooth on median tooth	1
			lateral gap	lateral gap on left lateral teeth	1
			fused teeth	fused first and second right lateral teeth	1
			Kohn gap	gaps on the median teeth	1

			Asymmetry	asymmetry between first lateral teeth	7
			fused teeth	fused fifth and sixth left lateral teeth	1
				fused lateral teeth	4
				fused third and fourth right lateral teeth	2
				fused fourth, fifth and sixth lateral teeth	1
				fused third and fourth left lateral teeth	
			missing teeth	missing sixth right lateral teeth and third to fifth left lateral teeth	12
				missing first right lateral teeth	4
			split tooth	split first left lateral teeth	4
				split median tooth	3
				split first right lateral teeth	2
			Kohn gap	gaps in the median teeth of the mentum	5
			Others	distortion in the basic configuration of mentum	1
		Chironomus sp. 1	extra tooth	extra tooth on the central median tooth	2
	Stone 3	*Dicrotendipes* sp.	Kohn gap	gaps in the median teeth of the mentum	1
	Stone 2	*Chironomus* sp. 1	Asymmetry	asymmetry between first lateral teeth	2
			fused teeth	fused fifth and sixth left lateral teeth	2
				fused lateral teeth	1
				fused third and fourth right lateral teeth	2
				fused fourth, fifth and sixth lateral teeth	3
				fused third and fourth left lateral teeth	1
			missing teeth	missing sixth right lateral teeth and third left lateral teeth	1
				missing first left lateral teeth	1
			split tooth	split first left lateral teeth	1
				split median tooth	2
				split first right lateral teeth	1
			Kohn gap	gaps in the median teeth of the mentum	1

			extra tooth	extra tooth on the central median tooth	2
	GSM 2	*Chironomus* sp. 1	missing teeth	missing central median tooth and second left lateral teeth	1
				missing sixth left lateral teeth	1
			fused teeth	fused fifth and sixth left lateral teeth	1
				fused fourth and fifth right lateral teeth	1
			lateral gaps	gaps in the left lateral teeth	1
			split tooth	split median tooth	1
	GSM 3	*Chironomus* sp. 1	split tooth	split median and first right lateral teeth	1
Site 4	Veg. 2	*Polypedilum* sp.	split tooth	split median tooth	1
	Veg. 3	*Polypedilum* sp.	split tooth	split median tooth	2
	Veg. 1	*Chironomus* sp. 1	fused teeth	fused fifth and sixth left lateral teeth	1
				all left lateral teeth fused with first lateral tooth	1
			extra teeth	two extra teeth on central median tooth	1
		Tanytarsus sp.	fused teeth	all left lateral teeth fused with first lateral tooth	1
	Stone 3	*Orthocladius* sp.	fused teeth	fused fifth and sixth left lateral teeth	1

Table D2: Number and description of mentum deformities recorded among chironomid taxa at the four sampling sites during summer 2009. Biotopes replicates are also shown. Biotopes: stone (stone-in-and-out-of-current), Veg (marginal and aquatic vegetation), GSM (grave, sand and mud). Number written in fron of each biotope indicate the replicate i.e. 1 (first replicate sample), 2 (second replicate sample) and 3 (third replicate sammple).

Sites	**biotope replicate**	**Genus**	**Deformity type**	**Description**	**No. of individual showing deformity**
Site 1	Stone1	*Cricotopus trifasciata* gr.	fused teeth	first and second right lateral teeth fused	1

			split tooth	split first lateral tooth	1
		Ablabesmyia sp.	split tooth	split inner tooth	1
	stone 2	*Cricotopus trifasciata* gr.	missing teeth	missing sixth lateral tooth	1
			fused teeth	fused fifth and sixth lateral teeth	1
	GSM2	*Cricotopus* sp. 1	missing teeth	missing fourth, fifth and sixth left lateral teeth	1
				missing fifth right lateral tooth	1
Site 2	Veg. 2	*Kiefferulus* sp.	split	split median tooth	1
			missing teeth	missing fifth left lateral tooth	1
		Dicrotendipes sp.	missing teeth	second left lateral tooth missing	1
				missing first and second right lateral teeth	1
				missing second right lateral tooth	1
			split tooth	split fourth left lateral tooth	1
	Veg. 1	*Kiefferulus* sp.	missing teeth	missing fifth left lateral tooth	1
		Dicrotendipes sp.	missing teeth	second right lateral tooth missing	2
				missing first and second right lateral teeth	1
				missing second right lateral tooth	1
			split tooth	split fifth left lateral tooth	2
	Veg. 3	*Dicrotendipes* sp.	missing teeth	sixth left lateral tooth missing	1
				missing first and second right lateral teeth	1
				missing second right lateral tooth	1
	GSM 1	*Tanypus* sp.	split tooth	split inner tooth	2
			fused teeth	fused outer and middle teeth	1
				fused outer and inner left tooth	1
			missing teeth	missing two outer teeth	1

		asymmetry	asymmetry between the two inner teeth	1
	Cricotopus trifasciata gr.	missing teeth	missing first and second right lateral teeth	1
	Chironomus sp.1	missing teeth	missing first left lateral tooth (fused fourth fifth and sixth lateral teeth)	1
GSM 2	*Tanypus* sp.	missing teeth	missing left inner tooth	2
			missing left outer tooth	1
		split tooth	split outer right tooth	4
GSM 3	*Chironomus* sp.1	fused teeth	fused fourth, fifth and sixth right lateral teeth	2
		missing teeth	missing outer median tooth	1
	Tanypus sp.	split tooth	split outer right tooth	3
			split outer left tooth	1
Stone 2	*Dicrotendipes* sp.	worn tooth	loss scleretization of the median tooth	1
		fused teeth	fourth and fifth left lateral teeth	1
		missing teeth	second right lateral tooth missing	1
	Chironomus sp.1	fused teeth	fourth, fifth and sixth right lateral teeth fused	1
	Chironomus sp.2	fused teeth	fused fourth and fifth left lateral teeth	1
Stone 1	*Chironomus* sp1	fused teeth	all three median teeth fused	1
		extra tooth	two extra median teeth	1
		split tooth	split right median and first and second left lateral teeth	1
	Cricotopus sp1	Kohn gap	Kohn gap	1
Stone 3	Dicrotendipes sp.	worn tooth	loss scleretization of the median tooth	1
		fused teeth	fourth and fifth left lateral teeth	1
		missing teeth	second right lateral tooth missing	1
			missing first left lateral tooth	2

			split tooth	split sixth left lateral tooth	4
			extra tooth	median tooth	2
			Kohn gap	Kohn gap	1
		Chironomus sp.1	fused teeth	fourth, fifth and sixth right lateral teeth fused	1
		Chironomus sp.2	missing teeth	missing sixth lateral tooth	2
Site 3	Stone 1	*Chironomus* sp.1	extra tooth	extra median tooth, missing tooth and fused tooth	2
			split tooth	split fourth left lateral tooth	2
				split second left lateral teeth	6
			asymmetry	asymmetry between right and left lateral teeth	2
		Chironomus sp.3	split tooth	split right third lateral teeth	2
		Chironomus sp. 2	split tooth	split central median tooth	2
			fused teeth	fused first and second lateral teeth	2
	Stone 2	*Chironomus* sp.1	Kohn gap	Kohn gap (twisted median tooth)	2
			split tooth	split first right lateral tooth	3
			fused teeth	fused fifth and sixth right lateral teeth	2
			missing teeth	missing sixth lateral tooth	4
		Chironomus sp.3	missing teeth	missing sixth and fifth left lateral teeth	9
	Stone 3	*Chironomus* sp.1	extra tooth	extra median tooth, missing tooth and fused tooth	2
			split tooth	split fourth left lateral tooth	4
				split second left lateral teeth	3
			asymmetry	asymmetry between right and left lateral teeth	2
			Kohn gap	Kohn gap	2
			fused teeth	fused third and fourth left lateral teeth	3
		Chironomus sp.3	split tooth	split right third lateral teeth	2

	Chironomus sp.2	split tooth	split central median tooth	2
		fused teeth	fused first and second lateral teeth	2
	Chironomus sp1	missing teeth	missing fourth, fifth and sixth left lateral teeth	2
			missing sixth lateral teeth	2
		split tooth	split first right lateral teeth	2
Veg. 1	*Chironomus* sp.1	missing teeth	missing sixth left lateral teeth	7
			missing third lateral teeth	2
		Kohn gap	Kohn gap (twisted median tooth)	5
		split tooth	split second left lateral teeth	2
		fused teeth	fused fourth and fifth right lateral teeth	2
	Dicrotendipes sp.	missing teeth	missing sixth left lateral teeth	1
Veg. 3	*Chironomus* sp.1	missing teeth	missing sixth left lateral teeth	4
			missing third lateral teeth	3
		Kohn gap	Kohn gap	3
		split tooth	split second left lateral teeth	4
		fused teeth	fused fourth and fifth right lateral teeth	2
		extra tooth	extra median tooth	1
GSM1	*Chironomus* sp.1	missing teeth	missing fourth left lateral teeth	1
			missing fourth and sixth left lateral teeth	1
			missing sixth left lateral tooth	4
		fused teeth	fused first and second lateral teeth	2
			fused fifth and sixth right lateral teeth	1
		Kohn gap	Kohn gap	2
		split tooth	split first right lateral teeth	1
		extra tooth	extra median tooth	1

		Chironomus sp.2	split tooth	split sixth lateral teeth	2
Site 4	GSM 2	*Chironomus* sp.1	missing teeth	missing sixth left lateral teeth	3
				sixth teeth on both sides of mentum missing	1
				missing left median tooth	1
			extra tooth	extra central median tooth	1
		Chironomus sp.2	fused teeth	fused fourth, fifth and sixth right lateral teeth	1
			Kohn gap	Kohn gap	2
		Chironomus sp.3	missing teeth	missing sixth lateral tooth	2
			split tooth	split right second lateral tooth	1
	GSM 1	*Chironomus* sp.1	Kohn gap	Kohn gap	1
			fused teeth	fused fifth and sixth left lateral teeth	1
		Chironomus sp.1	extra tooth	extra median tooth	1
	GSM3	*Chironomus* sp.1	split tooth	split outer left median tooth	1
			missing teeth	missing sixth left lateral tooth	2
		Chironomus sp.3	fused teeth	fused fourth and fifth lateral teeth	1
	Stone 1	*Chironomus* sp.1	missing teeth	missing fifth right lateral tooth	1
			split tooth	split first lateral tooth	1
	Stone 3	*Chironomus* sp.1	Kohn gap	Kohn gap	2

Table D3: Number and description of mentum deformities recorded among chironomid taxa at the four sampling sites during autumn 2010. Biotopes replicates are also shown. . Biotopes: stone (stone-in-and-out-of-current), Veg (marginal and aquatic vegetation), GSM (grave, sand and mud). Number written in fron of each biotope indicate the replicate i.e. 1 (first replicate sample), 2 (second replicate sample) and 3 (third replicate sammple).

Site	biotope replicate	Species	deformity type	Description	No. of individual showing deformity
Site 1	Stone 1	*Cricotopus trifasciata* gr.	missing teeth	missing second left lateral teeth	1
			fused teeth	fused fifth and sixth right lateral teeth	2
				fused third and fourth right lateral teeth	1
				fused third and fourth left lateral teeth	2
	Stone 3	*Cricotopus trifasciata* gr.	fused teeth	fused fifth and sixth left lateral teeth	1
	GSM 1	*Tanytarsus* sp.	fused teeth	fused fourth and fifth right lateral teeth	1
Site 2	Veg. 1				
		Chironomus sp.1	Kohn gap	Kohn gap	1
			fused teeth	fused fourth and fifth left lateral teeth	1
			missing teeth	missing left median tooth	1
			split tooth	split central median tooth	1
			extra teeth	extra median tooth	1
		Chironomus sp.2	split tooth	split first-third lateral teeth	1
	Veg. 2	*Chironomus* sp.1	missing teeth	missing third left lateral teeth	2
				missing left median tooth	1
	Veg. 3	*Chironomus* sp.1	fused teeth	fused third and fourth right lateral teeth	1
	Stone 1	*Chironomus* sp.1	split tooth	split first left lateral tooth	1
				split fourth left lateral tooth	1
				split left median and first lateral teeth	1

			fused teeth	fused fifth and sixth right lateral teeth	1
		Kiefferulus sp.	fused teeth	fused first and second right lateral teeth	1
		Microchironomus sp.	missing teeth	missing sixth left lateral tooth	1
	Stone 3	*Dicrotendipes* sp.	fused teeth	fused sixth, fifth and fourth right lateral teeth	1
		Chironomus sp.1	missing teeth	missing second right lateral tooth	1
			fused teeth/ missing teeth	fused first and second right lateral teeth, missing fourth left lateral tooth	1
			split tooth	split first right lateral tooth	1
		Tanypus sp.	twisted tooth	twisted outer lateral tooth	1
	GSM 2	*Tanypus* sp.	split tooth	split outer tooth	2
			twisted tooth	twisted outer lateral tooth	1
Site 3	Veg. 1	*Chironomus* sp.1	missing teeth	missing left median tooth	2
				missing first right lateral tooth	2
				missing first and second right lateral teeth	1
				missing right median tooth and twisted first right lateral teeth	1
				missing third right lateral tooth	1
				missing second lateral tooth	1
				missing second left lateral tooth	1
			Kohn gap	Kohn gap	1
			fused teeth	fused sixth, fifth and fourth lateral teeth	1
			split tooth	split first left lateral tooth	1
				split second first lateral tooth	1
	Veg. 2	*Chironomus* sp.2	split tooth	split left median tooth	1
		Chironomus sp.1	extra teeth	extra central median tooth	1

		missing teeth	missing first left lateral tooth	1
			missing right median tooth	1
		Kohn gap	Kohn gap	1
	Chironomus sp.3	missing teeth	missing fourth right lateral tooth	1
		others	distortion in overall configuration	1
Stone 2	*Chironomus* sp.1	missing teeth	missing central median tooth	1
			missing fifth left lateral teeth	4
			missing first left lateral and fourth right lateral tooth	2
			missing first right lateral tooth	1
		extra teeth	extra median tooth	4
		fused teeth	fused first and second left lateral teeth	1
Stone 1	*Chironomus* sp.1	missing teeth	missing first and second right lateral tooth	1
			missing third right lateral tooth	1
			missing fifth right lateral tooth	1
		Kohn gap	Kohn gap	1
		split tooth	split left median tooth	1
			split first left median tooth	1
			split central median tooth	1
			split second left lateral tooth	1
		extra teeth	extra median tooth	1
	Chironomus sp.2	missing teeth	missing sixth left lateral tooth	1
	Chironomus sp.3	missing teeth	missing first right lateral teeth	1
Stone 3	*Chironomus* sp.1	extra teeth	extra third left lateral teeth	2
			extra median tooth	2

			missing teeth	missing sixth lateral teeth	1
				missing second left lateral teeth	2
				missing second right lateral teeth	1
				missing third and fourth right lateral teeth	2
			fused teeth	fused second and third left lateral teeth	3
				fused fifth and sixth left lateral teeth	1
			Extra teeth/fused teeth	extra median tooth, fused third and fourth right lateral teeth	2
			Kohn gap	Kohn gaps	4
		Chironomus sp.2	missing teeth	missing first lateral teeth	1
			extra tooth	extra median tooth	3
	Veg. 3	*Chironomus* sp.1	Kohn gap	Kohn gap	1
			fused teeth	fused fourth, fifth and sixth right lateral teeth	1
			missing teeth	missing fifth left lateral tooth	1
				missing sixth left lateral tooth	1
			split tooth	split second left lateral tooth	1
Site 4	GSM 2	*Chironomus* sp.1	fused teeth	fused fifth and sixth right lateral teeth	1
				fused fourth, fifth and sixth left lateral teeth	1
	Veg. 2	*Chironomus* sp.1	fused teeth	fused third and fourth left lateral teeth	1

Table D4: Number and description of mentum and ligula deformities recorded among chironomid taxa at the four sampling sites during winter 2010. Biotopes replicates are also shown. . Biotopes: stone (stone-in-and-out-of-current), Veg (marginal and aquatic vegetation), GSM (grave, sand and mud). Number written in fron of each biotope indicate the replicate i.e. 1 (first replicate sample), 2 (second replicate sample) and 3 (third replicate sammple).

Site	Biotope replicate	Genus	Deformity type	Description	No. of individual showing deformity
Site 1	Stone 1	*Tanytarsus* sp.	fused teeth	fused fourth and fifth right lateral teeth	1
	Veg. 1	*Polypedilum* sp.	Split tooth	right central median tooth	1
	GSM 3	*Tanytarsus* sp.	missing teeth	Fifth left lateral tooth	1
			fused teeth	fourth and fifth left lateral teeth	1
		Cricotopus trifasciata gr.	fused teeth	Fourth to sixth right lateral teeth	1
	GSM 2	*Cricotopus trifasciata* gr.	split tooth	median tooth	1
	GSM 1	*Tanytarsus* sp.	fused teeth	Fourth and fifth right lateral teeth	1
	Veg. 3	*Tanytarsus* sp.	fused teeth	Fourth and fifth right lateral teeth	1
Site 2	Stone 3	*Chironomus* sp.1	fused teeth	fused median teeth	1
	GSM 2	*Chironomus* sp. 1	fused teeth	Fused fifth and sixth right lateral teeth	2
Site 3	GSM 1	*Chironomus* sp.1	missing teeth	Sixth left lateral tooth	2
				Sixth right lateral tooth	1
				Fifth and sixth left lateral teeth	2
				third left lateral tooth	2
			fused teeth	fifth and sixth left lateral teeth	1
		Chironomus sp.2	missing teeth	first right and sixth left lateral teeth	1
			split tooth	third right lateral tooth	1
	Stone 2	*Chironomus* sp.1	missing teeth	third right lateral tooth	1
				first left and fifth right lateral teeth	1

			sixth right lateral tooth	4
		fused teeth	central and left median teeth, split central median tooth	2
		Extra teeth	left median tooth	4
		split tooth	split median tooth	2
			second left lateral tooth	4
	Chironomus sp. 2	fused/ missing teeth	fused first and second left lateral teeth / missing fifth left lateral tooth	1
		split tooth	split first left lateral tooth	1
		missing teeth	fifth and sixth left lateral teeth	1
Stone 1	*Chironomus* sp.1	missing teeth	right median teeth	2
			third and fourth left lateral tooth	3
		extra tooth	left median tooth	1
		fused teeth	fifth and sixth left lateral teeth	2
			fused third and fourth right lateral teeth	2
		extra / missing teeth	extra right median tooth / missing sixth left lateral tooth	1
Stone 3	*Chironomus* sp. 1	split tooth	first left lateral teeth	**2**
			first and fourth left lateral teeth	**1**
		missing teeth	sixth right lateral teeth	**5**
	Chironomus sp.2	missing teeth	first right lateral and fourth left lateral teeth	**1**
Veg. 3	*Chironomus* sp.1	missing teeth	sixth left lateral tooth	1
			fifth left lateral tooth	1
			second left lateral tooth	1
		split tooth	central median tooth	1
		fused teeth/ extra teeth	median teeth/ extra third left lateral teeth	1

		Chironomus sp.2	split tooth	first left lateral tooth	1
	Veg. 2	*Chironomus* sp.1	missing teeth	missing left median teeth	2
			fused teeth	left median and central teeth	2
				fifth and sixth right lateral teeth	3
			Kohn gap	mentum gaps	1
	Veg. 1	*Chironomus* sp. 1	missing /split teeth	missing third left lateral tooth and split first left lateral tooth	1
			missing teeth	missing right median teeth	2
				missing sixth left lateral teeth	3
Site 4	GSM 3	*Chironomus* sp. 1	missing teeth	sixth left lateral tooth	2
			split teeth	first right lateral tooth	1
			fused teeth	first and second right lateral teeth	1
				fourth to sixth left lateral teeth	1
		Chironomus sp. 3	fused teeth	fused median teeth	1
	GSM 2	*Chironomus* sp.1	Split teeth	first left lateral tooth	1
			split/ missing teeth	second left lateral tooth / missing sixth left lateral tooth	1
			Kohn gap	mentum gap	1
			Kohn gap/ missing teeth	mentum gap/ missing right median tooth	2
	GSM 1	*Chironomus* sp.1	missing teeth	central median tooth	3
				fourth left lateral tooth	2
				second left lateral tooth	1
		Chironomus sp. 2	fused teeth	left and central median teeth	1
	Veg. 2	*Chironomus* sp.1	missing teeth	third left lateral tooth	1
				sixth lateral teeth on both sides of mentum	1

			Kohn gap	mentum gap	1
			extra teeth	median tooth	1
			split tooth	left median tooth	1
	Veg. 1	*Chironomus* sp.1	missing teeth	sixth left lateral tooth	1
			Asymmetry	Between left and right first lateral teeth	1
			split teeth	third right lateral tooth	1
	Veg. 3	*Chironomus* sp.1	split tooth	central median tooth	2
				left median tooth	1
			missing teeth	second right lateral tooth	2
				sixth right lateral teeth	3
	Stone 2	*Chironomus* sp.1	fused teeth	first and second right lateral teeth/ fourth and fifth right lateral teeth	1
				fourth and fifth right lateral teeth	2
	Stone 1	*Chironomus* sp. 1	extra teeth	right median tooth	2
			missing teeth	right median tooth	1
	Stone 3	*Chironomus* sp.1	missing teeth	missing fifth left lateral teeth	2
			missing / split teeth	missing sixth lateral tooth/ split left median tooth	1

Table D5: Number and description of mentum and ligula deformities recorded among chironomid taxa at the four sampling sites during spring 2011. Biotopes replicates are also shown. Biotopes: stone (stone-in-and-out-of-current), Veg (marginal and aquatic vegetation), GSM (grave, sand and mud). Number written in fron of each biotope indicate the replicate i.e. 1 (first replicate sample), 2 (second replicate sample) and 3 (third replicate sammple).

Site	**biotope replicate**	**Species**	**deformity type**	**description**	**No. of individual showing deformity**
Site 1	Stone 2	*Cricotopus* sp1	fused teeth	fused fifth and sixth lateral teeth	2
	Stone 1	*Tanytarsus* sp.	missing teeth	missing 5th left lateral tooth	3
		Ablabesmyia sp	split tooth	split left inner tooth	1

	Veg 1	*Dicrotendipes* sp	split tooth	split median tooth	4
			fused teeth	fused 2nd and 3rd left lateral teeth	1
		Polypedilum sp	missing teeth	missing 4th right lateral teeth	1
	Veg 2	*Dicrotendipes* sp	split tooth	split left outer median tooth	2
		Ablabesmyia sp.	split tooth	split right outer tooth	1
	GSM 2	*Ablabesmyia* sp.	split tooth	split right outer tooth	1
Site 2	Stone 1	*Cricotopus* sp 1	Split tooth	Split median tooth	3
			Missing teeth	missing 4th and 5th left lateral teeth	2
		Cricotopus trifasciata gr.	Fused teeth	fused median and 1st left lateral teeth	2
			Kohn gap	Kohn gap	2
		Dicrotendipes sp	Missing teeth	Missing 5th left and right lateral teeth	1
				Missing 5th right lateral teeth	3
				Missing 5th left lateral teeth	2
	Stone 3	*Dicrotendipes* sp	Fused teeth	fused 4th and 5th right lateral teeth	1
		Chironomus sp 1	Missing teeth	Missing 6th right lateral teeth	3
			Extra teeth	extra median tooth	2
		Orthocladius sp	Fused teeth	Fused 3rd and 4th right lateral teeth	1
	Veg 1	*Cricotopus trifasciata* gr.	split tooth	split first lateral teeth	1
		Dicrotendipes sp	missing teeth	fifth and sixth left lateral teeth missing	2
	Veg 2	*Dicrotendipes* sp	Missing teeth	2nd left lateral teeth	2
	GSM 1	*Cricotopus* sp 1	Split tooth	Split median tooth	2
		Cricotopus trifasciata gr.	Split tooth	Split median tooth	3
		Chironomus sp 1	Missing teeth	Missing left median tooth	1
Site 3	Stone 1	*Chironomus* sp 1	Asymmetry	asymmetry between first lateral teeth	3
			fused teeth	fused fifth and sixth left lateral teeth	3

				fused third and fourth left lateral teeth	4
				fused fourth, fifth and sixth lateral teeth	1
				fused median teeth	3
			missing and split teeth	missing sixth and split second median teeth	2
			missing teeth	missing first right lateral teeth	2
				Missing 5th left lateral teeth	1
				Missing left median tooth	3
			Kohn gap	Kohn gap	1
		Chironomus sp 2	missing teeth	Missing 6th right lateral teeth	3
			split tooth	split first left lateral teeth	1
				split median tooth	1
				split first right lateral teeth	1
			fused teeth	Fused all three median teeth	2
			extra teeth	extra median tooth	2
	Stone 2	*Chironomus* sp 1	missing teeth	missing right median tooth	3
				missing 6th left lateral teeth	6
				Missing 6th right lateral teeth	3
			fused teeth	fused median teeth	3
			fused and extra teeth	Extra median tooth and fused third and fourth left lateral teeth	1
			extra teeth	Extra median tooth	2
				extra second left lateral teeth	3
			Asymmetry	asymmetry between first lateral teeth	2
		Chironomus sp 2	missing teeth	Missing 4th left lateral teeth	4
				Missing 4th and 6th left lateral teeth	2
	Veg 1	*Chironomus* sp 1	Extra teeth	extra teeth on left lateral teeth	3
			fused teeth	fused outer median and first lateral teeth	3
			split tooth	split median tooth	3
			Extra and split teeth	Extra median tooth and split 3rd right lateral teeth	2

.			missing teeth	Missing median tooth	3
				Missing 3rd left lateral teeth	2
		Chironomus sp 2	fused teeth	fused fourth, fifth and sixth left and right lateral teeth	2
			missing teeth	missing sixth left lateral teeth	2
			split tooth	split median tooth	3
		Dicrotendipes sp	split tooth	split median tooth	2
			Split and missing teeth	Split median tooth and missing 5th left lateral tooth	1
			Fused teeth	Fused median and 1st right lateral teeth	1
			missing teeth	Missing 4th left lateral teeth	2
	Veg 2	*Chironomus* sp 1	missing teeth	missing outer median tooth	2
				missing fifth left lateral tooth	1
				missing sixth left lateral teeth	4
				missing first right lateral teeth	1
			Extra teeth	extra tooth on median tooth	4
			fused teeth	fused first and second right lateral teeth	2
			kohn gap	gaps on the median teeth	1
			missing and split teeth	missing second right and split median teeth	2
	Stone 3	*Chironomus* sp 1	Asymmetry	asymmetry between first lateral teeth	5
				asymmetry in the entire left and right lateral teeth	2
			fused teeth	fused fifth and sixth left lateral teeth	1
				fused lateral teeth	4
				fused third and fourth right lateral teeth	2
				fused fourth, fifth and sixth lateral teeth	1
				fused third and fourth left lateral teeth	2
			missing teeth	missing sixth right lateral teeth and third to fifth left lateral teeth	12
				missing first right lateral teeth	4
			split tooth	split first left lateral teeth	4

				split median tooth	3
				split first right lateral teeth	2
			Kohn gap	Kohn gap	5
			extra tooth	extra tooth on the central median tooth	2
		Dicrotendipes sp 1	Kohn gap	Kohn gap	2
	GSM 1	*Chironomus* sp 1	Asymmetry	asymmetry between first lateral teeth	2
			fused teeth	fused fifth and sixth left lateral teeth	2
				fused lateral teeth	2
				fused third and fourth right lateral teeth	2
				fused fourth, fifth and sixth lateral teeth	3
				fused third and fourth left lateral teeth	1
			missing teeth	missing sixth right lateral teeth and third left lateral teeth	1
				missing first left lateral teeth	1
				missing 6th left lateral teeth	2
				Missing 6th right lateral teeth	3
			split tooth	split first left lateral teeth	3
				split median tooth	4
				split first right lateral teeth	2
			Kohn gap	gaps in the median teeth of the mentum	2
			extra tooth	extra tooth on the central median tooth	2
		Chironomus sp 2	missing teeth	missing central median tooth and second left lateral teeth	2
				missing sixth left lateral teeth	1
			fused teeth	fused fifth and sixth left lateral teeth	1
			missing and fused teeth	Missing left median tooth and fused 5th and 6th right lateral teeth	2
			Fused teeth	fused fourth and fifth right lateral teeth	1
			split tooth	split median tooth	1
	GSM 2	*Chironomus* sp 1	split tooth	split median and first right lateral teeth	4

			missing teeth	Missing 4th and 6th left lateral teeth	3
				missing 4th and 1st left lateral teeth	2
			extra teeth	Extra median teeth	5
			fused teeth	fused median teeth	2
	GSM 3	*Chironomus* sp 1	missing teeth	Missing 2nd and 3rd right lateral teeth	1
				Missing 4th right lateral teeth	2
				missing 6th left lateral teeth	5
			extra teeth	Extra median teeth	4
				Extra 5th left lateral teeth	4
			Asymmetry	Asymmetry	2
Site 4	GSM 1	*Chironomus* sp 1	fused teeth	Fused 5th and 6th right lateral teeth	2
			Missing teeth	Missing 5th and 6th left lateral teeth	2
				Missing 5th lateral teeth	1
			Extra teeth	Extra median teeth	1
			Split teeth	Split 1st right lateral teeth	4
		Dicrotendipes sp.	Extra teeth	Extra median teeth	2
		Cricotopus sp 1	fused teeth	Fused 4th and 5th right lateral teeth	2
	GSM 2	*Chironomus* sp 1	Kohn gap	Kohn gap	2
			Missing and split teeth	Missing 5th and split 2nd left lateral teeth	1
			Missing teeth	Missing left median tooth	3
				missing 4th right lateral teeth	3
				Missing 6th lateral teeth on both sides of mentum	1
			Extra teeth	Extra median teeth	6
				Extra left median teeth	1
				Extra teeth on both sides of median teeth	2
			fused teeth	Fused 5th and 6th right lateral teeth	3
	GSM 3	*Dicrotendipes* sp.	Missing teeth	missing 2nd left lateral teeth	1

		Split teeth	split 2nd and 3rd right lateral teeth	2
Veg 1	*Chironomus* sp 1	Missing teeth	Missing 4th and 5th left lateral teeth	1
			Missing 4th left lateral teeth	3
			Missing median teeth	1
			Missing 6th right lateral teeth	2
		fused teeth	fused fifth and sixth left lateral teeth	2
			all left lateral teeth fused with first lateral tooth	1
		extra teeth	two extra teeth on central median tooth	1
		fused teeth	all left lateral teeth fused with first lateral tooth	1
	Chironomus sp 2	fused teeth	fused fifth and sixth left lateral teeth	3
			Fused median teeth	3
	Dicrotendipes sp	fused teeth	Fused 1st and 2nd left lateral teeth	3
		Missing teeth	Missing 4th and 5th left lateral teeth	1
		Split teeth	Split median tooth	3
	Chironomus sp. 2	Missing teeth	Missing 5th right lateral teeth	2
Stone 1	*Cricotopus trifasciata* gr.	Missing teeth	Missing 2nd right lateral teeth	2
		Kohn gap	Kohn gap	2
		Split teeth	Split median tooth	1
	Chironomus sp 1	Kohn gap	Kohn gap	5
		Missing teeth	Missing right median tooth	4
			Missing 4th left lateral teeth	3
			Missing 6th right lateral teeth	5
		fused teeth	Fused 5th and 6th right lateral teeth	2
			Fused median teeth	1
	Dicrotendipes sp.	Missing teeth	Missing 1st right lateral teeth	3
			Missing 1st left lateral teeth	2
		fused teeth	Fused 1st and 2nd left lateral teeth	2
	Cricotopus sp 1	fused teeth	Fused median and 1st right lateral teeth	2

		Cricotopus trifasciata gr.	fused teeth	Fused median and 1st right lateral teeth	3
			Missing teeth	Missing 5th right lateral teeth	2
	Stone 2	*Dicrotendipes* sp.	Split teeth	Split median tooth	3
			Fused and split teeth	Split median tooth and split 2nd right lateral teeth	1
		Chironomus sp 2	Missing teeth	Missing left median tooth	1
		Chironomus sp 1	Extra teeth	Extra median teeth	3

Table D6: Number and description of mentum and ligula deformities recorded among chironomid taxa at the four sampling sites during summer 2011. Biotopes replicates are also shown. Biotopes: stone (stone-in-and-out-of-current), Veg (marginal and aquatic vegetation), GSM (grave, sand and mud). Number written in fron of each biotope indicate the replicate i.e. 1 (first replicate sample), 2 (second replicate sample) and 3 (third replicate sammple)

	Biotope replicate	**Species**	**Deformity type**	**Description**	**No. of individual showing deformity**
Site 3	Stone 1	*Chironomus* sp. 1	split tooth	Split 1st left and right lateral teeth	2
				split 1st left lateral teeth	3
				split 6th right lateral teeth	3
			asymmetry	asymmetry between 1st and right lateral teeth	3
			extra and split teeth	extra median tooth and split 1st left lateral teeth	1
				extra median teeth	6
			missing teeth	missing 6th and 4th left lateral teeth	2
				missing left median tooth	3
			kohn gap	Kohn gap	3
		Chironomus sp 2	missing teeth	missing 6th right lateral teeth	3
	Stone 2	*Chironomus* sp 1	missing teeth	Missing 4th right lateral teeth	4
			kohn gap	Kohn gap	3

			kohn gap and missing teeth	Kohn gap and missing 6th left lateral teeth	2
			Extra teeth	extra central median teeth	1
				extra median and split teeth	3
			Fused teeth	Fused 4th and 5th right lateral teeth	1
				fused 5th and 6th right lateral teeth	2
			asymmetry	asymmetry between 3rd and right lateral teeth	2
			split tooth	split median teeth	2
		Chironomus sp 2	missing teeth	missing 6th left lateral teeth	1
			fused teeth	fused median teeth	1
		Chironomus sp 3	split teeth	split median teeth	1
	Stone 3	*Chironomus* sp 2	fused teeth	fused median teeth	2
			extra and missing teeth	extra median and missing 6th lateral teeth	2
		Chironomus sp 1	fused teeth	fused left median and central teeth	2
				fused median teeth	1
			missing teeth	missing left median teeth	8
				missing 4th right lateral teeth	2
				missing 6th left lateral and 4th right lateral teeth	2
				Missing 3rd right lateral teeth	6
				Missing 3rd left lateral teeth	5
			Kohn gap	Kohn gap	3
			split tooth	split median teeth	1
			split tooth	split right median teeth	3
	GSM 1	*Chironomus* sp.1	missing teeth	missing left median teeth	4
				missing right median teeth	2
				missing 6th left lateral teeth	3
			Kohn gap	Kohn gap	1
		Chironomus sp 2	split tooth	Split median teeth	2
			fused teeth	Fused 5th and 6th right lateral teeth	1

			extra tooth	extra median tooth	3
	GSM 3	*Chironomus* sp 1	split and missing teeth	Split median and missing 6th left lateral teeth	1
			missing teeth	missing 2nd left lateral teeth	6
				missing 2nd right lateral teeth	3
				missing 6th left lateral teeth	5
				missing 6th right lateral teeth	2
		Chironomus sp 2	missing teeth	missing 6th right and left lateral teeth	1
Site 2	Veg 2	*Dicrotendipes* sp	messing teeth	second left lateral tooth missing	1
				missing first and second right lateral teeth	1
				missing second right lateral tooth	1
			Fused teeth	fused 4th and 5th left lateral teeth	1
	GSMS 1		Fused teeth	fused 4th and 5th left lateral teeth	1
	Stone 1	*Cricotopus* sp 1	Split tooth	Split median tooth	1
Site 4	GSM 3	*Chironomus* sp1	fused teeth	fused median and 1st left lateral teeth	3
			missing teeth	missing 2nd left lateral tooth	1
				missing 5th left lateral tooth	2
				missing 5th left lateral and 6th right lateral teeth	3
				missing left median teeth	4
				missing 4 and 6 right lateral teeth	3
		Chironomus sp 2	fused teeth	fused median and 1st left lateral teeth	1
	GSM 1	*Chironomus* sp 1	fused teeth	fused 3rd, 4th and 5th right lateral teeth	1
			Kohn gap and split teeth	Kohn gap and split median tooth	1
			Extra teeth	extra median teeth	1
		Dicrotendipes sp	fused teeth	Fused 1st and 2nd right lateral teeth	1
	Stone 1	*Dicrotendipes* sp	fused teeth	fused 1st and 2nd right lateral teeth	2
			Split and missing teeth	Split 1st left lateral and missing 2nd right lateral teeth	1
		Chironomus sp. 1	Fused teeth	fused median and 1st left lateral teeth	3

				fused 6th and 5th left lateral teeth	4
			Kohn gap	Kohn gap	2
			Extra teeth	extra left median tooth	3
			extra teeth and missing teeth	extra left median tooth and missing 5 right lateral tooth	1
			missing teeth	missing 4th left lateral teeth	5
				missing 3 left lateral teeth and 6 left lateral teeth	2
	stone 2	*Chironomus* sp 1	Missing teeth	missing 3 right lateral teeth	2
				missing 3 left lateral teeth	3
				Missing right median teeth	4
		Dicrotendipes sp	missing teeth	missing 5 left lateral teeth	4
				missing 4 right lateral teeth	4
			Split teeth	Split median teeth	5
			fused teeth	fused 4th and 5th right lateral teeth	3
		Kiefferulus sp	missing teeth	Missing 4 left lateral teeth	1
				missing 3 right lateral teeth	3
		Chironomus sp 2	missing teeth	missing 4th left lateral teeth	2
			Missing and fused teeth	missing 6th and fused 4th and 3rd left lateral teeth	1
Site 1	Stone 1	*Tanytarsus* sp	Missing teeth	missing 3rd left lateral teeth	1
		Ablabesmyia sp.	Split tooth	Split central median tooth	1

Table D7: Number and description of mentum and ligula deformities recorded among chironomid taxa at the four sampling sites during autum 2012. Biotopes replicates are also shown. . Biotopes: stone (stone-in-and-out-of-current), Veg (marginal and aquatic vegetation), GSM (grave, sand and mud). Number written in fron of each biotope indicate the replicate i.e. 1 (first replicate sample), 2 (second replicate sample) and 3 (third replicate sammple).

Site	Biotope replicate	Species	Deformity type	Description	No. of individual showing deformity
Site 3	Veg 1	*Dicrotendipes* sp.	split tooth	Split 3rd right lateral teeth	1
				median and 1st right lateral teeth	1
			Fused teeth	1st left and 2nd lateral teeth	1
			missing teeth	missing 1st and 2nd left lateral teeth	1
				missing 1st left median tooth	4
			kohn gap	Kohn gap	3
			kohn gap and missing teeth	Kohn gap and missing 5th left lateral teeth	2
			Extra teeth	Extra median teeth	1
		Chironomus sp. 1	missing teeth	Missing right median teeth	4
				Missing 6th left lateral teeth	3
			Kohn gap	Kohn gap	2
			Extra teeth	Extra median teeth	3
	Veg 2	*Chironomus* sp. 1	Fused teeth	fused 5th and 6th right lateral teeth	3
				Fused 3rd and 4th right lateral teeth	2
				Fused 3rd and 4th right lateral teeth, and split median teeth	1
			Split teeth	Split central median teeth	5
	Stone 1	*Chironomus* sp. 1	missing teeth	Missing 6th right lateral teeth	4
				Missing right median teeth	3
			Fused teeth	fused 2nd and 3rd left lateral teeth	3
				fused 5th and 6th right lateral teeth	6
			Split teeth	Split central median teeth	2
		Chironomus sp. 2	Fused teeth	fused median teeth	1

			missing teeth	Missing left median teeth	2
	GSM 1	*Dicrotendipes* sp	missing teeth	missing 5th left lateral teeth	1
				missing 4th right lateral teeth	2
			split tooth	split right median teeth	3
		Dicrotendipes sp.	missing teeth	missing 4th right lateral teeth	2
	Stone 2	*Dicrotendipes* sp	missing teeth	missing 5th left lateral teeth	2
			Asymmetry	4th right and 4th left lateral teeth	1
		Chironomus sp.1	Asymmetry	left and right median teeth	3
			missing teeth	Missing 3rd left lateral teeth	2
Site 2	stone 2	*Dicrotendipes* sp	missing teeth	second left lateral tooth missing	2
				missing first and second right lateral teeth	1
				missing second right lateral tooth	1
			Fused teeth	fused 1st and 2nd left lateral teeth	1
	GSMS 1		Fused teeth	fused 4th and 5th left lateral teeth	1
	Stone 2	*Cricotopus* sp 1	Split tooth	Split median tooth	4
Site 4	Veg 1	*Dicrotendipes*	Fused teeth	fused 3rd and 4th left lateral teeth	1
			Split teeth	Split 3rd right lateral teeth	1
		Dicrotendipes sp	Extra teeth	extra 5th left lateral teeth	1
			Missing teeth	6th left lateral teeth	1
			Fused teeth	1st and 2nd left lateral teeth	1
	GSM 1	*Dicrotendipes* sp.	Split teeth	Median tooth	1
				4th left lateral and median teeth	1
	GSM 2	*Dicrotendipes* sp.	missing teeth	missing 5 left lateral teeth	5
				missing 4 righ lateral teeth	1
			Split teeth	Split median teeth	3
			fused teeth	fused 4th and 5th right lateral teeth	1
	GSM 3	*Dicrotendipes* sp.	missing teeth	missing 4th left lateral teeth	5

			Missing and fused teeth	missing 6th and fused 4th and 3rd left lateral teeth	1
	Stone 2	*Dicrotendipes* sp.	fused teeth	fused 1st and 2nd right lateral teeth	4
	Stone 1	*Cricotopus* sp. 1	fused teeth	fused 4th and 3rd left lateral teeth	1
			missing teeth	6th left lateral teeth	1
		Orthocladius sp.	Fused teeth	Fused 4th and 5th left lateral teeth	1
		Cricotopus trifasciata gr.	Fused teeth	fused median and 1st left lateral teeth	1
				fused 6th and 5th right lateral teeth	2
Site 1	Stone 3	*Polypedilum* sp.	Split teeth	Split right median teeth	1
		Polypedilum sp.	fused teeth	Fused 5th and 6th right lateral teeth	1
	Veg 1	*Polypedilum* sp.	fused teeth	Fused 5th and 6th right lateral teeth	1
	Stone 1	*Cricotopus trifasciata* gr.	Split teeth	Split 2nd right lateral teeth	1
	Veg 3	*Tanytarsus* sp.	fused teeth	fused 3rd-5th right lateral teeth	1
	GSM 1	*Virgatanytarsus* sp.	Missing teeth	Missing 1st left lateral teeth	1

Table D8: Number and description of mentum and ligula deformities recorded among chironomid taxa at the four sampling sites during spring 2012. Biotopes replicates are also shown. . Biotopes: stone (stone-in-and-out-of-current), Veg (marginal and aquatic vegetation), GSM (grave, sand and mud). Number written in fron of each biotope indicate the replicate i.e. 1 (first replicate sample), 2 (second replicate sample) and 3 (third replicate sammple).

Site	**Biotope replicate**	**Species**	**Deformity type**	**Description**	**No. of individual showing deformity**
Site 3	Veg 3	*Dicrotendipes* sp.	Fused teeth	1st and 2nd right lateral teeth, 5th and 6th right lateral teeth	1
				1st and 2nd lateral teeth on both side of the mentum	3
			Split tooth	3rd left lateral teeth	1
				median tooth	1
		Chironomus sp. 1	missing teeth	6th lateral, 4th-6th left lateral teeth	1

				right median teeth	2
				3rd left lateral teeth	3
			kohn gap	Kohn gap	3
			Extra teeth	Extra median teeth	2
	Veg 2	*Chironomus* sp. 1	Fused teeth	fused 5th and 6th right lateral teeth	2
				Fused 3rd and 4th right lateral teeth	1
			Extra teeth	Extra 6th right lateral teeth	2
			Split tooth	Split central median teeth	5
			missing teeth	Missing left median teeth	3
		Dicrotendipes sp.	Fused teeth	Fused 2nd and 3rd right lateral teeth	2
			missing teeth	Missing 5th right lateral teeth	1
				missing 5th left lateral teeth	2
		Chironomus sp. 2	missing teeth	missing median teeth	1
	GSM 2	*Dicrotendipes* sp.	missing teeth	missing 5th left lateral teeth	2
				missing 3rd right lateral teeth	3
			Extra teeth	Extra median teeth	2
			split tooth	split right median teeth	3
			Fused teeth	Fused 3rd and 4th right lateral teeth	3
		Dicrotendipes sp	missing teeth	missing 4th right lateral teeth	2
		Kiefferulus sp.	missing teeth	Missing 6th right lateral teeth	4
				Missing 2nd right lateral teeth	1
		Chironomus sp. 1	kohn gap	Kohn gap	2
			Asymmetry	left and right median teeth	2
			Extra teeth	Extra median teeth	5
				6th right lateral teeth	2
			missing teeth	1st right median teeth	2
	Stone 1	*Chironomus* sp. 1	missing teeth	Missing 6th right lateral teeth	8
				Missing right median teeth	1
			Fused teeth	fused 2nd and 3rd left lateral teeth	2

				fused 5th and 6th right lateral teeth	6
			Split tooth	Split central median teeth	2
		Chironomus sp. 2	Fused teeth	fused median teeth	5
			missing teeth	Missing left median teeth	3
	Stone 2	*Dicrotendipes* sp.	missing teeth	missing 5th left lateral teeth	5
			Asymmetry	4th right and 4th left lateral teeth	1
		Chironomus sp. 1	Asymmetry	left and right median teeth	2
			missing teeth	Missing 3rd left lateral teeth	2
Site 2	Veg 1	*Dicrotendipes* sp.	Split tooth	2nd right lateral teeth	1
		Cricotopus trifasciata gr	Extra teeth	1st left lateral teeth	1
	Veg 2	*Dicrotendipes* sp.	Fused teeth	1st and 2nd right lateral teeth	3
				5th and 6th left lateral teeth	1
				1st and 2nd left lateral teeth	1
			missing teeth	2nd right lateral teeth	1
	Veg 3	*Dicrotendipes* sp.	Fused teeth	1st and 2nd right lateral teeth	1
				1st and 2nd left lateral teeth	1
				4th and 5th left lateral teeth	1
Site 4	stone 1	*Chironomus* sp. 1	Extra teeth	extra median teeth	1
		Dicrotendipes sp.	split teeth	4th right lateral teeth	1
				median and 1st right lateral teeth	1
				3rd right lateral teeth	1
				median teeth	8
				median, 1st and 3rd right lateral teeth	1
				1st and 2nd left lateral teeth	1
			Fused teeth	1st and 2nd left lateral teeth	1
	Veg 1		split teeth	median, 1st right and left lateral teeth	10

	Veg 2		split teeth	median teeth	4
		Chironomus sp. 1	fused teeth	fused median and 1st left lateral teeth	1
	GSM 1	*Chironomus* sp. 1	fused teeth	fused 2nd, 3rd and 4th right lateral teeth	2
		Dicrotendipes sp.	fused teeth	Fused1st and 2nd right lateral teeth	2
	Stone 2	*Dicrotendipes* sp.	fused teeth	fused 1st and 2nd right lateral teeth	4
		Cricotopus trifasciata gr.	Fused teeth	fused median and 1st left lateral teeth	1
				fused 6th and 5th right lateral teeth	2
	GSM 2	*Dicrotendipes* sp.	missing teeth	missing 5 left lateral teeth	5
				missing 4 righ lateral teeth	1
			Split teeth	Split median teeth	7
			fused teeth	fused 4th and 5th right lateral teeth	9
	GSM 3	*Dicrotendipes* sp.	missing teeth	missing 4th left lateral teeth	6
			Missing and fused teeth	missing 6th and fused 4th and 3rd left lateral teeth	3
	GSM 2	*Virgatanytarsus* sp.	Fused teeth	4th and 5th left lateral teeth	1
Site 1					

Appendix E: Macroinvertebrate taxa collected in the model stream ecosystems during the experimental duration (August 2012 – May 2013)

Table E1: Abundance of macroinvertebrate collected in the model streams after 90 and 165 days of the experiment.

Time (day) of sampling after start of experiment	**Day 90**									**Day 165**								
Concentration	**Control**			**50% effluent**			**100% effluent**			**Control**			**50% effluent**			**100% effluent**		
Replicate stream	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3
Taxon																		
Oligochaeta	18	91	10	75	134	18	1	1	4	67	104	0	298	60	110	39	128	6
DIPTERA																		
Chironomidae	312	292	723	68	107	189	224	154	177	0	1	0	122	509	123	21	18	31
Culicidae	26	10	103	80	89	15	7	11	39	0	0	0	7	19	11	379	159	292
Muscidae	3	81	18	1	2	0	1	0	2	10	7	9	39	74	46	29	7	2
Ceratopogonidae	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Psychodidae	0	0	0	98	103	5	9	1	68	0	0	0	8	7	3	569	347	146
Ephydridae	0	0	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0	0
Syrphidae	0	0	0	0	0	0	0	0	0	0	0	0	4	0	0	1	1	0
EPHEMEROPTERA	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Caenidae	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
COLEOPTERA																		
Hydrophilidae	3	1	0	0	0	1	0	0	1	0	0	0	0	0	0	0	0	0
Dytiscidae	0	0	1	0	0	0	0	0	0	0	1	2	0	0	0	0	0	0
Gyrinidae	5	3	5	0	0	0	0	0	0	0	0	0	0	1	1	0	0	0
Elmidae	0	0	0	0	0	0	0	0	0	2	0	1	0	0	0	0	0	0
Hydraenidae	0	0	0	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0
GASTROPODA																		
Physidae	2	4	0	0	0	0	0	0	0	150	108	91	1	2	5	0	0	0

Ancylidae	0	1	0	0	0	0	0	0	0	1	0	0	0	72	0	0	0	0
Lymnaeidae	0	0	0	0	0	0	0	0	0	0	0	0	0	1	8	0	0	0
Planorbinae	0	30	0	0	0	0	0	0	0	34	95	13	0	0	0	0	0	0
Hydracarina	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0
LEPIDOPTERA																		
Pyralidae	0	0	0	0	0	0	0	0	0	0	0	0	0	5	0	0	0	0
ODONATA																		
Libellulidae	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0

Table E2: Abundance of macroinvertebrate collected in the model streams after 207 and 235 days of the experiment.

Time (day) of sampling after start of experiment	**Day 207**									**Day 235**								
Concentration	**Control**			**50% effluent**			**100% effluent**			**Control**			**50% effluent**			**100% effluent**		
Replicate stream	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3
Taxon																		
Oligochaeta	0	11	0	80	43	69	111	31	25	18	4	11	42	136	186	17	25	7
DIPTERA																		
Chironomidae	77	61	72	63	75	69	59	14	18	147	73	61	28	189	265	128	41	118
Culicidae	1	0	1	86	49	4	124	85	14	4	0	0	239	187	97	103	256	207
Muscidae	2	1	2	31	6	2	52	7	13	2	5	1	22	25	11	5	0	6
Ceratopogonidae	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Psychodidae	1	0	2	11	9	2	236	319	73	1	0	0	36	190	27	178	473	437
EPHEMEROPTERA																		
Baetidae	0	1	2	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0
COLEOPTERA																		
Hydrophilidae	0	0	0	0	0	0	0	2	0	1	2	3	0	0	0	0	0	0
Dytiscidae	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Gyrinidae	1	4	6	0	1	0	0	0	0	0	0	2	0	0	0	0	0	0

Hydraenidae	0	0	0	0	0	0	0	0	0	1	0	2	0	0	0	0	1	0
GASTROPODA	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Physidae	104	101	57	4	3	1	0	0	0	95	89	110	0	0	1	0	0	0
Ancylidae	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0
Lymnaeidae	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Planorbinae	11	10	7	0	0	0	0	0	0	14	16	12	0	0	0	0	0	0
LEPIDOPTERA	0	0	0		0	0	0	0	0	0	0	0	0	0	0	0	0	0
Pyralidae	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Table E3: Abundance of macroinvertebrate collected in the model streams after 263 and 284 days of the experiment.

Time (day) of sampling after start of experiment	**Day 263**									**Day 284**								
Concentration	**Control**			**50% effluent**			**100% effluent**			**Control**			**50% effluent**			**100% effluent**		
Replicate stream	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3
Taxon																		
Oligochaeta	0	0	2	38	208	94	45	71	32	1	0	26	33	111	26	9	15	18
DIPTERA	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Chironomidae	175	320	478	340	282	576	47	138	60	170	147	45	389	342	254	76	53	315
Culicidae	0	0	0	77	27	94	406	259	211	0	1	1	45	24	28	109	116	236
Muscidae	1	0	0	7	3	2	18	10	3	2	1	4	3	3	4	3	0	0
Psychodidae	2	1	16	341	397	624	540	322	401	1	0	0	110	133	169	136	303	211
COLEOPTERA																		
Hydrophilidae	1	0	1	2	0	0	0	1	0	0	0	1	2	0	0	0	0	0
Dytiscidae	1	0	0	0	0	0	1	0	0	0	0	0	0	0	0	1	0	0
Gyrinidae	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Elmidae	0	0	0	0	0	0	0	0	0	0	0	1	0	1	0	0	0	0
Hydraenidae	0	0	0	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0
GASTROPODA																		

Physidae	42	6	47	0	0	0	0	0	0	29	22	59	0	0	0	0	0	0
Lymnaeidae	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Planorbinae	6	18	41	0	0	0	0	0	0	23	10	15	0	0	0	0	0	0

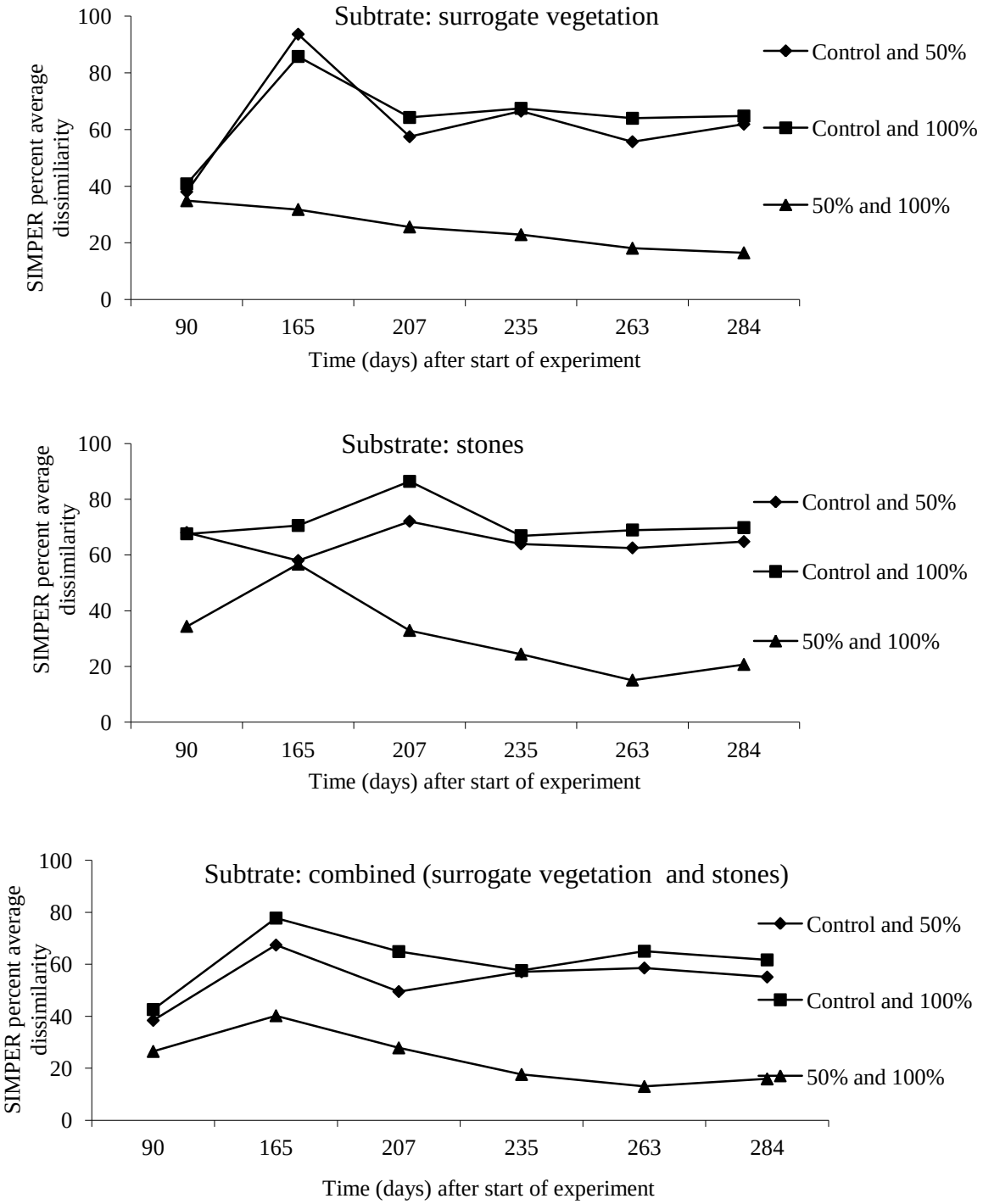

Figure E1: SIMPER analysis showing percent average dissimilarities between the model streams during the experimental period (August 2012 – May 2013) for macronibertebrate collected from the surrogate vegetation, stones and the combined substreates, showing that highest percent dissimilarity consitently occurred between the control and 100% effluent-treated streams, whereas the lowest occurred between the 50% and 100% effluent-treated streams.

Appendix F: Relating observed macroinvertebrate response in the model stream to the Swartkops River

To relate the observed macroinvertebrate community response in the model streams to those in the Swartkops River, the PRC was used to analyse the macroinvertebrate response in the river. The analysis was undertaken on macroinvertebrate taxa common to both the model streams and the river sites. By restricting the analysis on the river data to only macroinvertebrate taxa that occurred in the model streams, it was possible to investigate whether important taxa discriminating between the control and treatment streams would also discriminate between Site 1 and Sites 3 and 4 in the river. Macroinvertebrate data at Site 2 were not subjected to this analysis because the site had no effluent influences as it is located upstream of the effluent discharge point. The PRC had previously been used to analyse biomonitoring data to investigate the macroinvertebrate community response to pesticide effect in the Namoi River, New South Wales, Australia (Leonard *et al.*, 2000). In addition, the method has been further developed to accommodate biomonitoring data (van den Brink, 2009).

The results revealed that Site 3, located just downstream of the discharge point of the WWTW had greatest deviations from Site 1 during most of the sampling occassions. The PRC analysis indicated that differences in water physico-chemical conditions (treatment) between the sampling sites accounted 52.49% of the total variance, whereas differences between sampling time explained 31.33% of the variance captured. The remaining 16.18% is attributed to differences between replicate samples collected at each sampling sites on each sampling occasion. The first axis of the PRC with Eigen value 0.226 was statistically significant ($P < 0.05$) and it explained 42.96% of the variance captured by differences in water quality conditions between the sampling sites. The second axis with Eigen value 0.109 was not statistically significant and it explained only 20.84% of the variance captured by the differences in water quality condition between the sites.

The PRC taxa weights showed that the abundance of Physidae, Gyrinidae, Lymnaeidae, Ceratopogonidae and Ancylidae decreased at Sites 3 and 4 compared with Site 1. On the other hand, the abundance of Chironimidae, Culidae, Muscidae and Dytiscidae increased at Sites 3 and 4 relative to Site 1. The remaining taxa not shown had weights near zero and were deemed to have shown no response or a response unrelated to the PRC.

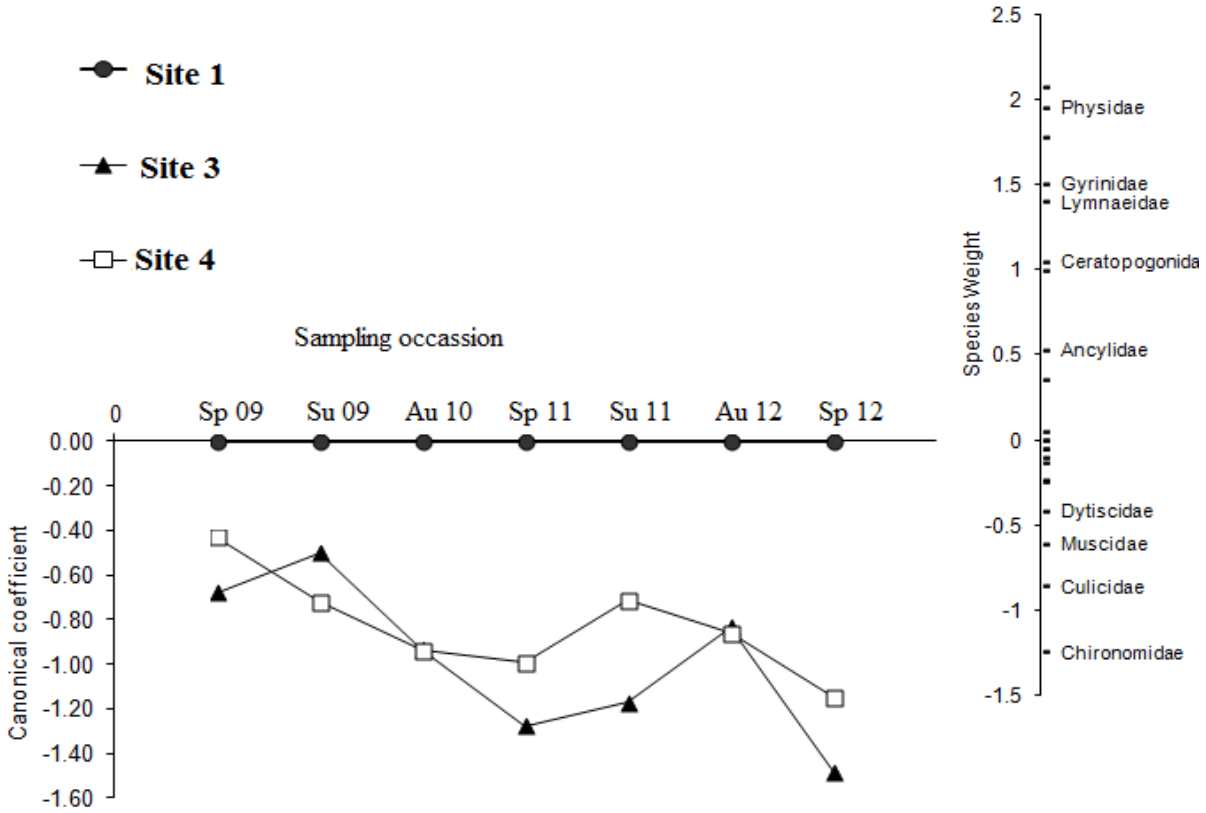

Figure F1: Principal response curve with taxa weights showing the response of the macroinvertebrate community collected at Sites 1, 3 and 4 in the Swartkops River during the study period (August 2009 – September 2012). Taxa with weights between 0.5 and -0.5 has been omitted for clarity. Sampling occasions: Sp 09 (spring 2009), Su 09 (summer 2009), Au 10 (autumn 2010), Sp 11 (spring 2011), Au 12 (autumn 2012) and Sp 12 (spring 2012).

References

Leonard, A.W., Hyne, R.V., Lim, R.P., Pablo, F. and van den Brink, P.J. (2000). Riverine endosulfan concentrations in the Namoi River, Australia: link to control field runoff and macroinvertebrate population densities. *Environmental Toxicology and Chemistry* **19 (6)**:1540 - 1551.

van den Brink, P.J., den Besten, P.J., de Vaate, A. and terBraak, C.J.F. (2009). Principal response curves techniques for the analysis of multivariate biomonitoring time series. *Environmental Monitoring and Assessment* **152**: 271 - 281.