# TAXONOMY- AND TRAIT-BASED RESPONSES OF CHIRONOMID ASSEMBLAGE STRUCTURE TO POLLUTION IN SELECTED URBAN RIVERS, EASTERN CAPE, SOUTH AFRICA

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# ABSTRACT

Urbanisation, occasioned by an increasing human population and the proliferation of industries, is a major threat to freshwater resources and has been implicated as a cause of stream water quality deterioration, habitat degradation, and the loss of aquatic biodiversity. Pollution of freshwater systems resulting from urban-related activities severely alters stream ecosystem structure and function. To effectively protect and sustainably manage freshwater resources, it is important to develop biomonitoring tools which are both sensitive to changes in water quality conditions and can provide an understanding of the mechanisms by which urban pollution impacts freshwater ecosystems. In South Africa, the macroinvertebrate-based South African Scoring System version 5 (SASS5) is routinely used for water quality assessment. This tool is developed at the family level of taxonomic resolution, with the exception of a few taxa. This raises the question as to whether a species-level taxonomic approach, complemented with a trait-based approach could add additional value. This question was addressed comparatively by developing and applying a taxonomy- and trait-based approach using the taxonomically and functionally diverse Chironomidae family.

Fifteen sites across three urban river systems (Buffalo, Bloukrans and Swartkops River systems) in the Eastern Cape Province of South Africa were purposefully selected for this study. Based on the predominant land use within the catchment of the sites and water quality variables, the study sites were grouped into five site categories. The least-impacted sites (REF) had 78.53% mean forested area, 5.98% mean urban area and 13.49% mean agricultural land. Sites that received diffuse pollution but were upstream from the effluent discharge point of wastewater treatment works (DP) had 59.07% mean forested area, 32.53% mean urban area and 6.20% mean agricultural land. Sites that received point source pollution from wastewater treatment works (PP) had 63.66% mean forested area, 26.26% mean urban area and 7.39% mean agricultural land. Sites further downstream from the wastewater treatment works that received impacts from both point source and diffuse pollution (AG) had 65.95% mean forested area, 18.24% mean urban area and 12.5% mean agricultural land. Sites selected for exploring the potential system recovery of the study rivers (RECV) had 77.21% mean forested area, 9.12% mean urban area and 10.7% mean agricultural land. Macroinvertebrates and physicochemical variables were sampled at sites in the Bloukrans and Buffalo rivers over four sampling events (spring, summer, winter, and autumn) between November 2021 and June 2022 using the SASS5 protocol. Historical chironomid and physicochemical data from the Swartkops River collected between 2009 and 2012 were also used in this study.

The SASS5 family-level biotic index classified the water quality condition at the least impacted site (REF) of the Swartkops River as minimally impaired 80% of the time, compared to the chironomid-based multimetric index (CUMMI), which indicated that the water quality condition at this site was near-natural 50% of the time. The chironomid-based multimetric index and the SASS5 were divergent in their classification of water quality conditions at the DP and AG sites but were 100% in agreement regarding water quality conditions at the PP site as critically/severely modified. The SASS5 scores classified the water quality condition at the AG site as critically/severely modified 100% of the time, whereas the CUMMI index classified the water quality condition at this site as critically/severely modified 90% of the time but moderately modified 10% of the time. The results indicate that species-level and family-level indices tend to be in agreement for heavily impacted sites, but the same was not true for least or moderately impacted sites.

Traits such as the possession of tracheal gills, very large body size, burrowing, whole-body undulation, construction of rigid tubes, possession of three tracheae, completion of lifecycle in more than one year, production of more than 1000 eggs per egg mass, preferences for fine detritus, and bivoltinism were deemed tolerant of urban pollution. Traits such as cuticular respiration, medium body size, predator feeding mode, completion of lifecycle within one year, and a preference for stone biotope were deemed sensitive to urban pollution. An approach was developed to classify chironomids into those that are potentially vulnerable and those that are resilient to urban pollution. The abundances of vulnerable species correlated positively with increasing dissolved oxygen and negatively with increasing turbidity, electrical conductivity, nitrite-nitrogen, ammonium-nitrogen, and orthophosphate-phosphorus. The relative abundance of the highly vulnerable species and that of the highly tolerant species responded significantly to urban pollution and differentiated between the site categories. The relative abundance of the highly vulnerable species was significantly different between the DP and PP site categories. The richness of vulnerable and highly vulnerable species was significantly different between the REF sites and the impacted site categories (DP, PP, and AG). These results indicated that the developed approach successfully predicted chironomid responses to urban pollution.

Overall, the study makes important contributions to the field of freshwater biomonitoring. First, the study highlighted that species-level identification is necessary to differentiate sites which may be considered moderately impacted. Both family and species-level tools were sensitive to water quality conditions for least impacted and highly impacted sites, but the differences between the family-level and species-level indices were pronounced for sites considered moderately impacted. Second, a trait-based approach provided a mechanism for developing predictive tools, and in the case of this study, the potential resilience or vulnerability of chironomids was reliably predicted.

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**APPENDIX B3**: Boxplots of chironomid species abundances showing differences between the site categories for each trait attribute per trait category.

## ABBREVIATIONS AND ACRONYMS

| | |
|---|---|
| ANOSIM | Analysis of similarity |
| AG | Sites that received impacts from point source and diffuse pollution |
| ANOVA | Analysis of Variance |
| ASPT | Average score per recorded taxon |
| AUSRIVAS | Australian River Assessment System |
| BCMM | Buffalo City Metropolitan Municipality |
| BEAST | Benthic Assessment of Sediment - |
| BMWP | Biological Monitoring Working Party System |
| CA | Correspondence analysis |
| CABIN | Canadian Aquatic Biomonitoring Network |
| CCA | Canonical correspondence analysis |
| CMAs | Catchment management agencies |
| CUMMI | Chironomid multimetric index |
| DCA | Detrended correspondence analysis |
| DO | Dissolved oxygen |
| DP | Sites that were upstream from wastewater treatment works that received diffuse pollution |
| DWAF | Department of water and forestry |
| DWS | Department of water and sanitation |
| EC | Electrical conductivity |
| EPOT | Ephemeroptera, Plecoptera, Odonata and Trichoptera |
| EPT | Ephemeroptera, Plecoptera and Trichoptera |
| EWQ | Environmental water quality |
| FFGs | Functional feeding groups |
| FR | Functional redundancy |
| GSM | Gravel, sand, and mud |
| IQRs | Interquartile ranges |
| KOH | Potassium hydroxide |
| MIRAI | Macroinvertebrate Response Assessment Index |
| NMBMM | Nelson Mandela Bay Metropolitan Municipality |

| | |
|---|---|
| NWA | National Water Act |
| NWRS | National water resources strategy |
| PAHs | Polyaromatic hydrocarbons |
| PCA | Principal component analysis |
| PCBs | Polychlorinated biphenyls |
| POP | Persistent organic pollutants |
| PP | Sites that received impacts point source pollution |
| RCC | River continuum concept |
| RDA | Redundancy analysis |
| RDMs | Resource Directed Measures |
| RECV | Recovery site |
| REF | Least impacted site |
| REMP | River Eco-status Monitoring Programme |
| RIVPACS | River Invertebrate Prediction and Classification System |
| RLQ | R, site-physicochemical variables matrix; L, site-species abundance matrix; Q, traits-taxa matrix |
| RQOs | Resource quality objectives |
| RSA | Republic of South Africa |
| SASS | South African Scoring System |
| SASS5 | South African Scoring System version 5 |
| SD | Standard deviations |
| SDCs | Source Directed Controls |
| SIMPER | Similarity percentage |
| Stats SA | Statistics South Africa |
| TBA | Trait-based approach |
| TDS | Total dissolved solids |
| TP | Trait plasticity |
| WWTWs | Wastewater treatment works |

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# DEDICATION

This thesis is dedicated to my father, Yahuah, and to all who seek the good of man on the earth.

# CHAPTER 1 – GENERAL INTRODUCTION AND LITERATURE REVIEW

## 1.1 Introduction

Freshwater ecosystems such as rivers and streams are vital for the sustenance of life (Booth & Bledsoe, 2009). Freshwater ecosystems supply crucial ecosystem services such as provisioning services (including water for human consumption), regulating and supporting services (including water purification, primary production and nutrient cycling) and cultural services (such as recreation, aesthetics and spiritual activities), which are all necessary to human health and well-being (Dudgeon et al., 2006; Grizzetti et al., 2019). Although some organisms may inhabit and utilise water of naturally poor quality (that is, poor due to geological factors (Baba & Gündüz, 2017)), the majority of aquatic lifeforms depend on good-quality water for their biological and ecological needs (UNEP-GEMS, 2008; Lin et al., 2022). Thus, the importance of good-quality water to human and aquatic health cannot be overemphasised. Nevertheless, freshwater ecosystems across the globe are facing an increasing decline in water quality and quantity, with consequent effects on aquatic biodiversity and the ecosystem services they provide, upon which humans depend.

The increasing human population occasioned by the advent of the industrial age has been implicated as a major contributor to the critical water challenges currently experienced in the world (Schwarzenbach et al., 2010; Mangadze et al., 2019; Boretti & Rosa, 2019). Anthropogenic factors including urbanisation, water abstraction, flow alteration through damming of river channels and habitat degradation associated with pollution from urban-industrial and agricultural areas all contribute to the loss of aquatic biodiversity. For example, the increase in human populations near freshwater systems has been known to cause deterioration in water quality and the loss of aquatic biota (Masese & Mcclain, 2012; Caro-Borrero et al., 2021). Consequently, the supply of valuable aquatic resources and services by freshwater systems are becoming increasingly hindered by anthropogenic pressures such as urbanisation (Wang et al., 2011). Thus, there is a need for sustainable water resource management to protect and conserve aquatic life and assure the supply of crucial ecosystem services on which humans depend.

In some parts of Africa, water quality managers and policymakers have made efforts to assess and tackle the impacts of urbanisation on freshwater ecosystems (Edegbene et al., 2020b;

Hamache et al., 2021; Masese et al., 2023). For example, the South Africa National Water Act (NWA) of No. 36 of 1998 (RSA, 1998) emphasises the need for the sustainability of water resources through water resource assessment and monitoring (RSA, 1998). Various biomonitoring tools have been developed and implemented to monitor freshwater ecosystem health and the impact of pollutants on aquatic biota (Dickens & Graham, 2002; Akamagwuna, 2021; Edegbene et al., 2022). However, the effort to manage water resources is cost-intensive, and some of the developed methods for freshwater biomonitoring are either only at a coarse taxonomic level or are not sufficiently responsive to the effects of a wide range of pollutants on biota to effectively inform management decisions regarding the protection of freshwater resources (van der Meer, 2016; Ko et al., 2020; Elias, 2021).

Although South Africa has made good progress regarding water quality monitoring, most management strategies for large-scale monitoring of freshwater pollution in the country rely only on the assessment of physicochemical and biological variables, which may not provide a critical understanding of the mechanistic response pathways of taxa to urban pollution (Mangadze et al., 2019; Odume, 2022). For example, most indices for urban pollution monitoring in the country are based on the presence/absence of information regarding aquatic biota, such as macroinvertebrates, at a coarse taxonomic resolution (Odume, 2014). The lack of a detailed exploration of mechanisms by which urban pollution alters freshwater ecosystems may hinder the sustainable management of aquatic resources in South Africa. This is because urban pollution is usually associated with stressors such as elevated sediment input, low dissolved oxygen concentrations, flow alteration and elevated contaminant levels, which differentially affect organisms based on the traits they possess. In essence, organisms that do not possess the right combination of traits may not be able to survive in polluted water. **Thus, this study applied an integrated water chemistry and chironomid-based taxonomy- and trait-based approach to investigate the effects of urban pollution on the ecological health and functioning of selected urban rivers in South Africa**.

The remainder of this chapter presents a critical literature review of the water quality challenges in South Africa, and the current protection and management of water resources through government instruments such as the South African National Water Act and the National Water Resource Strategy. It also provides a detailed review of environmental water quality and macroinvertebrate-based biomonitoring, including taxonomy- and trait-based approaches. The chapter concludes with the rationale, aim and objectives of the study, and the thesis structure.

## 1.2 Key drivers of water quality deterioration in South Africa

South Africa is a water-scarce country that is facing many water-related changes, including recurrent droughts, water infrastructure decay, poor water and sanitation services delivery, inequities in access to water and sanitation services, and water quality deterioration (Mangadze et al., 2019; DWS, 2022a). The South African General Household Survey of 2014 indicated that more than 60% of households in the country had access to piped water (Stats SA, 2014). However, more recently, a report published by Statistics South Africa (Stats SA) in 2021 showed that access to water in provinces such as Eastern Cape and Limpopo has significantly declined since 2014 (DWS, 2022a). South Africa depends largely on surface water resources such as rivers and streams for the supply of water for human and ecological needs (Basson, 2011). However, the deterioration of water quality in rivers and streams contributes significantly to the water challenges in South Africa, and negatively impacts the economy, human wellbeing, and the healthy functioning of aquatic ecosystems (Mangadze et al., 2019). Water quality deterioration reduces the amount of raw water available for human use and increases the water quantity needed to dilute polluted streams to acceptable standards (DWS, 2022a). Key drivers of water quality deterioration in South Africa include rapid population growth, land use change, and point and diffuse pollution sources such as agriculture, mining, wastewater treatment works and urban stormwater runoff.

The growth of modern industry has resulted in the increase of human population densities in built-up areas (World Economic Forum, 2016; França et al., 2019). Compared to countries in Europe, most African countries are categorised as developing countries (Kutor, 2014; Pelizzo et al., 2018). Urban hubs play an essential role in ensuring economic growth and improving livelihoods and are often considered the future of prosperity in the developing world (Saghir & Santoro, 2018). Although opportunities for economic development may be created, rapid population growth negatively impacts the quantity and quality of adjacent freshwater resources (Boretti & Rosa, 2019; Strokal et al., 2021). This is because rapid human population growth is typically associated with increased demand for resources and an enormous generation of domestic waste which end up in waterways (Bwapwa, 2018).

Visible features of urban areas include the conversion of indigenous land cover to built-up area with high human population densities, some degree of imperviousness such as roads and pavements, and the presence of industries and wastewater treatment infrastructure (McGrane, 2016). Urban areas are usually characterised by varying degrees of population, economic, social, cultural and built environment indicators (McGrane, 2016). Strokal et al. (2021) categorised urban areas into low, moderate, and highly urbanised, based on increasing human populations and the presence of sewer connections. Rapid urbanisation in South Africa began post World War II, when informal settlements constituting Black South Africans and people of mixed races developed in the urban periphery (Harrison, 1992). Thus, urban areas in South Africa comprise well-serviced formal settlements and often less organised or poorly planned informal settlements (Drimie et al., 2013; Gqomfa et al., 2022). Land cover changes significantly impact riparian characteristics of streams and alter food sources, physicochemical properties and biological components of freshwater bodies (Namugize, 2017; Camara et al., 2019; Jaureguiberry et al., 2022). Namugize (2017) assessed the effects of land use and land cover changes on the water quality of the Upper uMngeni River in KwaZulu-Natal Province, South Africa and found that a decline in natural vegetation occurred as land areas were converted to cultivated lands, urban built-up areas and degraded lands. Namugize (2017) also found elevated concentrations of *Escherichia coli* and nutrients attributable to dysfunctional sewage systems, poor or lack of sanitation facilities in the informal settlements, effluents from wastewater treatment works, agricultural activities, and runoff from livestock farming and stormwater.

Rivers in South African urban catchments are experiencing water quality impairment owing to both diffuse and point sources of pollution such as stormwater runoff, urban agriculture and discharges from wastewater treatment works (WWTWs). Urban stormwater runoff was identified as a significant source of riverine pollution in South Africa in 1986 (Grobler & Toerien, 1986). Stormwater runoff carries sediments and contaminant mixtures into streams (Grobler et al., 1987). Robertson et al. (2019) investigated the composition of pollutants in stormwater runoff and sediments from an urban highway in Cape Town, South Africa. These authors found elevated concentrations of metals, phosphorus, fats, oils, and greases in sediments from the highway compared to environmental levels. Moreover, metals, total phosphorus and total suspended solids were significantly greater in the highway runoff compared to rainwater from the same sampling locations. Gqomfa et al. (2022) investigated the impact of the Dunoon informal settlement on the water quality of the Diep River in South Africa. They observed elevated chemical oxygen demand, electrical conductivity, turbidity, salinity and ammonia levels as well as low dissolved oxygen in the water samples. Effluents from wastewater treatment plants are also known to significantly contribute pollutants such as metals, dissolved solids, organic matter and nutrients to receiving water bodies (Dickens & Graham, 1998; Agoro et al., 2020; Enns et al., 2023).

### 1.2.1 Urbanisation as a key contributor of pollution in South Africa

Water and environmental resource management are among the top five urbanisation-related challenges in Sub-Saharan Africa (World Economic Forum, 2016). Freshwater systems in urban landscapes are threatened by multiple urban stressors (Paul & Meyer, 2001). Rapid urbanisation causes significant changes in the physical (e.g., hydrology, temperature, and geomorphology), chemical (e.g., reduced dissolved oxygen, excessive nutrients, and heavy metals), and biological parameters (e.g., the diversity, richness, and composition of aquatic assemblages) of freshwater resources (Paul & Meyer, 2001; Booth & Bledsoe, 2009). These changes to the physical, chemical, and biological parameters of freshwater systems are collectively referred to as the “Urban Stream Syndrome” (Walsh et al., 2005). Major urban stressors that alter aquatic ecosystem health include eutrophication, salinisation and toxins (DWS, 2017a).

Eutrophication is the consequence of nutrient enrichment of surface water (Harding, 2015). Nutrient influx into rivers and streams may originate from natural or anthropogenic sources (van Ginkel, 2011). High nutrient concentrations and water stagnation for prolonged periods in reservoir dams, with suitable temperature, oxygen concentration and light are the main factors driving eutrophication (Griffin et al., 2014). Eutrophication results in undesirable production of algae and aquatic macrophytes. Severe algal blooms which result from eutrophication negatively affect water aesthetics and quality. Some species of Cyanobacteria (blue-green algae) produce intracellular toxins which can affect drinking water posing a significant human health risk (Frost & Sullivan, 2010; Matthews & Bernard, 2015). Macrophyte blooms also have severe ecological consequences, and may lead to blockages of drainage ways and prevent access to dams (van Ginkel, 2011; Griffin et al., 2014). Excessive algal growth and aquatic macrophytes often characterise urban-impacted sites.

Salinity is the property of water resulting from the combination of various mineral salts (Mgaba, 2018). Common contributors to the salinity profile of streams include calcium, potassium, magnesium, sodium, bicarbonate and chloride ions. Salinity in surface waters may be caused by natural processes, such as the interaction between water and the underlying geology of a river, stream, or reservoir, or anthropogenic activities, such as urban agriculture, mining, industry, and effluent from urban-related wastewater treatment works (Griffin et al., 2014; Mabidi et al., 2018; Mgaba, 2018). Salinisation of water resources may also increase during drought, when there is limited water for diluting excess salts in streams (Mgaba, 2018). Anthropogenic input of salts into rivers through effluent discharge from urban wastewater treatment works constitutes the main form of pollution in most South African rivers (Gqomfa et al., 2022). Diffuse inputs of salts from urban stormwater runoffs and irrigated lands are more difficult to monitor and control than those from from urban wastewater treatment works, and effects on aquatic biota may only become apparent after prolonged exposure (Griffin et al., 2014). Nevertheless, increased water salinity negatively affects freshwater biota. Mabidi et al. (2018) exposed crustacean resting eggs to different salinity levels (0.5 $gL^{-1}$, 2.5 $gL^{-1}$, 5 $gL^{-1}$ and 10 $gL^{-1}$) for 28 days. These authors observed a significant decrease in the emerged taxa richness and abundance at salinities of 2.5 $gL^{-1}$ and above. Mgaba (2018) also observed a decline in the richness and diversity of macroinvertebrates and diatoms in response to a gradient of salinity in the Kat River, South Africa. Although salinisation is regarded as a water quality issue in South Africa, regulatory tools for effective management of water salinisation are yet to be clarified and refined (Griffin et al., 2014).

Toxins from acid mine drainage and organic compounds such as phthalate esters, herbicides, pesticides, fungicides, persistent organic pollutants (POP), polychlorinated biphenyls (PCBs), and polyaromatic hydrocarbons (PAHs) are pollutants of emerging concern in South Africa’s freshwater systems (Olujimi et al., 2010; Feris & Kotze, 2015; Olisah et al., 2021). Mine water contains heavy metals such as manganese, copper, aluminium, radium, and uranium, which threaten aquatic life (Maree et al., 2004). Tailing dams for mine water can continue to threaten freshwater systems even after being decommissioned (DFFE, 2012). Moreover, metals from urban industrial activities such as building construction, textiles, wood and paper processing, motor construction and repairs, coal and fuel combustion and sewage are often carried in stormwater run-off into streams (Tchounwou et al., 2012; Adedeji & Olayinka, 2013; Agoro et al., 2020; Rezapour et al., 2022). Endocrine-disrupting chemicals have also been identified as threats to water quality in South Africa’s freshwater systems (DFFE, 2012). Adeyinka et al. (2018) assessed the presence of PCBs in the Msunduzi River in Pietermaritzburg, South Africa. These authors found higher concentrations of PCB congeners in sediment samples than in surface water. They also observed that winter samples showed higher total concentrations of PCBs (214.21–610.45 ng/g) compared to samples collected in the spring season (30.86–444.43

ng/g). In general, data on the presence and distribution of these toxicants is extremely sparse, and this may be due to the cost of analysis, the scarcity of appropriate laboratory techniques, the number of potential pollutants, and a general lack of resources (Vosloo & Bouwman, 2005; Griffin et al., 2014). The impacts of these pollutants of emerging concern on aquatic biota, with a particular focus on ecosystem health and the sustainable supply of ecosystem services, is largely underexplored in Africa.

Urbanisation results in severe declines in aquatic biodiversity and ultimate degradation in ecosystems functions (Gao et al., 2022). For example, the loss of aquatic biodiversity due to urban-related stressors may adversely impact the food web of freshwater systems and hamper key ecosystem processes such as nutrient cycling in streams (Gao et al., 2022; Paz et al., 2022). The need to develop highly sensitive and predictive biomonitoring tools to effectively manage the impacts of urban pollution on freshwater resources in South Africa, due to the projected increase of urbanisation in South Africa (Kok & Collinson, 2006), constitutes an important objective of this study.

## 1.3 Water resource management in South Africa

### 1.3.1 The South African National Water Act

The South African National Water Act (NWA) Act No. 36 of 1998 recognises the critical role of the nation’s water as a scarce natural resource that various users depend on for the performance of their daily activities (RSA, 1998). It emphasises the need to manage and protect aquatic ecosystems in order to ensure an ecologically sustainable development of water resources. As such, the NWA accentuates the importance of an integrated approach to water resources management by considering the interactions between water quality, quantity, and the social-ecological imperatives of water use and demand. It also provides the authority upon which institutions may be set up for the sustainable and strategic management of South Africa’s water resources (RSA, 1998; Odume, 2014).

The NWA confers power on the Minister of the Department of Water and Sanitation to develop a national water resources strategy (NWRS) that provides a framework for the protection, management, and control of water resources of the country, after consultation with all the relevant stakeholders (Chapter 2 of NWA, 1998). To ensure the progressive management of water resources in the country, the NWA mandates the minister to review the NWRS at intervals of not more than five years. Moreover, the NWA also provides for the establishment of catchment management agencies (CMAs) which will develop catchment management strategies and work in tandem with the framework set out by the NWRS. The purpose of establishing the CMAs is to ensure a holistic and integrated management of catchments within a water management area (RSA, 1998).

Chapter 3 of the NWA speaks directly to the protection of water resources and lays out the principles to be considered in the development of the NWRS. It divides the process of water resources protection into five interdependent parts which encompass the (i) classification of water resources in the country, (ii) setting of resource quality objectives, (iii) determination of the Reserve, (iv) prevention of water resources pollution, and (v) remediation of water resources pollution (RSA, 1998). These aspects of the protection of South Africa's water resources are elaborated in the NWRS.

### 1.3.2 The National Water Resource Strategy

The National Water Resource Strategy (NWRS) is a legally binding instrument through which all authorities, personnel and institutions associated with South Africa's water resources act and implement the objectives of the National Water Act (NWA) (Act No. 36 of 1998). The NWRS provides the framework for the protection, use, development, conservation, management and control of water resources for the country, and influences the strategies developed by catchment management agencies for the equitable allocation and sustainable protection of water resources at different local and regional scales (DWAF, 2013).

Following from the first NWRS (NWRS1) which set out the policies, strategies, guidelines, and procedures for the management of water resources in South Africa, the second NWRS (NWRS2) aims to ensure the sustainable management of the nation's water resources towards achieving the growth, development, and socio-economic priorities in an equitable and sustainable manner (DWAF, 2013). To achieve a balance between water use and protection, the NWRS provides two regulatory approaches: the Resource Directed Measures (RDMs) and the Source Directed Controls (SDCs) (RSA, 1998; Palmer et al., 2004; DWAF, 2013). RDMs comprise four critical components: (i) a national water resource classification system, (ii) determination of ecological categories, (iii) the determination of the Reserve, and (iv) setting of resource quality objectives (RQOs) (DWAF, 2013). A water resource classification system enables the categorisation of water resources into three management classes: Class I, Class II, and Class III (Palmer et al., 2004; DWAF, 2010; Ginsburg et al., 2010). A water resource is assigned Class I if it is to be kept in a near-natural condition; Class II if it is slightly impacted by human activities with little deviations from natural conditions; and Class III if it is heavily used with significant deviations from natural conditions (DWAF, 2010). Water resources are further classified into ecological categories A–F, representing a gradient of natural to severely modified water resources (Palmer et al., 2004; Kleynhans & Louw, 2008; Odume, 2014). Water resources in ecological category A are regarded as being in a near-natural or pristine condition, those classified as ecological category B are regarded as being minimally altered, and those classified in ecological category C are regarded as being moderately altered (DWS, 2022b). Water resources in ecological category D are regarded as being heavily altered, those in ecological category E as being critically modified, and those in ecological category F represent severely modified water resources (DWS, 2022b). Based on the management class and ecological category for each water resource, the Reserve and the resource quality objectives (RQOs) for that water resource can be prescribed.

The Reserve consists of two parts, the basic human need Reserve and the ecological Reserve (RSA, 1998). The basic human need Reserve relates to the quality and quantity of water resources required for the provision of the essential needs of individuals, such as water for drinking, preparing food and personal hygiene (RSA, 1998). The ecological Reserve relates to the quality, quantity and reliability of the supply of water required to protect and maintain the structure and function of aquatic ecosystems (Palmer et al., 2004; DWAF, 2013; Thirion, 2016). The Reserve is awarded priority before any allocation of water resources for use is made. The Resource Quality Objectives (RQO) provide qualitative and quantitative benchmarks of the desired level of protection for a water resource as defined by its management class (DWAF, 2004; DWAF, 2013). The RQOs specify the goals required to ensure the sustainable protection and utilisation of water resources. Water resources for human and agricultural needs may be categorised as ideal, acceptable, tolerable or unacceptable depending on the RQOs set for the resource (Palmer et al., 2004; Mgaba, 2018), whereas water resources for ecological needs are categorised in the ecological categories A–F (DWS, 2022b).

Source Directed controls (SDCs) define and impose limits and restrict the use of water resources to achieve the desired levels of protection. The SDCs are complementary to RDMs and are designed to control human and industrial water-use activities at the source of impact through tools such as licenses, general authorisations, permits, and the polluter pays principle, amongst other tools (DWAF, 2013). Source Directed Controls are the essential links between protecting water resources and regulating their use (Ginsburg et al., 2010; Odume, 2014).

Although South Africa has very good laws and policy instruments to ensure the protection of freshwater resources, there remains a critical challenge in the implementation and enforcement of these laws (Schreiner, 2013). Inequality, poverty, and mismanagement or malpractices are amongst the significant hindrances to the implementation of the National Water Act No. 36 of 1998 (Hughes et al., 2023). Sewer pipe vandalism in search of valuables (such as lost necklaces, rings or ornaments for trade), occasioned by chronic poverty, results in the flow of untreated sewage into streams.

The continuous vandalism of sewer pipes obstructs the management of freshwater systems through the RQOs set by managers and implies additional repair cost for which little or no budget may have been allocated. This triggers a reaction of apathy in managers who become clueless as to what intervention plans may prevent the locals from vandalising the sewer pipes. Moreover, the dysfunctional state of many wastewater treatment works owing to outdated or overloaded infrastructure limits the implementation of the laws and strategies (DWS, 2017b). Poor cooperative governance, inadequate implementation of best land-use management practices, inappropriate licence conditions and a lack of compliance and enforcement capacity, and the lack of precautionary planning, regulation and enforcement impact the execution of both RDMs and SDCs in South Africa (Schreiner, 2013; DWS, 2017b).

The lapses in the implementation of the RDMs and SDCs result in severe water quality and physical habitat impairment that require a holistic approach to be effectively managed. As such, environmental water quality (EWQ) tools such as biomonitoring, ecotoxicology and physicochemistry have been developed and applied for the implementation of both the RDMs and SDCs. For the purpose of this study, EWQ is briefly reviewed below, and the focus of the current study is articulated within the concept of EWQ.Environmental Water Quality

Environmental water quality (EWQ) is an integrated approach that enables the understanding of the complex interactions between the abiotic and biotic components of water resources (Palmer et al., 2004). The EWQ combines information from water physicochemistry, ecotoxicology and biomonitoring to protect water resources. In South Africa, water physicochemistry is the traditional method for assessing water quality (Huizenga et al., 2013). Water physicochemistry involves assessing the physical and chemical attributes of water as indicators of water quality without considering the effects of measured variables on biota. Biomonitoring uses the presence, absence, and abundance of instream biota to provide information on aquatic ecosystem health (Rabeni & Wang, 2001). Ecotoxicology evaluates the multi-level effects of specific toxicants on the biology and functioning of aquatic biota, with the potential for establishing causal linkages (Odume, 2014). Griffin et al. (2014) recommended the direct measurement of biotic responses in relation to a complex interaction of physicochemical variables. Thus, the EWQ approach provides a good platform for generating information regarding effective water resources management by aiding the decision-making process of what RQOs must be set for water resources in a catchment. Of the three EWQ pillars, the main focus of the current study is biomonitoring, although water physicochemistry was integrated into the study to provide correlational information.

## 1.4 Biomonitoring and biological indicators

Aquatic biomonitoring is the assessment of freshwater ecosystem health based on the responses of resident biota to changes in environmental variables (Erasmus et al., 2021; Mezgebu, 2022). It enables the identification of perturbation thresholds beyond which significant changes to biotic assemblages occur and is applicable across different levels of biological organisation (Gray, 2016; Akamagwuna et al., 2019a; van der Meer et al., 2022). The South Africa National River Eco-status Monitoring Programme (REMP) was established as one of the sources of input data to feed into the management decision on water quality (DWS, 2019). Thus, biomonitoring informs the water resource management action to be taken and has been employed in South Africa since 1994 (Dickens & Graham, 2002; Palmer et al., 2004; Akamagwuna, 2021). Organisms that enable the identification of changes (positive or negative) in aquatic conditions are called biological indicators (bioindicators) (Azrina et al., 2006; Suzzi et al., 2022). Indicator organisms form the bedrock of aquatic biomonitoring. Bioindicators also reflect physical habitat structure, flow regime, channel fragmentation and food sources (Martins et al., 2021). The responses of bioindicators, in the form of presence, absence, abundance, diversity and behaviour, may reflect instream water quality conditions (Gray, 2016). Common bioindicators used in biomonitoring river ecosystem health include fish, algae, diatoms and macroinvertebrates (Heino et al., 2017; Carmignani & Roy, 2017; Szabolcs et al., 2022).

Aquatic macroinvertebrates are arguably the most widely used group of organisms in freshwater biomonitoring (Tampo et al., 2021). Their unique characteristics, such as their ubiquity and diversity, make them suitable bioindicators of environmental perturbations in freshwater systems (Bruno et al., 2019). Macroinvertebrates spend most or all their lifecycle in water and have been used to provide an indication of stressor intensity and frequency over the duration of ecosystem health monitoring (Akamagwuna et al., 2019a; Odume, 2020; Arimoro & Keke, 2021). Moreover, their diversity and species richness offer differential responses to the various stressors of urban origin (Tampo et al., 2021). Macroinvertebrates are crucial elements in aquatic food webs and play critical roles in organic matter processing, nutrient cycling, and even the bioaccumulation and biomanipulation of toxicants (Akamagwuna, 2021; van der Meer et al., 2022). Thus, macroinvertebrate-based biomonitoring approaches and tools (e.g., taxonomy- and trait-based approaches) have been developed and applied to the monitoring of various streams receiving impact from agricultural, industrial, and urban sources (Ferreira et al., 2011; Ruiz-Picos et al., 2017; Paz et al., 2022). In this study, macroinvertebrates were used as bioindicators to monitor pollution in selected urban rivers in the Eastern Cape, South Africa.

## 1.5 Approaches to biomonitoring

### 1.6.1 Taxonomy-based approach

Taxonomic approaches to biomonitoring are based on the response of community attributes (e.g., diversity, species richness, abundance and composition measures) to environmental variables (Culp et al., 2011). The biotic assemblages of macroinvertebrates have been used to provide integrative instream ecological changes to anthropogenic disturbances. Taxonomy-based approaches to biomonitoring include multivariate models, single biotic indices and multimetric indices.

Single biotic indices are based on the perceived sensitivity or tolerance of selected aquatic macroinvertebrate families to water quality deterioration (Odume, 2014; Tampo et al., 2021). Notable single biotic indices include Hilsenhoff's Biotic Index in the United States, the Biological Monitoring Working Party System (BMWP) in the UK, the Belgian Biotic Index, and the South African Scoring System (SASS) in South Africa (Hilsenhoff, 1988; Chutter, 1998; Dickens & Graham, 2002; Ruiz-Picos et al., 2017). These indices attempt to relate the presence or absence of macroinvertebrate taxa to organic or environmental pollution. In essence, sensitive taxa are expected to be absent in highly impacted sites (Hilsenhoff, 1988; Cairns & Pratt, 1993).

Multivariate techniques employ a comparison-based analysis of multiple environmental variables between different sites to indicate water quality status. Multivariate models make use of the reference condition approach where taxa abundance matrices (or scores from single biotic indices) and environmental parameters of the study site are modelled and compared with modelled results of macroinvertebrate assemblages from several reference sites (Guerrero-Aguilar et al., 2022). A reference condition is defined as the physical, chemical and biological conditions that are representative of a group of reference sites with minimal or no anthropogenic influence (Dallas, 2000; Ramulifho et al., 2023). Compared to an upstream/downstream (site-specific) reference site scenario, a reference condition is derived from several sites with similar characteristics. Therefore, parameters that define a particular reference condition are subject to the ecological quality of the constituent reference sites and are sometimes prone to error or set too high (McDowell et al., 2013). Some multivariate tools that are widely used today include the River Invertebrate Prediction and Classification System (RIVPACS) (Clarke et al., 2003), the Canadian Aquatic Biomonitoring Network (CABIN) (Trajkovic, 2016), the Benthic Assessment of Sediment (BEAST) (Reynoldson et al., 1995), and the Australian River Assessment System (AUSRIVAS) (Sudaryanti et al., 2001). The logic behind multivariate models is that significant deviation in species assemblage patterns between the study site and reference site can be used to indicate water quality deterioration (Clarke et al., 2003). However, multivariate techniques rely solely on the accurate identification of reference sites within the catchment or region of the study to obtain reliable results. The challenge associated with obtaining pristine freshwater sites in an increasingly industrialised and urbanised world forms part of the limitations of multivariate models based on the reference condition approach (Chessman, 2021). Nevertheless, the reference condition approach does NOT require pristine reference sites any more than the single biotic indices or the multimetric indices require them.

Multimetric indices combine metrics representing several aspects of macroinvertebrate ecology (e.g., structure and function) to indicate ecosystem health (Bonada et al., 2006; Baptista et al., 2007; Edegbene et al., 2019). Thus, the individual metrics that constitute a multimetric index respond to increasing or decreasing human influence and can indicate different aspects of stream ecology, such as the diversity and evenness of macroinvertebrate communities (Plafkin et al., 1989). Multimetric indices provide a numerical score that estimates the quality of the biotic assemblage, which reflects the quality and health of the stream (Li et al., 2010; Lau & Lauer, 2015). Examples of multimetric indices include the Benthic Index of Biological Integrity (Kerans & Karr, 1994), the Piabanha-Paquequer-Preto Multimetric Index (Baptista et al., 2011), and the Macroinvertebrate Response Assessment Index (MIRAI) used in South Africa (Thirion, 2008, 2016). Multivariate and multimetric approaches are deemed more sophisticated than single biotic indices (Cuffney et al., 2013).

In South Africa, the most widely used tool for biological water quality assessment is the South African Scoring System (SASS). It is a single biotic index that was developed based on macroinvertebrate taxa (Chutter, 1998). The SASS method has undergone several modifications (Dallas, 1995, 1997) and is currently in its fifth version (SASS5) (Dickens & Graham, 2002). The method provides information on not only water quality but also the present ecological state of macroinvertebrate assemblages (DWAF, 2008). The strengths of the SASS5 method have made it a widely-used rapid bioassessment tool in South Africa (Dallas, 2021). Some of the strengths of the SASS5 method include its simple and easy operability, cost-effectiveness, non-destructive sampling procedure (except where representative samples are collected for laboratory analysis), and easy interpretation of results. However, there are some limitations to the SASS5 method. The SASS5 method does not provide accurate results for recently flooded rivers, and caution must be exercised when using it in ephemeral streams (Dickens & Graham, 2002). The SASS5 was developed at the family-level resolution and does not account for the differential responses of species within diverse macroinvertebrate families such as the Chironomidae (Lenat & Resh, 2001). This weakness imposes a limitation on relying on the SASS5 for biodiversity protection, particularly when keystone species are involved. With regards to the management of freshwater resources, tools such as SASS5, may be sensitive at the two extremes of pollution (minimal/near pristine situation or heavily impacted condition) but may be less sensitive with regard to reflecting the true state of ecological change in between the two extremes (Waterhouse & Farrell, 1985). This raises a concern as to the reliability of such tools in informing water quality management decisions. Several authors have argued for biomonitoring tools which are developed at the genus or species level in order to reduce the amount of information loss when genus and species information are aggregated at the family level (Resh & Unzicker, 1975; Simpson & Bode, 1980; Schmidt-Kloiber & Nijboer, 2004). As such aquatic insects belonging to the orders Ephemeroptera, Plecoptera and Trichoptera (EPT) have been used to explore the impact of disturbances at the genus/species level (Akamagwuna et al., 2019a; Valente-Neto et al., 2021). Macroinvertebrates of the EPT orders are frequently used as indicators of water quality because of their sensitivity to a variety of aquatic stressors, in that they are usually among the first groups of aquatic invertebrates to disappear from impaired sites (Elias, 2021; Valente-Neto et al., 2021). Species of the EPT orders are also frequently used in biomonitoring programmes because they are easy to identify to genus and species level (Akamagwuna et al., 2019a). Because most species of the EPT taxa are among the first to disappear at highly impacted sites, they were considered unsuitable for the assessment of urban pollution in this study. The absence of EPT taxa from disturbed sites may limit some information about the relationship between tolerant taxa and anthropogenic impact. Moreover, compared to chironomids, numerous studies have explored the responses of EPT taxa (often their intolerance) to anthropogenic pressure (Suhaila & Che Salmah, 2017; Sivaruban et al., 2022; Tubić et al., 2024). Although identifying macroinvertebrates to genus or species level comes with high costs, Whittier and Van Sickle (2010) noted that assemblage tolerance indices developed at the finest taxonomic resolution can better discriminate between impacted and unimpacted sites than those developed at coarser taxonomic resolution. **Thus, in this study, chironomid-based tools were developed at the species level and compared to SASS5 results**.

Taxonomic approaches offer rapid assessment measures needed for the immediate management of water resources. Moreover, they can aid the combination of different aspects of macroinvertebrate assemblage structure, including composition, abundance, and richness, to provide an ecological understanding of the response patterns of aquatic communities to anthropogenic pressures (Lam-Gordillo et al., 2021). Despite these advantages, taxonomic approaches are limited because they only provide an indication of changes at the structural level with little or no insights into the changes at the functional level. Thus, taxonomy-based tools do not permit the exploration of the mechanistic links between species assemblage structure and environmental impairment (Verberk et al., 2008a; Merritt et al., 2017). Because organisms can only survive in an environment based on the traits they possess, it is imperative that the trait composition of an ecosystem be evaluated to provide an in-depth understanding of the impacts of pollutants on aquatic ecosystem function (Culp et al., 2011). This has spurred the development of trait-based biomonitoring tools and approaches that are complementary to taxonomic approaches, and can elucidate the mechanistic pathways of pollutant effect on aquatic organisms (Culp et al., 2011; Odume et al., 2018).

### 1.6.2 Trait-based approach (TBA)

In addition to the conventional taxonomy-based approaches, trait-based approaches are increasingly used to assess stream ecosystem health and function (Menezes et al., 2010; Merritt et al., 2017; Mathers et al., 2019). Traits are morphological, behavioural, physiological or life-history attributes of organisms which are inherent and measurable at the individual level without making reference to the external environment (McGill et al., 2006; Violle et al., 2007). The product of the direct interaction between an organism and its external environment is referred to as an ecological preference (Odume, 2022). Examples of ecological preferences include the preference for specific habitats (e.g., stone, vegetation, mud or silt), channel depth, food and flow velocity (Edegbene et al., 2021; Odume et al., 2023). Traits and ecological preferences are functional attributes which may confer resilience, resistance, adaptability, or vulnerability on an individual organism (Culp et al., 2011; Pyne & Poff, 2017; Odume et al., 2018).

The application of trait-based approaches to assess the impacts of water quality and physical habitat deterioration on stream ecosystem function is on the rise (Kuzmanovic et al., 2017; Edegbene et al., 2020b). Trait-based approaches have impact diagnostic potential and can help to explore trait-stressor relationships (Verberk et al., 2013; Odume, 2020, 2022). Trait-based approaches can provide an understanding of the mechanistic pathways by which organisms are eliminated in an ecosystem (Dolédec & Statzner, 2008; Culp et al., 2011; Jiang et al., 2021). Trait-based approaches can also help to predict what species will be filtered out by a stressor, based on the combination of traits they possess (Menezes et al., 2010; Collins & Fahrig, 2020). Richards et al. (1997) predicted the response of macroinvertebrates to catchment- and reach-scale filters and discovered that life-history and behavioural traits best related to habitat predictors such as cross-sectional area, depth, slow-water habitats and fine particulate matter. However, the trait-based approach has remained underexplored in South Africa, regardless of its potential usefulness in diagnosing and predicting species-stressor relationships and informing water quality management.

Southwood (1977, 1988) provided a theoretical basis for exploring the interactions between the biotic and abiotic components of an ecosystem and hypothesised that habitats with similar environmental conditions would comprise organisms that possess similar characteristics. The habitat template concept is based on the constraints and selection of traits imposed on organisms by external environmental factors (Southwood, 1977; Townsend & Hildrew, 1994). For example, an increase in nutrient concentrations due to anthropogenic activities may impact the dissolved oxygen concentrations of a stream and filter out organisms which cannot adapt to such changes. Thus, the habitat template concept implies a deviation from the descriptive assessment of population and community dynamics offered by species taxonomic composition to a mechanistic one (Merritt et al., 2017; Odume, 2022).

Two approaches to trait-based biomonitoring can be found in the literature: i) an analysis of a single trait response to a given stressor (Akamagwuna et al., 2019b; Collins & Fahrig, 2020; Paz et al., 2022) and ii) an analysis of trait combinations for individual species and their responses to a particular environmental stressor (Verberk et al., 2008b; Heino, 2008; Pilière et al., 2016). The general assumption underlying the use of the single trait-based approaches along a gradient of environmental perturbation is that a single trait is responsible for conferring resilience or vulnerability on the individual (Tomanova et al., 2008). This approach is popular in trait-based biomonitoring, and authors have successfully used it to explore the effects of anthropogenic stressors on macroinvertebrate trait assemblages and ecosystem functioning. For example, Paz et al. (2022) used this approach to predict community changes in stream sites disturbed by urbanisation and industrial pollution. They noted that traits including possession of breathing apparatus such as spiracles, cylindrical body shape and oviparity were prominent in severely impacted sites while traits considered sensitive to disturbance included possession of gills, preference for algae and associated materials (grazers) and crawlers (Paz et al., 2022).

In contrast to the single trait-based approach, the trait combination approach proposes that organisms do not respond to a specific stressor using a single trait but rather a combination of traits, which must be clustered and their interactions analysed as a collective response to environmental disturbances (Poff et al., 2006; Menezes et al., 2010; Verberk et al., 2013). Hence, the relationship between a combination of traits and a given environmental factor could be due to trait syndromes, which collectively determine species adaptation, resilience, sensitivity and responses to environmental disturbance (Poff et al., 2006; Verberk et al., 2008a; Verberk et al., 2013). The combined traits approach provides a more in-depth understanding of how traits interact, through trade-offs or spin-offs, to influence the response of an organism to anthropogenic stressors (Verberk et al., 2008b). The underlying framework of the combined traits approach suggests that trait syndromes prevent the isolation or independence of certain traits for use in biomonitoring. Hence, environment-trait relationships using the single trait approach may be insufficient in expounding mechanistic responses to stressors, and may lead to a misinterpretation of results due to confounding factors resulting from trait syndromes (Culp et al., 2011; Pilière et al., 2016). In this study, the single and combined trait-based approaches were used to explore traits that are responsive to urban pollution and to elucidate the vulnerability or resilience of chironomids to urban pollution.

Some authors have attempted to link macroinvertebrate functional performance to persisting environmental conditions (Belmar et al., 2019; Chen et al., 2019). Metrics such as functional richness, Rao's quadratic entropy, functional evenness, functional redundancy and functional dispersion based on organism traits have been used to describe macroinvertebrate assemblage structure to disturbance and the effect of altered ecosystem function (Belmar et al., 2019; Bruno et al., 2019; Chen et al., 2019). However, these metrics (or functional indicators) were not employed in this study because of the overall purpose of this study to explore how urban pollution impacts the taxonomic and trait distribution of chironomids and predict the vulnerability or resilience of chironomids to urban stressors. Nevertheless, these metrics may find essential application in studies related to how the ecological functioning of chironomids may be impacted by anthropogenic impact. Moreover, care must be exercised in effectively unpacking and extrapolating the ecological meaning of such functional metrics in biodiversity-ecosystem functioning research.

Despite the numerous advantages of the TBA in freshwater biomonitoring, it comes with several challenges (Culp et al., 2011; Green et al., 2022). As with taxonomy-based approaches, the taxonomic resolution at which traits are applied is a challenge in trait-based biomonitoring of water quality. This challenge is mostly associated with the cost and difficulty of identifying some macroinvertebrate groups such as the Chironomidae to the species level. Hence, some researchers have opted for the use of macroinvertebrate taxa which are easy to identify and can distinguish anthropogenic disturbance at study sites. For example, Gayraud et al. (2003) compared the effects of different taxonomic resolutions (species, genus, and family levels) on macroinvertebrate trait-based assessment of human impact in large European rivers, and concluded that genus or family-level trait information is sufficient for large river biomonitoring, despite the fact that members of Diptera and Oligochaeta were only identified to family level. However, some researchers have suggested that the level of information gained by identifying macroinvertebrates at the species level outweighs the cost (Resh & Unzicker, 1975; Lenat & Resh, 2001; Schmidt-Kloiber & Nijboer, 2004). This is because species are regarded as an ecologically meaningful unit for studying the effects of stressor-related impacts on ecological processes (Jones, 2008). Species traits define what organisms do in an environment, and the inherent differences in species-level responses to environmental impacts can be used to improve the sensitivity and discriminatory power of biomonitoring tools. Thus, this study employed a species-level taxonomic resolution to explore the effects of urban pollution on chironomid traits assemblage structure and distribution patterns.

The level at which traits are measured and assigned constitutes another challenge to the use of trait-based biomonitoring approaches. The premise that traits can aid the exploration of anthropogenic impacts without the need for taxonomy is a subject of concern among researchers (Culp et al., 2011). For example, some researchers are of the opinion that since traits are measured at the individual level, there may be no need for taxonomy in applying organism traits in biomonitoring (see Schmera et al., 2017). Although some traits such as body size and body shape can be measured without the need for taxonomic identification (Odume, 2022), some life history traits such as those related to reproduction and dispersal may be tied to the taxonomic identity of species (Resh & Unzicker, 1975; Lenat & Resh, 2001; Bouchard et al., 2005; Storey & Quinn, 2011). This highlights the need for the taxonomic identification of species in the construction of trait databases for biomonitoring programmes.

A given trait may take different forms/modalities within a taxon (Serra et al., 2016; Lourenço et al., 2023; Odume et al., 2023). For example, the trait mobility can take the form of burrowing, crawling or swimming depending on the species considered. Thus, inconsistencies in the assignment of trait information are bound to occur where species/genus-specific traits are collapsed into a family-level taxonomic resolution. For example, fuzzy-coding the traits of chironomids (which are known to be very diverse) may present statistical challenges such as defining what affinity score (i.e. a score that describes the relationship between a species and a trait attribute) to assign to the group for multiple trait modalities expressed at the species level. Other challenges of the trait-based approach include problems associated with quantifying trait syndromes through trade-offs and spin-offs, the selection of what traits to include/exclude during biomonitoring, and the lack of elaborate autecological information on macroinvertebrates species in readily accessible databases (Culp et al., 2011; Schmera et al., 2015; Pilière et al., 2016). In view of these challenges, this study presents an attempt to investigate the effects of pollution on the ecological health of selected urban rivers in Eastern Cape South Africa, using a combination of taxonomy- and trait-based approaches based on the species of the family Chironomidae. By focusing on a single but highly diverse group such as chironomids, a focused investment into the taxonomy of this group can be made to advance species-level biomonitoring, since the diversity of chironomids alone exceeds that of other macroinvertebrate groups combined.

### 1.6 Chironomid-based assessment of water quality

Aquatic ecologists have recommended the use of the highest possible taxonomic classification in ecological studies to provide a more accurate representation of biotic responses to changing environmental conditions (Usseglio-Polatera et al., 2000; Lenat & Resh, 2001). In an effort to reduce cost, most biomonitoring approaches are developed using the family-level taxonomic and trait-based information of macroinvertebrates (Voß & Schäfer, 2017; Edegbene et al., 2020a). Biomonitoring tools developed at the family level have been reported to offer rapid bioassessment of the ecological health of freshwater systems and require little or no taxonomic expertise (Maseti, 2005). However, they do not consider the diversity, richness, and varied responses of macroinvertebrate species to changing environmental conditions. Moreover, family-level bioassessment tools may display high sensitivity to water quality impairment at pollution extremes (i.e. highly impacted versus unimpacted water quality) but may not be sensitive enough to discriminate among levels of water quality between these extremes. In contrast, species-level tools account for the diversity and species-specific responses of macroinvertebrates to perturbation, which is often masked by the family-level tool, and can provide information about the exact ecological condition at a site (Bouchard et al., 2005; Jones, 2008). Moreover, conclusions based on results from species-level biomonitoring can be made with a high level of confidence (Lenat & Resh, 2001). The limitations of macroinvertebrate species-level identification include the requirement of taxonomic expertise, the small sizes of macroinvertebrate instars which can be difficult to handle, and the cost implications in terms of time and resources needed to process species samples (Bouchard et al., 2005). Nevertheless, the potential for species-level tools to detect water quality differences between sites within the bands of pollution extremes makes them valuable for diagnosing and predicting the effects of disturbances, and aiding management decisions in the protection, restoration or conservation of freshwater resources (Lenat & Resh, 2001).

Of the aquatic macroinvertebrates, Chironomidae constitutes the most diverse family of aquatic insects (Serra et al., 2017a; Houri et al., 2020; Villamarín et al., 2021). Chironomids are generally referred to as non-biting midges in their adult stage (Houri et al., 2020; Reyes-Maldonado et al., 2021). Since the 1900s, chironomids have been used as indicators of water quality deterioration in Europe (Kolkwitz & Marsson, 1909) and have found application in trophic dynamics and ecosystem functioning research (Armitage et al., 1995). Larval chironomid assemblages have been used to discriminate impacted sites from pristine sites, temporary from permanent waters, and to indicate hydrological variability and drought

(Cañedo-Argüelles et al., 2016; Serra et al., 2017a; Rossaro et al., 2022). However, this group of macroinvertebrates has remained underexplored in Africa, particularly in South Africa, where the protection and sustainable use of water resources is driven by the National Water Act No. 36 of 1998 (Harrison, 2004; Odume & Muller, 2011; Odume et al., 2014).

Chironomid larvae are considered tolerant of a wide range of environmental and toxicological stressors (Isidoro, 2014) and are often included in field and laboratory experiments in aquatic ecotoxicology. For example, morphological alterations in the mouthparts of chironomid larvae have been used to investigate the effects of organic pollution and heavy metals in aquatic ecosystems (Odume et al., 2016; Arimoro et al., 2018; Akyildiz et al., 2018; Jonsson, 2019). The possession of certain morphological and physiological features has been known to confer some level of tolerance to certain chironomids in heavily polluted environments. For instance, Nguyen & Donini (2010) noted that two mechanisms of ion regulation occur in the anal papillae of *Chironomus riparius*. Studies have also noted changes in the length and size of the anal papillae of chironomids in relation to salinity and dissolved solid concentrations in water. Kefford et al. (2011) exposed *Chironomus oppositus* to five levels of salinity concentrations (≈0.1 mS/cm, 1mS/cm, 2.5 mS/cm, 5mS/cm and 15mS/cm) for 21 days and observed that the anal papillae lengths of the species differed significantly between the treatment levels. Moreover, certain chironomids have been shown to tolerate low dissolved oxygen concentrations in naturally oxygen-poor and polluted rivers because they possess haemoglobin in their haemolymph (Lencioni et al., 2012; Villamarín et al., 2021). Thus, in addition to the discriminatory potential of chironomid taxonomic assemblage, chironomid traits can also help ecologists to understand more deeply the effects of pollution on aquatic biota. Therefore, this study applied both chironomid-based taxonomy- and trait-based approaches to investigate the effects of pollution in selected urban rivers in the Eastern Cape of South Africa.

Using species belonging to the family Chironomidae has its own challenges. Chironomidae is regarded as a difficult taxon to work with because of the need for taxonomic expertise and the time cost associated with identifying vastly abundant taxa to genus and/or species level (Lenat & Resh, 2001). Nevertheless, as earlier stated, the increased sensitivity to water quality changes provided by a species-level biomonitoring tool outweighs the cost required, and the diversity and varied responses of chironomid taxonomy and traits make them ideal bioindicators for the development of a species-level bioassessment tool for urban pollution monitoring. In this study, chironomid larvae were identified to species group (morphotypes) level using the relevant keys described by Wiederholm (1983), Harrison (2004) and Cranston (2014). Species groups were used because of the limited information on chironomid systematics and distribution in South Africa. Because it is impossible to effectively manage a resource for which little or no information exists, this study contributes data on chironomid distribution and responses to urban pollution in the study rivers to aid management efforts in freshwater resources management and conservation.

## 1.7 The rationale and significance of the study

Urbanisation is an important driver of ecological change in rivers and streams. Pollution arising from urban activities impact on river ecosystem structure, function, and biotic assemblages. Biomonitoring tools and approaches have been developed to monitor biotic response to urban effects and to determine the status of riverine ecosystem health. This study utilises water physicochemistry, macroinvertebrate-based South African Scoring System version 5 (SASS5) index and chironomid taxonomy- and trait-based approaches to assess urban pollution effect in selected rivers in the Eastern Cape of South Africa. While family-level biomonitoring tools such as the SASS5 are useful for routine biomonitoring, they mask taxonomic and functional diversity of species within families. Further, Lenat and Resh (2001) revealed that family level tools may not be very responsive to sites where water quality conditions are not severely impacted. Thus, this study analyses the responses of chironomids at the species level to urban pollution, comparing the results to the family level SASS5. Such comparative assessment can provide insights into what might be missed when using tools developed at the coarse versus fine taxonomic resolutions. A mechanistic approach underpinned by trait-based ecology is useful for predicting biotic responses to environmental stressors, as traits mediate organism-environment interactions. This study therefore develops a trait-based approach for predicting the resilience and vulnerability of chironomid species to urban pollution.

## 1.8 Aim of the study

The aim of this study was to investigate the taxonomy- and trait-based responses of chironomids to pollution in selected urban rivers in Eastern Cape, South Africa.

## 1.9 Research objectives

1. To develop, apply and compare a chironomid-based index to a family-level index in assessing water quality deterioration in selected urban river systems.

2. To analyse the distribution patterns of chironomid traits and ecological preferences in relation to urban pollution.

3. To develop a trait-based approach for predicting the resilience and vulnerability of chironomid taxa to urban pollution.

### 1.10 Thesis structure

This thesis consists of six chapters, namely, a general introduction and literature review chapter (Chapter 1), a study area and methods description section (Chapter 2), three results chapters (Chapters 3 to 5), and a discussion and conclusion chapter (Chapter 6).

**Chapter 1**: This chapter covers the general introduction to the study, background information on urban pollution in freshwater, and a review of river management in South Africa. The chapter introduces the biomonitoring of water quality deterioration, explicitly focusing on chironomid-based biomonitoring. The conclusion includes the study rationale, and the research aim and objectives.

**Chapter 2** provides a description of the study area from catchment-scale characteristics to selected sampling sites. Methods used for collecting and analysing environmental and biological data are described in this chapter.

**Chapter 3** is the first results chapter of the thesis. It presents results on the spatial and temporal distribution patterns of chironomid communities and the development of a chironomid-based index for assessing pollution in the selected urban rivers. Chironomid-based indicators of urban pollution are identified and an index for long-term monitoring of urban riverine pollution is developed. The results of the new index are compared with those of the routinely used SASS5.

**Chapter 4** explores the distribution patterns of chironomid traits and ecological preferences in relation to urban pollution in the study rivers. Here, sensitive chironomid traits which can potentially serve as indicators of urban riverine pollution are identified.

**Chapter 5** provides a trait-based predictive approach for assessing the potential vulnerability or resilience of chironomids to urban pollution based on the type of traits, trait interactions and the combinations identified.

**Chapter 6** provides the general discussion, recommendations, and conclusion of this study. A synthesis of research findings and the implications of the results for water management in

South African in urban landscapes are provided. The limitations of the research and recommendations for further research are also provided.

# CHAPTER 2 – STUDY AREAS DESCRIPTION AND GENERAL MATERIALS AND METHODS

## 2.1 Introduction

This chapter provides a description of the study area and the general materials and methods. The chapter also highlights the land-use activities influencing the selected study sites and concludes with a general description of selected chironomid traits and attributes.

## 2.2 Study areas description

Samples were collected from three rivers for this study, the Buffalo River, the Bloukrans River and the Swartkops River. The rivers were selected because they are situated within urban catchments within the Eastern Cape Province of South Africa. The rivers are impacted by runoff from urban settlements, stormwater runoff, effluent discharge from wastewater treatment works, industrial effluents, agricultural activities and weirs. Thus, they serve as critical case studies to investigate the effects of urbanisation on the structural and functional assemblage of chironomids.

### *Buffalo River*

The Buffalo River is in the Buffalo City Metropolitan Municipality (BCMM) in the Eastern Cape Province, South Africa. It is the largest river in the Amatole system situated on the east coast of South Africa (Mantel et al., 2015) and drains a total area of 1,353 km$^2$ (Palmer, 1991a) out of the 2,753 km$^2$ established boundary of the municipality (MDB, 2021). The Buffalo River is a fourth-order (Strahler, 1957; 1:250000) river that rises at 1,300 m above sea level in the indigenous forested area of the Amatole mountains (Rowntree et al., 2000) and empties into the Indian Ocean at East London. The river stretches 125 km (Slaughter et al., 2014) and flows through major urban settlements including King William's Town, Zwelitsha, Mdantsane and East London. The Izele, Mgqakwebe and Yellowwoods Rivers are the main tributaries of the Buffalo River (O’Keeffe et al., 1996). These tributaries enter the Buffalo River at various locations along its length (Figure 2.1). The Buffalo River consists of four main reservoirs, the Maden, Rooikraans, Laing and Bridle Drift dams. The Maden and Rooikraans dams are in the upper reaches, while the Laing and Bridle Drift dams are found in the river's middle reaches.

The reservoirs serve as water sources for domestic and commercial use during the dry winter months and buffer the effects of crises in the catchment, such as drought. The Buffalo River serves as a valuable resource for religious activities, conservation, agriculture, recreation (e.g., swimming, picnicking and hiking) and a source of livelihood to fishermen.

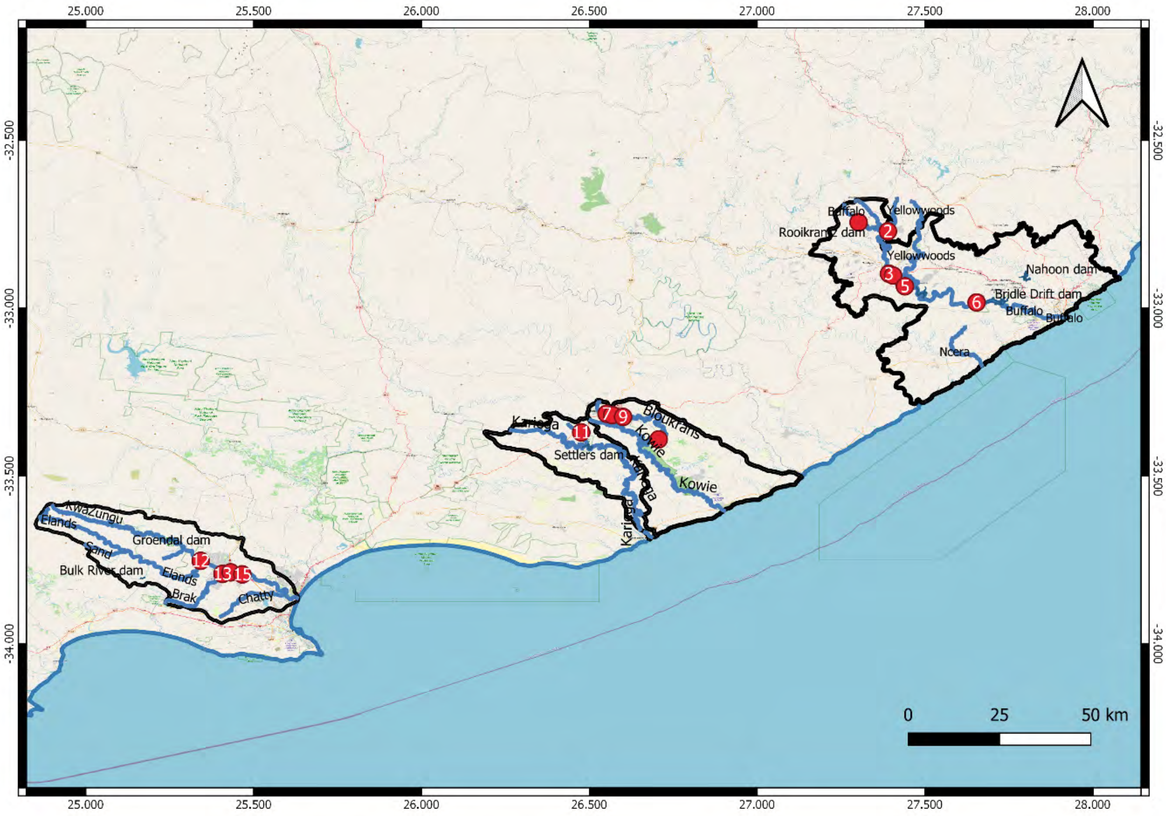


Figure 2.1 Map of the study areas showing the locations of sampling sites and the geographical location of the Buffalo, Bloukrans and Swartkops River Catchments within Eastern Cape Province, South Africa

### *Bloukrans River*

The Bloukrans River catchment is located in the Makana local municipality under the Sarah Baartman District Municipality in the Eastern Cape Province of South Africa (Stats SA, 2016). It is a small perennial river of approximately 59 km in length and originates from the lowlands approximately 4 km north of Makhanda (formerly called Grahamstown) and drains a catchment of about 230 km$^2$ (Mgaba, 2014; Gininda, 2016; Nel et al., 2018). The river, a tributary of the Kowie River (Figure 2.1), flows through the urbanised area of Makhanda and then through agricultural areas and areas of natural vegetation to Port Alfred, where it eventually empties into the Indian Ocean (Mgaba, 2014). This river is economically and culturally significant as it supplies water to the Belmont Valley agricultural farms and provides sites for rituals (Mafuta et al., 2011; Mgaba, 2014; Gininda, 2016).

***Swartkops River***

The Swartkops River is located in the Nelson Mandela Bay Metropolitan Municipality (NMBMM) in the Eastern Cape of South Africa, and originates in the foothills of the Groot Winterhoek Mountains (Odume et al., 2012). The river rises from the confluence of two rivers, the KwaZunga River to the north and the Elands River to the southwest. The Swartkops River, which is about 155 km in length (Olisah et al., 2020), drains a catchment of about 1,555 km$^2$ and runs through the urban and industrial areas of Uitenhage and Despatch before discharging into the Indian Ocean at Algoa Bay in Port Elizabeth (Odume, 2014). The river comprises small tributaries, the Brak River, which joins the Swartkops River at Uitenhage and the Chatty River, which empties into the Swartkops River just below the tidal limit at Perseverance (Odume, 2014). Other minor ephemeral drainage features in the southern catchment of the Swartkops River include the Sand and the Bulk Rivers, which join the Elands River at different locations along its length (Maclear, 1996). The Groendal dam is situated in the lower reaches of the KwaZungu River (Geldenhuys, 1989; DWS, 2019), while the Sandriver and Bulkriver dams are situated in the middle reaches of the Sand and Bulk Rivers, respectively (Figure 2.1).

### 2.2.1 Biophysical characteristics of the catchments

***Climate***

The climate of the Buffalo River catchment is characterised by high spatial and temporal variabilities. Temperatures in the Buffalo River catchment are generally cold to warm throughout the year (O’Keeffe et al., 1996; Owolabi et al., 2020). The mean annual temperature in the catchment ranges from 13.5°C to 22.3°C, with maximum daily temperatures in the summer months (December–February) and minimum daily temperatures in the winter months (May–July) (Owolabi et al., 2020). The mean annual rainfall in the Buffalo River catchment is 736 mm (O’Keeffe et al., 1996). However, the upper catchment area of the Buffalo River receives predominantly high precipitation (within the ranges of 1500–2000 mm) compared to the middle and lower catchment areas, which receive 500–625 mm and 700–800 mm, respectively (O’Keeffe et al., 1996; Slaughter et al., 2014). Seasonal variation in rainfall occurs in the Buffalo River catchment, with peak rainfall during the spring and summer months, although rainfall may occur year-round.

In contrast, temperatures in the Bloukrans River catchment are higher and vary between 18.9˚C in July during winter and 26.6˚C in February during summer (Mgaba, 2014). The Bloukrans River catchment receives an average annual rainfall of 680 mm (Makana Local Municipality, 2017), with a minimum of 16 mm in the winter months and a maximum of 57 mm in the summer months (Mafuta et al., 2011; Gininda, 2016).

The climate in the Swartkops River catchment is warm and temperate with a mean daily temperature of about 6˚C in July and 27˚C in January (Odume, 2014). Although rainfall patterns are highly variable, the catchment receives rain throughout the year with at least a minimum monthly average of 60 mm (Odume, 2014). Average annual rainfall ranges from 1000 mm in the upper catchment area, 435 mm at Uitenhage, and 700 mm in the coastal areas (Maclear, 1996; Mmachaka, 2022). In all the study areas, flooding may occur due to high precipitation over several days.

### *Geology and soils*

The bed of the Buffalo River and its catchment is composed of the Lower Beaufort Series rocks of the Karoo system, which are predominantly sandstones, mudstones and intrusive diabase (Palmer, 1991). The doleritic intrusions are prevalent within the upper areas of the Buffalo River catchment. The soils in the catchment include red and black clay from dolerites and sand (O'Keeffe et al., 1996). The geological composition of the Buffalo River catchment accounts for the naturally high level of salts in the river (O'Keeffe et al., 1996; Palmer, 1991).

Similar to the geology of the Buffalo River, the Bloukrans catchment is underlain by sandstones and shales of the Beaufort series group. Shallow and weakly developed soils derived from the quartzite and shale of the Witteberg Group of the Cape Supergroup can be found in the Makhanda area (Sinchembe & Ellery, 2010; Makana Local Municipality, 2017). Topsoil textures vary from rich loamy sand to sandy clay loam suitable for intensive agricultural cultivation along river channels (Gininda, 2016; Makana Local Municipality, 2017).

The upper reaches of the Swartkops River and its tributaries is dominated by the highly folded, faulted and jointed quartzitic Table Mountain sandstone (Maclear, 1996). Uitenhage and Despatch lie on alluvium and the Kirkwood and Sundays River Formations of the Uitenhage Group (Maclear, 1996). Soils of the Swartkops River catchment consist mainly of fine sand, loam and clay of consolidated dune sands and lime-rich sandy clay derived from the Uitenhage group, which are easily erodible (Veevers et al., 1994; Maclear, 1996; Odume, 2014). Thus, naturally high concentrations of dissolved salts may be found just immediately after the confluence of the KwaZungu and the Elands Rivers and throughout the length of the Swartkops River.

***Vegetation***

The natural vegetation of the Buffalo River catchment includes small areas of false Macchia at the summit of the Amatole Mountains, Afro-montane yellowwood forest on the slopes of the mountains, false thornveld dominated by grassland and Acacia karoo in the middle catchment areas from below Rooikrans to Bridle Drift dam, valley bushveld from below the Bridle Drift dam, and coastal forest in the lower reaches (O'Keeffe et al., 1996). Much of the natural vegetation areas have been converted to urban and industrial landscapes, particularly in the middle catchment areas of the Buffalo River. The invasive black wattle tree (*Acacia mearnsii*) dominates the middle reaches of the river catchment. The riparian vegetation of the middle reaches of the river just before the Liang dam is dominated by the grass *Miscanthus capensis* (Palmer, 1991). Aquatic macrophytes and reeds are rare in the Buffalo River and its riparian zone.

The Bloukrans River catchment falls within the Albany Thicket biome of South Africa (Rebelo et al., 2006). Natural vegetation in this biome include the Kowie Thicket, Bisho Thornveld, Suurberg Shale Fynbos, Suurberg Quartzite Fynbos, Grahamstown Grassland Thicket, Albany Thicket and Suurberg Grassy Fynbos (Rebelo et al., 2006). Large areas of the natural vegetation around the Belmont Valley wastewater treatment works (WWTWs) and the golf club have been cleared for agricultural purposes (Mgaba, 2014; Makana Local Municipality, 2017). Areas around the Belmont Valley golf club are heavily infested by alien vegetation (mainly *Acacia mearnsii*). Unlike the Buffalo River catchment, common reeds (*Phragmites australis*) can be found growing in the site immediately downstream of the Belmont Valley WWTWs effluent discharge point (Mgaba, 2014).

Natural vegetation in the Swartkops River catchment include Dune thicket, Mesic succulent thicket, valley thicket, Xeric succulent thicket coastal grassland, eastern thorn bushveld, limited grassy fynbos, mountain fynbos, southwest coast Renosterveld and Afromontane forest (Kleynhans et al., 2005). The upper catchment is dominated by mountainous fynbos while the bushveld and succulent thicket dominate the lower areas of the catchment (Kleynhans et al., 2005; Odume, 2014). Alien invasive vegetation which negatively impact on stream flow, bank stability and sedimentation in the catchment include prickly pear (*Opuntia* spp), Kariba weed (*Salvinia molesta*), cluster pine (*Pinus* spp), gum trees (*Eucalyptus* spp), water hyacinth (*Eichornia crassipes*), and wattle (*Acacia* spp) (Odume, 2014). The riparian vegetation of the Swartkops River is also characterised by extensive and dense growths of reeds (*Phragmites australis*) (Odume, 2014).

### 2.2.2 Socio-economic characteristics of the catchments

The Eastern Cape is South Africa’s poorest province, with a consistently lower provincial economy and a higher unemployment level than the national average (Sinchembe & Ellery, 2010; ECSECC, 2020; Nelson Mandela Bay Municipality, 2022). In South Africa, inequities stemming from the colonial and apartheid era resulted in the development of formal settlements (referred to as ‘towns’), that are predominantly occupied by Whites, and informal settlements (referred to as ‘townships’) that are mostly occupied by Blacks and peoples of mixed races. Formal settlements are generally areas where industrial activities are concentrated, with appreciable levels of urban planning and high housing costs (Nkoane, 2019), whereas rapid proliferation of low-income housing and shanty towns characterise informal settlements (Department of Human Settlements, 2021). As such, around the vicinity of formal industrial areas are clusters of poorly planned and uncontrolled informal settlements which have been constructed as shelter alternatives for the poor (Nkoane, 2019). The differences in urban settlement types and resource allocation in South Africa indicate significant distinctions in service delivery and urban planning (Nkoane, 2019; Department of Human Settlements, 2021). For example, services such as waste management and refuse collection are challenged by the lack of proper access roads in informal settlements (COGTA, 2018; COGTA, 2020a; Nelson Mandela Bay Municipality, 2022). Thus, illegal refuse dumps are common in the catchments of the study rivers.

The Buffalo River catchment comprises regions typical of rural settlements, peri-urban settlements, and those that show remarkable urban development (COGTA, 2020a; Khumalo, 2021). Major towns and townships in the Buffalo City Metropolitan Municipality are Berlin, Beacon Bay, Bisho, Breidbach, Dimbaza, King William's Town, Schornville, Mdantsane, Zwelitsha, Kidd's Beach, Phakamisa, Potsdam, and East London (COGTA, 2020a). The main urban areas in the BCMM are King Williams Town, Schornville, Mdantsane, Zwelitsha and East London (COGTA, 2020a). Based on the survey conducted in 2019, the human population in the BCMM is 893,157 people, with an annual population growth average of 1.0% (COGTA, 2020a; BCMM, 2021). The 2016 Community Survey set the number of households in the BCMM at 253,478 households and increasing (BCMM, 2021).

Makana Local municipality is situated in a smaller geographical area than the BCMM. Based on the 2019 Community Survey, the human population in the Makana Local Municipality is 91,200 people, with an annual population growth average of 0.26% (COGTA, 2018; Makana Local Municipality, 2020). Ninety percent (90%) of the inhabitants of Makana Local Municipality reside in Makhanda (Von Der Meden et al., 2004; Makana Local Municipality, 2021). The Makana Local Municipality has 24,104 households (COGTA, 2018). In 2020, a total number of 17,500 people were unemployed in Makana local Municipality (Makana Local Municipality, 2020).

The Swartkops catchment is occupied by a human population of about 1.2 million people, with an annual population growth average of 1.47% (COGTA, 2020b; Odume et al., 2022). Major cities, towns and townships in the catchment include the industrial town of Uitenhage, residential towns of Despatch and Perseverance, KwaNobuhle, Zwide, and Motherwell (Odume et al., 2022). Over 150,000 households are found within the catchment (COGTA, 2020b). Household income in these towns and townships is much lower than that in the nearby city of Port Elizabeth (Odume et al., 2022).

### 2.2.3 Anthropogenic impacts on the study rivers

Diffuse and point sources of pollution along the study rivers include urban run-off from formal and informal settlements, raw sewage disposal, rubbish dumpsites, and effluent from wastewater treatment facilities. Effluent discharges from wastewater treatment works (WWTWs) are major contributors to changes in water quality in rivers. The compliance of WWTWs with regulatory standards is essential to water resources protection. There are fifteen WWTWs in the catchment of the Buffalo River, three in the Swartkops River catchment, and one in the Bloukrans River catchment. Six of the fifteen WWTWs in the Buffalo River catchment discharge effluent into the Buffalo River, while the others discharge into irrigation ponds or directly to sea. Based on the 2022 Green Drop report, which provides an indication of wastewater management services in the different municipalities, the WWTWs that discharge into the Buffalo River were compliant with recommended standards except those that were hydraulically overloaded (DWS, 2022c). The Schornville WWTW has a design capacity of 5 ML/day but currently operates at 6.8 ML/day (DWS, 2022c). Of the three WWTWs in the

Swartkops catchment, only the Kelvin Jones WWTW is located within the sampling sites. The Kelvin Jones WWTW in Uitenhage has a design capacity of 24 ML/day, but currently operates at 17.04 ML/day (DWS, 2022c). Thus, wastewater treatment operations are handled without being over-capacitated. The Belmont Valley WWTW in the Bloukrans River catchment is overloaded (DWS, 2022c). It has a design capacity of 5.5 ML/day, but currently operates at 7.48 ML/day (DWS, 2022c).

In addition to WWTWs, industrial activities also contribute to water quality deterioration in the study rivers. Companies such as Da Gama Textiles in Zwelitsha and King Tanning Company in King Williams Town dispose untreated effluent unto agricultural plots next to the Buffalo River. This practice has existed for more than 10 years and poses a potential threat to water resources in the Buffalo River as a result of run-off in the rainy seasons (O’Keeffe et al., 1996). The ageing services infrastructure, vandalism and theft are also part of the main contributors to the increased incidents of sewage overflows that threaten the Buffalo River (BCMM, 2020). The tannery industry and wool processing factories in Uitenhage are potential sources of indirect pollution in the Swartkops River. Effluents from these activities are discharged into evaporation ponds from where seepage into the river is believed to occur (Odume, 2014). The Transnet Cuylor Manor Plant in Uitenhage uses settling dams where effluent is treated with alum before further discharging into the Swartkops River (Odume, 2014). Sand mining which occurs in the Brak River in the Swartkops River catchment portends a significant source of increased sediment load in the Swartkops River (DWS, 2019).

Although chemical factories are not presently found in Makhanda, the Bloukrans River has historically been impacted by elevated nutrient levels from domestic activities such as laundry (Mgaba, 2014). Moreover, untreated wastewater from leaking or vandalised sewer pipes and litter are carried into the Bloukrans River through stormwater runoff. Agricultural activities such as fodder cultivation and livestock farming contribute to in-stream nutrient levels through surface run-off from farmlands (Gininda, 2016; Mangadze, 2019). The combined impact of pollutants from point and diffuse sources have generally contributed to deteriorating water quality and ecosystem health in the study rivers.

## 2.3 Description of sampling sites

Fifteen study sites were purposefully selected based on the predominant land-use in the catchment of the sites and water quality variables in order to capture a gradient of anthropogenic disturbance (Table 2.1). Land cover digital elevation model for each study river catchment was downloaded from the United States Geological Survey website (http://ned.usgs.gov). A vector shapefile of 3 km radius buffer zone around each site was overplayed on the land cover raster file to clip out the land cover area within the catchment of the sites. The semi-automatic classification plugin in the QGIS software (version 3.28.15) (QGIS development Team, 2022) was used to conduct a supervised classification of the land use within the catchment of the sites. Percentages of each land use cover type were summarised into forested area, urban area and agricultural area (Table 2.1). The forested area included indigenous forest, grassland, thicket, shrubs, woodland and dense bush. The urban area included urban commercial, industrial, informal, village and built-up areas. The agricultural area included cultivated commercial fields and pivots, cultivated permanent commercial lands, cultivated orchards and vines, and plantations/woodlots.

Each of the sites sampled covered an area of approximately 50 $m^2$ (5 m by 10 m), provided that the river reach was safe to sample in accordance with the South African Scoring System (version 5; SASS5) protocol. The sampling sites were selected based on the predominant land use occurring within the catchment of the site to reflect an anthropogenic disturbance gradient along the stretch of the study rivers. Data collection was conducted four times at each site of the Buffalo and Bloukrans Rivers (once in November 2021, January, March and June 2022) to account for seasonality. Historical data from sites in the Swartkops River were collected eight times between 2009 and 2012. Samples were collected seasonally from the sites.

Table 2.1 Summary of the land use characteristics of the sampling sites

| Site | Forested (%) | Urban (%) | Agriculture (%) | Depth (cm) | Width (m) | Turbidity (NTU) | Dissolved oxygen (mg/l) | Anthropogenic disturbance |
|---|---|---|---|---|---|---|---|---|
| Site 1 | 75,44 | 3,00 | 17,94 | 19,41 | 14,00 | 4,09 | 8,80 | Dam, Picnic |
| Site 2 | 75,17 | 11,29 | 13,28 | 16,14 | 4,76 | 22,22 | 8,13 | Bridge |
| Site 3 | 47,94 | 39,10 | 12,45 | 14,96 | 18,84 | 22,20 | 7,64 | Human settlement |
| Site 4 | 47,42 | 37,65 | 14,20 | 18,98 | 15,08 | 21,91 | 5,02 | WWTW |
| Site 5 | 44,82 | 27,43 | 26,29 | 21,26 | 11,38 | 29,15 | 5,92 | Mixed |
| Site 6 | 71,75 | 18,24 | 4,61 | 24,34 | 40,20 | 23,66 | 8,38 | Bridge |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Site 7 | 74,33 | 19,40 | 6,16 | 20,83 | 2,50 | 21,51 | 5,99 | Sewage, Human settlement |
| Site 8 | 78,49 | 15,10 | 6,29 | 20,12 | 3,10 | 23,25 | 2,72 | WWTW |
| Site 9 | 91,01 | 0,09 | 8,69 | 9,32 | 5,38 | 15,60 | 2,54 | Agriculture, upstream WWTW |
| Site 10 | 82,67 | 0,00 | 16,79 | 19,70 | 6,13 | 5,03 | 8,33 | Cultural activities |
| Site 11 | 96,56 | 0,01 | 3,06 | 16,38 | 1,93 | 3,67 | 8,95 | Cultural activities |
| Site 12 | 66,92 | 9,60 | 19,67 | | | 6,15 | 6,07 | Dam |
| Site 13 | 54,94 | 39,08 | 0,00 | | | 6,15 | 6,99 | Human settlement |
| Site 14 | 65,06 | 26,02 | 1,68 | | | 115,63 | 3,26 | WWTW |
| Site 15 | 62,01 | 27,21 | 2,52 | | | 9,35 | 2,17 | Mixed |

### *Buffalo River sites*

#### ***<u>Site 1</u>***

Site 1 (32°44'38.3"S, 27°18'08.2"E), the headwater sampling site of the Buffalo River, is located downstream of a small impoundment, the Maden dam (Figure 2.2). This site, surrounded by indigenous forest, was the only least impacted site that was accessible for sampling. About 50% of the runoff of the Buffalo River is generated by the catchment of this site (O'Keeffe et al., 1996). Water supply, forestry, conservation and recreation (including hiking, trout fishing, cultural and picnic activities) constitute the primary use of this part of the river and its catchment (Palmer, 1991a). Instream conditions included over 2 metres of white-water rapids, a mixture of pool, run and riffle biotopes, and more than 2 metres of marginal vegetation. No algal presence was observed at this site. All the biotopes were available at this site. The Rooikrans dam, which serves as a major reservoir for water supply to the King Williams Town and the Zwelitsha area, is about 3 kilometres downstream of this site.

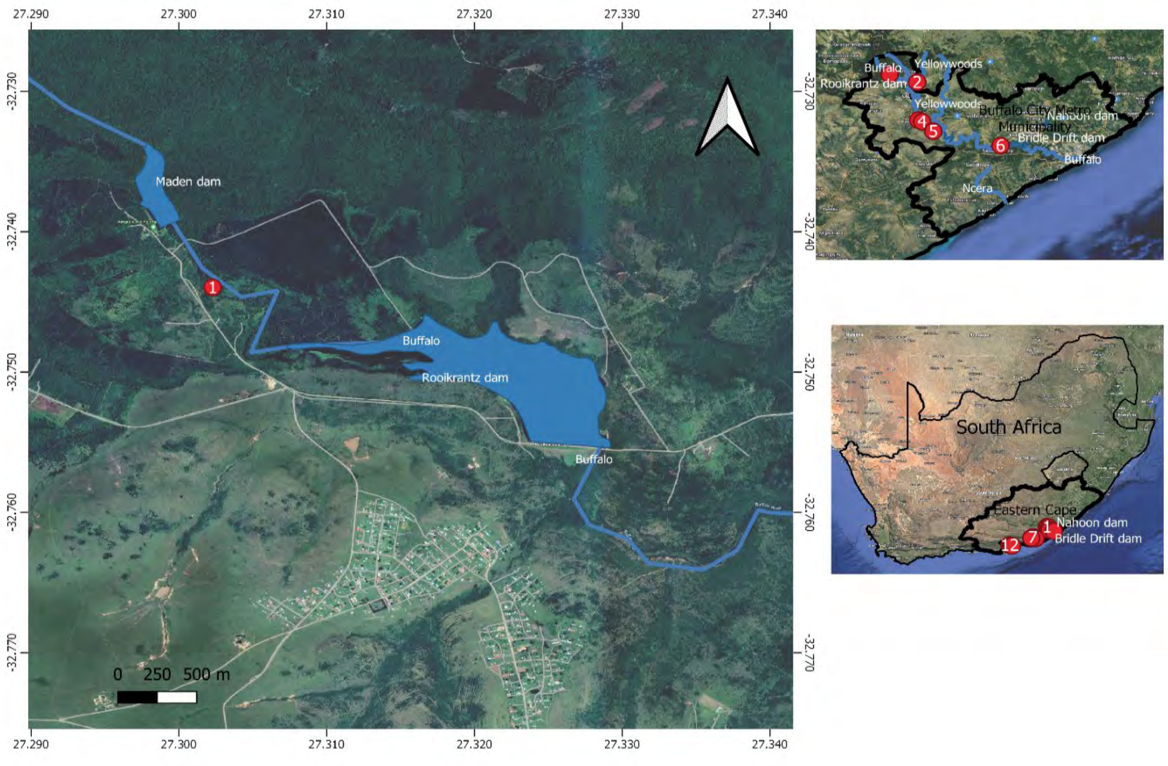


Figure 2.2 Map of study area showing the location and forested area of Site 1 in the Buffalo River, Eastern Cape Province, South Africa.

### ***Site 2***

Site 2 (32°46'14.1"S, 27°23'22.7"E) is in the Izele River (Figure 2.3). The Izele River is a second-order stream, and one of the Buffalo River tributaries. This site was selected as a second least impacted site because the water level in the Rooikrans dam was very low and appeared to be insufficient to generate enough discharge into the downstream sites of the Buffalo River. This observation corresponds with the Buffalo River water quality and flow analysis by O'Keeffe et al. (1996). Hence, much of the flow in the forested areas, headstream of Buffalo River was supplied by tributaries (Izele and Nyatela rivers) during the site survey in November 2021. Instream conditions at this site included moderately opaque water caused by the natural geomorphology of the river, slow flow velocity and an absence of algal growth. A mixed riparian vegetation consisting of shrubs and grasses was visible at this site. The main land use in the catchment of this site is small-scale animal rearing associated with rural settlements.

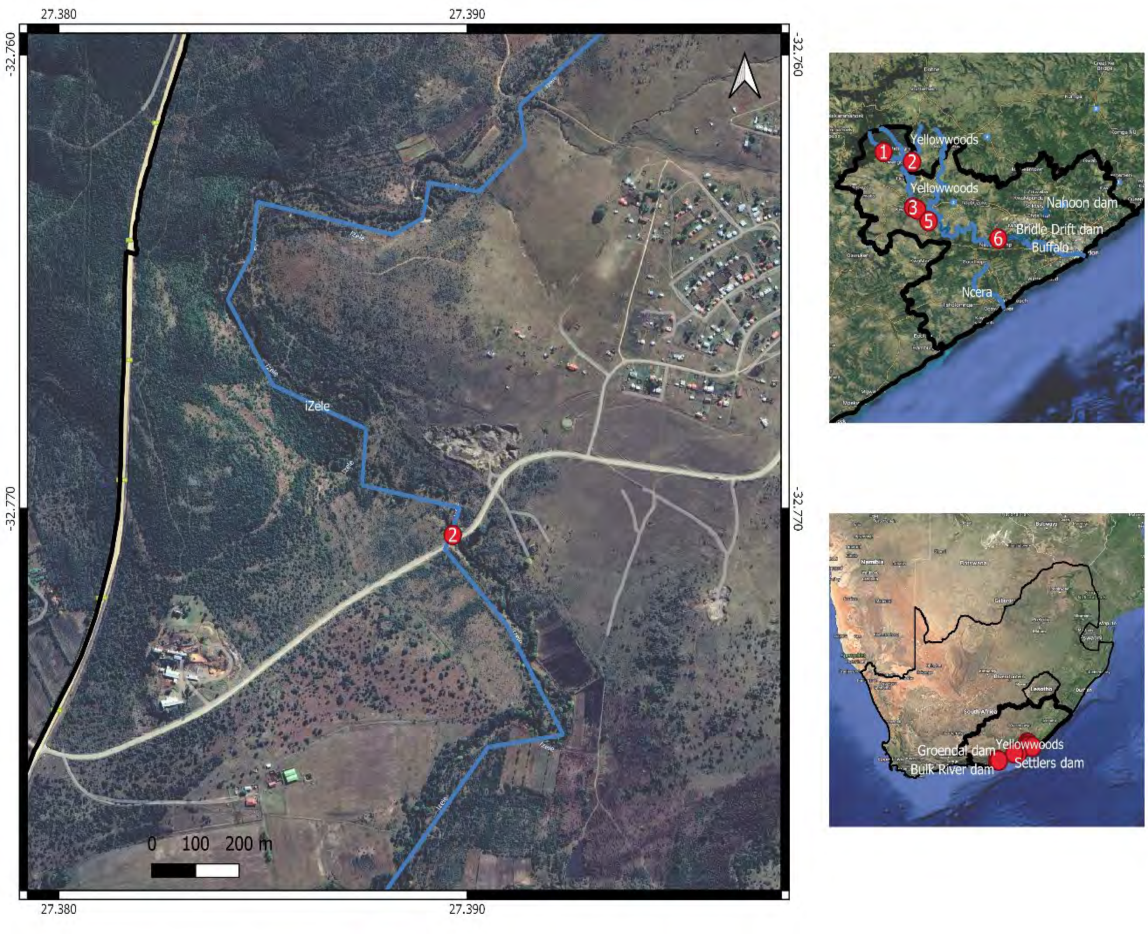


Figure 2.3 Map of study area showing the location and landscape of Site 2 in the Buffalo River Catchment, Eastern Cape Province, South Africa

## ***Site 3***

Site 3 (32°53'49.0"S, 27°23'34.2"E) is situated in the middle reaches of the Buffalo River catchment (Figure 2.4). The site is impacted by diffuse pollution from rubbish dumps, raw sewage flow into the site and pollutants from urban run-off. Instream morphology was characterised by submerged stones, gravel, and sand. Flow was generally moderate, and there was evidence of channel modification and deforestation. The site had little canopy cover and impacted riparian vegetation. Water was discoloured and moss could be seen growing on submerged stones. All the biotopes were sampled during the sampling season except in autumn and winter when the vegetation biotope was absent.

Figure 2.4 Map of study area showing the location and urbanised catchment of Site 3 in the Buffalo River Catchment, Eastern Cape Province, South Africa

## ***Site 4***

Site 4 (32°54'08.6"S, 27°24'18.5"E) is situated downstream of the Schornville WWTW effluent discharge point at King Williams Town (Figure 2.5). Instream conditions included discoloured water with high flow velocity, occasional sewage sludge deposition, and algae on some submerged stones. Channel morphology alterations were evident at this site. All the biotopes were sampled at this site.

Figure 2.5 Map of study area showing the location and catchment area of Site 4 in the Buffalo River Catchment, Eastern Cape Province, South Africa. The purple tile indicates the location of the Schornville WWTW.

## ***Site 5***

Site 5 (32°56'03.9"S, 27°26'25.2"E) is situated downstream of the Zwelitsha area and the Mlakalaka traditional village (Figure 2.6). This site receives diffuse pollution from the settlements, and run-off from agricultural activities and rubbish dumps in the catchment of the site. Immediately downstream of the site is the Zwelitsha WWTW effluent discharge point. However, the impact of effluent from the WWTW was not captured in this study because downstream of site 5 was inaccessible and the biotopes could not be sampled for SASS5. Flow velocity at this site was generally high, and there was evidence of channel modification due to the Mlakalaka weir. Instream morphology was characterised by submerged stones with mosses. The site had very little canopy and water depth was shallow. All the biotopes were available here.

Figure 2.6 Map of study area showing the location and catchment area of Site 5 in the Buffalo River Catchment, Eastern Cape Province, South Africa. The purple tile indicates the irrigation lands for effluents from the Da Gama Textiles industry.

## ***Site 6***

Site 6 (32°58'57.2"S, 27°39'14.1"E) is located upstream of the Bridle Drift dam in the Potsdam area (Figure 2.7). This site was selected to explore the system recovery potential of the Buffalo River under the stress of urbanisation. The major impacts at this site are an overhead bridge which receives very sparse vehicular traffic, and run-off from the resettlement camp upstream from the site. Flow velocity at this site was high and there was evidence of channel modification owing to the constructed bridge. Moreover, significant changes to channel depth and width were observed during summer. The changes were due to a high rainfall event which damaged a bridge further upstream of this site. There was no canopy cover at this site and riparian vegetation was characterised by grasses. No excessive algal growth was observed throughout the sampling seasons. All the biotopes were sampled at this site.

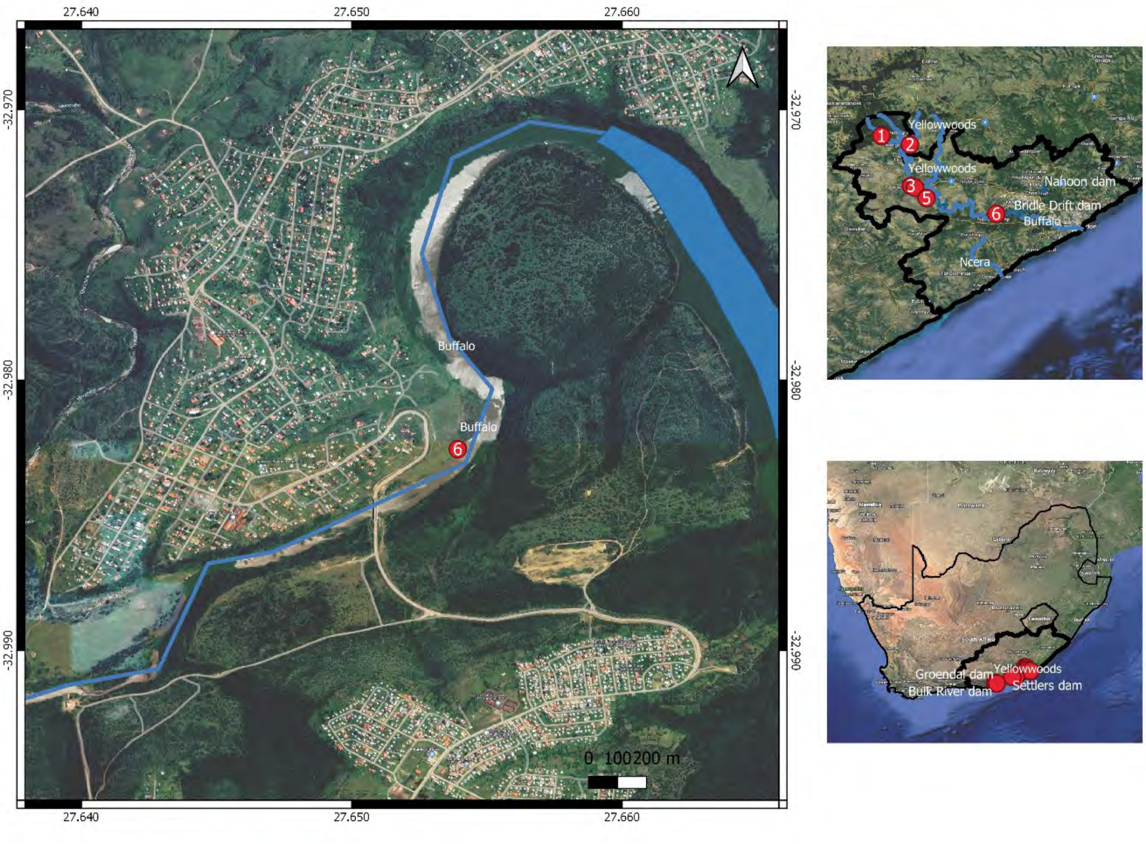


Figure 2.7 Map of study area showing the location and catchment area of Site 6 in the Buffalo River Catchment, Eastern Cape Province, South Africa

### ***Bloukrans River sites***

#### ***Site 7***

This site (33°18'51.3"S, 26°33'06.0"E) drains the entire formal and informal settlement areas of Makhanda (Figure 2.8). Water in this site was odorous and highly turbid with the presence of litter, debris and evidence of overgrazing by cattle. Because of the failing municipal infrastructure in Makhanda, leaking sewer pipes release untreated sewage into the environment. Thus, organic pollutants are carried in storm water run-off from the formal and informal settlements into this site. Sampled biotopes included submerged stones, marginal riparian vegetation and gravel, sand and mud (GSM).

Figure 2.8 Map of study area showing the location and urban catchment upstream from Site 7 in the Bloukrans River Catchment, Eastern Cape Province, South Africa

## ***Site 8***

This site (33°19'05.3"S, 26°34'05.7"E) is about 500 metres downstream of the effluent discharge point of the Belmont Valley Wastewater Treatment Works (WWTW) (Figure 2.9). It is characterised by high water velocity, very little canopy, and evidence of channel modification. Fodder and livestock farming are located upstream of the site. Effluent discharge, irrigation, livestock faecal contamination, and runoff from informal settlements are the main sources of impact in this site. The riparian vegetation of the river reach at this site was characterised by common reeds.

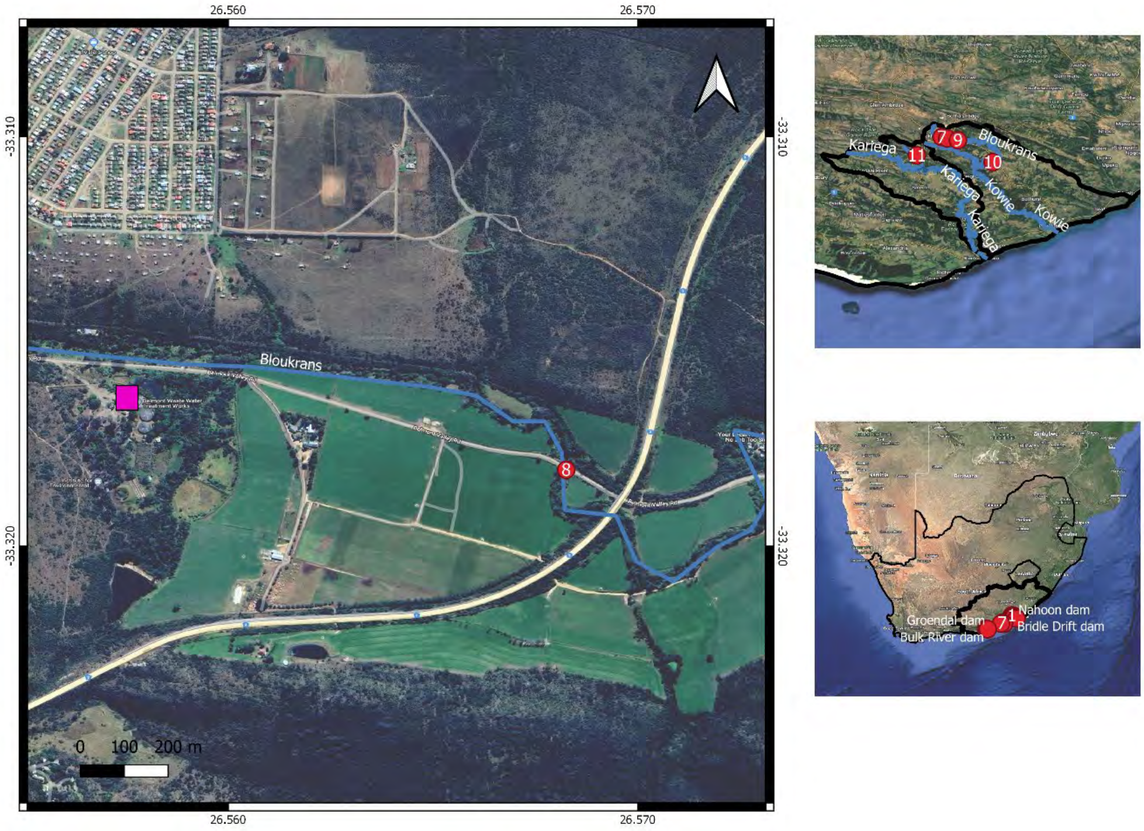


Figure 2.9 Map of study area showing the location of Site 8 in the Bloukrans River Catchment, Eastern Cape Province, South Africa. The purple tile indicates the location of the Belmont Valley WWTW.

## ***Site 9***

This site (33°19'25.6"S, 26°36'02.5"E) is situated approximately 1 km downstream of Site 8 (Figure 2.10). It is surrounded by agricultural activities (crop and fodder production farmed land) and is extensively modified due to irrigation. The site is located underneath an overhead bridge and is adjacent to an unpaved road. Flow alteration and run-off of agrochemicals such as fertilisers and pesticides were deemed likely to be important factors determining macroinvertebrate assemblage structure. Due to flow alteration, vegetation biotope was not available in all the sampling seasons at this site.

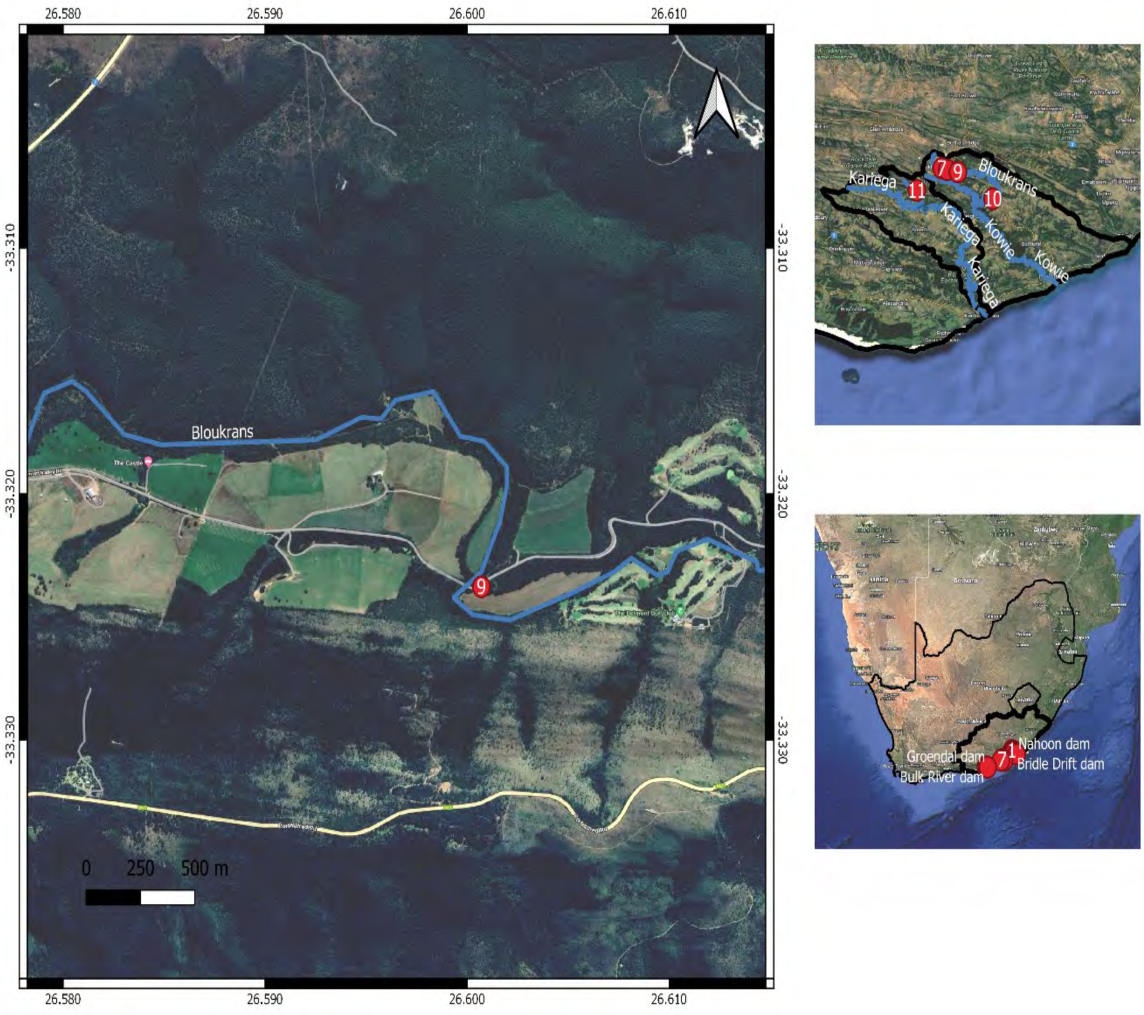


Figure 2.10 Map of study area showing the location and agricultural plots upstream from Site 9 in the Bloukrans River Catchment, Eastern Cape Province, South Africa

## ***Site 10***

Site 10 (33°23'26.3"S, 26°42'23.9"E) is situated approximately 36 kilometres downstream of the WWTW effluent discharge point. It is located just upstream of the pool in the Blaauwkrantz Local Authority Nature Reserve (Figure 2.11). This site is a recovery site that is used for religious and cultural purposes. Instream morphology is dominated by mixed bedrock and gravel, and side bars indicating the potential channel width increase during high flow.

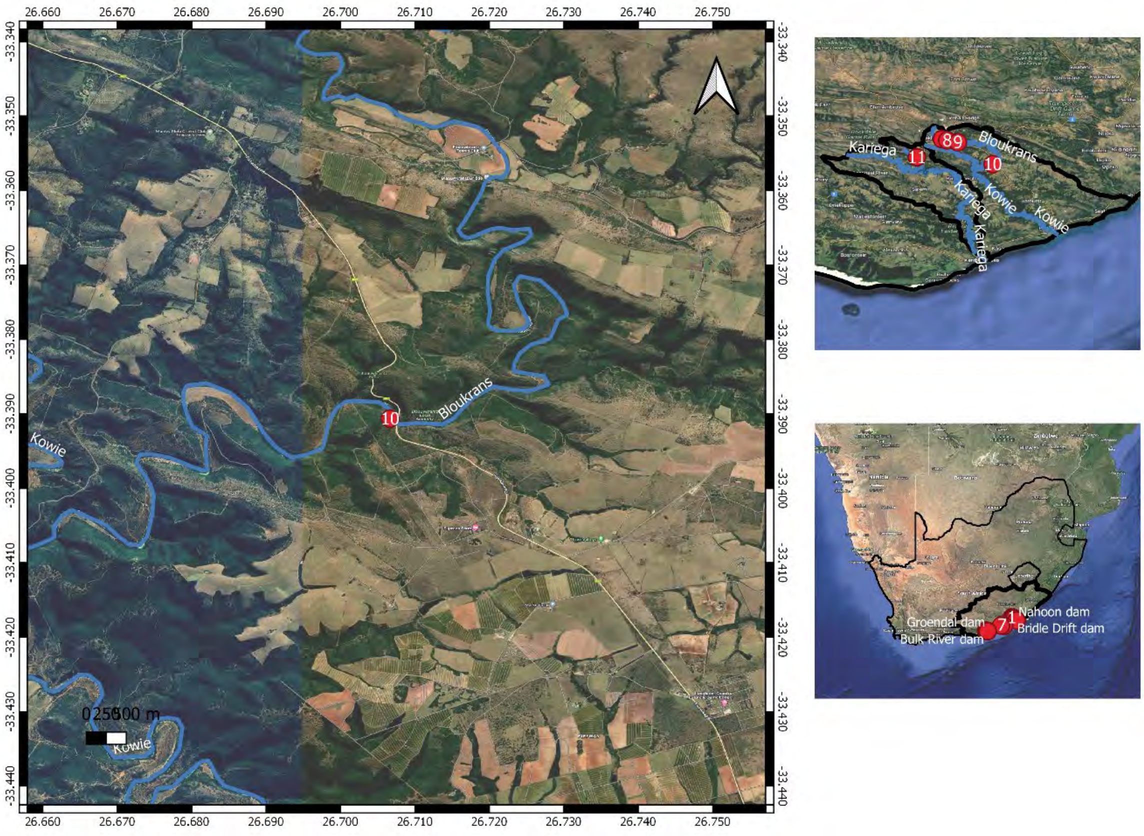


Figure 2.11 Map of study area showing the location and forested catchment of Site 10 in the Bloukrans River Catchment, Eastern Cape Province, South Africa

## ***Site 11***

Site 11 (33°22'10.9"S, 26°28'33.6"E) is about 10 km southwest of Makhanda. Because the headwater region of the Bloukrans River is urbanised, it was necessary to select a reference site in a river within the same ecoregion as the Bloukrans River (Figure 2.12). Site 11, located in the Palmiet River, is in the same ecoregion as the Bloukrans River. It was selected as a reference site to enable comparison of macroinvertebrate assemblages between sampling sites of the same ecoregion, because of the natural variation in geomorphology and instream conditions between different ecoregions (Kleynhans & Louw, 2007; Dallas & Day, 2007). Apart from minor and occasional religious rituals carried out at this site, no evidence of urban impact was observed. All the biotopes were sampled at this site.

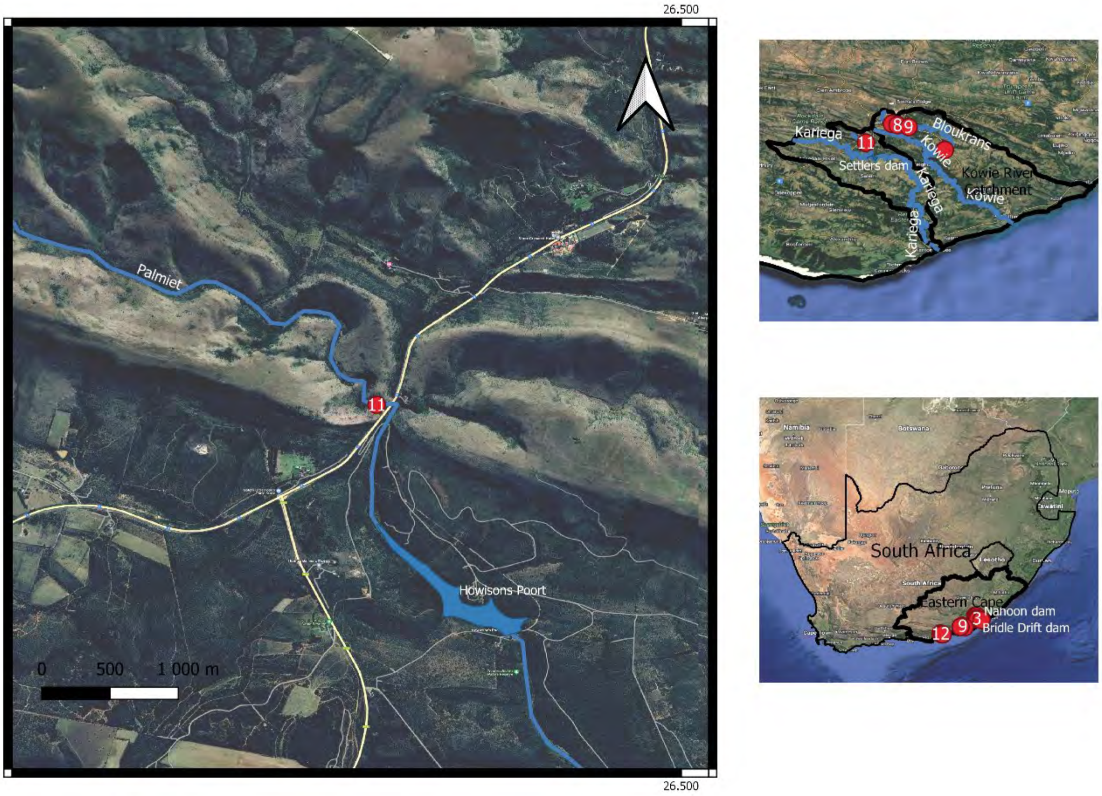


Figure 2.12 Map of study area showing the location and near-natural catchment of Site 11 in the Bloukrans River Catchment, Eastern Cape Province, South Africa

### ***Swartkops River sites***

### ***Site 12***

Site 12 (33°45'08.4"S, 25°20'32.6"E) is situated upstream of Uitenhage and was selected to represent the least impacted site. This site was determined to be a least impacted site because it is located 16.4 km downstream of the Groendal dam. Such an impoundment could impact on the macroinvertebrate composition of the site, however, this site represented the best available condition in the river. Presently, much of the catchment area at this site has been converted to agricultural land (Figure 2.13).

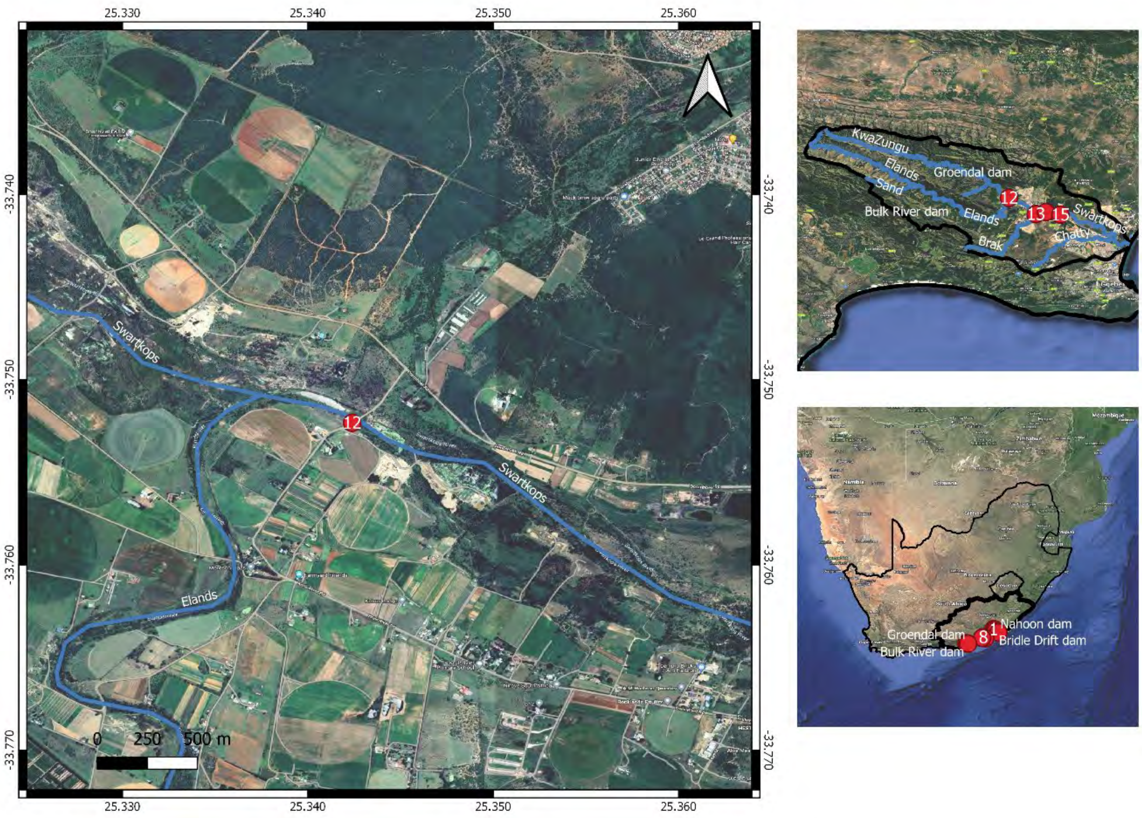


Figure 2.13 Map of study area showing the location and agricultural lands in the catchment of Site 12 in the Swartkops River Catchment, Eastern Cape Province, South Africa

### ***Site 13***

Site 13 (33°47'29.0"S, 25°24'26.4"E) is in the industrial town of Uitenhage (Figure 2.14). The site receives run-off from roads and informal settlements, livestock farming and other agricultural practices. Part of the river reach at the site was covered with water hyacinth and other aquatic weeds between 2009 and 2010. However, the flooding event of 2011 cleared them away (Odume, 2014). There was evidence of moderate habitat modification on the riparian zone. All the sampling biotopes were available at this site.

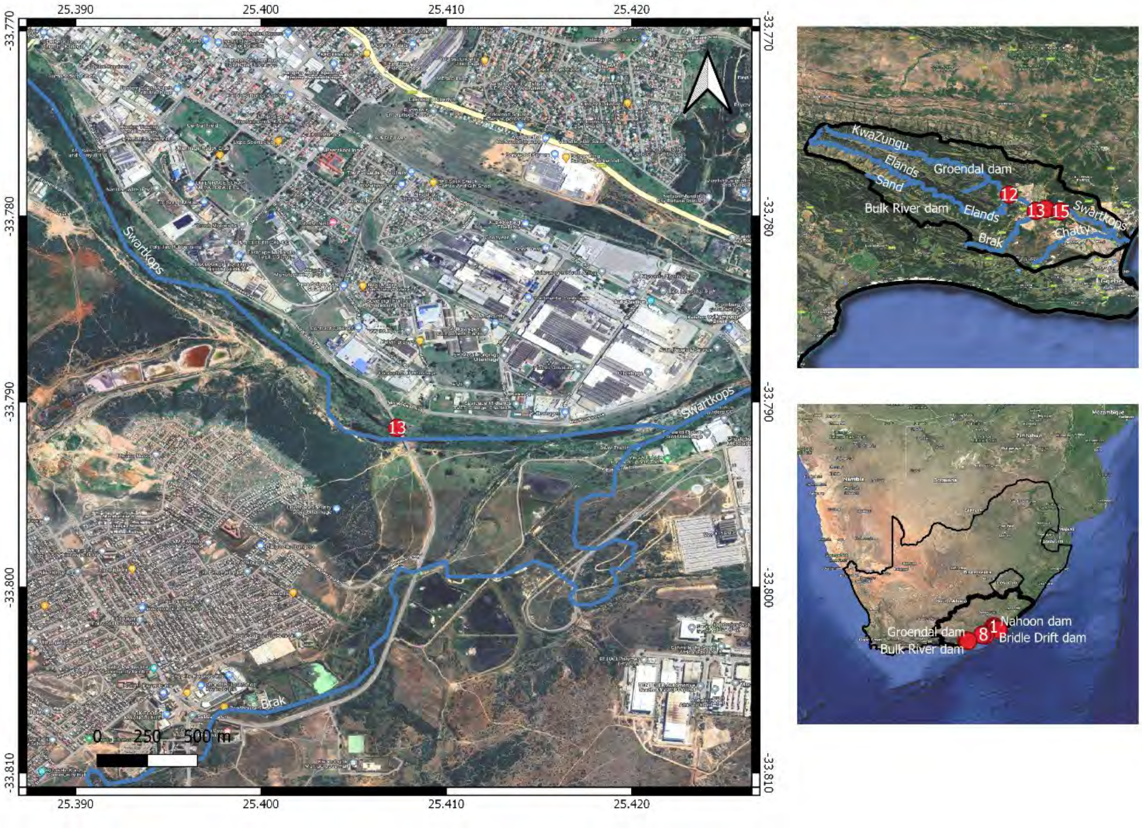


Figure 2.14 Map of study area showing the location and urban catchment at Site 13 in the Swartkops River Catchment, Eastern Cape Province, South Africa

### ***Site 14***

Site 14 (33°47'11.8"S, 25°25'53.97"E) is immediately downstream of the Kelvin Jones WWTW effluent discharge point. The site is also within the industrial town of Uitenhage, but further downstream of Site 13 (Figure 2.15). The main impacts at this site include industrial and wastewater effluent discharges, run-off from road networks, and agricultural activities. Similar to site 13, almost the entire river reach at this site was covered with water hyacinth, common reed and Kariba weed between 2009 and 2010. The flooding event of 2011 also cleared them (Odume, 2014). Water at this site was turbid and had low aesthetic value.

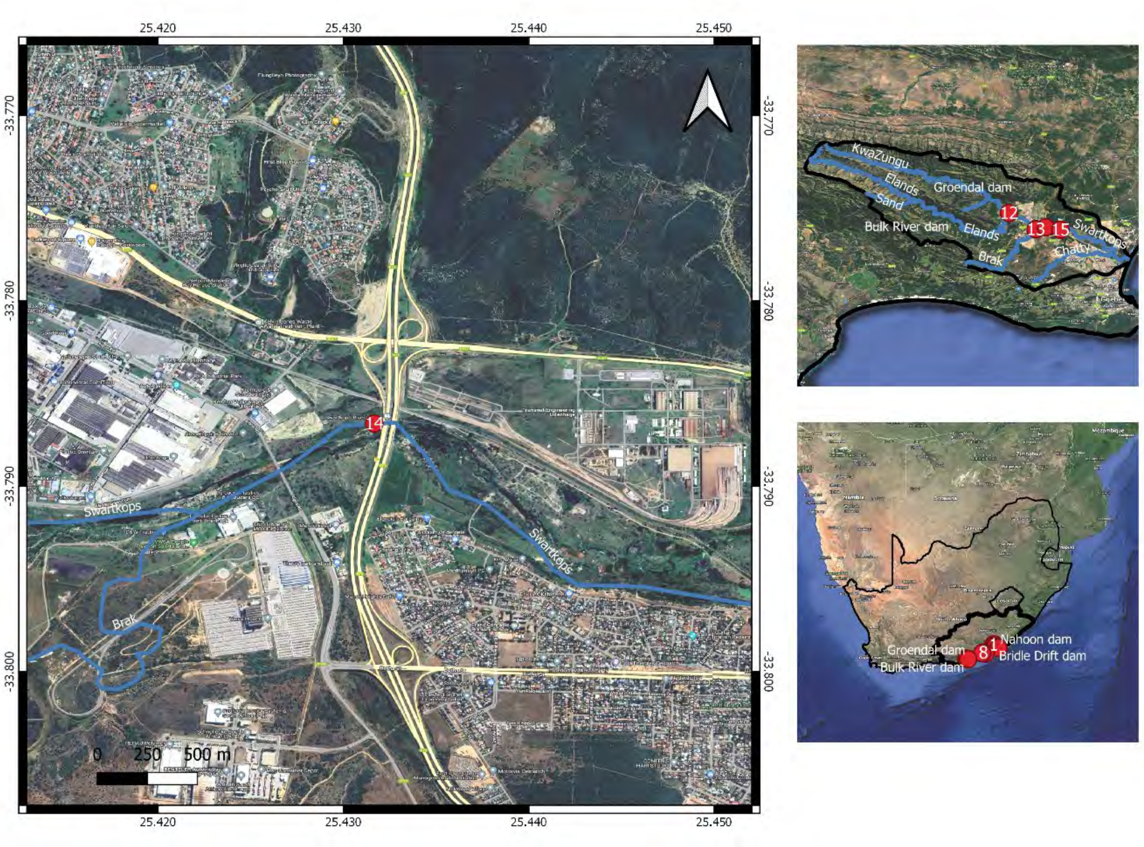


Figure 2.15 Map of study area showing the location and urban catchment at Site 14 in the Swartkops River Catchment, Eastern Cape Province, South Africa

## ***Site 15***

Site 15 (33°47'34.0"S, 25°27'58.7"E) is in Despatch and is downstream of Site 14 (Figure 2.16). Activities in the catchment of this site including sand and gravel mining as well as municipal run-off, contribute to water quality degradation at this site. River flow was modified by a small access road with a culvert built across the site. There was evidence of riparian habitat degradation and the impacts of wastewater effluent discharges from the Kelvin Jones WWTW along the river reach (Odume, 2014). The aquatic weeds which were observed at this site between 2009 and 2010 were cleared by the floods in 2011. All the biotopes were available at this site. Selecting another site further downstream to monitor for potential system recovery was not possible because of the tidal limit and the influence of estuarine effects on freshwater macroinvertebrate assemblages (Odume, 2014).

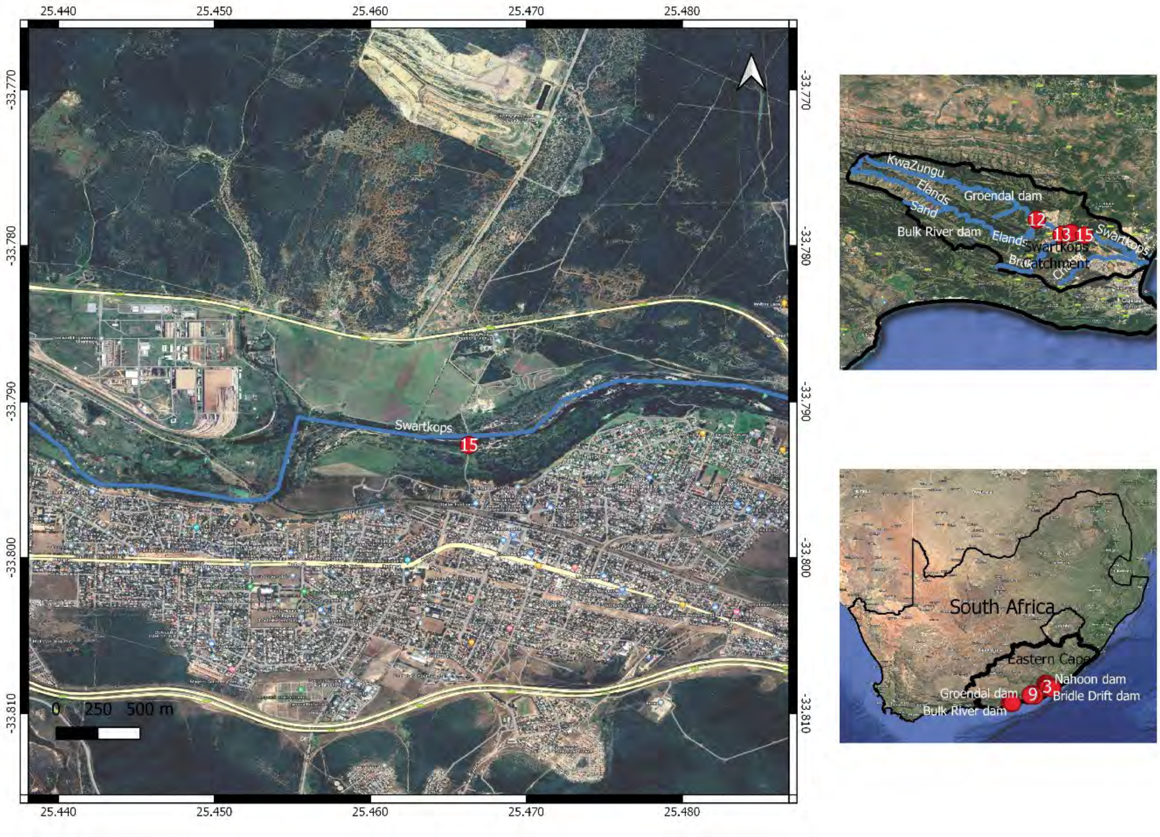


Figure 2.16 Map of study area showing the location and urban catchment Site 15 in the Swartkops River Catchment, Eastern Cape Province, South Africa

### 2.3.1 Classification of sampling sites

The sampling sites were purposefully selected based on the predominant land-use in the river catchments (Table 2.1), and classified into five groups: i) least impacted sites (REF sites; 78.53% mean forested area, 5.98% mean urban area and 13.49% mean agricultural land), ii) sites that received diffuse pollution but were upstream from the effluent discharge point of WWTWs (DP sites; 59.07% mean forested area, 32.53% mean urban area and 6.20% mean agricultural land), iii) sites that received primarily point source pollution from WWTWs (PP sites; 63.66% mean forested area, 26.26% mean urban area and 7.39% mean agricultural land), iv) sites that are further downstream from the WWTW that received impacts from both point source and diffuse pollution (AG sites; 65.95% mean forested area, 18.24% mean urban area and 12.5% mean agricultural land) and, v) sites that were selected for exploring the potential system recovery of the study rivers (RECV sites; 77.21% mean forested area, 9.12% mean urban area and 10.7% mean agricultural land). Thus, Sites 1, 2, 11 and 12 were classified as

REF sites, sites 3, 7 and 13 were DP sites, Sites 4, 8 and 14 were PP sites, Sites 5, 9 and 15 were AG sites, and Sites 6 and 10 were regarded as RECV sites.

## 2.4 Sample collection, processing, and analysis

Measurements of physicochemical variables and collections of water and macroinvertebrates for the Buffalo and Bloukrans rivers were done once per season from spring (November 2021) to winter (June 2022). Historical data collected seasonally between 2009–2012 for the Swartkops River were also used in this study.

### 2.4.1 Water sampling and physicochemical measurements

Physicochemical variables measured on-site at the Bloukrans and Buffalo River sites included temperature, dissolved oxygen (DO), electrical conductivity (EC), turbidity, pH, flow velocity, canopy cover, depth and width. Dissolved oxygen (DO), temperature and EC were measured using the multiparameter probe model H198. Turbidity was measured using the Orbeco-Helliage 966 portable turbidity metre, while pH was measured using a Cyberscan pH 110 metre. Flow velocity measurements in metres per second (m/s) were taken using an OTT portable flow meter. Depth (in centimetres) was determined using a graduated measuring rod. Width (in metres) was measured with a measuring tape. For canopy cover measurements, hemispherical images were taken at different points of the site using a camera. Collected images were analysed using the Image Processing and Analysis in Java (ImageJ) software (version 1.53k) (Burger & Burge, 2007). Canopy cover measurements were expressed in percentages.

Surface water samples for nutrient and total suspended solids analyses were collected from the Bloukrans and Buffalo River sites using separate 250 ml acid-washed plastic bottles. Additional water samples for heavy metals analysis were collected using acid-washed 500 ml glass bottles. Collected water samples were transported on ice to the water quality laboratory of the Institute for Water Research, Rhodes University, where further analyses were undertaken. Samples were analysed within 24 hours of collection for nitrate-nitrogen ($NO_3$-N), nitrite-nitrogen ($NO_2$-N), orthophosphate-phosphorus ($PO_4$-P), and ammonium-nitrogen ($NH_4$-N). Nitrate-nitrogen ($NO_3$-N) and $NO_2$-N were analysed according to APHA (2005) using a Biotek microplate reader at 540 nm. Orthophosphate phosphorus ($PO_4$-P) and $NH_4$-N were analysed using Merck spectroquant® phosphate and ammonium concentration test kits, catalogue numbers 1.14752.0001 and 1.14848.0001, respectively. Analyses were conducted in triplicate for each sample and averaged. Water samples were analysed for metals, including aluminium, zinc, copper, chromium, nickel and iron, using the Palintest photometer model 5000. Analyses were done in accordance with the manufacturer's instructions.

Physicochemical variables data for the Swartkops River samples collected between 2009 and 2012 included temperature, dissolved oxygen (DO), electrical conductivity (EC), turbidity, pH, nitrate-nitrogen ($NO_3$-N), nitrite-nitrogen ($NO_2$-N), orthophosphate-phosphorus ($PO_4$-P), and ammonium-nitrogen ($NH_4$-N).

### 2.4.2 Macroinvertebrate sampling

Macroinvertebrate samples were collected seasonally from the Buffalo and Bloukrans rivers between November 2021 and June 2022 at each sampling site using the South African Scoring System version 5 (SASS5) protocol (Dickens & Graham, 2002). Briefly, macroinvertebrate samples were collected from three biotope groups, stones (stones-in-current and stones-out-of-current), GSM (gravel, sand, and mud) and vegetation (marginal and aquatic), using a kick net (frame: 30 x 30 with mesh size 1 mm) (Dickens & Graham, 2002). The stone-in-current included free/loose pebbles and cobbles with 2–25 cm average size and boulders or large sheets of rock greater than 25 cm situated in current which prevented the deposition of fine silt or sediments. Stones-out-of-current included pebbles, cobbles, and boulders in pools that allowed for the deposition of fine sediments. Gravels were deemed to be small stones, usually less than 2 cm in diameter, while sand and mud were smaller, less than 2 mm and 0.06 mm, respectively. Marginal vegetation included vegetation hanging into the river or growing on the edge of the river, whereas aquatic vegetation included submerged vegetation not confined to the riverbanks, filamentous algae and the roots of floating macrophytes. Following the protocol, stones were sampled for 2 minutes, GSM for 1 minute, and samples for vegetation biotope were collected from a total length of approximately 2 metres (Dickens & Graham, 2002). Only one sample per available biotope group was collected on each sampling occasion at each of the sampling sites. Although a 500 µm mesh size may capture many chironomids, the 1 mm mesh size of the SASS5 kick net was considered sufficient to enable the comparison between the results of the SASS5 and results based on chironomid assemblage structure without any need for a different sampling procedure. Thus, the decision to use the SASS5 protocol to sample chironomids was to ensure that no effects due to sampling effort may influence the interpretation of the results.

Using identification keys by Gerber and Gabriel (2002), macroinvertebrate taxa were identified on-site to family level (Dickens & Graham, 2002). Macroinvertebrates collected per biotope at each sampling site were identified for a maximum of 15 minutes, and identification was stopped if no new taxa was seen following the SASS5 protocol (Dickens & Graham, 2002). The identified macroinvertebrate families were recorded on a SASS5 sheet together with estimates of their abundances. Once field identification was completed, samples were preserved in 70% ethanol, and transported to the laboratory for sorting, mounting, identification and abundance counts of chironomids.

### 2.4.3 Chironomid processing and identification

Sorted chironomid larvae kept in 70% ethanol were processed and mounted for taxonomic identification. Chironomid larvae were transferred from the 70% ethanol into a 10 ml pill vial containing 10% (w : v) cold potassium hydroxide (KOH) solution and then left in the solution for about 10–20 minutes to clear (Odume, 2014). After clearing, larvae were dehydrated in 96% ethanol for 5 minutes and finally put in absolute ethanol for 2 minutes. The dehydrated larvae were transferred into a solution of xylene for about 10 minutes. Larvae were removed from the xylene and placed on a mounting slide containing a drop of Canada balsam for microscopy. Under a dissecting microscope and using a pair of fine forceps and dissecting needles, the head capsule, ventral side up, was dissected from the body segment. Using a pair of forceps, a cover slip was placed over the head capsule with the ventral side still up and the body segment placed on the side. A gentle rotary force was applied carefully to flatten the head capsule and expose the mouthparts (Odume, 2014). Slides were left for about five to eight days to air dry. Mounted specimens were examined under an Olympus compound microscope equipped with an Ultra 20 soft imaging system digital camera. Magnifications between X4 and X40 were used, depending on the size of the specimen being examined. Image analysis and photographs were taken using the analySIS Five soft imaging system. Chironomid larvae were identified to genus and species morphotype level using the keys described by Wiederholm (1983), Cranston (2014) and Harrison (2004).

## 2.5 Chironomid trait selection and analysis

Chironomid traits were selected based on their mechanistic link to urban-related stressors. The literature was reviewed to identify stressors related to the urban stream syndrome. The main stressors of urban rivers are storm water runoff with increased fine sediment loads, elevated nutrients and heavy metal concentration from wastewater treatment works and raw sewage disposal, dissolved oxygen depletion, elevated concentration of dissolved solids and changes in sediment concentrations and flow regimes (Paul & Meyer, 2001; Walsh et al., 2005; Dolédec & Statzner, 2008) (Table 2.2).

Chironomid traits and ecological preferences were selected based on their potential mechanistic links to the identified urban stressors, the ease of measuring the trait and the availability of information in the literature. In this study, fifteen (15) categories of traits and ecological preferences, namely, maximum body size, voltinism, reproduction type, number of eggs per egg mass, tube construction, haemoglobin, respiration, number of tracheae, mobility, lifecycle duration, body armour, substrate relation, feeding mode, substrate preference and food type were selected (Table 2.2). Chironomid trait information was obtained from visual observations, available published literature (e.g., Collins & Fahrig, 2020; Hershey, 1987; Karima, 2010; Poff et al., 2006), and the South Africa macroinvertebrate trait database (Odume et al., 2023), supplemented with the trait database for European chironomids (Serra et al., 2016).

Trait variability and plasticity between chironomid species were accounted for using a fuzzy coding technique (Chevenet et al., 1994). A fuzzy coding system of 0–5 was used to assign affinity scores to chironomid species in relation to trait and ecological preferences. Fuzzy coding values of 0, 1, 3, and 5 were used, corresponding to no affinity, low affinity, moderate affinity, and high affinity, respectively (Chevenet et al., 1994).

Table 2.2 Selected chironomid traits and ecological preferences with the rationale for their selection

| **Trait category** | **Trait attributes** | **code** | **Rationale** |
|---|---|---|---|
| Haemoglobin | Present<br>Absent | H1<br>H2 | Urban pollution results in input of high organic matter into receiving rivers and streams. High levels of organic matter may result in low dissolved oxygen. Haemoglobin in chironomids has been reported to confer resilience on species in low oxygenated water bodies due to the high affinity of haem groups |

|  |  |  |  |
|---|---|---|---|
|  |  |  | to dissolved oxygen in aquatic systems (Walshe, 1950). Thus, chironomids that possess haemoglobin are likely to be more resilient to low dissolved oxygen compared with those without haemoglobin (Jiang et al., 2019). |
| Respiration | Tracheal gills<br>Cuticular respiration<br>Aerial respiration | R1<br>R2<br>R3 | Urban pollution is associated with increased surface runoff, which can carry sediment and organic matter from urban catchments into freshwater systems. Elevated organic matter processing and sediment accretion result in reduced dissolved oxygen concentrations, and may put species which utilise tracheal gills and cuticular respiration at risk of abrasion and clogging (Jones et al., 2012). Thus, chironomids with an adaptation for aerial respiration may be more adaptable to increasing urban pollution (Odume, 2014). |
| Tube construction | Tube absent<br>Tube without shape<br>Tube rigid | TC1<br>TC2<br>TC3 | Urban pollution results in elevated dissolved solids, sediment, and metal concentrations. Tube construction limits the exposure of chironomids to the effects of dissolved solids and metals (Halpern et al., 2002). It can also enable the avoidance of the impact |

| | | | |
|---|---|---|---|
| | | | of sedimentation on morphological and physiological features (Armitage et al., 1995; van Kleef et al., 2015; Serra et al., 2017b). |
| Substrate relation | Burrower<br>Free-living<br>Miner<br>Fixed | SR1<br>SR2<br>SR3<br>SR4 | Sediment accretion associated with urban pollution coupled with the loss of riparian habitats can affect aquatic habitats. Organisms which are permanently attached to the substrate (fixed) are likely to be more affected because they lack the ability to escape during adverse environmental conditions (Armitage et al., 1995). |
| Number of tracheae | 12 tracheae<br>6 tracheae<br>3 tracheae | RP1<br>RP2<br>RP3 | Urban pollution leads to low dissolved oxygen concentrations in freshwater. Dissolved oxygen is required for the performance of biological and ecological functions. Cuticular respiration depends on the uptake efficiency of dissolved oxygen and its distribution within the body of the organism (Armitage et al., 1995; Serra et al., 2017a). Thus, organisms possessing a higher number of tracheae are likely to be favoured in environments with low dissolved oxygen. |

|  |  |  |  |
|---|---|---|---|
| Mobility | Swimmer/ Sprawler<br>Crawler/Climber<br>Whole-body undulator | M1<br>M2<br>M3 | The type of mobility an organism possesses can determine whether a species can move into refugia during disturbance and re-establish when conditions become favourable (Townsend & Hildrew, 1994; Lamouroux et al., 2004). Chironomids which exhibit slow movement such as crawling/climbing may be vulnerable to urban pollution impact. |
| Feeding mode | Collector-filterer<br>Collector-gatherer<br>Shredder<br>Scraper/grazer<br>Predator-engulfer<br>Predator-piercer | FM1<br>FM2<br>FM3<br>FM4<br>FM5<br>FM6 | Elevated nutrient concentrations and sediment associated with organic pollution can alter the quality and quantity of food. Therefore, collectors (filterers and gatherers) and grazers are likely to be favoured by increased particulate organic matter and the formation of biofilms in highly disturbed sites. Predators are less likely to be favoured in such environments due to the requirement for low turbid water to acquire prey (Akamagwuna, 2021). |
| Maximum body size (mm) | < 2.5 (very small)<br>> 2.5 – 5 (small)<br>> 5 – 10 (medium) | BS1<br>BS2<br>BS3 | Urban pollution alters water quality conditions. Cuticular exposure to contaminants may predispose chironomids to severe impacts due to the influence of |

| | | | |
|---|---|---|---|
| | > 10 – 20 (large)<br>> 20 – 40 (very large) | BS4<br>BS5 | body surface area to volume ratio on contaminant uptake. Thus, individuals with reduced body surface area to volume ratio may increase in urban impacted sites (Paul & Meyer, 2001; Dolédec & Statzner, 2008). |
| Voltinism (number of generations per year) | 1<br>2<br>3<br>> 3 | V1<br>V2<br>V3<br>V4 | Voltinism determines the recovery rate of a species from adverse urban perturbations (Dolédec & Statzner, 2008). Disturbances in riverine environment may select for individuals with an increased number of reproductive cycles per year and therefore higher recovery rates (Groenendijk, 1999; Lamouroux et al., 2004; Serra et al., 2017b). |
| Lifecycle duration (year) | ≤ 1<br>> 1 | D1<br>D2 | Lifecycle duration is critical because it determines the exposure duration of a species to harsh environmental conditions. Species with early maturation are likely to be selected in urban impacted sites (Groenendijk, 1999; Serra et al., 2017b). |
| Reproduction type | Egg in clutches<br>Asexual reproduction<br>Free isolated eggs | RT1<br>RT2<br>RT3 | Urban pollution results in elevated pollutant concentrations which can modify gene expressions in chironomids and cause damage at the genetic level (Arambourou et |

|  |  |  |  |
|---|---|---|---|
|  |  |  | al., 2020). Chironomid species which lay sexually produced eggs in impacted water are vulnerable to urban pollution impact, whereas those which can reproduce asexually may be resilient to urban pollution. |
| Number of eggs per egg mass | < 100<br>100–500<br>500–1000<br>> 1000 | EM1<br>EM2<br>EM3<br>EM4 | With increasing urban perturbation, eggs of individuals may be vulnerable to a highly toxic environment occasioned by metals and elevated dissolved solids, with the threat of high egg mortality (Edegbene et al., 2021). Chironomids which produce more eggs per egg mass are more likely to be resilient in polluted environments. |
| Substrate preference | Stone, cobble, boulders<br>Gravel, sand, mud, silt<br>Macrophyte & filamentous algae<br>Microphytes (microscopic algae)<br>Twigs, roots<br>Fine organic matter<br>Wood | SP1<br>SP2<br>SP3<br>SP4<br>SP5<br>SP6<br>SP7<br>SP8 | Urban pollution can result in the homogenisation of aquatic habitats and negatively impact macroinvertebrate assemblage structure. The absence of stable substrates diminishes the availability of refugia (Serra et al., 2017b). Thus, chironomids which are unable to inhabit different substratum may be adversely affected. |

| | | | |
|---|---|---|---|
| | Moss | | |
| Body armour | Present<br>Absent | BA1<br>BA2 | Sediment and contaminants increase in freshwater systems due to urban pollution. Sediment can cause abrasion on body surface Chironomids with body armour are less exposed to contaminant uptake and abrasion than those without body armour (Collins & Fahrig, 2020). |
| Food type | Fine sediment<br>Debris < 1mm<br>Plant debris > 1 mm<br>Living microphytes<br>Living macrophytes<br>Dead animals<br>Living microinvertebrates<br>Living macroinvertebrates<br>Wood | FT1<br>FT2<br>FT3<br>FT4<br>FT5<br>FT6<br>FT7<br>FT8<br>FT9 | Urban-influenced sediment accretion can increase turbidity, which reduces the light penetration available for photosynthetic organisms to function. This may affect the trophic status and the organic content of streams. Impact on the riparian zones of streams may reduce the availability of allochthonous food sources (Edegbene et al., 2021). The burial of plant debris by sediment accretion may also reduce the coarse detrital materials available as food for organisms (Akamagwuna, 2018). Thus, only organisms which are generalist feeders may survive the impact of urban pollution on the availability of food materials. |

# CHAPTER 3 – DEVELOPING AND APPLYING A CHIRONOMID-BASED INDEX FOR ASSESSING WATER QUALITY IN SELECTED URBAN RIVER SYSTEMS, EASTERN CAPE, SOUTH AFRICA

## 3.1 Introduction

Multiple water-use demands are rising due to a growing human population (McGrane, 2016; Martins et al., 2020). Thus, access to good quality water for human and aquatic biota has become a global challenge and is especially crucial in developing African countries like South Africa. Similar to other countries in Africa and Asia, the water crisis in South Africa is mainly caused by deteriorating river health occasioned by poorly managed urban municipal facilities (e.g., wastewater treatment works), unstainable human exploitation of the environment, and run-off from informal settlements (Lemley et al., 2014; Agoro et al., 2020; Gqomfa et al., 2022). The declining water quality in rivers and streams portends a severe threat to aquatic taxa. Therefore, the complexity of impacts, mainly due to human activities such as urbanisation and pollution, on aquatic systems makes the development of advanced ecologically relevant tools a necessity for freshwater resource management (McGrane, 2016; Agboola, 2017; Martins et al., 2020; Ko et al., 2020).

Several tools have been developed to aid the assessing and monitoring of anthropogenic pressures on riverine systems. Some of these methods are based solely on the physical and chemical characteristics of water (e.g., Rahman et al., 2021), while others were designed to reflect the biological assemblages and attributes that constitute aquatic ecosystems (e.g., Obubu et al., 2021). In contrast to physicochemical assessment of stream water quality, biological assessment tools offer insights on the influence of abiotic factors on aquatic biodiversity and vice versa. Therefore, human disturbances on a river can be detected by assessing the assemblage structure and response of instream fauna present at different sites within a river.

Aquatic macroinvertebrates constitute a major source of food for many aquatic organisms (Rodríguez-Lozano et al., 2015). They are diverse and abundant in most streams and can integrate instream conditions (Masese et al., 2013). They exhibit a wide range of sensitivities to disturbances and respond differently to human pressures, and as such, they are seen as biological indicators of the environment in which they live (Masese et al., 2013; Akamagwuna, 2021). Thus, macroinvertebrates are extensively used in stream biomonitoring programmes worldwide (Edegbene et al., 2019; Odume, 2020; Beck et al., 2022).

In South Africa, the macroinvertebrate-based South African Scoring System version 5 (SASS5) is the most widely used water quality monitoring tool (Dickens & Graham, 2002; Gordon et al., 2015; Agboola, 2017). The SASS5 is a single biotic index based on the perceived sensitivities of aquatic macroinvertebrate taxa to water quality deterioration. The index is simple to use, and a water quality category can be determined based on its two component metrics, the SASS5 score and the average score per recorded taxon (ASPT) value (Dallas, 2007).

While the SASS 5 index is a rapid assessment tool, its family-level resolution masks the various species-level responses of macroinvertebrates to anthropogenic disturbances and may potentially limit its interpretative and discriminatory power. This is especially evident where macroinvertebrate families consist of various species with similar biological attributes but different adaptive features, ecological preferences and response patterns to perturbation. For example, species of the family Chironomidae are very diverse and inhabit an extensive ecological range (Rossaro et al., 2022). However, this family is awarded a very low sensitivity score in the SASS5 index (Dickens & Graham, 2002), indicating that all chironomids are tolerant to all manner of pollution, which does not reflect the functional diversity of the chironomids (Odume & Muller, 2011). This raises the question of whether allocation of sites to an ecological category (Section 1.3.2 of Chapter 1 gives a detailed description of water resources classification in South Africa) based on such coarse taxonomic resolution of the SASS5 is accurate enough to ensure that sites are not either under- or over-protected, which may have implications on the management and sustainable utilisation of aquatic resources.

Various studies (Lenat & Resh, 2001; Masese et al., 2023) have highlighted the need for a species-level bioassessment tool to address the problems potentially associated with under or over-protection of biodiversity, reflecting a more accurate health condition of the system. Applying macroinvertebrate species-level data in biomonitoring comes with certain challenges, such as selecting the group of species that would accurately reflect the extent of disturbance at minimal cost. In the Afrotropical region, one of the most critical challenges is the shortage of taxonomic expertise as well as sparse knowledge on the life-history of most macroinvertebrate species. For these reasons, biomonitoring studies in Afrotropical regions are mainly at the coarse family-level taxonomic resolution. The few studies which have attempted to undertake such studies at the generic or species level have focused mainly on taxa such as Ephemeroptera, Plecoptera and Trichoptera (EPT). When compared to the family Chironomidae, the EPT taxa occupy a more restricted ecological range and are less functionally diverse. Chironomids have been found to dominate highly polluted sites where the relative abundance and richness of other macroinvertebrate taxa were low (Gresens et al., 2007; Gouissi et al., 2019). In addition, chironomids have been employed in various biomonitoring programmes (Deliberalli et al., 2018; Ziajahromi et al., 2019; Muñiz-González et al., 2021) and have been used to elucidate the disparities between temporary and permanent Mediterranean streams (Serra et al., 2017a). Thus, the wide ecological range and the pollution sensitivity spectrum occupied by chironomids make them good candidate species for the development of bioassessment tools for water quality monitoring (Mezgebu et al., 2019).

The assemblage structure of chironomid species in response to urban river pollution was explored in this study. It is argued that applying a species-level chironomid-based biomonitoring tool may make it possible to elucidate the extent to which important ecosystem health and ecological information may be masked. For example, in the SASS5 protocol, the family Chironomidae is assigned a score of 2, implying a very high tolerance to water quality disturbances, despite studies elsewhere indicating that certain species within the family have a wide sensitivity/tolerance range. This raises the question of what might be lost by a family-level taxonomic resolution, both in terms of the ecology of the group and practical water resource management and biodiversity protection. To this end, a chironomid species-based multimetric index for assessing water quality deterioration was developed in this study and compared with the results of the SASS 5 index. Multimetric indices are considered more robust than single biotic indices because they are comprised of various metrics (indices) condensed to an index value to reflect community response to environmental perturbation (Agboola, 2017; Martins et al., 2020). They are perceived to be more reliable and robust as they incorporate information across the levels of organisation from individual to ecosystem level, and therefore provide a more relevant assessment of water quality impairment (Agboola, 2017; Obubu et al., 2021; Tampo et al., 2021). Macroinvertebrate multimetric indices based on family-level data have been found to perform better than single biotic indices (Jun et al., 2012; Odume, 2014). However, a species-based multimetric index may provide a better picture of biotic responses to riverine pollution.

This chapter addresses Objective one of this study, which is to develop, apply and compare a chironomid species-based tool to a family-level index for assessing water quality deterioration in selected urban rivers in the Eastern Cape, South Africa.

## 3.2 Methods

### 3.2.1 Measurement of water physicochemical variables and metals

Detailed descriptions of the study area, sites, sampling procedures and laboratory analyses are provided in Chapter 2. Samples were collected at eleven study sites spread across the Buffalo and Bloukrans rivers. Physicochemical variables, including dissolved oxygen (DO), canopy cover, stream width, depth, flow velocity, total suspended solids (TSS), electrical conductivity (EC), turbidity, temperature, pH, nitrate-nitrogen ($NO_3$-N), nitrite-nitrogen ($NO_2$-N), ammonium-nitrogen ($NH_4$-N) and orthophosphate-phosphorus ($PO_4$-P) were measured for each site per sampling occasion as described in Section 2.4.1 of Chapter 2. Selected metals, including aluminium (Al), copper (Cu), nickel (Ni), chromium (VI) ($Cr^{+6}$), zinc (Zn) and iron (Fe) were also analysed in water samples for each of the sampling sites, seasonally. Physicochemical variables collected for the historical Swartkops River data between 2009 and 2012 included dissolved oxygen (DO), electrical conductivity (EC), turbidity, temperature, pH, nitrate-nitrogen ($NO_3$-N), nitrite-nitrogen ($NO_2$-N), ammonium-nitrogen ($NH_4$-N) and orthophosphate-phosphorus ($PO_4$-P).

Physicochemical data for the Bloukrans and Buffalo rivers were pooled based on the site categories and presented as mean ± standard deviation. Physicochemical data based on the site categories for the historical Swartkops River data were presented as mean ± standard deviation. The Kruskal-Wallis multiple comparison test was used to test for significant differences ($p < 0.05$) in the medians of the measured water physicochemical variables between the site categories. The Kruskal-Wallis test was used because the physicochemical data violated the assumption of normality required for a parametric one-way ANOVA. The pairwise Conover-Iman post hoc test was used to indicate sites that differed when the Kruskal-Wallis test indicated a statistically significant difference. For other statistical analyses, physicochemical data from the different sites were combined as replicates based on the site classification categories used in this study (see section 2.3.1 of Chapter 2).

### 3.2.2 The South African Scoring System version 5 (SASS5)

Macroinvertebrate samples collected at the sampling sites were analysed on-site using the SASS5 protocol (Dickens & Graham, 2002). The SASS5 scores, average score per taxon (ASPT) value and the total number of taxa were used to assess the water quality of the sampling sites. The SASS5 score was calculated by summing the scores of all the recorded taxa, while the ASPT value was obtained by dividing the total SASS5 score by the number of taxa recorded per sampling event and site. The interpretation of the SASS5 scores and ASPT values was done following the guidelines set by Dallas (2007). The guidelines stipulate ranges of SASS5 scores and ASPT values for the different ecological categories, which are reflective of both the water quality conditions and inherent macroinvertebrate biota of rivers according to their ecoregions (Dallas, 2007). The Buffalo River sites are within the south-eastern uplands (lower zone) and the eastern coastal belt (lower zone) ecoregions, while the Bloukrans and Swartkops Rivers are within the southern folded mountains, and the southern eastern coastal belt (lower zone) ecoregions, respectively (Kleynhans et al., 2005). The ranges of SASS5 scores and ASPT values for these zones were used to interpret the SASS5 data in this study (Table 3.1). The SASS5 scores and ASPT values for macroinvertebrate taxa in the Swartkops River sites were collected between 2009 and 2012. The SASS5 scores, ASPT values, and number of macroinvertebrate taxa for all the study rivers were pooled based on the site categories and summarised using boxplots.

### 3.2.3 Spatial and temporal distribution patterns of chironomid assemblage structure

The spatial-temporal distribution of chironomids based on pooled data for the study site categories was assessed using the two-way analysis of similarity (ANOSIM), followed by a pairwise post hoc permutation test, with the contributions of each species to differences among the site groupings indicated by a Similarity percentage (SIMPER) analysis. The ANOSIM test was used to assess the differences in chironomid assemblages between the site categories and between the sampling seasons. Analysis of similarity (ANOSIM) is a multivariate non-parametric permutation procedure that detects differences between defined sample groups based on a rank similarity matrix (Clarke & Warwick, 1994; Clarke & Warwick, 2001).

Table 3.1 Biological Bands/Ecological categories for interpreting SASS5 data for the different ecoregions (Dallas, 2007)

| **Ecoregion** | **Ecological category** | **A** | **B** | **C** | **D** | **E/F** |
|---|---|---|---|---|---|---|
| | Ecological category name | Natural | Good | Fair | Poor | Seriously modified |
| | Description | Unmodified natural | Largely natural with few modifications | Moderately modified | Largely modified | Seriously modified |
| Southeastern uplands | SASS5 | >171 – 260 | >138 – 171 | >111 – 138 | >91 – 111 | 0 – 90 |
| | ASPT | >7.45 – 9 | >6.65 – 7.45 | > 6 – 6.65 | >5.25–6 | 0 – 5.25 |
| Eastern coastal belt | SASS5 | >94 – 140 | >69 – 94 | >50 – 69 | >40 – 50 | 0 – 40 |
| | ASPT | >7.6 – 9 | >6.21 – 7.6 | >5.4 – 6.21 | >4.15–5.4 | 0 – 4.15 |
| Southern folded mountains | SASS5 | >104 –180 | >71 – 104 | >53 – 71 | >42 – 53 | 0 – 42 |
| | ASPT | >5.8 – 8 | >4.8 – 5.8 | >4.5 – 4.8 | >4.2 – 4.5 | 0 – 4.2 |
| Southern Eastern coastal belt | SASS5 | >149 – 180 | >100 – 149 | >82 – 100 | >63 – 82 | 0 – 63 |
| | ASPT | >7.1 – 8 | >6.0 – 7.0 | >5.4 – 5.9 | >5.1 – 5.3 | 0 – 5.1 |

The similarity percentage (SIMPER) analysis indicates the percentage contribution by each species to similarity and dissimilarity between groups (Legendre & Legendre, 2012). The analysis of similarity (ANOSIM) and similarity percentage (SIMPER) were used to assess the spatial distribution of chironomids and to compare the similarities between chironomid assemblages between the site categories. The ANOSIM test was also used to assess the differences in chironomid assemblages between the four sampling seasons, with the contributions of each species to differences between the seasons indicated by a similarity percentage (SIMPER) analysis. The Global R-value produced by the ANOSIM test indicates the degree to which the samples are similar or dissimilar. An R-value of 1 indicates a complete separation of groups, whereas an R-value near 0 implies little or no segregation. The similarity percentage is a factor of species abundance, i.e., the more abundant a species is, the more it contributes to the dissimilarity between groups (Clarke & Warwick, 2001). Both the ANOSIM and the SIMPER were undertaken using the Bray-Curtis distance coefficient. The R statistical package (R Core Team, 2022) was used to compute the ANOSIM and SIMPER analyses, while the post hoc permutation test was performed using the Paleontological Statistics software package (PAST version 4.03).

### 3.2.4 Developing a multimetric index for assessing pollution in urban rivers

The chironomid multimetric index (CUMMI) for monitoring pollution in urban rivers was developed based on chironomid data collected from the Buffalo and Bloukrans rivers and applied to the historical chironomid data from the Swartkops River. Chironomid data from the Buffalo and Bloukrans rivers was divided into two groups for index development and testing. Data collected during spring and summer were used to develop the index, while the autumn and winter data were used to validate the index. Two least-impacted REF sites (Site 1 and Site 11; see Section 2.3 of Chapter 2 for details on site descriptions) were selected for developing the multimetric index while the last REF site in the Buffalo River (Site 2) was included in the validation of the index. Although two least-impacted sites may be considered insufficient for the development of reference conditions for a multimetric index, the pseudo-replicate samples collected over four seasons enabled a robust statistical analysis. Moreover, (Ruaro et al., 2024) noted that the criteria upon which reference sites are selected, rather than the number of reference sites used, affected the responsiveness of multimetric indices to anthropogenic disturbance. Seventy-seven (77) candidate metrics (Appendix A, Table 1) were selected for testing based on the data collected and the existing literature on chironomid ecology (Reynolds & Ferrington, 2002; Milošević et al., 2018a, 2018b; Vega et al., 2021). The metrics were selected to reflect the abundance, richness, composition, tolerance and diversity of chironomid species in the study rivers. Richness metrics were calculated as the total number of species and genera in the Chironomidae family, whereas diversity measures (such as the Shannon-Weiner diversity, Menhinick’s diversity and Simpson diversity) were defined following Clarke and Warwick (2001) as the relationship between species richness and the composition of taxa. Abundance measures were determined as the absolute number of sampled taxa within a sample (Edegbene et al., 2022), and composition measures were calculated as relative abundances of chironomid species (Appendix A, Table 1). Chironomid pollution sensitivity/tolerance measures were extracted from the relevant literature (Al-Shami et al., 2010; Odume et al., 2016; Miliša et al., 2022).

A four-step procedure was followed to determine the suitability of each metric for inclusion in the multimetric index by testing their (i) discriminatory potential, that is, their potential to differentiate impacted sites from least impacted sites, (ii) seasonal stability, to check whether or not they significantly changed seasonally, (iii) repeatability, to check whether the metrics were affected by sampling variation or by water quality conditions, and (iv) redundancy, in order to eliminate metrics that provided similar information about chironomid assemblage structure. Only metrics which passed all the tests were standardised and integrated into the chironomid multimetric index for urban rivers (CUMMI). Metrics were calculated and captured in Microsoft Excel, 2019 and imported into R version 4.2.0 (R Core Team, 2022), which was used to perform the tests.

### ***Metric discriminatory test***

Metrics were categorised into two groups (sensitive and insensitive metrics), based on their potential to discriminate between least impacted sites and highly impacted sites (Baptista et al., 2007). Metric sensitivity was visualised using boxplots and deemed satisfactory based on the degree of overlap between the interquartile ranges (IQRs) of the plots. The criteria set for selecting metrics as sensitive were: (i) there is no overlap between the IQRs of the least impacted sites and the highly impacted sites; and (ii) where there is some overlap of the IQRs, one of the medians do not overlap.

Screened metrics were tested for normality using the Shapiro-Wilks normality test. As metrics were non-normally distributed, the Kruskal-Wallis non-parametric test was used to confirm the sensitivity of metrics. Thus, only metrics that were statistically significant ($p < 0.05$) in discriminating sites were selected for subsequent testing. Boxplots were produced using the ggplot2 package within the R environment (Wickham, 2016; R Core Team, 2022). No further testing was conducted for metrics that were deemed insensitive ($p > 0.05$).

### ***Metric seasonality test***

Metrics that passed the sensitivity test were evaluated for seasonal stability. Moya et al. (2011) and Chen et al. (2019) documented the importance of adjusting metrics for natural variation to account for the natural response of biological metrics to environmental gradients. Thus, only REF sites were used for this test to avoid the effects of confounding factors of urban pollution on the test result (Odume et al., 2012; Edegbene et al., 2022). Boxplots were used to visualise the seasonal variation of metrics for the sampling seasons (spring, summer, autumn, and winter). Kruskal-Wallis test was used to test the significance of variation at $p < 0.05$. Only metrics that were not significantly different between the four seasons were considered seasonally stable and retained for further analysis.

### *Metric repeatability test*

Metrics repeatability was calculated following Stoddard et al. (2008). Metrics were tested based on the ratio of variances between metrics of the entire data set (signal, S) and a subset (noise, N). Briefly, the variance of the retained metrics for all the study sites (S) was divided by the variance of metric values for the least impacted sites (N). Metrics with high signal-to-noise ratios ($S{:}N \geq 2$) were deemed relatively repeatable, and those with low signal-to-noise ratios were considered to be less precise (Stoddard et al., 2008; Edegbene et al., 2022).

### *Metric redundancy test*

Retained metrics were tested for multi-linearity using Spearman's rank correlation test. Metrics with high correlation at $r > |0.75|$ AND $p < 0.05$ were considered redundant. Redundant metrics were further assessed for ecological relevance based on the existing literature (Milošević et al., 2018b), and the most ecologically relevant metric of the highly correlated pair of metrics was retained for the development of the index (Odume et al., 2012; Akamagwuna, 2021).

### *Metric scoring and integration into the multimetric index*

The numerical values of retained metrics were standardised to place the metrics on the same measurement and scoring scale (Cuffney et al., 2013). Standardisation of metrics was done using the minimum value, lower quartile (25%), mid-quartile (50%), upper quartile (75%) and maximum value of each metric data set, following Baptista et al. (2007). Minimum values, lower, mid, and upper quartile values, and the maximum values for the least impacted sites (REF) were extracted from the data matrix in R (R Core Team, 2022).

Depending on the predicted response of metrics to increasing urban pollution (Appendix A, Table 1) and the numeric value of the metrics for the highly impacted sites, metric values were assigned scores of either 5, 3 or 1, representing low, moderate or high deviation from the values of the least impacted sites. Regarding metrics that increased with increasing pollution, a score of 5 was assigned for metrics expected to increase in numeric value if the numeric value at the highly impacted sites (sites receiving diffuse and point source pollution) was lower than the upper quartile (75%) of the least impacted sites. If the numeric value was between the upper quartile and the maximum value of the least impacted sites, a score of 3 was assigned, and a score of 1 was assigned if it was greater than the maximum value of the least impacted sites.

For metrics predicted to decrease with increasing pollution (Appendix A, Table 1), if the numeric value of a metric at the highly impacted site was greater than the lower quartile (25%) of that for the least impacted sites (REF), it was scored 5, and if it was between the minimum and the lower quartile of the metric for the least impacted sites, it was scored 3. A score of 1 was awarded if the value was less than the minimum value for the least impacted sites for that metric (Baptista et al., 2007; Odume, 2014). Although an alternative method of scoring was developed by Stoddard et al. (2008), the method by Baptista et al. (2007) was utilised in this study because categorical thresholds based on the quartiles of the metric values represented the response of each metric to urban degradation. This scoring method by Baptista et al. (2007) avoids the subjective approach in the original index of biotic integrity developed by Karr (1981). The scoring method also provides an easy interpretation of the index scores without the need for rescaling to a continuous scale as performed by Stoddard et al. (2008).

The final CUMMI score was computed following the method described in Baptista et al. (2007) by summing the scores of the integrated metrics and dividing the value range of the index into five ecological categories A, B, C, D, and E/F indicative of natural, good, fair, poor and very poor water quality, respectively. The rationale for sectioning the value range of the CUMMI scores into five categories was to enable the comparison of the CUMMI index scores and the SASS5 and ASPT values on a similar scale.

### *Correlating metrics with environmental variables and metrics validation*

A multivariate redundancy analysis (RDA) was used to examine the influence of environmental variables on the individual metrics integrated into the CUMMI. Metric values were Hellinger transformed prior to the ordination. Environmental variables that were highly multi-collinear, as indicated by the variance inflation factor, were not included in the RDA model. A Monte Carlo permutation test of global significance with 999 permutations was used to ascertain the level of significant differences ($p < 0.05$) between the first two RDA axes. The RDA and Monte Carlo tests were performed using the vegan package in R statistical software (R Core Team, 2022).

The developed CUMMI index was validated using the data from the Buffalo and Bloukrans River sites collected in autumn and winter. The index performance was evaluated by computing the degree of correspondence between the CUMMI index result and the SASS 5 and ASPT classifications. For example, congruence between the CUMMI and SASS 5 was determined by evaluating the percentage of sites classified into the same ecological categories as the SASS 5 scores and ASPT values. The responsiveness of the developed CUMMI index was assessed by comparing the final CUMMI scores for the different site categories using the Kruskal-Wallis non-parametric test followed by a Conover post hoc test with Holm correction (Martins et al., 2020). Prior to the responsiveness test, CUMMI scores were assessed for normality using the Shapiro-Wilks test. Although a $p$-value of 0.05 may be considered quite restrictive as a cut-point for biological data (Wasserstein & Lazar, 2016), it was the ideal cut-point for the data collected in this study after statistical hypothesis testing at different $p$-values of 0.01, 0.05 and 0.10. The significance level of 0.05 was set for all the hypothesis testing in this study following Kwak (2023) and Greenland et al. (2016).

## 3.3 Results

### 3.3.1 Physicochemical variables and selected metals

The means and standard deviations of the measured physicochemical water quality variables taken at the site categories for the Bloukrans and Buffalo rivers during the four sampling seasons are presented in Table 3.2. Means and standard deviations of physicochemical variables from the historical Swartkops River data are presented in Table 3.3.

The mean values for dissolved oxygen (DO) were highest at the least impacted sites (REF) of the Bloukrans and Buffalo rivers and lowest at the PP sites just immediately downstream of the Wastewater Treatment Works (WWTWs) effluent discharge points of the Bloukrans and Buffalo rivers (Table 3.2). The Kruskal-Wallis test indicated a statistically significant difference in DO concentration between the site categories (REF, DP, PP, AG and RECV). However, the pairwise comparison using the Conover-Iman post-hoc test revealed that DO did not differ significantly between the REF and RECV sites, indicating that system recovery was taking place in the Bloukrans and Buffalo rivers (Table 3.2). By contrast, no significant difference was observed for the DO concentrations between the REF and the DP sites of the Swartkops River (Table 3.3). Temperature and pH did not significantly vary between the site categories in any of the study rivers (Table 3.2 and 3.3). Depth and width showed no significant variation between the site categories in the Buffalo and Bloukrans rivers (Table 3.2), while EC was not significantly different between the site categories in the Swartkops River (Table 3.3).

Table 3.2 Mean ± standard deviation for the physicochemical variables in the different site categories of the Bloukrans and the Buffalo River. P-values are indicated by the Kruskal-Wallis test. Different superscripts indicate significant differences ($p < 0.05$) revealed by the Conover-Iman post-hoc test per variable per the different groups of sites. The same superscript between the groups per variable indicates no significant difference.

| Variable | REF<br>*(n = 12)* | DP<br>*(n = 8)* | PP<br>*(n = 8)* | AG<br>*(n = 8)* | RECV<br>*(n = 8)* | *p*-value |
|---|---|---|---|---|---|---|
| Dissolved oxygen (mg/L) | $8.62 \pm 1.07^{a}$ | $6.81 \pm 1.74^{b}$ | $3.87 \pm 2.27^{c}$ | $4.23 \pm 2.66^{c}$ | $8.35 \pm 1.21^{ab}$ | 0.000 |
| Temperature (°C) | 19.7 ± 4.87 | 19.6 ± 5.33 | 20.3 ± 4.66 | 20.1 ± 4.95 | 20.8 ± 5.40 | |
| pH | 7.21 ± 0.62 | 7.03 ± 0.77 | 6.72 ± 0.57 | 7.00 ± 0.45 | 6.79 ± 0.43 | |
| Electrical conductivity (µS/cm) | $216.82 \pm 153.04^{a}$ | $4362.00 \pm 6300.70^{b}$ | $4166.25 \pm 6015.33^{b}$ | $4683.5 \pm 6460.26^{b}$ | $2885.5 \pm 5610.08^{b}$ | 0.000 |
| Width (m) | 6.90 ± 5.43 | 10.67 ± 9.07 | 9.09 ± 6.50 | 8.38 ± 3.58 | 23.17 ± 18.53 | |
| Depth (cm) | 17.31 ± 9.52 | 17.89 ± 9.16 | 19.55 ± 8.94 | 15.29 ± 7.57 | 22.02 ± 10.14 | |
| Flow velocity (m/s) | $0.23 \pm 0.12^{a}$ | $0.48 \pm 0.16^{bd}$ | $0.68 \pm 0.26^{b}$ | $0.48 \pm 0.21^{bc}$ | $0.35 \pm 0.20^{acd}$ | 0.001 |

| | | | | | | |
|---|---|---|---|---|---|---|
| Turbidity (NTU) | 9.99 ± 9.60$^{a}$ | 21.85 ± 10.49$^{b}$ | 22.58 ± 7.15$^{b}$ | 22.38 ± 18.53$^{ab}$ | 14.34 ± 13.19$^{ab}$ | 0.033 |
| Total suspended solids (TSS) (g) | 10.47 ± 12.90$^{a}$ | 34.06 ± 31.13$^{b}$ | 24.56 ± 15.81$^{ab}$ | 43.54 ± 56.81$^{ab}$ | 22.54 ± 22.81$^{ab}$ | 0.047 |
| Nitrate ($NO_3$-N) (mg/L) | 3.49 ± 5.63$^{a}$ | 11.94 ± 9.34$^{ab}$ | 20.68 ± 36.07$^{ab}$ | 60.49 ± 68.02$^{b}$ | 26.93 ± 39.01$^{ab}$ | 0.007 |
| Nitrite ($NO_2$-N) (mg/L) | 0.03 ± 0.04$^{a}$ | 0.88 ± 1.18$^{b}$ | 0.89 ± 1.64$^{b}$ | 2.20 ± 1.99$^{b}$ | 0.30 ± 0.37$^{b}$ | 0.000 |
| Ammonia ($NH_4$-N) (mg/L) | 0.00 ± 0.00$^{a}$ | 0.02 ± 0.03$^{b}$ | 0.03 ± 0.03$^{b}$ | 0.04 ± 0.04$^{b}$ | 0.00 ± 0.00$^{ac}$ | 0.000 |
| Orthophosphate ($PO_4$-P) (mg/L) | 0.61 ± 0.97$^{a}$ | 6.09 ± 6.36$^{b}$ | 9.23 ± 8.49$^{b}$ | 7.54 ± 7.06$^{b}$ | 2.96 ± 3.43$^{ab}$ | 0.002 |
| %Canopy cover | 55.62 ± 13.48$^{a}$ | 0.00 ± 0.00$^{b}$ | 36.83 ± 6.19$^{c}$ | 44.51 ± 34.66$^{ac}$ | 23.23 ± 25.74$^{bc}$ | 0.000 |

The mean values of flow velocity in the Bloukrans and Buffalo rivers were higher at the highly impacted site categories (DP, PP, and AG) compared to the least impacted (REF) and recovery sites (RECV) (Table 3.2). Electrical conductivity (EC) in the Bloukrans and Buffalo rivers was significantly lower in the REF sites compared to the other site categories (Table 3.2). Although mean EC values were higher in the highly impacted sites of the Swartkops River, no significant difference was indicated between the site categories by the Kruskal-Wallis test (Table 3.3). Turbidity significantly differed between the least impacted and highly impacted sites but not between the least impacted (REF) and recovery (RECV) sites in the Buffalo and Bloukrans River areas (Table 3.2). In the Swartkops River, turbidity was significantly higher in the PP site immediately downstream of the Kelvin-Jones WWTW effluent discharge point but was not significantly different between the site categories receiving diffuse pollution (DP) and the least impacted site (REF) (Table 3.3). The concentration of nutrients ($NO_3$-N, $NO_2$-N, $NH_4$-N and $PO_4$-P) differed significantly between the site categories for all the study rivers (Table 3.2–3).

Seasonally, the dissolved oxygen concentration, electrical conductivity, turbidity, canopy cover, flow velocity, total suspended solids, pH, nitrate-nitrogen, nitrite-nitrogen, ammonium-nitrogen and orthophosphate-phosphorus did not differ significantly between the sampled seasons for each of the site categories ($p > 0.05$). Water temperature differed significantly between the seasons for only the REF sites (KW-H = 10.9186, $p = 0.01$) and the AG sites (KW-H = 8.2725, $p = 0.04$).

Table 3.3 Mean ± standard deviation for the physicochemical variables in the different site categories of the historical Swartkops River data. P-values are indicated by the Kruskal-Wallis test. Different superscripts indicate significant differences ($p < 0.05$) revealed by the Conover-Iman post-hoc test per variable per the different groups of sites. The same superscript between the groups per variable indicates no significant difference.

| Variable | REF<br>*(n = 4)* | DP<br>*(n = 4)* | PP<br>*(n = 4)* | AG<br>*(n = 4)* | *p*-value |
|---|---|---|---|---|---|
| Dissolved oxygen (mg/L) | $6.07 \pm 1.13^{a}$ | $6.99 \pm 1.84^{a}$ | $3.26 \pm 0.42^{b}$ | $2.17 \pm 1.30^{b}$ | 0.008 |
| Temperature (°C) | 17.88 ± 4.44 | 17.71 ± 7.72 | 20.13 ± 4.49 | 18.55 ± 5.43 | |
| pH | 6.61 ± 1.17 | 7.12 ± 1.08 | 7.36 ± 0.42 | 7.27 ± 0.56 | |
| Electrical conductivity (µS/cm) | 325.75 ± 46.94 | 1442.50 ± 2106.41 | 2127.75 ± 799.49 | 2097.75 ± 1279.95 | |
| Turbidity (NTU) | $6.15 \pm 2.78^{a}$ | $6.15 \pm 1.47^{a}$ | $115.63 \pm 139.21^{c}$ | $9.35 \pm 11.22^{a}$ | 0.048 |
| Nitrate ($NO_3$-N) (mg/L) | $0.04 \pm 0.06^{a}$ | $0.92 \pm 1.36^{ab}$ | $1.45 \pm 0.71^{b}$ | $2.20 \pm 2.29^{b}$ | 0.031 |

| | | | | | |
|---|---|---|---|---|---|
| Nitrite ($NO_2$-N) (mg/L) | 0.01 ± $0.01^a$ | 0.07 ± $0.09^{ab}$ | 0.21 ± $0.12^b$ | 0.17 ± $0.18^b$ | 0.028 |
| Ammonia ($NH_4$-N) (mg/L) | 0.12 ± $0.16^a$ | 0.57 ± $0.81^a$ | 5.54 ± $2.24^c$ | 3.97 ± $1.99^c$ | 0.012 |
| Orthophosphate ($PO_4$-P) (mg/L) | 0.00 ± $0.91^a$ | 1.11 ± $0.56^b$ | 7.46 ± $4.05^{bc}$ | 7.58 ± $1.35^c$ | 0.005 |

Generally, the concentration of metals in water sampled from the Bloukrans and Buffalo River site categories was low and not significantly different, except for chromium (VI) ($Cr^{+6}$) (Table 3.4). However, nickel (Ni) and aluminium (Al) were significantly different across the sampling seasons, as indicated by the Kruskal-Wallis test (KW-H = 9.2183, $p = 0.02652$ and KW-H = 12.357, $p = 0.006256$ respectively). The mean concentration of all the metals were relatively higher in the highly impacted sites (DP, PP, and AG) than in the least impacted and recovery sites (Table 3.4). Except for the mean concentration of Ni at the PP sites, the mean values of iron (Fe) across the highly impacted site categories (DP, PP, and AG) were relatively higher than the mean concentration of other metals for the same sites (Table 3.4). Amongst the analysed metals, only the concentrations of aluminium differed significantly between the sampling seasons and at the REF sites (KW-H = 8.3199, $p = 0.04$).

Table 3.4 Mean ± standard deviation of the analysed metals in the groups of sites in the Bloukrans and Buffalo Rivers. P-values are indicated by the Kruskal-Wallis test. Different superscripts indicate significant differences ($p < 0.05$) revealed by the Conover-Iman post-hoc test per variable per the different groups of sites. The same superscript between the groups per variable indicates no significant difference.

| Metal | REF *(n = 12)* | DP *(n = 8)* | PP *(n = 8)* | AG *(n = 8)* | RECV *(n = 8)* | *p*-value |
|---|---|---|---|---|---|---|
| Aluminium (Al) (mg/L) | 0.01 ± 0.02 | 0.05 ± 0.07 | 0.03 ± 0.04 | 0.02 ± 0.03 | 0.01 ± 0.03 | |

| | | | | | | |
|---|---|---|---|---|---|---|
| Copper (Cu) (mg/L) | 0.04 ± 0.05 | 0.20 ± 0.37 | 0.15 ± 0.20 | 0.14 ± 0.22 | 0.06 ± 0.11 | |
| Zinc (Zn) (mg/L) | 0.00 ± 0.00 | 0.02 ± 0.04 | 0.14 ± 0.28 | 0.12 ± 0.35 | 0.01 ± 0.02 | |
| Chromium ($Cr^{+6}$) (Cr) (mg/L) | $0.08 \pm 0.05^{a}$ | $0.15 \pm 0.07^{b}$ | $0.17 \pm 0.09^{b}$ | $0.16 \pm 0.09^{b}$ | $0.11 \pm 0.05^{ab}$ | 0.032 |
| Iron (Fe) (mg/L) | 0.16 ± 0.23 | 0.26 ± 0.30 | 0.28 ± 0.27 | 0.20 ± 0.18 | 0.11 ± 0.22 | |
| Ni (mg/L) | 0.10 ± 0.14 | 0.19 ± 0.38 | 0.28 ± 0.38 | 0.13 ± 0.14 | 0.13 ± 0.16 | |

### 3.3.2 The South African Scoring System version 5 (SASS5)

The SASS5 scores showed marked differences in the water quality conditions between the site categories (Figure 3.1). The SASS5 scores for the least impacted sites (REF) were consistently higher than those at the highly impacted sites during the sampling seasons. The ASPT values exhibited a similar trend to the SASS5 scores (Figure 3.2). The ASPT values indicated that the water quality conditions at the recovery sites were similar to the water quality conditions at the least impacted sites (REF) and sometimes even better. The number of taxa recorded were consistently higher at the REF sites than at the DP, PP and AG sites (Figure 3.3). Generally, the medians of the SASS5 scores, ASPT values and the number of recorded taxa were lower in the highly impacted sites (DP, PP, and AG) than in the least impacted and recovery sites (Figure 3.1–3). According to the SASS5 results, the composition of macroinvertebrate families varied more markedly between the site categories than between the seasons. Macroinvertebrate taxa which characterised the REF and RECV sites included Baetidae, Leptophlebiidae, Coenagrionidae, Corixidae, Pisulidae, Caenidae, Hydropsychidae and Aeshnidae. Taxa which characterised the highly impacted sites were Oligochaeta, Hirudinea, Culicidae, Chironomidae, Muscidae, Psychodidae and Simulidae.

Based on the SASS5 scores, 50% of the time, the REF sites of the Bloukrans and Buffalo rivers were classified in the E/F water quality category, indicative of severely modified water quality condition at the sites (Figure 3.4). Similarly, the ASPT values classified the REF sites of the Bloukrans and Buffalo rivers in the E/F water quality category more than 30% of the time (Figure 3.4). Moreover, both the SASS5 scores and ASPT values classified the DP and PP sites in the E/F category for more than 70% and 80% of the time, respectively (Figure 3.4). Similar to the PP sites, the water quality at the AG sites was critically impaired, as indicated by the SASS5 scores and the ASPT values (Figure 3.4). The SASS5 scores classified the RECV sites as having pristine water quality (category A) and slightly modified (Category B) 10% of the time, whereas the ASPT values classified the sites as moderately modified (category C) and heavily modified (Category D), 37% of the time (Figure 3.4).

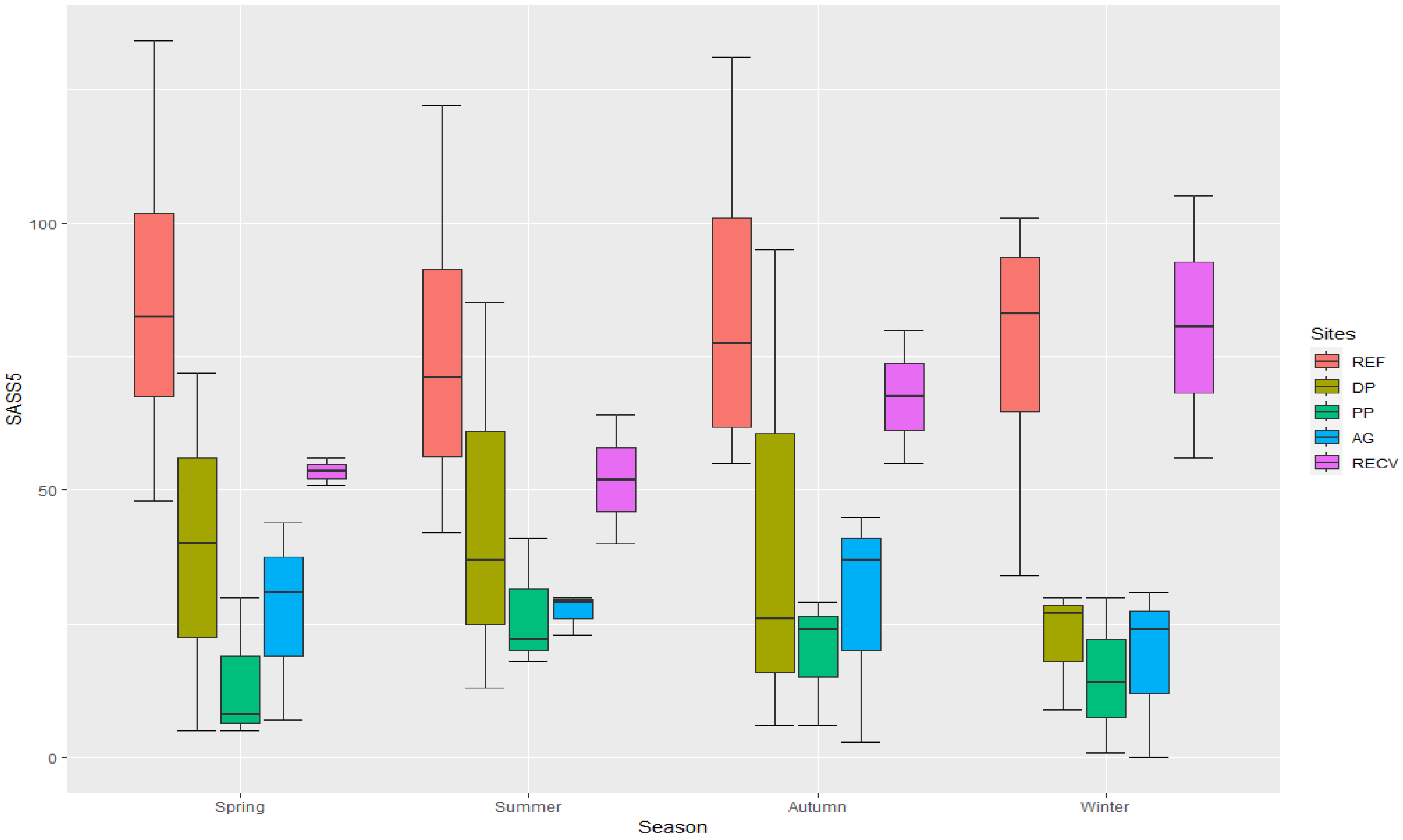


Figure 3.1 Boxplots of the pooled SASS5 scores for the site categories in all the study rivers

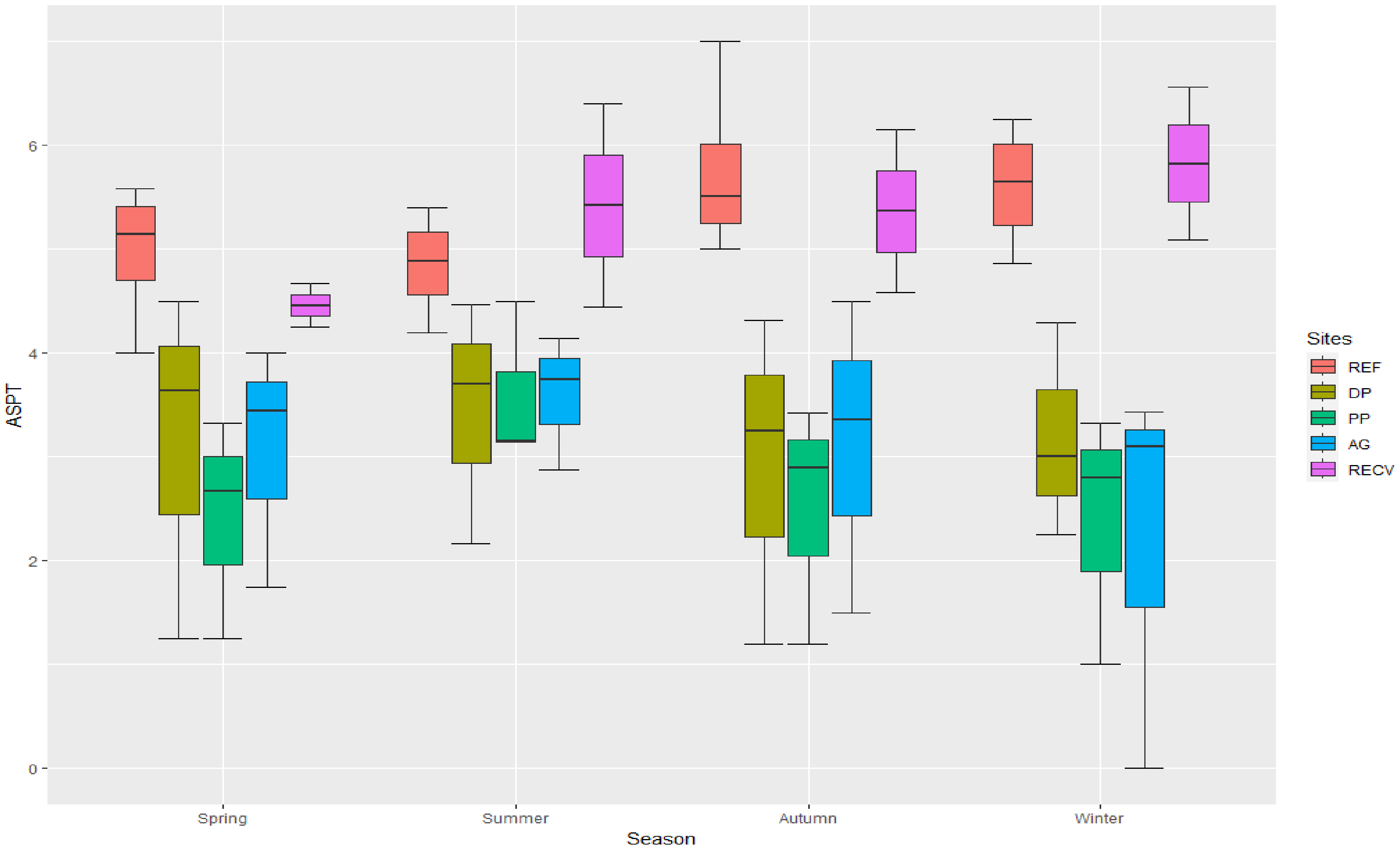


Figure 3.2 Boxplots of the pooled average score per recorded taxon (ASPT) for the site categories in all the study rivers

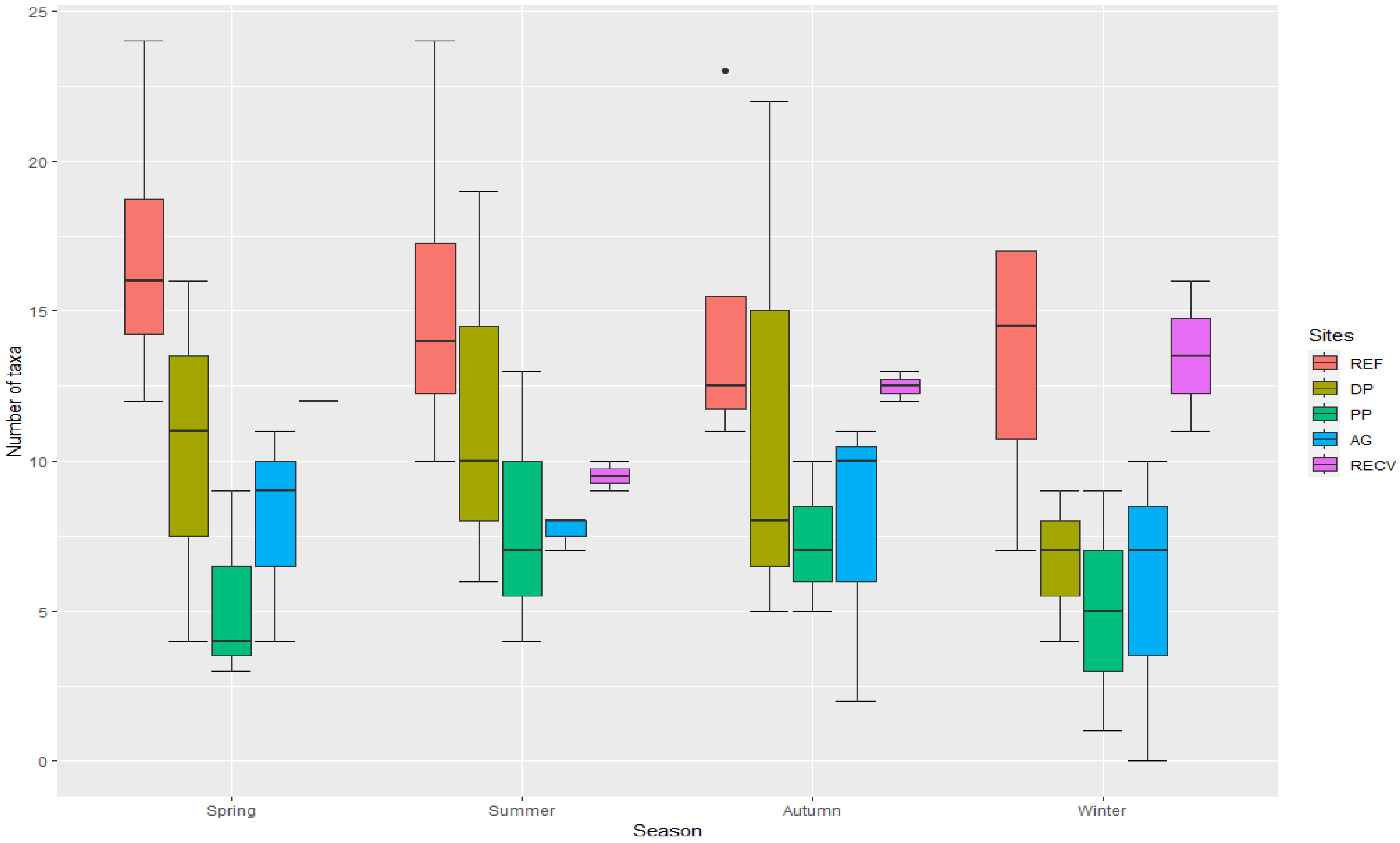


Figure 3.3 Boxplots of the pooled number of taxa recorded for the site categories in all the study rivers

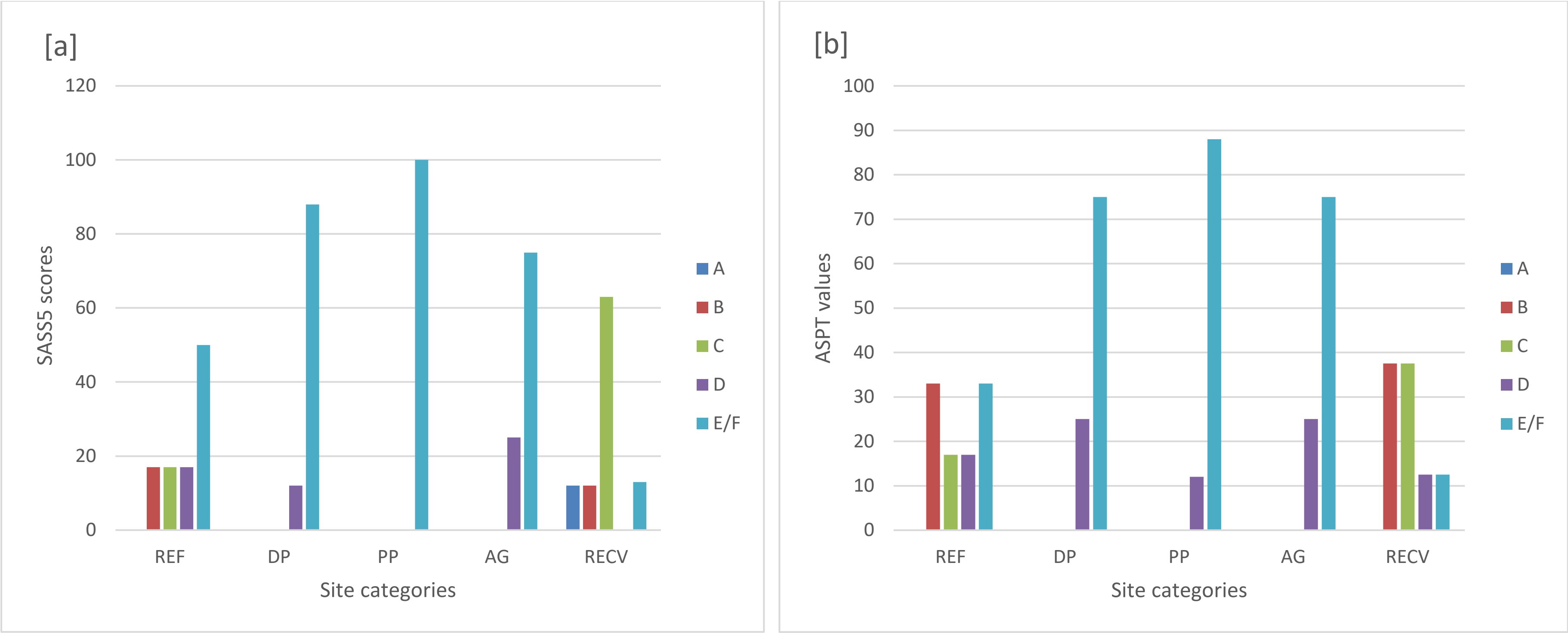


Figure 3.4 Percent number of sample data points per site category classified in each water quality category by the [a] SASS5 scores and [b] ASPT values for pooled data collected from the Bloukrans and Buffalo rivers. The water quality categories represent A- near natural or pristine, B-slightly modified, C-moderately modified, D- heavily modified, E/F- Critically/severely modified water quality.

Overall, the SASS5 score and ASPT values were more accurate than the physicochemical analysis in detecting water quality deterioration at the highly impacted sites, but fared worse in terms of accurately reflecting the ecological condition at the REF and RECV sites of the Bloukrans and Buffalo rivers (Table 3.2).

### 3.3.3 Spatial and temporal distribution patterns of chironomid assemblage structure

A total of one hundred and four chironomid (104) species belonging to three Chironomidae subfamilies, Tanypodinae, Orthocladiinae and Chironominae, were recorded during the study period (Appendix A, Table 2). Because taxa richness is a function of sampling effort and number of individuals collected, the number of individuals collected and chironomid species richness information for each site is provided in Table 3.5. The relative abundances of chironomid species at the REF sites were dominated mainly by *Cricotopus trifasciata* gr., *Cricotopus* sp.1, *Tanytarsus* sp., *Polypedilum tuberculum*-type and *Rheotanytarsus curtistylus*-type across the sampling seasons (Appendix A, Table 3). Although all the subfamilies recorded in this study were represented throughout the study, species of the tribe Chironomini of the subfamily Chironominae dominated the chironomid assemblage at the REF sites during spring, summer, and autumn. In winter, the chironomid assemblage at the REF sites was dominated by species of the subfamily Orthocladiinae. The species richness of chironomids at the REF sites was highest in spring compared to the other seasons.

Table 3.5 Number of chironomid individuals per sample and species richness per site per season

| Site | Metric | Spring | Summer | Autumn | Winter | category |
|---|---|---|---|---|---|---|
| Site 1 | Number of individuals | 68 | 49 | 20 | 53 | REF |
| | Species richness | 12 | 13 | 10 | 16 | |
| Site 2 | Number of individuals | 44 | 29 | 31 | 7 | REF |
| | Species richness | 14 | 5 | 13 | 2 | |
| Site 3 | Number of individuals | 7004 | 586 | 209 | 310 | DP |
| | Species richness | 3 | 12 | 6 | 7 | |
| Site 4 | Number of individuals | 10690 | 422 | 361 | 156 | PP |
| | Species richness | 4 | 7 | 9 | 13 | |
| Site 5 | Number of individuals | 832 | 801 | 116 | 292 | AG |
| | Species richness | 4 | 10 | 5 | 5 | |

| | | | | | | |
|---|---|---|---|---|---|---|
| Site 6 | Number of individuals | 102 | 107 | 48 | 38 | RECV |
| | Species richness | 11 | 13 | 11 | 11 | |
| Site 7 | Number of individuals | 5 | 8352 | 880 | 334 | DP |
| | Species richness | 2 | 2 | 2 | 1 | |
| Site 8 | Number of individuals | 1 | 3887 | 9 | 0 | PP |
| | Species richness | 1 | 2 | 1 | 0 | |
| Site 9 | Number of individuals | 1530 | 1125 | 3 | 0 | AG |
| | Species richness | 4 | 12 | 2 | 0 | |
| Site 10 | Number of individuals | 49 | 49 | 37 | 120 | RECV |
| | Species richness | 8 | 5 | 13 | 8 | |
| Site 11 | Number of individuals | 47 | 182 | 97 | 31 | REF |
| | Species richness | 14 | 11 | 8 | 8 | |
| Site 12 | Number of individuals | 319 | 149 | 137 | 244 | REF |
| | Species richness | 18 | 9 | 9 | 10 | |
| Site 13 | Number of individuals | 329 | 714 | 284 | 28 | DP |
| | Species richness | 10 | 11 | 7 | 2 | |
| Site 14 | Number of individuals | 537 | 637 | 472 | 518 | PP |
| | Species richness | 3 | 4 | 4 | 4 | |
| Site 15 | Number of individuals | 45 | 139 | 24 | 445 | AG |
| | Species richness | 9 | 5 | 2 | 5 | |

The relative abundance of chironomid species at the DP sites was dominated by *Chironomus* sp.1, *Chironomus* sp.2, *Tanypus* sp., *Dicrotendipes* sp., *Rheotanytarsus curtistylus*-type and *Polypedilum* spp. (Appendix A, Table 3). Species belonging to the subfamilies Orthocladiinae and Chironominae were predominant at the DP sites. Of the species belonging to the subfamily Chironominae, the species of the tribe Chironomini were more dominant than species of the tribe Tanytarsini in all the sampling seasons. The species of the subfamily Tanypodinae were not recorded in spring and winter.

In contrast to REF and DP sites, species of the subfamily Tanypodinae were not recorded at the PP sites in any of the sampling seasons. The relative abundance of chironomid species at the PP sites was dominated by *Chironomus* spp., *Dicrotendipes* sp., *Cricotopus intersectus*-

type and *R. curtistylus*-type (Appendix A, Table 3). *Chironomus* spp. contributed over 80% of species relative abundance at the PP sites across the sampling seasons. Only two species, *Cladotanytarsus sp* and *R. curtistylus*-type, of the tribe Tanytarsini were recorded at the PP sites.

At the AG sites, the relative abundance of chironomid species was dominated by *Chironomus* sp.1, *Chironomus* sp.2, *Corynoneura lobata*, *R. curtistylus*, *Rheocricotopus* sp., *Orthocladius clarki*-type, *Dicrotendipes lobiger*-type, *Tvetenia* spp and *Polypedilum* spp. *Chironomus* spp. contributed over 50% of the relative abundance of chironomid species at the AG sites. Generally, species of the subfamily Orthocladiinae and the tribe Chironomini dominated the chironomid species assemblage at the AG sites. The only species representing the subfamily Tanypodinae at the AG sites was recorded in summer. The species richness of chironomids recorded at the AG sites (37 species) was higher than that of the DP and the PP sites, 34 and 25 species, respectively (Figure 3.5). Compared to the other downstream sites (DP, PP, and AG), the RECV sites had the highest species richness (42 species). The relative abundance of species at the RECV sites was dominated by *Polypedilum* spp., *Eukiefferiella gracei*-type, *R. curtistylus*-type and *Tanytarsus usmaensis*-type. The highest number of species in the tribe Chironomini was recorded in autumn, while the highest number of species in the subfamily Tanypodinae was recorded in spring. Similar to the other sites in this study, the species of the subfamilies Chironominae and Orthocladiinae dominated the chironomid species assemblage in the RECV sites. Generally, the REF sites supported more chironomid species than the other sites. Seasonally, the highest number of species was recorded in spring for REF sites, summer for DP and AG sites, winter for PP sites, and autumn for RECV sites (Figure 3.5). The number of species recorded throughout all the seasons showed a consistent pattern across the sites, as more species were recorded in the REF and RECV sites than in the urban pollution-impacted sites.

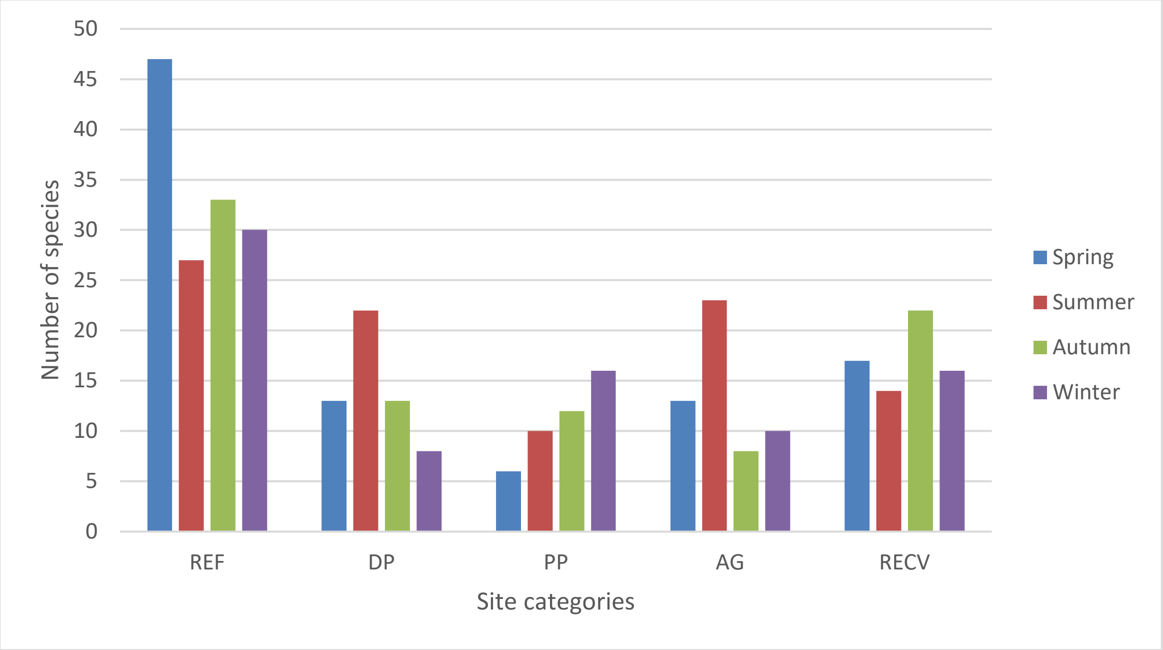


Figure 3.5 Pooled chironomid species richness per site category per season for all the study rivers

A two-way ANOSIM indicated a globally significant difference between the site categories (R = 0.3043, $p = 0.001$), but no significant difference between the sampling seasons. The pairwise post hoc permutation tests between the site categories indicated no significant difference between the chironomid assemblage of the highly impacted sites (DP, PP, and AG) (Table 3.6). The percent dissimilarity in chironomid assemblages was highest between the REF and PP sites, with an average percent dissimilarity of 97.84%, followed by the percent dissimilarity between the REF and AG sites and between the PP and RECV sites, with average dissimilarity percentages of 97.62% and 96.79%, respectively (Figure 3.6). The smallest average dissimilarity was between the PP and AG sites, suggesting that similar communities exist at these site categories. Overall, the species which contributed the most to the dissimilarities between the sites were *Chironomus* sp.1, *R. curtistylus*-type, *Tanypus* sp, *Cricotopus* sp.1, *Cricotopus trifasciata* gr, *Polypedilum* spp., *E. gracei*-type, *Chironomus* sp.2 and *Tanytarsus usmaensis*-type. No significant difference was found in the chironomid assemblage between the REF and RECV sites (Table 3.6). No significant difference was detected in the chironomid assemblages between the sampling seasons. There was also no significant interaction between the site categories and the sampling seasons.

Table 3.6 Summary statistics of ANOSIM and SIMPER analyses showing the average dissimilarities in chironomid assemblage structure between the pairs of sites. Bolded numbers show statistically significant p-values.

| **Site category pairs** | **R-statistic** | ***p-value*** |
|---|---|---|
| REF, DP | 0.4185 | **0.001** |
| REF, PP | 0.5005 | **0.001** |
| REF, AG | 0.3711 | **0.001** |
| REF, RECV | 0.00834 | 0.377 |
| DP, PP | -0.0404 | 0.812 |
| DP, AG | -0.04661 | 0.848 |
| DP, RECV | 0.6165 | **0.001** |
| PP, AG | -0.03956 | 0.797 |
| PP, RECV | 0.6579 | **0.001** |
| AG, RECV | 0.4184 | **0.003** |

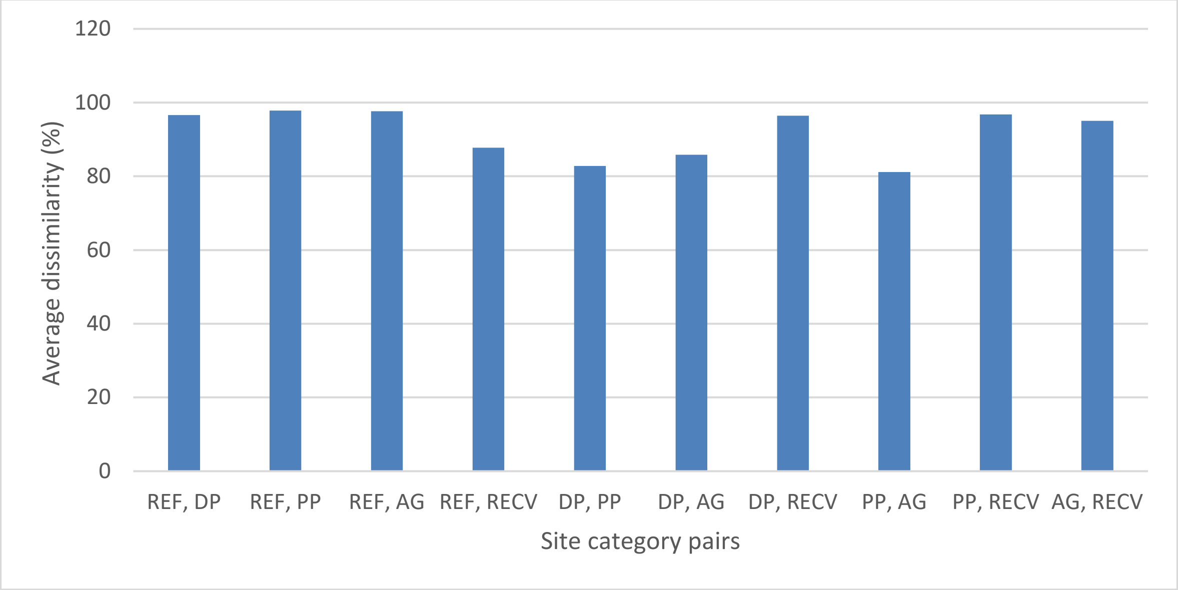


Figure 3.6 Percent average dissimilarity of pooled chironomid species abundances between the site category pairs

### 3.3.4 Developing the CUMMI multimetric index for assessing pollution in urban rivers

***Metrics discriminatory, seasonal stability, repeatability, and redundancy tests***

The testing of chironomid-based metrics occurred in a stepwise manner, in that only metrics which passed a test were retained for further testing until all the tests were complete and the final metrics scored and integrated into the index. Of the seventy-seven (77) metrics that were tested for their discriminatory potential, 23 of them satisfactorily discriminated the least impacted sites (REF) from the highly impacted sites (DP, PP, and AG) and were retained for seasonal stability testing (Appendix A; Figure 1). The 23 metrics that were retained for the seasonal stability test proved to be seasonally stable by not varying significantly between the seasons and were retained for the repeatability test. Only 15 metrics passed the repeatability test and were retained for the redundancy test. Finally, the Spearman rank correlation test revealed that 6 metrics were deemed non-redundant ($r < |0.75|$; $p > 0.05$) and were retained for integration into the multimetric index (Figure 3.7). The six (6) metrics retained for integration into the final multimetric index included the relative abundance of *Chironomus* species (Cmus.), the abundance of *Tanytarsus* species (Tsus_Abun), total chironomid species richness (Spe_rich), the number of species belonging to tribe Tanytarsinii (Tan_sp), Shannon-Weiner diversity (Sha_inde) and the ratio of the sum of *Chironomus* and *Cricotopus* species richness to the total chironomid species richness at a site (Chi_Cric.spe) (Appendix A, Table 1; Appendix A, Figure 1; Figure 3.7).

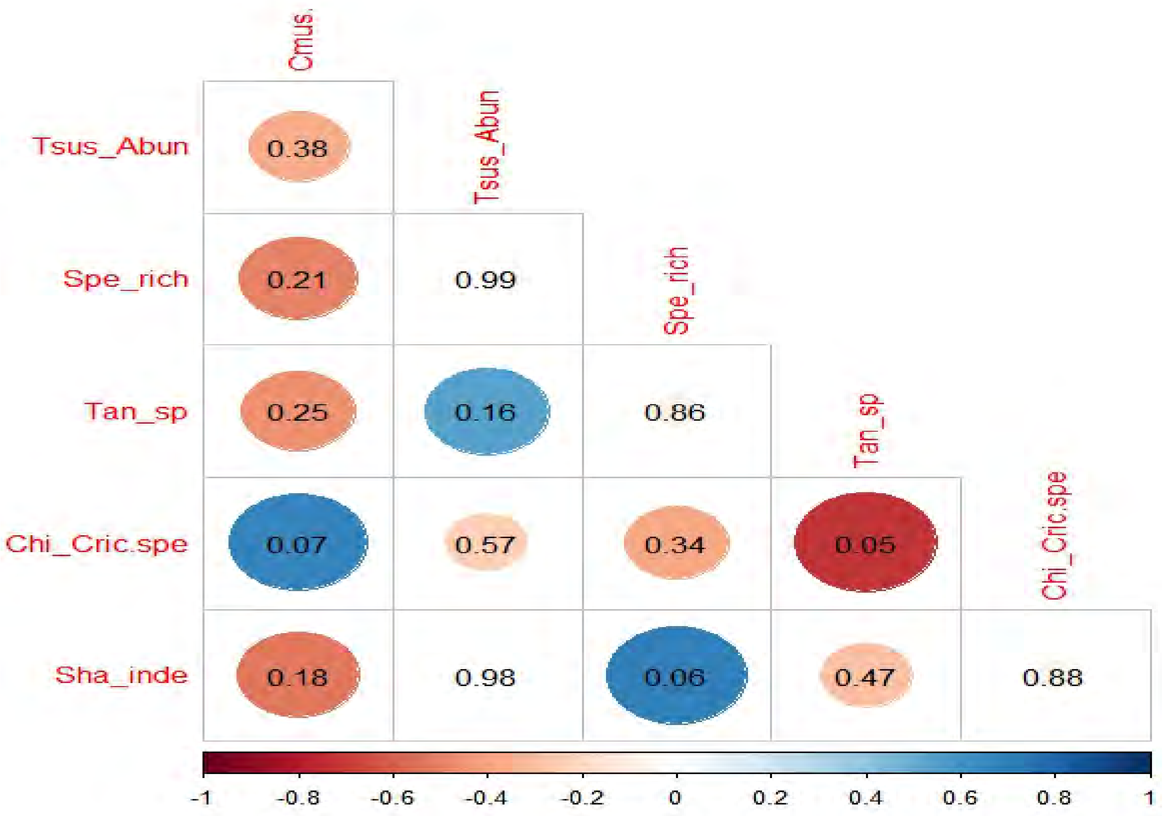


Figure 3.7 Spearman correlation of the six (6) non-redundant metrics integrated into the CUMMI index. The blue colour indicates a positive correlation while the red colour indicates a negative correlation. The *p*-value of the correlation between metrics is indicated by the number in each cell. Chi_Cric.spe - ratio of the sum of *Chironomus* and *Cricotopus* species richness to the total chironomid species richness, Spe_rich - total chironomid species richness, Cmus. - relative abundance of *Chironomus* species, Tsus_Abun - the abundance of *Tanytarsus* species, Tan_sp - the number of species belonging to tribe Tanytarsinii, Sha_inde - Shannon-Weiner diversity index

### *Scoring and integrating the metrics*

Metric scores were calculated using the minimum value, lower quartile (25%), mid-quartile (50%), upper quartile (75%) and maximum value of each metric for the REF sites (Table 3.7). The CUMMI was computed by summing the scores of the six metrics. The index value range (6–30) was realised, as six metrics were used (6 × 5 = 30). The index value range was divided to reflect five water quality categories: A, B, C, D, and E/F, indicative of natural, good, fair, poor and very poor water quality, respectively (Table 3.8).

Table 3.7 Scores and thresholds of the selected metrics integrated into the CUMMI index for urban pollution monitoring. Chi_Cric.spe - ratio of the sum of *Chironomus* and *Cricotopus* species richness to the total chironomid species richness, Spe_rich - total chironomid species richness, Cmus. - relative abundance of *Chironomus* species, Tsus_Abun - the abundance of *Tanytarsus* species, Tan_sp - the number of species belonging to tribe Tanytarsinii, Sha_inde - Shannon-Weiner diversity index

| **Metrics** | **Statistics** | | | | | **Scores** | | |
|---|---|---|---|---|---|---|---|---|
| | Min | 25th | 50th | 75th | Max | 5 | 3 | 1 |
| Spe_rich | 8.00 | 9.50 | 11.50 | 13.25 | 16.00 | >9.50 | ≥8.00–<9.50 | <8.00 |
| Tan_sp | 1.00 | 1.00 | 2.50 | 3.00 | 3.00 | >1.00 | >1.00–<1.00 | ≤1.00 |
| Chi_Cric.spe | 0.00 | 0.00 | 0.00 | 0.078 | 0.125 | ≤0.078 | >0.078–≤0.125 | >0.125 |
| Tsus_Abun | 0.00 | 0.00 | 2.50 | 6.0 | 10.0 | >0.00 | >0.00–<0.00 | ≤0.00 |
| Sha_inde | 1.48 | 1.67 | 2.00 | 2.13 | 2.24 | ≥1.67 | 1.48–<1.67 | ≤1.48 |
| Cmus. | 0.00 | 0.00 | 0.00 | 0.00 | 3.23 | ≤0 | >0–<3.23 | >3.23 |

Table 3.8 CUMMI index score range and associated water quality categories for urban pollution monitoring

| Water quality description | Very poor | Poor | Fair | Good | Natural |
|---|---|---|---|---|---|
| CUMMI score range | 6–10 | 11–15 | 16–20 | 21–25 | 26–30 |
| Ecological category | E/F | D | C | B | A |

### *Index validation*

Chironomid data collected in the autumn and winter seasons from the Buffalo and Bloukrans River site categories were used to validate the index. More than 70% of the time, the CUMMI index classified the water quality at the PP sites as critically/severely modified (Figure 3.8). The SASS5 scores and ASPT values classified the water quality at the PP sites as critically/severely modified 100% of the time. Both the CUMMI index and SASS5 scores classified the water quality conditions at the DP sites as critically/severely modified 100% of the time. The ASPT values and SASS5 scores classified the water quality conditions at the RECV sites as moderately modified 50% of the time, while the CUMMI index classified the water quality condition at the RECV sites as pristine or near natural 50% of the time. The water quality at the REF sites were classified as pristine or near natural 16% of the time, but this water quality category was not reflected by the SASS5 scores and ASPT values for the REF sites. The SASS5 scores classified the water quality condition at the least impacted (REF) sites as critically/severely modified 50% of the time, while the ASPT values also classified the quality as critically/severely modified 33% of the time. Contrarily, the CUMMI index did not classify the water quality condition at the REF site as critically/severely modified (Figure 3.8). The CUMMI index indicated that some of the sites in the REF site category were heavily impacted (Figure 3.8). However, no evidence of anthropogenic impact was observed at the REF sites and the natural seasonal variation in precipitation patterns and water level at headwater streams could be a reasonable explanation for the classification of least-impacted sites as heavily modified due to the limited biotope/substrate resulting from very low water level conditions. Overall, the correspondence between the CUMMI, SASS5 scores and ASPT values were higher for the water quality classification of the highly impacted sites. The SASS5 and ASPT values appeared to inaccurately classify the water quality condition at the REF sites.

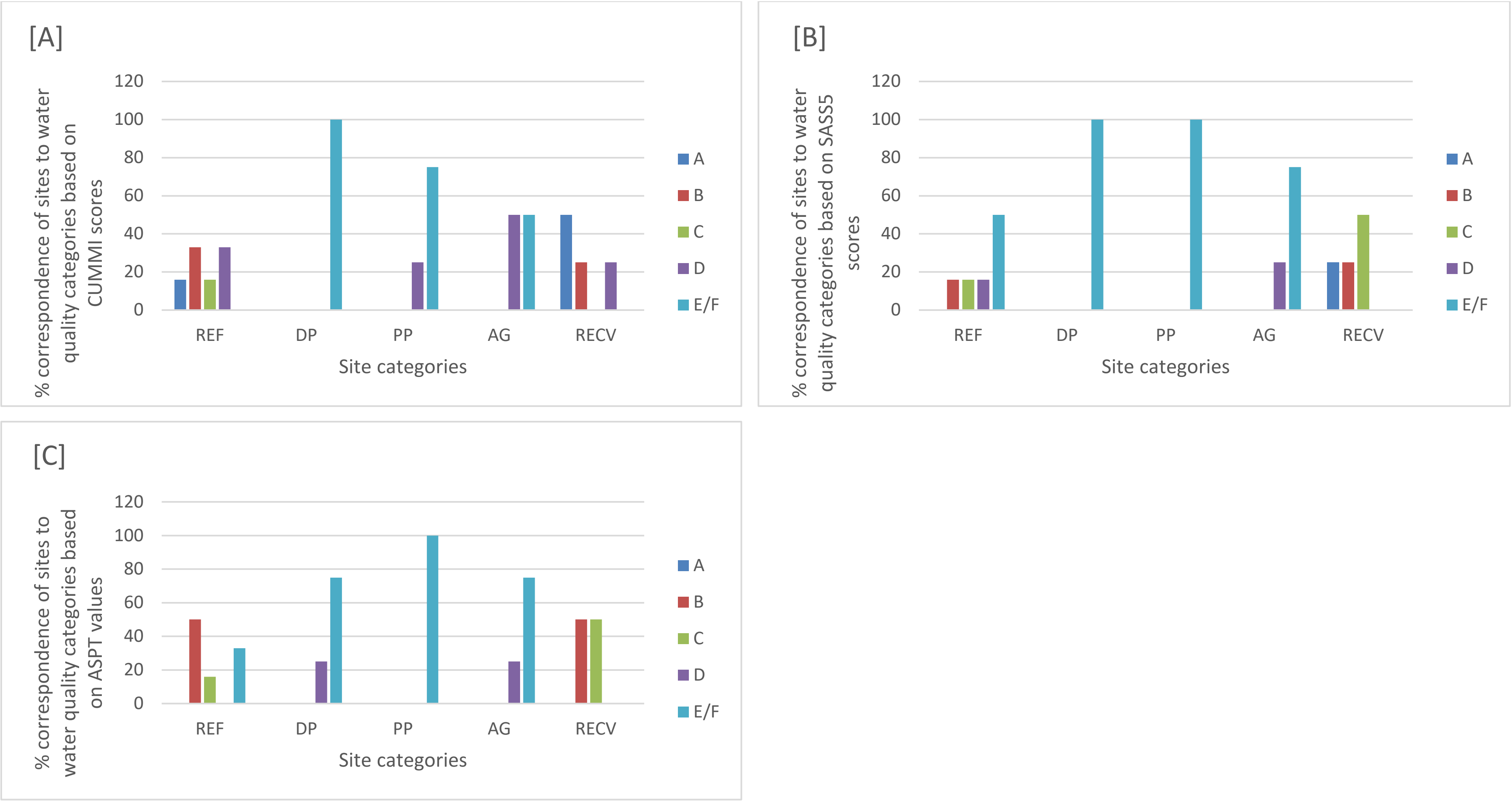


Figure 3.8 Percent correspondence of water quality classification by the [A] CUMMI index, [B] SASS5 scores, and [C] ASPT values for data collected during autumn and winter seasons from the site categories of the Bloukrans and Buffalo rivers. Water quality categories A- natural, B- slightly modified, C- moderately modified, D- heavily modified, and E/F- critically/severely modified.

### 3.3.5 Relating the selected metrics integrated into the CUMMI to physicochemical variables

The RDA ordination plot showed that the abundance of *Tanytarsus* species (Tsus_Abun), total chironomid species richness (Spe_rich), the number of species belonging to tribe Tanytarsinii (Tan_sp) and Shannon-Weiner diversity (Sha_inde) metrics were positively correlated with the dissolved oxygen (Figure 3.9). The ratio of the sum of *Chironomus* and *Cricotopus* species richness to the total chironomid species richness at a site (Chi_Cric.spe), and the relative abundance of *Chironomus* species (Cmus.) were positively correlated with increasing zinc, copper, chromium, nickel, nitrate-nitrogen, nitrite-nitrogen, electrical conductivity, width and temperature (Figure 3.9). Overall, the CUMMI metrics displayed considerable sensitivity to deteriorating water quality in the Bloukrans and Buffalo rivers. The first two axes of the RDA explained 47.8% of the total variance between the physicochemical variables and the metrics. The Monte Carlo test did not reveal a statistically significant correlation in the model ($p > 0.05$).

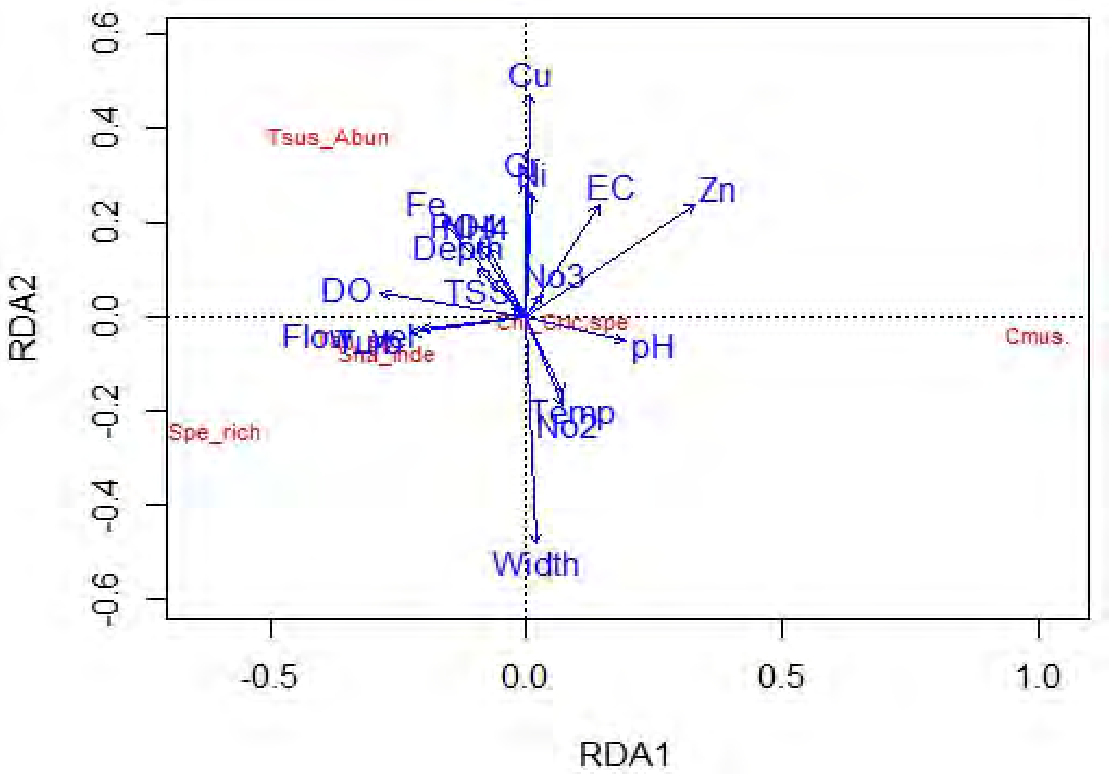


Figure 3.9 An RDA plot showing the correlations between metrics integrated into the CUMMI and selected physicochemical variables variables ($p = 0.05$). Chi_Cric.spe - ratio of the sum of *Chironomus* and *Cricotopus* species richness to the total chironomid species richness, Spe_rich - total chironomid species richness, Cmus. - relative abundance of *Chironomus* species, Tsus_Abun - the abundance of *Tanytarsus* species, Tan_sp - the number of species belonging

to tribe Tanytarsinii, Sha_inde - Shannon-Weiner diversity index. No3- Nitrate-nitrogen, No2- Nitrite-nitrogen, Cu- Copper, Zn- Zinc, Flow_vel- Flow velocity, DO- Dissolved oxygen, Temp- temperature, TSS- Total suspended solids, PO4- orthophosphate-phosphorus, NH4- ammonium-nitrogen, Fe- Iron, Ni- Nickel.

### 3.3.6 Congruence and divergence between the CUMMI and SASS 5 classifications of sites in the Swartkops River

The Kruskal-Wallis test confirmed the significant difference in CUMMI scores between the site categories of the Swartkops River (KW-H = 21.482, $p$ = 8.36e-05). The post hoc test showed that the developed index was responsive to subtle differences in impact categories by identifying significant differences between DP and PP sites and between PP and AG sites. No significant difference was found between CUMMI scores of the DP and AG sites of the Swartkops River.

The CUMMI index classified the water quality condition at the REF site as pristine or near natural 50% of the time (Figure 3.10), whereas the SASS5 scores awarded the same classification for this site 10% of the time. The ASPT values classified the water quality condition at the REF site as moderately modified 50% of the time. The CUMMI index classified the water quality condition at the DP site as critically/severely modified 50% of the time (Figure 3.10), whereas the SASS5 scores awarded the same classification for this site 60% of the time. The ASPT values classified the water quality condition at the DP site as moderately modified 85% of the time. The CUMMI, ASPT and SASS5 scores classified the water quality at the PP sites as critically/severely modified 100% of the time and were congruent (Figure 3.10). The SASS5 scores classified the water quality condition at the AG site as critically/severely modified 100% of the time, whereas the CUMMI index classified the water quality condition at this site as critically/severely modified 90% of the time and moderately modified 10% of the time (Figure 3.10). Overall, the CUMMI, SASS5 sores and ASPT values were divergent in classifying the water quality conditions at the REF sites and, particularly, at the DP and AG impacted site categories, but were congruent in classifying the conditions at the PP sites as critically/severely modified 100% of the time.

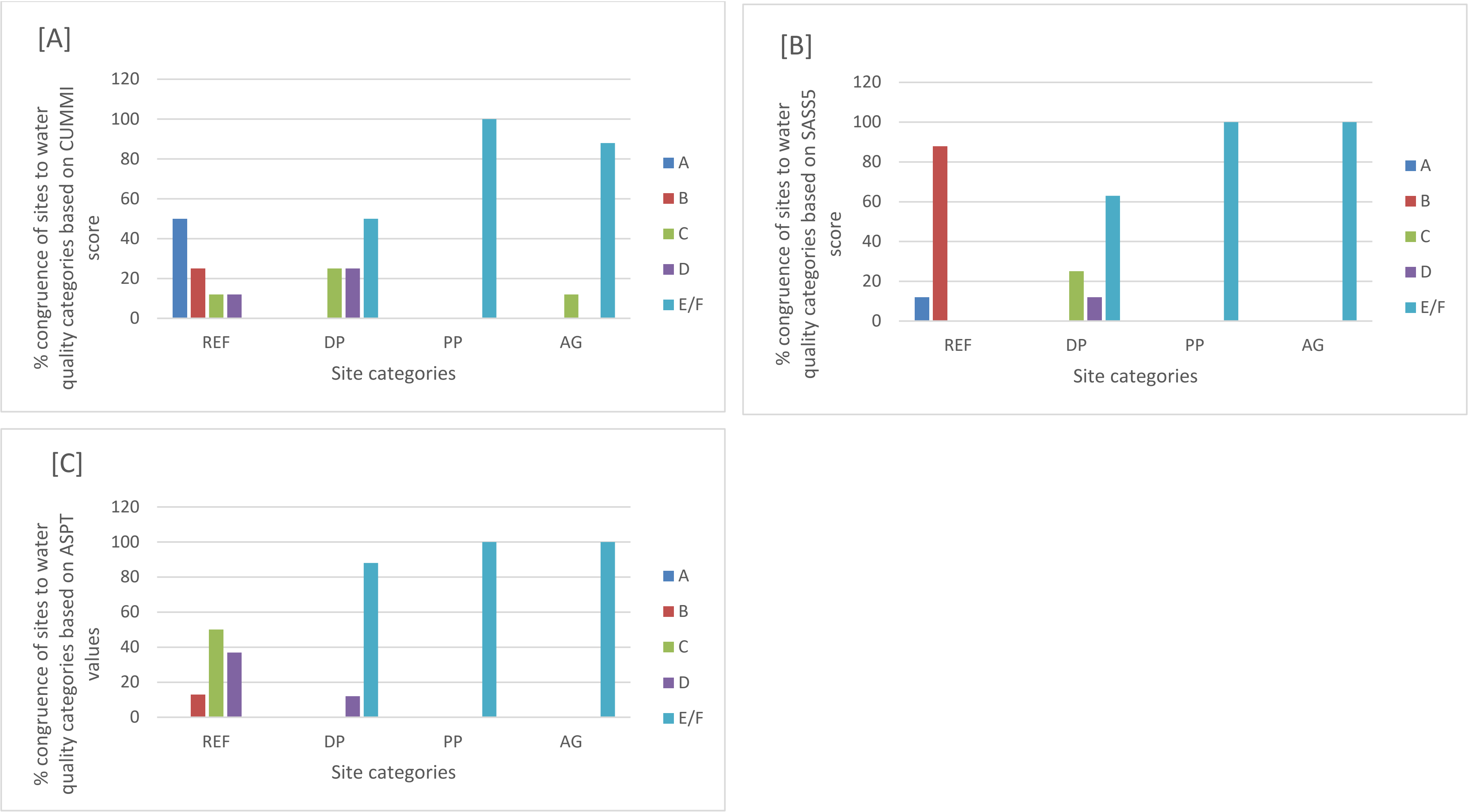


Figure 3.10 Percent congruence of water quality categories (A–F) for the site categories using data collected from the Swartkops River showing the differences between the [A] CUMMI index, [B] SASS5 scores, and [C] ASPT values classification. Water quality categories are A- natural, B- slightly modified, C- moderately modified, D- heavily modified, and E/F- critically/severely modified.

## 3.4 Discussion

### 3.4.1 Response of macroinvertebrates to urban pollution

Urban pollution alters the physicochemical makeup of freshwater systems and can negatively impact aquatic communities (Deliberalli et al., 2018; Edegbene, 2020; Xia et al., 2020). The results of this study showed that the dissolved oxygen (DO) concentration at the least impacted sites (REF) of the Buffalo and Bloukrans rivers differed significantly from that in the highly impacted site categories (DP, PP, and AG sites), but was similar to the DO concentrations at the recovery sites (RECV, Table 3.2). Similarly, the DO concentrations at the REF site of the Swartkops River differed significantly from the DO concentrations at the PP and AG sites (Table 3.3). Depleted dissolved oxygen concentrations in freshwater systems negatively affect aquatic organisms such as macroinvertebrates which depend on dissolved oxygen to carry out their biological and ecological activities (Lévesque et al., 2017).

The SASS5 and ASPT results showed that macroinvertebrate families exhibited varied responses to increasing anthropogenic pressures and deteriorating water quality (Figure 3.1–3). The SASS5 and ASPT score reflected water quality conditions and physical habitat structure. Some macroinvertebrates are biotope-specific and forage based on the characteristics of their preferred biotope. Where their preferred combination of habitat structure and water quality conditions are not available, they will not be found. For example, while species of the order Plecoptera prefer stone biotope and stones may be present at heavily degraded stream sites, the failure to meet the water quality requirements of Plecopterans will essentially eliminate them from such environment where there is ample number of stones. The number of taxa metric of the SASS5 method showed a consistently low number of recorded macroinvertebrate taxa at the highly impacted sites throughout the study seasons. Water physicochemical variables such as DO, electrical conductivity (EC), flow velocity, nutrient concentrations and temperature are among the key environmental variables that influence the structuring of aquatic communities (Palmer, 1991a; Lévesque et al., 2017; Alves et al., 2020). The low number of taxa at the highly impacted sites may be due to elevated dissolved solids, nutrient input and sediment, which negatively impact biota by modifying the set of environmental conditions within which they can survive. The findings of this study are congruent with Gininda (2016), who reported a significant decline in the SASS5 and ASPT scores of macroinvertebrates in sites impacted by urban pollution in the Bloukrans River.

Elevated nutrient concentrations in stormwater run-off, effluent from WWTWs and runoff from agricultural lands have been reported to affect the composition and distribution of macroinvertebrates (Tampo et al., 2021; Arimoro & Keke, 2021; Akamagwuna et al., 2022). Macroinvertebrate taxa which characterised the REF and RECV sites included Hydropsychidae, Baetidae, Pisulidae, Leptophlebiidae, Corixidae, Caenidae, Coenagrionidae and Aeshnidae, whereas taxa that characterised the highly impacted sites (DP, PP and AG) included Oligochaeta, Hirudinea, Culicidae, Chironomidae, Muscidae, Psychodidae and Simuliidae. Based on the SAS5 results, the macroinvertebrate assemblage structure at the highly impacted sites comprised mostly tolerant taxa. Diptera and Oligochaeta are known to dominate highly polluted rivers due to the wide range of sensitivities they possess (Odume & Muller, 2011; Edegbene et al., 2019). These taxa are largely underexplored, especially in South Africa where the availability of taxonomic expertise has remained a challenge. Researchers such as Milošević et al. (2018), Odume (2014) and Martins et al. (2021) have reported species-specific responses of chironomids to organic pollution and metals that were not captured by widely applied biomonitoring tool such as the SASS5. Thus, chironomids may offer a more accurate reflection of the effects of pollution on the ecology of freshwater systems.

### 3.4.2 A chironomid species-based multimetric index for monitoring urban pollution

The science of biomonitoring freshwater ecosystem health requires access to all or most of the information provided by the model organisms, and the taxonomic scale of developed tools is of utmost importance. Although members of the family Chironomidae have been reported to have high tolerance thresholds to anthropogenic disturbance such as effluents from urban sources, chironomid species exhibit a broad spectrum of sensitivities to pollution. Therefore, species-level responses may be interrogated to unpack information masked by family-level biomonitoring tools, as Lenat and Resh (2001) suggest.

A total of 104 chironomid species were identified in this study. The high number of species recorded in this study is not an uncommon observation when chironomids are identified to species level. Leszczyńska et al. (2021) recorded 61 chironomid species in lowland rivers in Poland, and Özkan et al. (2010) recorded 60 chironomid species in eight study sites in the Ergene River Basin. Chironomids are very diverse and occupy a broad ecological range (Gadawski et al., 2021).

The results of the two-way ANOSIM indicated a globally significant difference in the composition of chironomid species between the site categories (R = 0.3043, $p$ = 0.001), although no significant seasonal variation was observed.  The pairwise post hoc permutation tests revealed no significant difference between the chironomid assemblages of the highly impacted sites (DP, PP, and AG). The percent dissimilarity in chironomid assemblages was highest between the REF and PP sites, with an average percent dissimilarity of 97.84%, followed by the percent dissimilarity between the REF and AG sites and between the PP and RECV sites, with average dissimilarity percentages of 97.62% and 96.79%, respectively (Figure 3.6). The smallest average dissimilarity was between the PP and AG sites, suggesting that similar communities exist at these site categories than in other category pairs. Similar to the SASS5 and ASPT results, the ANOSIM provided an indication of chironomid responses to changing water quality conditions at the broad pollution extremes, by indicating that the chironomid assemblage did not differ between the highly impacted sites. Species which contributed the most to the dissimilarities between the least impacted and highly impacted sites were *Chironomus* sp.1, *Rheotanytarsus curtistylus*-type, *Tanypus* sp, *Cricotopus* sp.1, *Cricotopus trifasciata* gr, *Polypedilum* spp., E. *gracei*-type, *Chironomus* sp.2 and *Tanytarsus usmaensis*-type. The relative abundances of chironomid species at the REF sites were dominated mainly by *Cricotopus trifasciata* gr., *Cricotopus* sp.1, *Tanytarsus* sp., *Polypedilum tuberculum*-type and *R. curtistylus*-type., whereas *Chironomus* spp were consistently found to dominate the relative abundance of chironomids at the highly impacted sites (DP, PP, and AG). The dominance of *Chironomus* spp could be due to their possession of adaptive features such as tracheal gills and haemoglobin, which enable them to survive highly degraded environments (Płóciennik et al., 2016; Gadawski et al., 2021).

The lack of a significant seasonal difference in the taxonomic distribution of chironomids suggests that seasonality has little or no effect on the impact of anthropogenic disturbances on chironomid assemblage structure (See Section 3.3 of this Chapter). Thus, if untreated or poorly treated effluent from point source or runoff from diffuse sources reach a stream, a significant change in the distribution patterns of chironomids may be observed regardless of seasons. In essence, chironomid species taxonomic composition may reliably indicate urban-related disturbances irrespective of what season sampling occurred. Additionally, a plausible explanation for the lack of a significant seasonal difference could be that the anthropogenic stress was so high that seasonality played very minimal mediating effect on the assemblage structure of chironomids at the sites. Thus, future biomonitoring studies may maximise available resources by sampling multiple distinct sites once rather than sampling fewer sites seasonally if the aim is to simply establish a gradient of anthropogenic disturbance.

To address the second sub-objective in this chapter, seventy-seven (77) metrics based on chironomid taxonomic assemblages were examined for the development of a multimetric index for monitoring pollution in urban rivers. Of the 77 metrics evaluated, 6 metrics representing composition (relative abundance), richness and diversity were retained and integrated into the chironomid species-based multimetric index for monitoring pollution in urban rivers (CUMMI). These metrics responded predictably to urban pollution and significantly discriminated the site categories (Figure 3.9). Contrary to the *a priori* predictions that metrics integrated into the CUMMI would be exclusively drawn from the Tanypodinae or Orthocladiinae subfamilies as reported by Milošević et al. (2018a, 2018b), two of the sensitive metrics which discriminated between the site categories in the study rivers involved species of the *Chironomus* genera. Hilsenhoff (1988) noted that *Chironomus* spp. dominate severely organic polluted sites. Therefore, it was not surprising that the relative abundance of *Chironomus* species (Cmus.) and the ratio of the sum of *Chironomus* and *Cricotopus* species richness to the total chironomid species richness (Chi_Cric.spe) were positively correlated with nitrate-nitrogen, nitrite-nitrogen and metals (Figure 3.9). *Chironomus* spp. have been used in aquatic toxicity testing and are known to accumulate contaminants owing to their high organic pollution tolerance range (Hilsenhoff, 1987; Odume, 2014). Hence, changes in the richness and relative abundance of species in the *Chironomus* genera relative to the relative abundances and richness of other chironomid species can indicate increasing aquatic pollution (Nicacio & Juen, 2015; Corbi et al., 2019).

The other metrics integrated into the CUMMI which effectively discriminated between the site categories, e.g., total chironomid species richness and the number of species belonging to tribe Tanytarsinii, decreased with increasing organic pollution. Congruent with reports by Edegbene et al. (2020), Suriano et al. (2011) and Odume et al. (2012), the metrics in the richness category which were integrated into the CUMMI were sensitive to water quality deterioration. In this study, the Shannon-Weiner diversity index was also sensitive to urban pollution, with metric scores decreasing with increasing urban pollution. A similar study also reported diversity measures to have high discriminatory potentials, attesting to the usefulness of diversity measures in the development of biomonitoring tools (Edegbene et al., 2019).

Overall, the CUMMI index differentiated the water quality classifications for the site categories and reflected the intensity of pollution at the PP sites compared to the DP and AG sites (Figure 3.10). The performance of the developed CUMMI index using data from the Swartkops River showed that the index performed relatively better than the SASS 5 and ASPT metrics in classifying the river health condition of the REF and the RECV sites. The CUMMI index classified the water quality condition at the DP site as critically/severely modified 50% of the time (Figure 3.10), whereas the SASS5 scores awarded the same classification for this site 60% of the time. The ASPT values classified the water quality condition at the DP site as moderately modified 85% of the time. The CUMMI, ASPT and SASS5 scores were congruent in classifying the water quality at the PP sites. However, the post hoc test showed that the CUMMI index was responsive to the differences in water quality conditions between DP and PP sites and between PP and AG sites. Water quality managers rely on tools that accurately describe river health conditions for informed decision-making. The results of this study show that SASS5 and ASPT can potentially mask subtle changes in the assemblage structure and ecological condition of sites compared to the CUMMI. This may be because of differences in the taxonomic scale of the indices. Indeed, a species-level multimetric tool may prove more informative for long-term monitoring of urban rivers.

## 3.5 Conclusion

In the present study, chironomid species assemblages differed significantly between site categories of the study rivers, indicating taxon-specific responses of chironomids to urban pollution. Species such as *Chironomus* sp.1, *R. curtistylus*, *Tanypus* sp, *Cricotopus* sp.1, *Cricotopus trifasciata* gr, *Polypedilum* spp., *E. gracei*, *Chironomus* sp.2 and *Tanytarsus usmaensis* contributed significantly to the differences found between the study sites. In addition, a chironomid species-based multimetric index for monitoring pollution in urban rivers (CUMMI) was developed. Six metrics representing composition, diversity and richness were integrated into the final index. The CUMMI and SASS5 sores were divergent in classifying the water quality conditions at the DP and AG impacted sites of the Swartkops River but were congruent in classifying the conditions at the PP site as critically/severely modified, indicating that the conditions at the DP and AG sites were not as impaired as the SASS5 suggested. Although there is need for further testing and calibration of the index in other urban rivers in South Africa, the developed CUMMI proved effective as a riverine health biomonitoring tool for urban rivers and could be employed by freshwater quality managers for long-term monitoring of rivers and streams subjected to urban pollution. Also, although the reference conditions set based on data from two least-impacted sites was sufficient in accounting for the natural seasonal variability in species composition and discriminating between the different impact categories, the calibration of the CUMMI to incorporate multiple least-impacted sites may improve the sensitivity of the index. Overall, the findings of this study provide evidence to support the growing body of knowledge indicating that not all chironomid species are tolerant to urban pollution. Moreover, the results emphasise the relevance of a chironomid species-level urban pollution biomonitoring tool. Nonetheless, traits of species which mediate species-specific adaptations to anthropogenic stressors may enable an understanding of the mechanistic links between the biological and ecological responses of chironomids to urban pollution and may aid in the identification of trait-based indicators that can be used to develop traits-based tools complementary to taxonomic approaches, forming the basis of the next chapter of this thesis.

# CHAPTER 4 – EXPLORING THE DISTRIBUTION PATTERNS OF CHIRONOMID TRAITS AND ECOLOGICAL PREFERENCES IN SELECTED URBAN RIVERINE SYSTEMS

## 4.1 Introduction

Pollution of riverine systems due to urbanisation is one of the factors responsible for severe alterations in ecosystem processes (Pauchard et al., 2006; Mackenzie et al., 2022) and consequently, the supply of ecosystem services (Bohus et al., 2023). This is because riverine pollution is usually associated with the loss of aquatic biodiversity (O'Driscoll et al., 2010; Chadwick et al., 2012; Caro-Borrero et al., 2021), resulting in alterations in aquatic community assemblage structure (Kuzmanovic et al., 2017; Tampo et al., 2021).

Although taxonomy-based approaches to biomonitoring riverine pollution have been reported to effectively discriminate between least impacted sites and highly impacted sites, they are limited by their descriptive potential (Collins & Fahrig, 2020). For example, the composition and distribution patterns of species provide structural assemblage changes occurring within an ecosystem with respect to changing environmental conditions, but do not provide an understanding of the mechanisms by which these changes are occurring (Menezes et al., 2010). Thus, an impact-diagnostic tool which can provide an ecologically sound explanation for the observed responses of aquatic biota to disturbance is needed (Hamilton et al., 2020; Odume, 2022).

The trait-based approach (TBA) to biomonitoring is one of the approaches that has been proven to provide an understanding of aquatic macroinvertebrate responses to environmental perturbations (Wilkes et al., 2020; Juvigny-Khenafou et al., 2021; Paz et al., 2022). Because it is based on the collection of species traits present in the ecosystem rather than species identity (Verberk et al., 2013; Merritt et al., 2017; Zhao et al., 2018), it provides an opportunity to assess the impact of pollution on the functional integrity of riverine ecosystems.

Traits and ecological preferences encompass characteristics of an organism's physiology, behaviour, morphology, life history and habitat relation, which can be measured and used to provide insights into the effects of abiotic factors on species distribution and performance (McGill et al., 2006; Violle et al., 2007; Akamagwuna et al., 2022; Green et al., 2022).

Consequently, the environment acts as a filter through which traits that are suited to a set of abiotic conditions are selected. Therefore, an organism is only able to survive in an environment for which it possesses the right combination of traits (Pilière et al., 2016; Odume et al., 2023). Because traits influence the relationship between organisms and their environment, the distribution pattern of traits may be used as a proxy to reflect community responses to anthropogenic disturbances and to understand the effects of pollution on ecosystem functioning (Usseglio-Polatera et al., 2000; Gayraud et al., 2003). For example, invertebrates which possess a streamlined body with substrate-attachment traits have been found to dominate fast-flowing waters (Statzner, 2008; Wooster et al., 2011).

Trait-based approaches present several complementary advantages in freshwater biomonitoring (Martini et al., 2021). First, many traits such as body shape and body size do not require taxonomic expertise to be accurately described (Palmer et al., 1996; Cummins, 2019; Antczak-Orlewska et al., 2021). This is particularly important in the Afrotropical region, where taxonomic expertise is sparse. Second, traits can provide a means of indirect assessment of ecosystem function, e.g., through food and feeding habits (Ashford et al., 2021; El Yaagoubi et al., 2023). Third, traits tend to be less constrained by biogeography than taxonomy, implying that methods developed based on traits may be more widely applied than those based on taxonomy alone (Culp et al., 2011; Kunz et al., 2024). Fourth, traits mediate organisms' relationships with their external environment and thus can form the basis for predicting biotic responses to external environmental stressors (Verberk et al., 2008b; Mouillot et al., 2011). This can potentially lead to the development of predictive tools (Odume et al., 2018).

Chironomids are one of the most taxonomically and functionally diverse groups of aquatic macroinvertebrates (Lencioni et al., 2012; Beneberu et al., 2014). Although they may be difficult to work with due to their requirement for taxonomic expertise, chironomids are widely distributed (Mezgebu et al., 2019). Species of chironomids possess varying degrees of sensitivity to anthropogenic perturbations (Cranston, 1990; Serra et al., 2017b; Rodríguez Garay et al., 2020). They have been reported to dominate the assemblage structure of degraded environments (Molineri et al., 2020). Therefore, they can be explored to provide information about the biological traits and ecological preferences they possess that enable them to dominate impacted sites. For these reasons, they were adopted in this study for the trait-based analysis in relation to urban pollution. Therefore, the objectives of this chapter were to i) identify chironomid traits and ecological preferences which are mechanistically linked to urban pollution in the study rivers, ii) explore the distribution of chironomid traits in relation to urban pollution, and iii) identify potential chironomid trait-based indicators of urban pollution.

## 4.2 Materials and Methods

A detailed description of the study area and site classification are provided in Chapter 2. Briefly, the study was conducted at fifteen study sites spread across three urban rivers in the Eastern Cape province of South Africa. The sites were classified as least-impacted sites (REF sites), diffuse pollution-impacted sites (DP sites), sites immediately downstream of wastewater treatment (WWTW) effluent discharge points (PP sites), sites receiving impact from a combination of agricultural activities, stormwater runoff and effluent from WWTWs (AG sites), and sites further downstream of the length of the study rivers, which were selected for possible stream recovery (RECV sites) (see Section 2.3.1 of Chapter 2).

### 4.2.1 Chironomid and physicochemical variables

Detailed descriptions of sampling techniques and trait measurements are provided in Chapter 2. Historical chironomid and physicochemical variables data for sites in the Swartkops River were collected between 2009 and 2012. Chironomid and physicochemical data for the study rivers were pooled based on the site categories adopted in this study (Section 2.4.1 of Chapter 2).

### 4.2.2 Selection of chironomid traits and ecological preferences

Fifteen (15) categories of traits and ecological preferences were selected and resolved into 61 trait attributes (Table 4.1). The rationale for selecting these traits and ecological preferences is presented in Table 2.2 under Section 2.5 of Chapter 2. Briefly, ecological preferences and traits which are related to chironomid morphology and physiology (maximum body size, respiration, haemoglobin, body armour and number of tracheae), behaviour (tube construction, mobility, feeding mode and substrate relation), life history (voltinism, lifecycle duration, number of eggs per egg mass and reproduction type), and ecological preference (substrate preference and food type) were selected for exploring the effects of urbanisation on chironomid species traits and preferences.

Chironomid species identified in this study were assigned trait information at the genus and species level. When trait information was not available at the species level, information at the genus level was assigned. No chironomid species was assigned trait information at the family level. Information on traits and ecological preferences were obtained from the South African macroinvertebrate trait database (Odume et al., 2023), the European chironomid trait database (Serra et al., 2016), freshwater ecology database (Schmidt-Kloiber & Hering, 2015, 2023; Brabec et al., 2023), and other relevant literature (Walshe, 1950; Walshe, 1951; Forsyth & McCallum, 1978; Forsyth, 1979; Roback, 1981; Goddeeris, 1989; Nolte, 1992; Berg & Hellenthal, 1992; Dettinger-Klemm, 2003; Jacobsen & Bilyj, 2007; Moller Pillot, 2013; Rossaro et al., 2016; Imada, 2020), as well as laboratory measurements/observations. Moreover, the maximum body size of chironomid species was based on the fourth instar larvae measured from the posterior end of the mentum to the base of the last abdominal segment, excluding the antennae and anal setae. Chironomid species were deemed to possess body armour when larvae had transportable cases or could perform biological activities without leaving their cases. For example, species of the genus *Rheotanytarsus* live in cases and project arm-like extensions to catch suspended particles for food without leaving the case. Chironomid species which construct tubes but leave them partially or wholly during feeding were deemed not to possess body armour, due to the perceived increase in cuticular exposure to water quality conditions. Information for substrate preference, substrate relation, respiration, tube construction and food preference were obtained from the literature and databases and confirmed in the laboratory. Information regarding possession of haemoglobin, reproduction type, number of tracheae possessed, motility, feeding mode, voltinism, lifecycle duration and number of eggs per egg mass were obtained only from the databases and literature.

A fuzzy coding approach was used to link chironomid trait attributes to each species (Appendix B1). The fuzzy coding system is based on the assignment of affinity scores representing the association of a species to a specific trait attribute (Chevenet et al., 1994). Thus, the relationship between a species and the expression of a trait attribute is nominally categorised, enabling the association of more than one trait attribute of a particular trait (e.g., food preference or respiratory structure) to a single species (Bournaud et al., 1992; Chevenet et al., 1994; Tomanova et al., 2006). An affinity score ranging from 0–5 was used to describe chironomid species' affinities to trait attributes. A score of 0 represented no affinity of a species to a particular trait attribute, a score of 1 indicated low affinity, a score of 3 indicated moderate affinity, and a score of 5 indicated high affinity. Where species trait information was not available for all the attributes of a trait, a score of 0 was assigned following Tomanova et al.

(2008). Trait information was available for more than half of the selected traits and ecological preferences for 96 species, representing 92.3 % of the chironomid species sampled.

Predictions regarding the potential responses of chironomid traits and ecological preferences to urban stressors (e.g., organic pollution, sediment influx, habitat and flow alteration and nutrient enrichment) were made (Table 4.1). Traits which confer vulnerability to increasing urban stressors were predicted to be less abundant in highly impacted sites compared to traits that confer resilience (Odume et al., 2023). For example, chironomid species which neither possess tracheal gills nor haemoglobin for increased dissolved oxygen intake were predicted to be eliminated by decreasing dissolved oxygen concentrations associated with urban pollution (Townsend & Hildrew, 1994).

Table 4.1: Selected chironomid traits, trait attributes, their codes and predicted responses to urban stressors. Potential increase or decrease in the proportion of trait attributes with increasing urban perturbation is indicated with (+ or −), while ± indicates a varied response of the trait attribute to increasing urban disturbance.

| **Trait** | **Trait attributes** | **code** | **Predicted response** |
|---|---|---|---|
| Haemoglobin | Present | H1 | + |
| | Absent | H2 | - |
| Respiration | Tracheal/ventral gills | X1 | + |
| | Cutaneous | X2 | - |
| | Aerial | X3 | + |
| Reproduction type | Egg in clutches | RT1 | + |
| | Asexual reproduction | RT2 | + |
| | Free isolated eggs | RT3 | - |
| Tube construction | Tube absent | TC1 | - |
| | Tube without shape | TC2 | + |

| | | | |
|---|---|---|---|
| | Tube rigid | TC3 | + |
| Substrate relation | Burrower | SR1 | + |
| | Free-living | SR2 | - |
| | Miner | SR3 | + |
| | Fixed/attached | SR4 | - |
| Number of tracheae | 12 tracheae | RP1 | + |
| | 6 tracheae | RP2 | ± |
| | 3 tracheae | RP3 | - |
| Mobility | Swimmer/Sprawler | M1 | + |
| | Crawler/Climber | M2 | - |
| | Whole-body undulator | M3 | + |
| Feeding mode | Collector-filterer | FM1 | + |
| | Collector-gatherer | FM2 | + |
| | Shredder | FM3 | - |
| | Scraper/grazer | FM4 | + |
| | Predator-engulfer | FM5 | - |
| | Predator-piercer | FM6 | - |
| Maximum body size (mm) | < 2.5 (very small) | BS1 | + |
| | > 2.5–5 (small) | BS2 | + |
| | > 5–10 (medium) | BS3 | + |
| | > 10–20 (large) | BS4 | - |

| | | | |
|---|---|---|---|
| | > 20–40 (very large) | BS5 | - |
| Voltinism (number of generations per year) | 1 | V1 | - |
| | 2 | V2 | + |
| | 3 | V3 | + |
| | > 3 | V4 | + |
| Lifecycle duration (year) | ≤ 1 | D1 | + |
| | > 1 | D2 | - |
| Number of eggs per egg mass | < 100 | EM1 | - |
| | 100–500 | EM2 | ± |
| | 500–1000 | EM3 | + |
| | > 1000 | EM4 | + |
| Substrate preference | Stone, cobble, boulders | SP1 | + |
| | Gravel, sand, mud, silt | SP2 | ± |
| | Macrophyte & filamentous algae | SP3 | ± |
| | Microphytes (microscopic algae) | SP4 | + |
| | Twigs, roots | SP5 | ± |
| | Litter, fine organic matter | SP6 | + |
| | Wood | SP7 | - |
| | Moss | SP8 | + |
| Body armour | Present | BA1 | + |
| | Absent | BA2 | - |

| | | | |
|---|---|---|---|
| Food type | Fine sediment | FT1 | + |
| | Debris < 1mm | FT2 | + |
| | Plant debris > 1mm | FT3 | - |
| | Living microphytes | FT4 | + |
| | Living macrophytes | FT5 | - |
| | Dead animals | FT6 | + |
| | Living microinvertebrates | FT7 | ± |
| | Living macroinvertebrates | FT8 | - |
| | Wood | FT9 | - |

### 4.2.3 Statistical analysis

To analyse the chironomid community traits-based responses, the abundances of chironomid species for each site category (REF, DP, PP, AG, and RECV) per season were pooled to create a taxon-site table. A matrix containing trait information coded for each taxon was created (Appendix B1) and multiplied by the abundance of each species per season in each site category to create a table containing trait-site information (Appendix B2) as described in Tomanova et al. (2008). Percent relative abundances were calculated for each trait attribute per season for the different site categories. Significant differences in the median value of each trait attribute for each site category were tested using the Kruskal-Wallis non-parametric test followed by a Wilcox rank-sum test, for pairwise comparison of site categories.

The RLQ (R, site-physicochemical variables matrix; L, site-species abundance matrix; Q, traits-taxa matrix) analysis was used to examine the associations between ecological preferences and traits of chironomid species and the sampling sites. RLQ is a multivariate ordination analysis that simultaneously performs ordinations on three matrices (environmental variables-R, species abundance-L, and traits-Q) (Dolédec et al., 1996). It provides an efficient means of assessing the relationship between species traits and environmental variables. The ordination analyses each data matrix separately using principal component analysis (PCA) for the R matrix, correspondence analysis (CA) for the L matrix, and the Hill-Smith analysis for the Q matrix (Akamagwuna, 2021). The combination of the separate analyses in the RLQ is done using sites and species scores of the L matrix as links between the R and Q matrices. Thus, RLQ analysis represents partial inertia analyses of the different data matrices (R, L, and Q) (Dolédec et al., 1996). RLQ identifies the traits and physicochemical variables shaping the species assemblages at a site by calculating the contributions of each physicochemical variable to total inertia (Akamagwuna et al., 2019a; Odume, 2020). Following Akamagwuna et al. (2022) and Odume (2020), the RLQ analysis was applied to explore relationships between environmental variables and trait attributes of chironomid species assemblages. The RLQ involves a series of stepwise ordinations performed on the different data matrices (R, L, and Q). Firstly, a correspondence analysis (CA) is performed on the site-species abundance matrix (L-table), followed by a principal components analysis (PCA) on the site-physicochemical variables matrix (R-table), using the sample score results of the CA as row-weights. The traits-taxa matrix (Q-table) was analysed using a PCA with row weights extracted from species scores of the correspondence analysis performed on the site-species abundance matrix. Results of the separate analyses performed on the three matrices were combined in the final step of the RLQ analysis to measure the intensity of the link between species traits and physicochemical variables (Dray et al., 2014). A multivariate Monte Carlo permutation test was used to determine the statistical significance of the taxa-trait-physicochemical variable relationships in the RLQ analysis at 999 permutations and $p = 0.05$. A combination of two models (Model 2 and 4) in the permutation test was used to test the null hypothesis that species composition is neither influenced by physicochemical variables nor species traits (Dray et al., 2014).

The fourth-corner test is a multivariate permutational analysis which indicates the correlations between traits and ecological preferences, and physicochemical variables (Akamagwuna et al., 2019b; Edegbene, 2020). It indicates the traits and ecological preferences that either positively or negatively correlate to physicochemical variables. It allows for the exploration of significant bivariate associations between one single trait and one single environmental variable at a time (Dray et al., 2014). Fourth-corner analysis was used to test the bivariate associations between the individual traits and ecological preferences, and the physicochemical variables. Because the fourth-corner method is complementary to the RLQ analysis, the three matrices used in the RLQ analysis were also used to perform the fourth-corner analysis at 999 permutations. Traits and ecological preferences which were positively correlated with dissolved oxygen (DO) and negatively correlated with electrical conductivity (EC), turbidity and nutrients were deemed to be urban pollution-sensitive traits and ecological preferences, while those positively correlated with EC, turbidity and nutrients or negatively associated with DO were deemed to be urban pollution-tolerant traits and ecological preferences. Odume (2020) and Edegbene et al. (2020) applied a similar approach to identify signature macroinvertebrate traits and ecological preferences of urban pollution. The Monte Carlo permutation test at 999 permutations at alpha = 0.05 was used to test the statistical significance of the RLQ axes (Dray et al., 2014).

The RLQ and fourth-corner analyses were performed with $\log_{10}(x + 1)$ transformed species abundance data and nine (9) physicochemical variables, namely, temperature, turbidity, DO, EC, nitrate-nitrogen, nitrite-nitrogen, ammonium-nitrogen and orthophosphate-phosphorous. RLQ and fourth-corner analyses were performed using the ade-4 package in R statistical software version 4.2.0 (Dray & Dufour, 2007; Thioulouse et al., 2018; R Core Team, 2022).

## 4.3 Results

### 4.3.1 Distribution patterns of chironomid traits and ecological preferences in relation to urban pollution in the selected rivers

Chironomids which possess haemoglobin (H1) dominated the relative abundance of chironomid communities at the impact sites throughout the study seasons, compared to chironomids which do not possess haemoglobin (H2) (Figure 4.1). However, species which do not possess haemoglobin did not dominate the assemblage at the REF and RECV sites in any of the seasons. The relative abundances of species which possess haemoglobin were highest at the DP and PP sites throughout the study seasons compared to the other site categories. The relative abundances of species which do not possess haemoglobin were higher during summer and autumn compared to the spring season at the AG sites (Figure 4.1). The Kruskal-Wallis test revealed significant differences between the site categories for species which possess haemoglobin ($p = 0.016$, Appendix B3a).

Chironomid species which utilise cuticular respiration (X2) dominated the REF and RECV sites in all the seasons (Figure 4.2). Conversely, species which possess tracheal gills (X1) for respiration dominated the relative abundance of chironomids at the DP, PP and AG sites in all the seasons except in summer and autumn at the AG sites (Figure 4.2). There was no record of any chironomid species which utilise aerial respiration (X3) in this study (Appendices B2 and B4b). The possession of tracheal gills (X1) differed significantly between the site categories (*p*

= 0.007, Appendix B3b). The Kruskal-Wallis test revealed significant differences in the site categories for species which utilise cuticular respiration (*p* = 0.013, Appendix B3b).

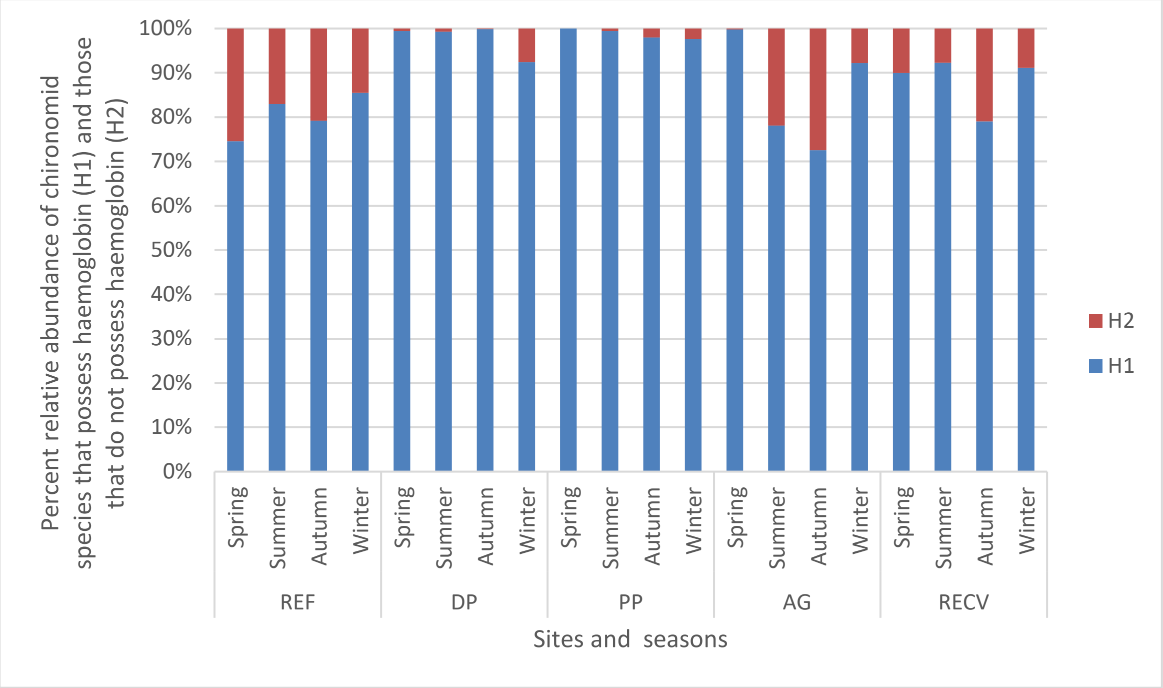


Figure 4.1 Percent relative abundance of chironomids which possess haemoglobin (H1) and those that do not possess haemoglobin (H2) per season per site category

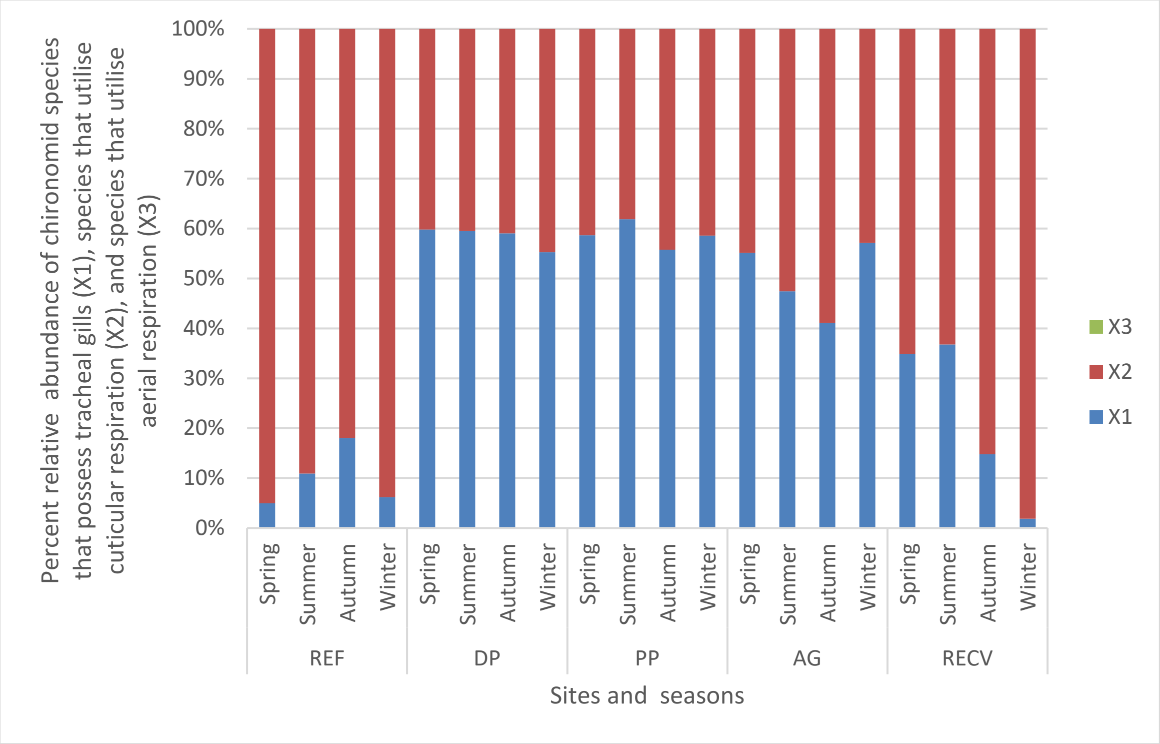


Figure 4.2 Percent relative abundance of chironomid species which possess tracheal gills (X1), species which utilise cuticular respiration (X2), and species which utilise aerial respiration (X3) per site categories per season

In terms of reproduction type, chironomid species which lay eggs in clutches (RT1) dominated the relative abundance of chironomid communities in the site categories in all the seasons (Figure 4.3). Species which reproduce asexually by parthenogenesis (RT2) were mostly present at the REF sites in all the seasons and at the RECV sites in spring and autumn. Species which reproduce asexually were not present at the DP and PP sites in any the seasons except in summer, where their relative abundance constituted 0.01% at the DP sites (Figure 4.3). The trait of laying free isolated eggs (RT3) by chironomid species was not recorded in this study (Appendix B1). The Kruskal-Wallis test revealed significant differences in the abundance of species which lay eggs in clutches between the site categories (*p* = 0.016, Appendix B3c). The abundances of chironomids which reproduce asexually differed significantly between the site categories (*p* = 0.0098, Appendix B3c).

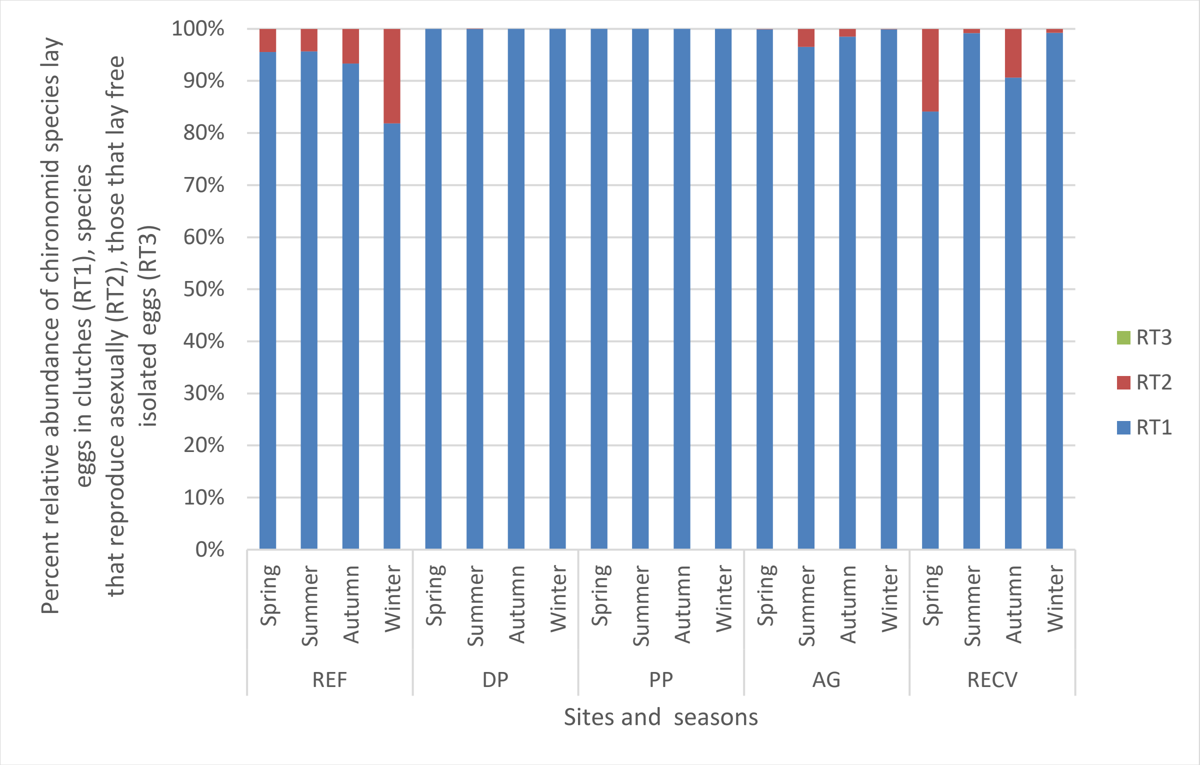


Figure 4.3 Percent relative abundance of chironomids which lay eggs in clutches (RT1), species which reproduce asexually (RT2), and those which lay free isolated eggs (RT3) per site categories and seasons

The relative abundances of chironomids which do not construct tubes (TC1) were generally low at the different site categories and in all the seasons (Figure 4.4). At the REF and RECV sites, chironomids which construct and live in irregularly shaped tubes (TC2) dominated the relative abundance of the chironomid community, except in spring at the RECV sites, where those which construct rigid tubes (TC3) were dominant. Chironomids which construct rigid tubes (TC3) dominated the relative abundances at the DP, PP and AG sites in all the seasons except in autumn at the AG sites (Figure 4.4). There was no significant difference between the abundances of chironomids which do not construct tubes (TC1) for the site categories ($p <$ 0.05, Appendix B3d). Moreover, there was no significant difference between the site categories for chironomids which construct irregularly shaped tubes (TC2).

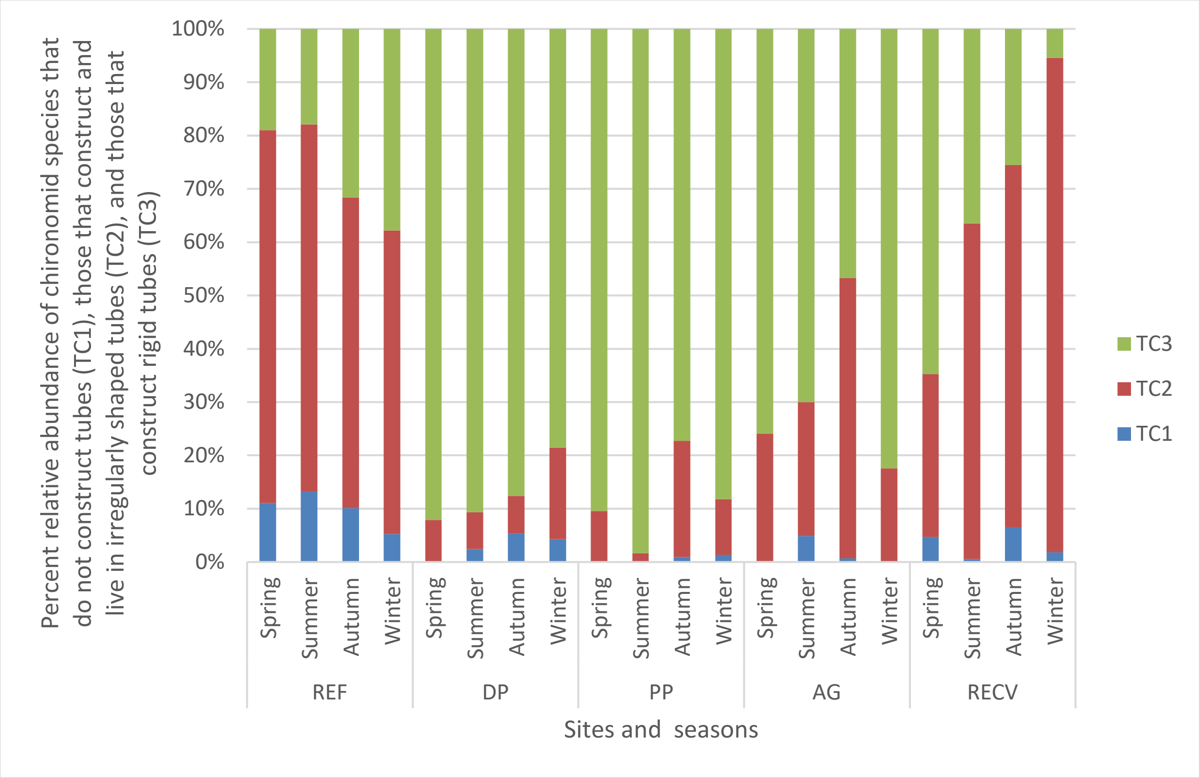


Figure 4.4 Percent relative abundance of chironomid which do not construct tubes (TC1), those which construct and live in irregularly shaped tubes (TC2), and those which construct rigid tubes (TC3) per season and site category

In terms of substrate relation, chironomids which are burrowers (SR1) were present at all the site categories and during all the seasons but were mostly dominant at the DP and PP sites (Figure 4.5). At the AG sites, the dominance of burrowers steadily declined across the seasons but took a sharp increase in winter (Figure 4.5). Miners (SR3) were dominant at the REF sites in all the seasons and the RECV sites in summer, autumn, and winter. The relative abundances of free-living chironomids were highest at the REF sites in spring and at the AG sites in summer. The relative abundance of chironomids which are fixed/attached to hard substratum (e.g., stones and aquatic plants; SR4) were highest at the AG and RECV sites. The Kruskal-Wallis test revealed significant differences between the site categories for the abundance of SR1 ($p$ = 0.014) and SR2 ($p$ = 0.037, Appendix B3e).

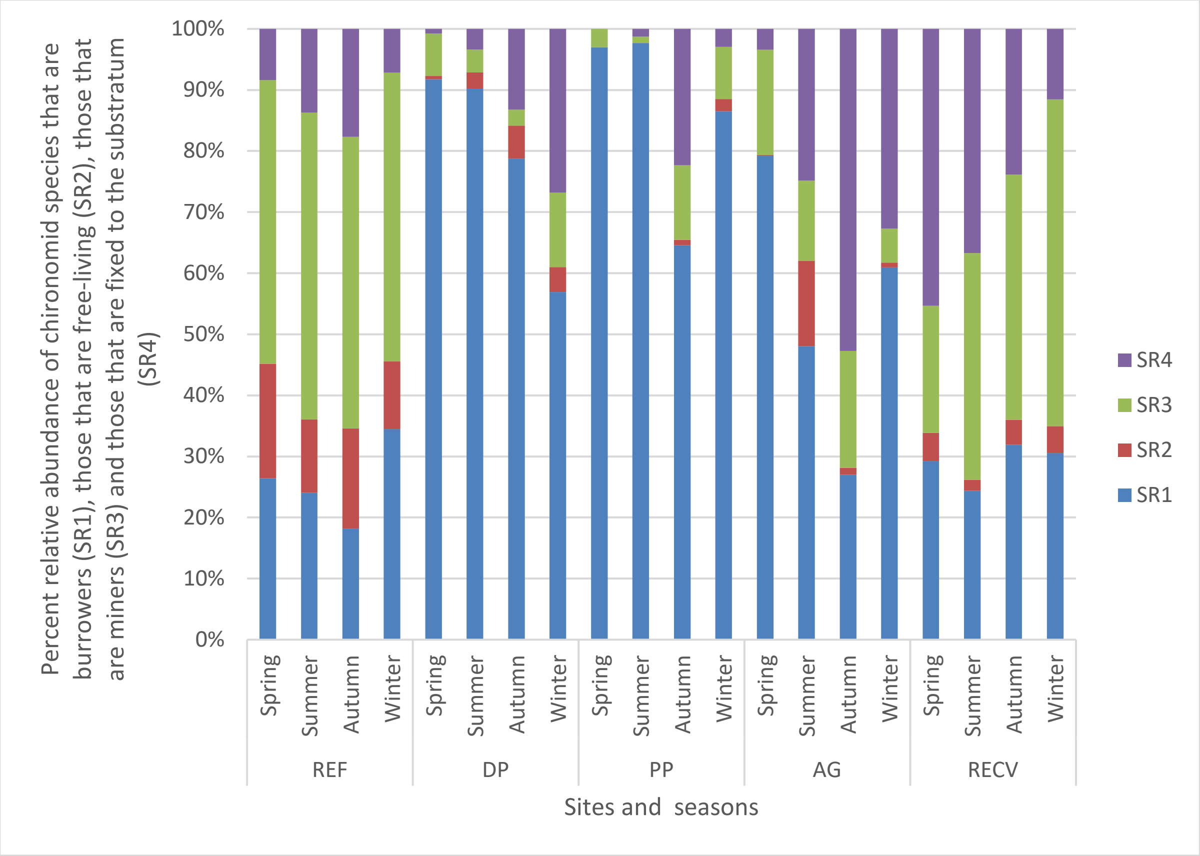


Figure 4.5 Percent relative abundance of chironomids which are burrowers (SR1), those which are free-living (SR2), those which are miners (SR3) and those which are fixed to the substratum (SR4) per season per site category

Chironomids which possess 12 tracheae (RP1) were more dominant at the REF sites compared to the other sites (Figure 4.6). Chironomids which possess 3 tracheae (RP3) dominated the relative abundance at the DP, PP, AG and RECV sites in all the seasons, except during spring at the RECV sites (Figure 4.6). The Kruskal-Wallis test did not detect any significant difference between the site categories for chironomids which possess 12 tracheae and those which possess 6 tracheae (RP2) (Appendix B3f). However, there was a significant difference between the site categories for chironomids which possess 3 tracheae ($p$ = 0.013).

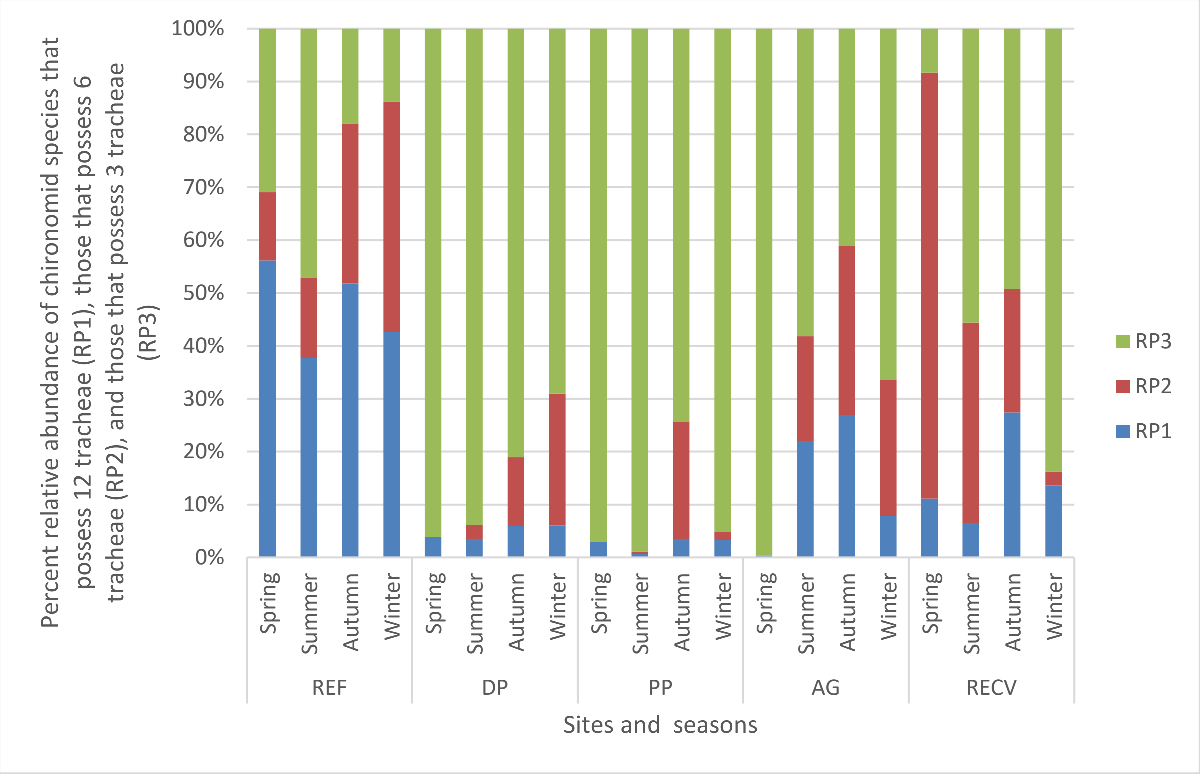


Figure 4.6 Percent relative abundance of chironomids which possess 12 tracheae (RP1), those which possess 6 tracheae, and those which possess 3 tracheae (RP3) per season per site category

The relative abundance of chironomids which crawl (M2) was dominant at the REF sites in all seasons and the RECV sites in summer, autumn and winter (Figure 4.7). Chironomids which swim (M1) were greatly represented at the REF sites, and their relative abundance exhibited a steady increase at the DP sites in summer, autumn and winter. The relative abundance of chironomids which utilise whole-body undulations (M3) was dominant at the DP, PP and AG sites in all seasons. The Kruskal-Wallis test detected significant differences between the site categories for chironomids which swim ($p$ = 0.036) and those which utilise whole-body undulations ($p$ = 0.0069), but there was no significant difference between the site categories for chironomids which crawl (Appendix B3g).

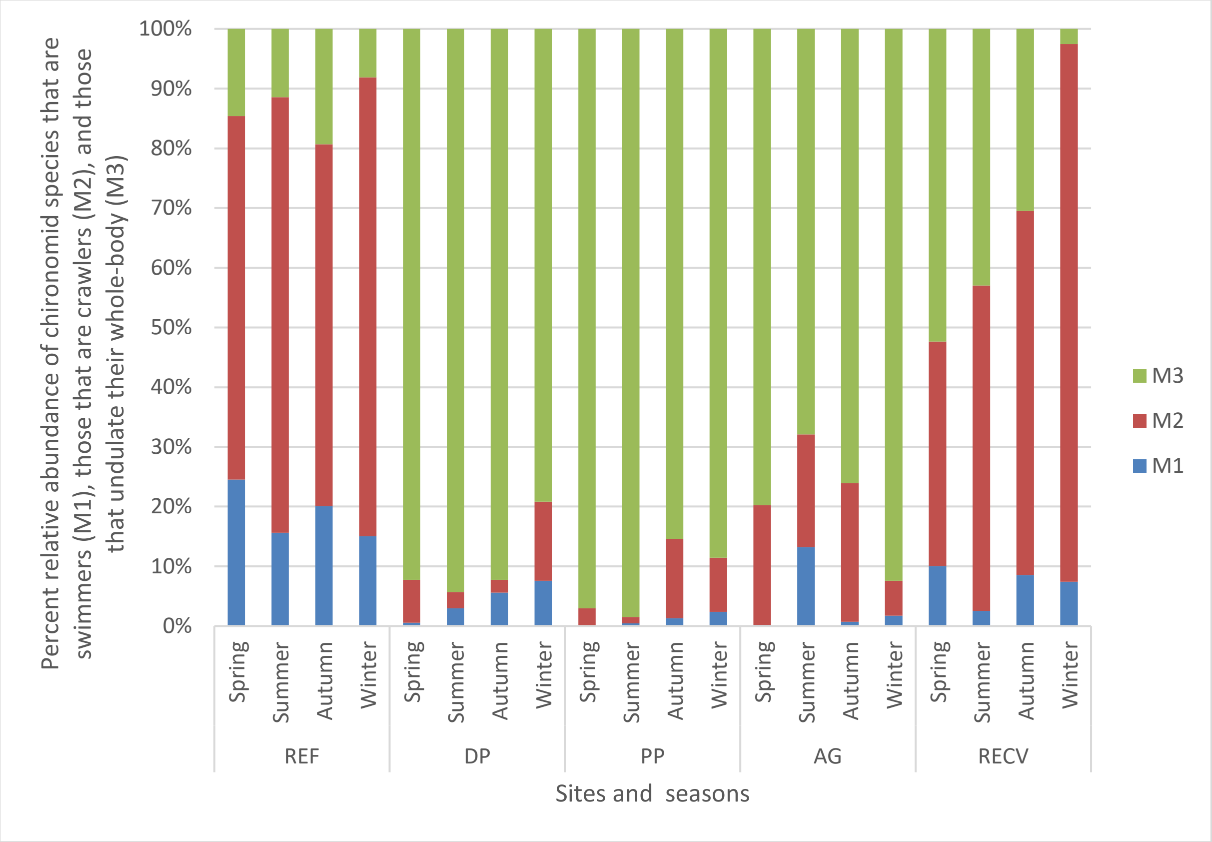


Figure 4.7 Percent relative abundance of chironomids which are swimmers (M1), those which are crawlers (M2), and those which undulate their whole body (M3) per season and site category

Of the five maximum body size trait attributes analysed, the relative abundance of chironomids which possess a very large body size (BS5; >20–40 mm) dominated the chironomid community at the DP, PP and AG sites in all the seasons except in autumn at the AG sites (Figure 4.8). Species which possess medium body size (BS3; >5–10 mm) dominated REF and RECV sites (Figure 4.8). The Kruskal-Wallis test showed that the abundances of chironomids which possess small body size trait attributes (BS2; >2.5–5 mm), medium body size (BS3; >5–10 mm), and large body size (BS4; >10–20 mm) did not significantly change across the site categories (Appendix B3h). However, the abundance of chironomids which possess very large body size attribute (BS5; >20–40 mm) showed significant differences between the site categories ($p = 0.0044$). There was no record of chironomids which possess very small (BS1; < 2.5 mm) maximum body size attributes in this study (Appendix B1).

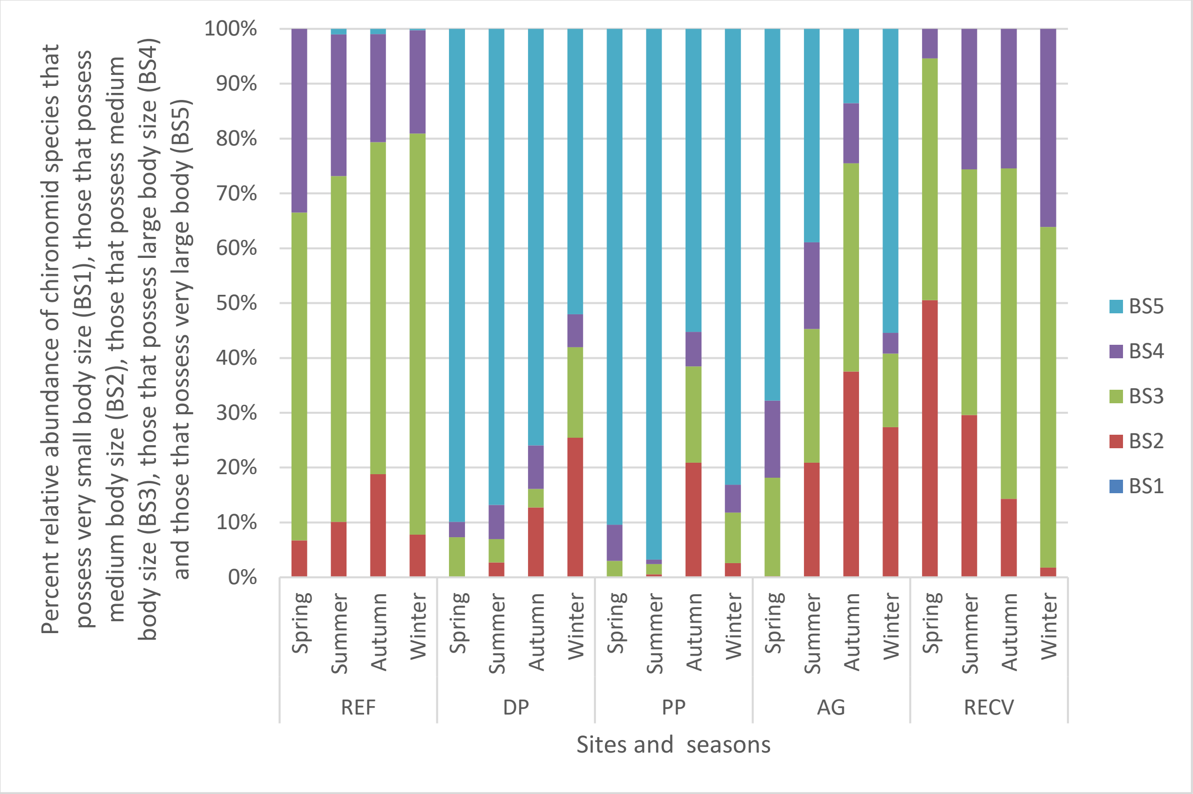


Figure 4.8 Percent relative abundance of chironomids which possess very small body size (BS1; < 2.5 mm), those that possess medium body size (BS2 = >2.5–5 mm), those which possess medium body size (BS3; >5–10 mm), those which possess large body size (BS4; >10–20 mm) and those which possess very large body size (BS5; >20–40 mm) per season per site category

Of the two body armour trait attributes that were analysed, the relative abundances of chironomids which do not qualify as possessing body armour (BA2) were dominant in all the site categories and across the seasons (Figure 4.9). The Kruskal-Wallis test indicated that there were significant differences in the abundance of chironomids which do not possess body armour (BA2) between the site categories (P= 0.017, Appendix B3i). There was no significant difference in the abundance of chironomids which possessed body armour between the site categories.

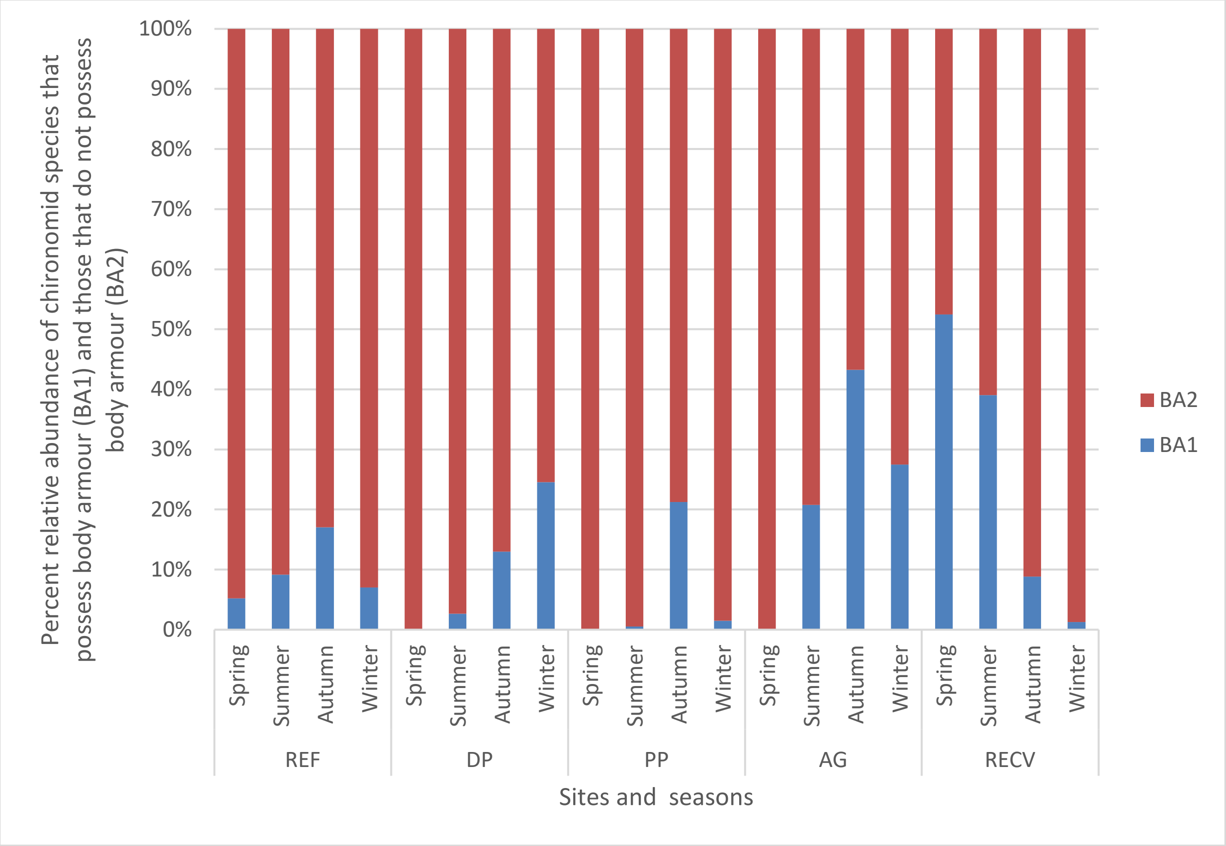


Figure 4.9 Percent relative abundance of chironomids which possess body armour (BA1) and those which do not possess body armour (BA2) per season and site category

Two attributes of chironomid lifecycle duration trait were analysed, and species which complete their lifecycle in less than or equal to one year (D1) dominated the relative abundances of chironomids at the REF and RECV sites in all the seasons (Figure 4.10). On the other hand, species which take more than one year to complete their lifecycle (D2) were dominant at the DP, PP and AG sites in all the seasons except during summer and autumn at the AG sites (Figure 4.10). The Kruskal-Wallis test indicated significant differences in the site categories for species which take more than one year to complete their lifecycle (*p* = 0.0054) but no significant difference in the site categories for species which complete their lifecycle in less than or equal to one year (*p* = 0.19; Appendix B3j).

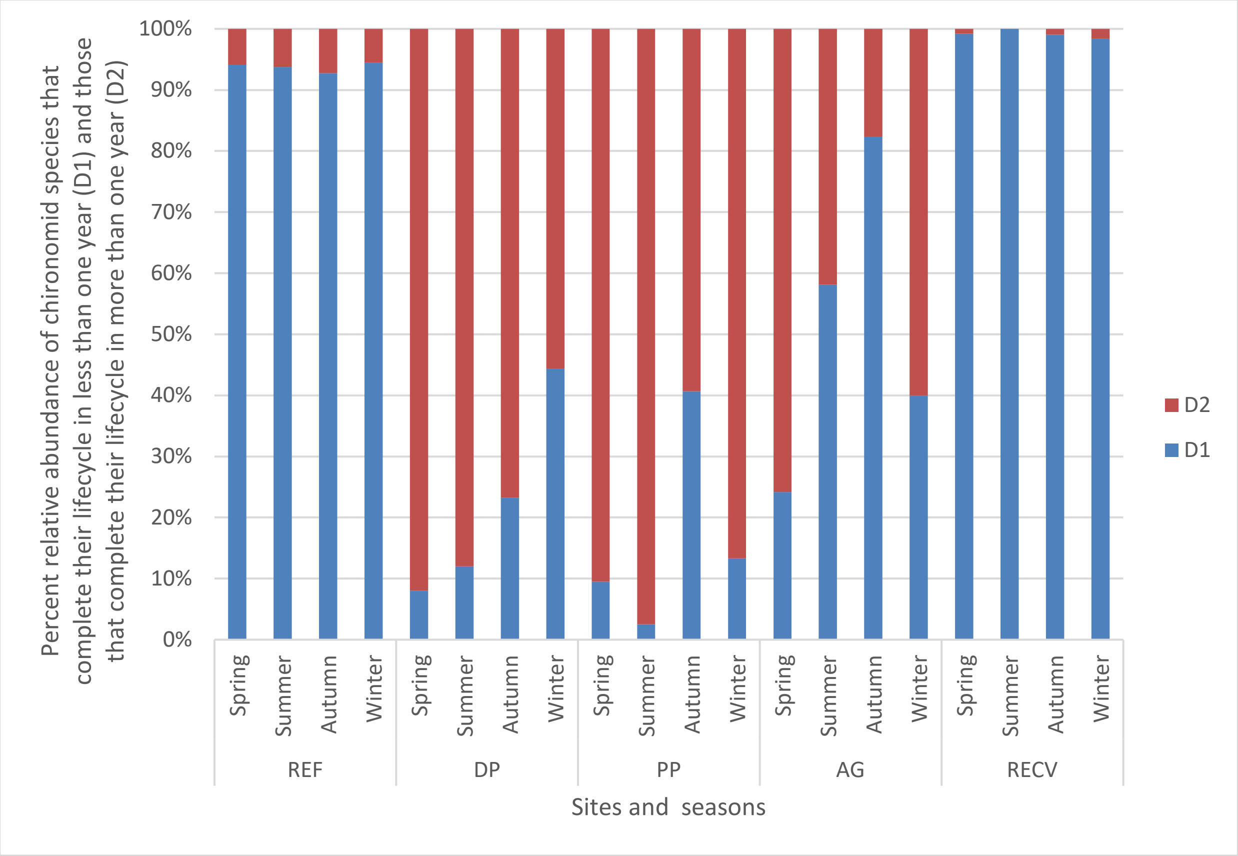


Figure 4.10 Percent relative abundance of chironomids which complete their lifecycle in less than one year (D1) and those which complete their lifecycle in more than one year (D2) per season per site category

Of the four attributes of the voltinism trait that were analysed, the relative abundances of species which produce two generations per year (V2) were dominant across the site categories and in all the seasons (Figure 4.11). At the RECV sites, the relative abundances of species which produce one generation per year (V1), those which produce two generations per year (V2) and those which produce three generations per year (V3) increased steadily during the seasons, whereas that of species which produce more than three generations per year (V4) declined across the seasons (Figure 4.11). The Kruskal-Wallis test showed that there were significant differences between the site categories for all the voltinism trait attributes analysed (V1, $p$= 0.04; V2, $p$= 0.011; V3, $p$= 0.024; and V4, $p$= 0.011; Appendix B3k).

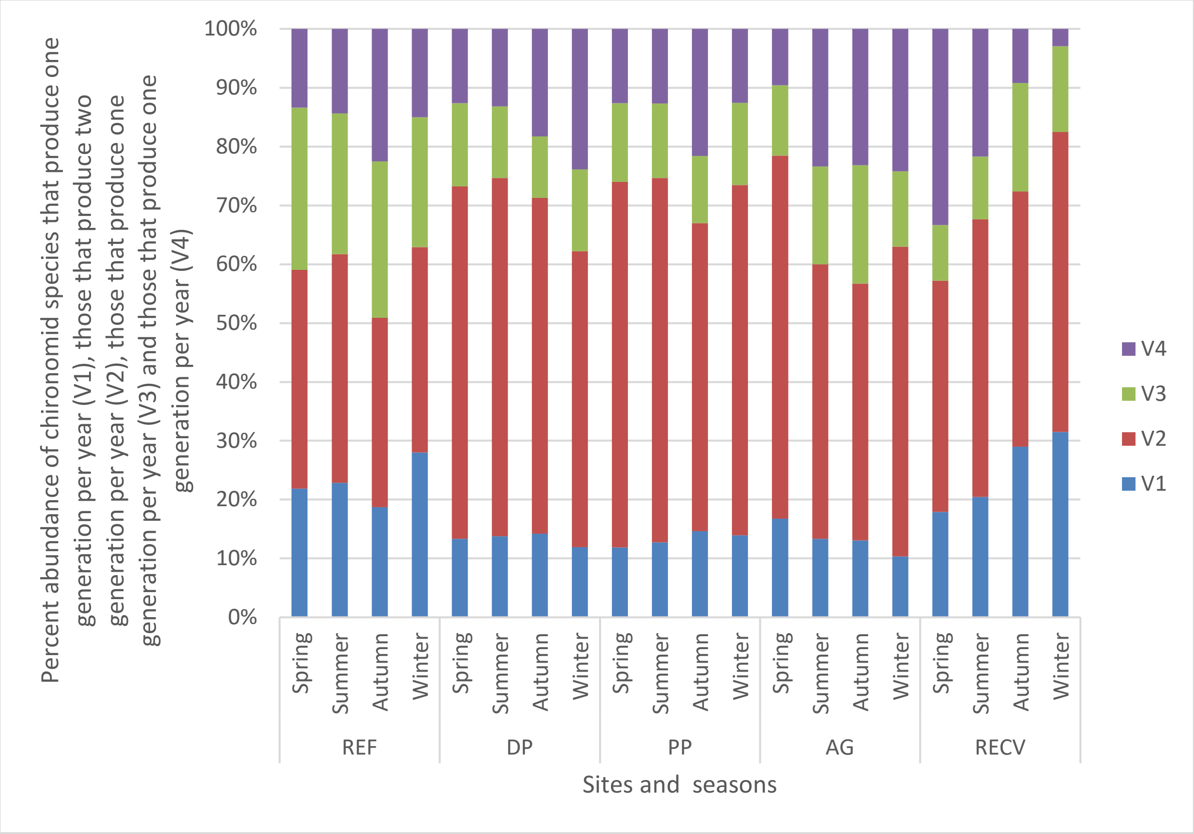


Figure 4.11 Percent abundance of chironomid species which produce one generation per year (V1), those which produce two generations per year (V2), those which produce three generations per year (V3) and those which produce more than three generations per year (V4) per season per site category

The number of eggs per egg mass trait had four trait attributes which were analysed. The relative abundance of chironomid species which lay 100–500 eggs per egg mass (EM2) dominated the chironomid community at all the site categories and in all the seasons except during spring at the RECV sites (Figure 4.12). The relative abundance of chironomid species which lay less than 100 eggs per egg mass (EM1) increased steadily across the seasons at the REF sites. The Kruskal-Wallis test indicated significant differences between the site categories for abundances of species which lay 100–500 eggs per egg mass ($p = 0.011$), those which lay 500–1000 eggs per egg mass ($p = 0.0093$), and those which lay more than 1000 eggs per egg mass (EM4; $p = 0.0065$), but no significant difference between site categories for species which lay less than 100 eggs per egg mass (Appendix B3l).

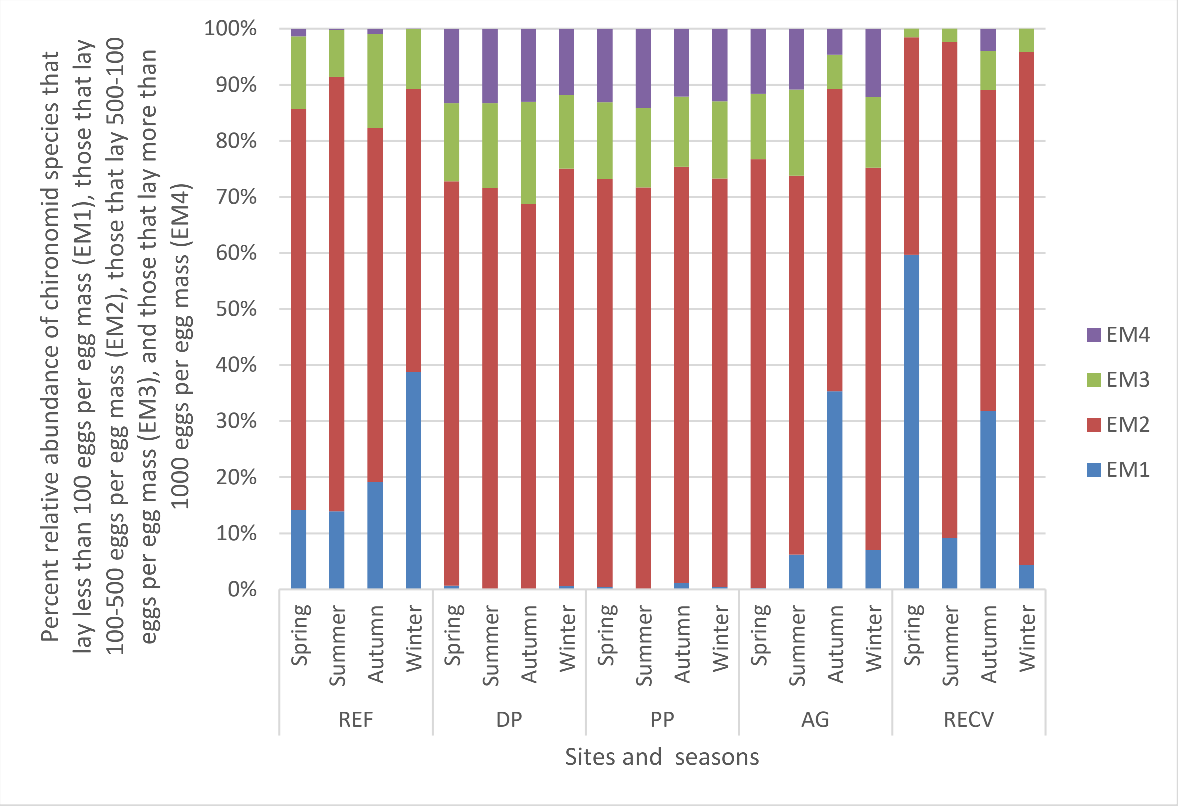


Figure 4.12 Percent relative abundance of chironomids which lay less than 100 eggs per egg mass (EM1), those which lay 100–500 eggs per egg mass (EM2), those which lay 500–1000 eggs per egg mass (EM3), and those which lay more than 1000 eggs per egg mass (EM4) per season per site category.

Although some urban streams may be naturally dominated by coarse substrates, others by fine substrates, macrophytes or diatoms, Figure 4.13 shows that each of the samples collected from the different site categories possessed not less than seven out of the eight biotopes analysed. Here, I explored the differences in the relative abundances of chironomid substrate preference between the site categories in relation to water quality deterioration and not the presence or absence of substrates. This was done because, while there may be available substrates at a site, the species which prefer those substrates may be eliminated by deteriorated water quality. Chironomids which preferred macrophytes and filamentous algae (SP3) dominated the relative abundances of the assemblages at the REF and RECV sites in all the seasons (Figure 4.13). The relative abundance of species preferring litter and fine organic matter substratum (SP6) increased at the DP, PP, and AG sites. Species preferring stone biotopes (SP1) were relatively more abundant in all seasons at the REF and RECV sites and during the summer, autumn and winter seasons at the AG sites (Figure 4.13). Chironomid species preferring the gravel, sand, silt and mud biotope (SP2) dominated the relative abundance of the assemblage at the DP and PP sites in all the seasons and the AG sites in spring (Figure 4.13). For all the substrate preference attributes analysed, the Kruskal-Wallis test revealed significant differences in the abundances of species between the site categories except for species preferring SP4 (microphytes), SP5 (twigs and roots) and SP7 (wood) (Appendix B3m).

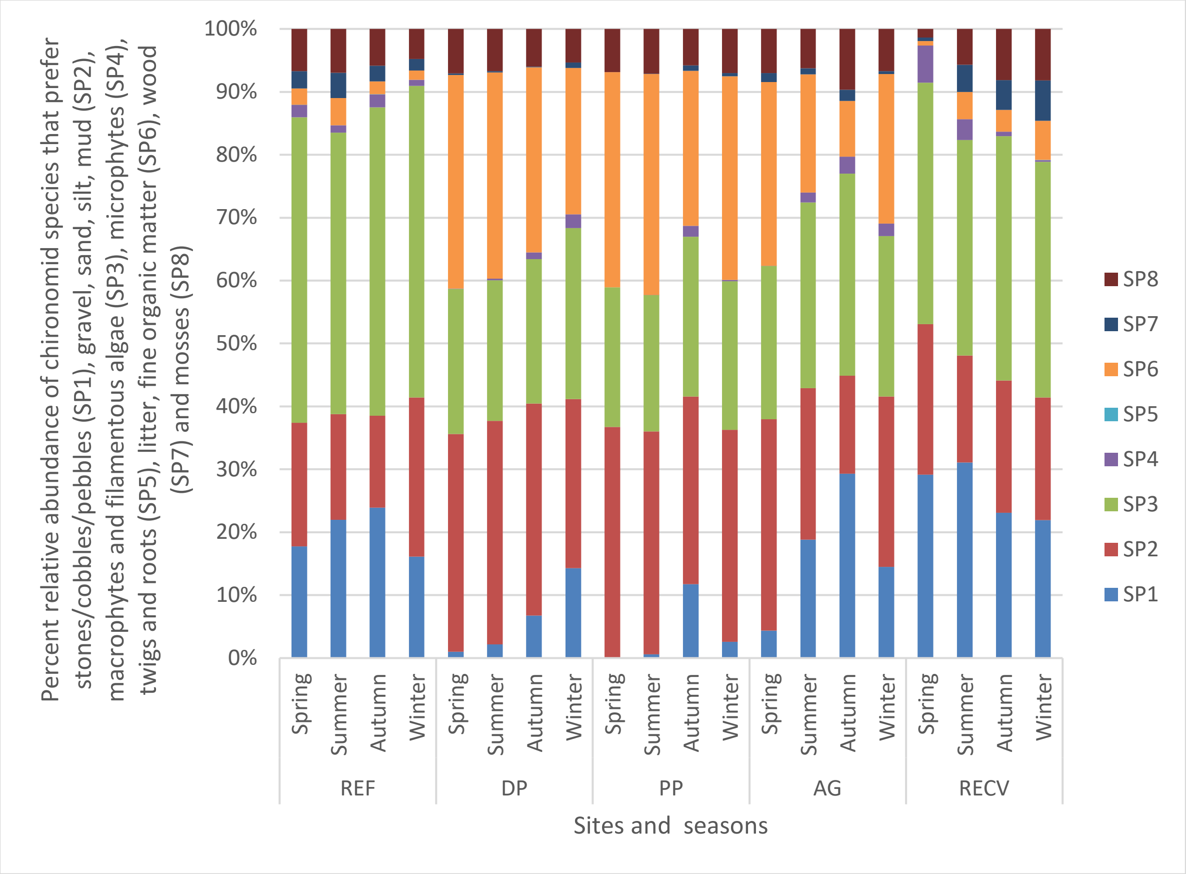


Figure 4.13 Percent relative abundance of chironomids that prefer stones/cobbles/pebbles (SP1), gravel, sand, silt, mud (SP2), macrophytes and filamentous algae (SP3), microphytes (SP4), twigs and roots (SP5), litter, fine organic matter (SP6), wood (SP7) and mosses (SP8) per season per site category

In terms of feeding habits and preferred food traits, chironomids which are collector-gatherers (FM2) and primarily feed on fine detritus (FT1) dominated the relative abundance of chironomid communities at the DP, PP and AG sites in all the seasons (Figure 4.14). The relative abundance of the shredders (FM3), which primarily consume living plant material (FT5), was markedly higher at the REF and RECV sites than at the impacted sites (DP, PP and AG) (Figure 4.14Figure 4.15). Chironomids which are predators (piercers, FM5 and engulfers, FM6) were present at the REF sites throughout the sampling season, compared to spring and winter at the DP site and PP sites, where they were completely absent (Figure 4.14). The relative abundance of collector-filterers (FM1) was highest during spring at the RECV sites but significantly declined across the seasons (Figure 4.14). However, the Kruskal-Wallis test only detected a significant difference in the abundance of species which are collector-gatherers (FM2) ($p$ = 0.0068) and predator-engulfers (FM2) ($p$ = 0.049) between the site categories (Appendix B3n). The Kruskal-Wallis test showed significant differences between the site categories in the abundance of species which prefer FT1 ($p$ = 0.015), FT2 ($p$ = 0.01), FT3 ($p$ = 0.011), FT4 ($p$ = 0.013), FT5 ($p$ = 0.038), FT6 ($p$ = 0.0044), FT7 ($p$ = 0.0067) and FT8 ($p$ = 0.049) (Appendix B3o).

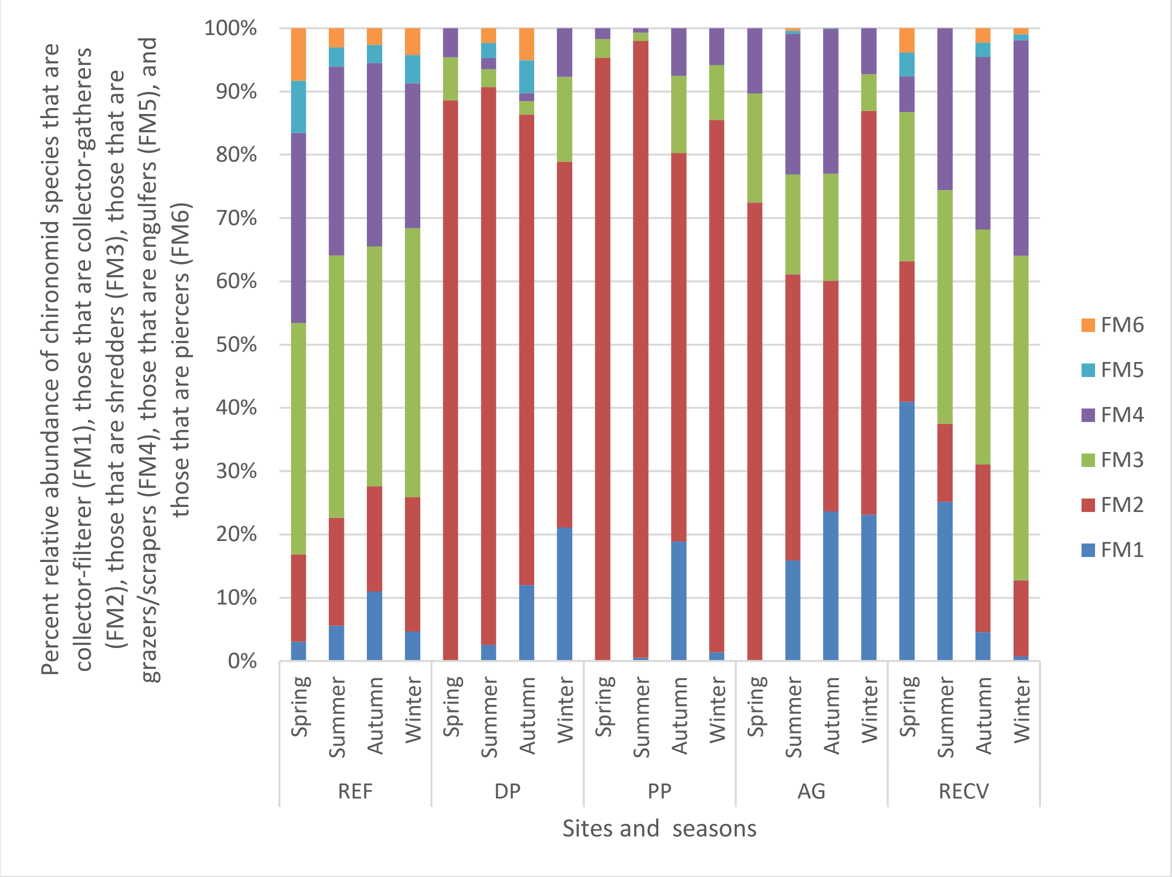


Figure 4.14 Percent relative abundance of chironomids which are collector-filterers (FM1), those which are collector-gatherers (FM2), those which are shredders (FM3), those which are grazers/scrapers (FM4), those which are engulfers (FM5) and those which are piercers (FM6) per season per site category

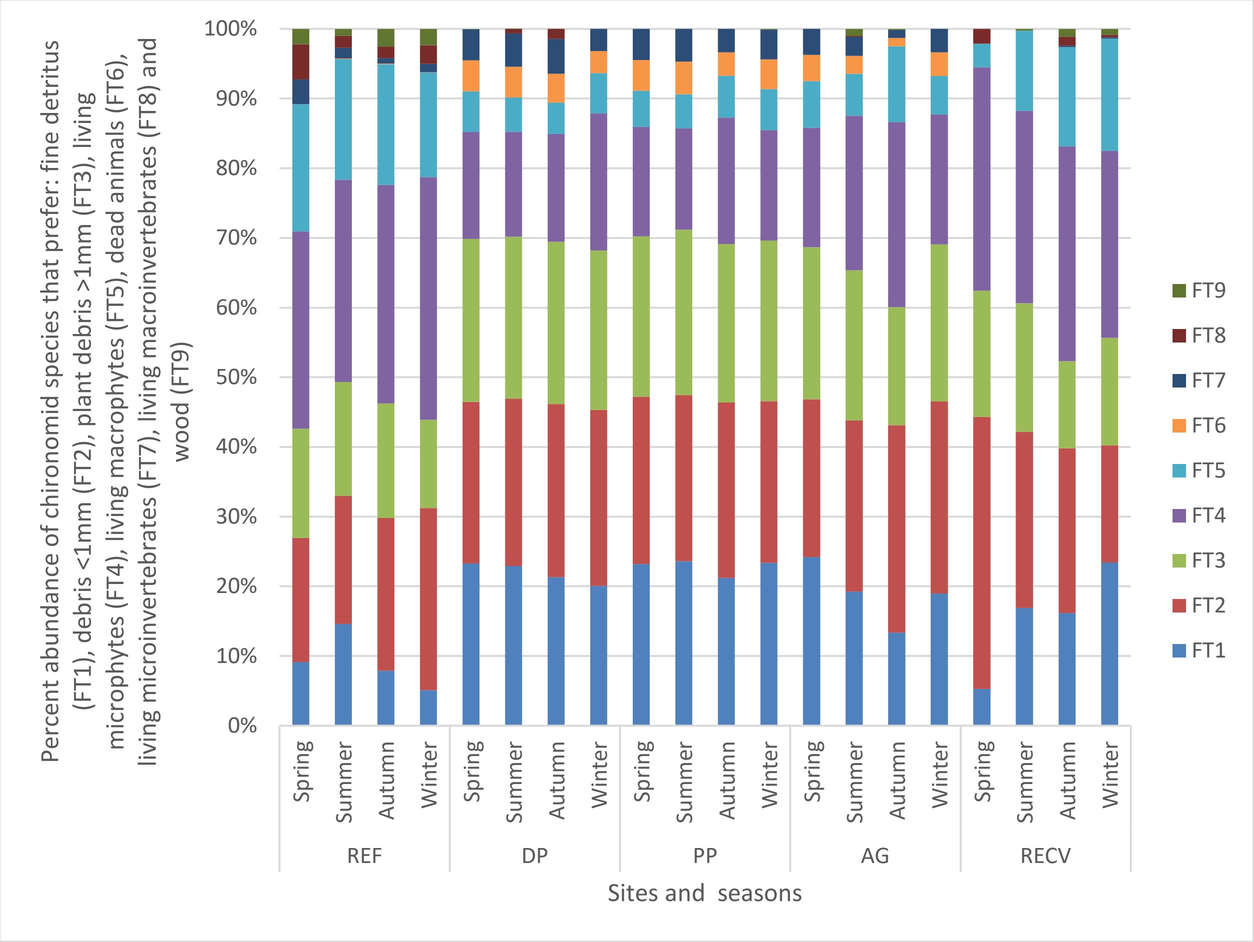


Figure 4.15 Percent relative abundance of chironomid species that prefer fine detritus (FT1), those that prefer debris < 1mm (FT2), those that prefer plant debris > 1mm (FT3), those that prefer living microphytes (FT4), those that prefer living macrophytes (FT5), those that prefer dead animals (FT6), those that prefer living microinvertebrates (FT7), those that prefer living macroinvertebrates (FT8) and those that prefer wood (FT9) per season per site category.

### 4.3.2 Relating chironomid traits and ecological preferences to urban pollution in the selected rivers

Trait attributes belonging to 104 chironomid species were analysed in this study. The first two axes of the RLQ summarised the relationship between traits, chironomid compositions and physicochemical variables, explaining 92.62% and 4.87% for axes 1 and 2, respectively, and a cumulative sum of 97.49% of the covariances between the traits and the physicochemical variables (Table 4.2). The Monte-Carlo test revealed that the relationships between traits, chironomid compositions and physicochemical variables were statistically significant for model 2 (*p* = 0.001), which tests whether the environment influences the distribution of species with fixed traits, and model 4 (*p* = 0.001), which tests whether the traits influence the composition of species assemblages within the given environmental conditions.

Table 4.2 RLQ ordination properties showing the percent variations explained by Axes 1 and 2, the eigenvalues, covariance and correlation and projected variances explained by the first two axes of the analysis

| **RLQ Properties** | **Axis 1** | **Axis 2** |
|---|---|---|
| Variance (%) | 92.62 | 4.87 |
| Cumulative variance (%) | 92.62 | 97.49 |
| Eigenvalue | 10.74 | 0.56 |
| Covariance | 3.28 | 0.75 |
| Correlation | 0.61 | 0.28 |
| Variance R/RLQ (%) | 95.60 | 90.46 |
| Variance L/RLQ (%) | 66.09 | 32.69 |
| Variance Q/RLQ (%) | 97.06 | 80.21 |

The RLQ analysis indicated three site clusters (Figure 4.16). The sites were distinctly clustered based on the study rivers and the impact received at the sites rather than seasonality. The first identifiable cluster includes data points from the REF and RECV sites (Cluster A), followed by the cluster of highly impacted DP, PP and AG sites (Cluster B), and then AG and DP sites, which make up the third cluster (Cluster C) (Figure 4.16). Chironomid traits associated with Cluster A include large body size (>10–20mm; BS4), laying of less than 100 egg per egg mass (EM1), asexual reproduction (RT2), possession of 12 tracheae (RP1), swimming behaviour (M1), cuticular respiration (X2), crawling behaviour (M2), absence of tube construction (TC1) or irregularly shaped tubes (TC2), grazing behaviour (FM4), free-living behaviour (SR2), lifecycle duration of less than or equal to one year (D1), production of three generations per year (V3), a preference for plant fibres (FT9) and predator feeding modes (FM5 and FM6). These trait attributes were positively associated with decreasing urban disturbance (Figure 4.17).

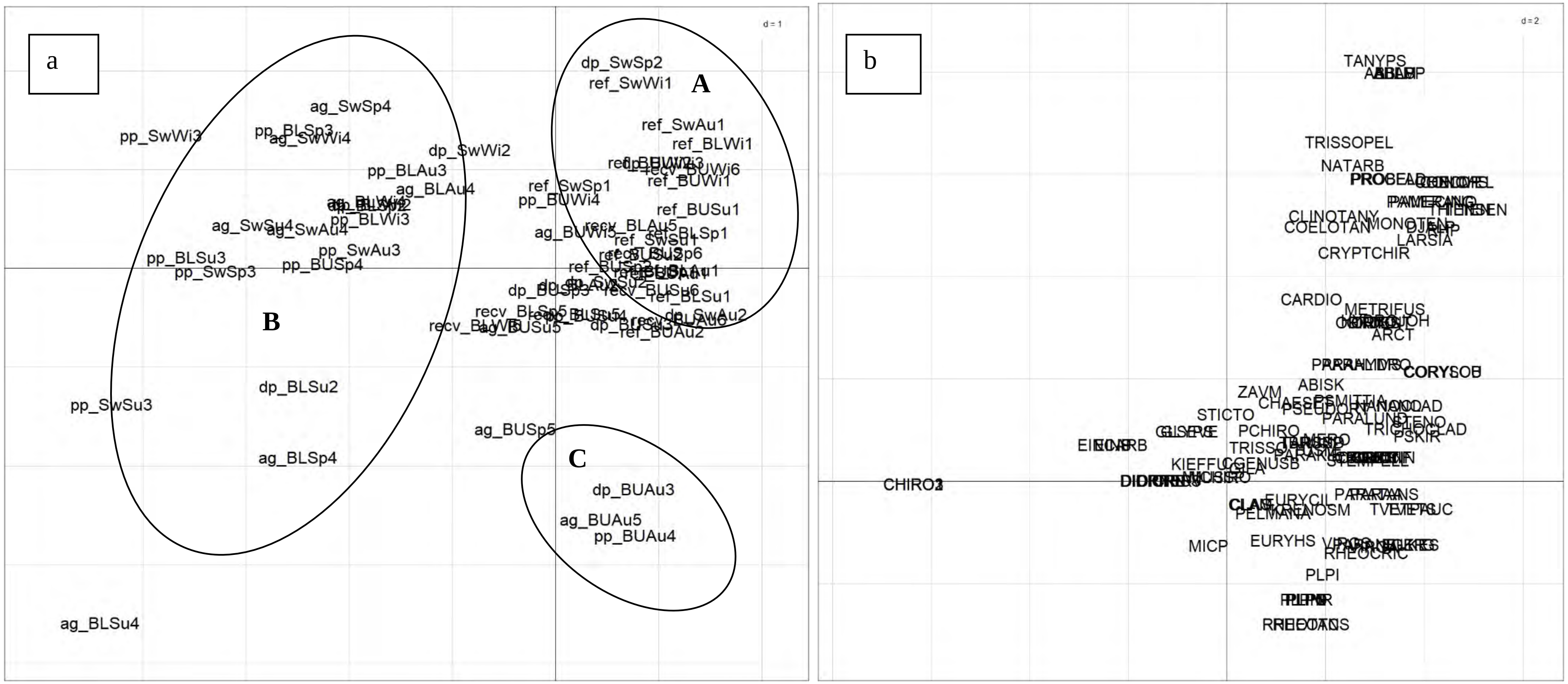


Figure 4.16 RLQ analysis showing the ordination of [a] site categories during the sampling seasons showing the clusters (A, B, and C) and [b] the distribution of chironomid species in relation to the sites. Site categories: ref = REF (least impacted sites), dp = DP (diffuse pollution impacted sites), pp = PP (point pollution impacted sites), ag = AG (agriculture + diffuse pollution impacted sites), recv = RECV (recovery sites). Seasons: Sp-spring, Su-summer, Au-autumn, Wi-winter. Study rivers: BL-Bluokrans, BU-Buffalo, SW-Swartkops. Species: CHIRO2-*Chironomus* sp2, MICP-*Microtendipes pedellus-type*, STICTO-*Stictochironomus* sp, DICRN-*Dicrotendipes notatus*-type, EURYHS-*Euryhapsis* sp, CLINOTANY-*Clinotanypus* sp, CLAM-*Cladotanytarsus mancus*-type, CARDIO-*Cardiocladius* sp, TANYPS-*Tanypus* sp, GLYPS-*Glyptotendipes* sp, EINP-*Einfeldia pagana*-type, PLPI-*Polypedilum illinoense*-type, ABLP-*Ablabesmyia phatta*-type, ZAVM-*Zavreliella marmotata*-type, PLPT-*Polypedilum tuberculum*-type, RHP-*Rheopelopia* sp, TUSM-*Tanytarsus usmaensis*-type, CRYPCHIR-*Cryptochironomus* sp, ABISK-*Abiskomyia* sp. See Appendix A, Table 2 for chironomid species abbreviations.

Chironomid species representing cluster A include *Rheopelopia sp*, *Orthocladius clarki*-type, *Zavreliella marmotata*-type, *Stenochironomus sp*, *Parametriocnemus lundbecki*-type, *Ablabesmyia* spp, *Arctopelopia* sp, *Orthocladius oliveri*-type, *Larsia sp*, *Corynoneura lobata*-type, *Abiskomyia sp, Thienemannimyia geijskesi*-type, *Monopelopia tennicalcar*-type, *Coelotanypus sp, Trissopelopia sp, Clinotanypus sp*, *Cryptochironomus sp*, *Cardiocladius sp*, *Nanocladius rectinervis*-type, *Hydrobaenus johannseni*-type, *Chaetocladius setosipennis*-type, *Metriocnemus fuscipes*-type, *Conchapelopia* spp, *Thienemannimyia senata*-type, *Pseudorthocladius sp*, *Paratanytarsus sp*, *Natarsia baltimoreus*-type, *Pseudochironomus sp*, *Djalmabatista pulcher*-type, *Tvetenia* spp, *Paramerina anomalus*-type, *Pseudosmittia sp* and *Tanypus sp* (Figure 4.16).

Traits such as the possession of haemoglobin (H1), possession of tracheal gills (X1), burrowing (SR1), whole-body undulation (M3), production of more than 1000 eggs per egg mass (EM4), collector-gathering feeding behaviour (FM2), possession of very large body size (> 20–40 mm; BS5), completion of lifecycle in more than one year (D2), possession of 3 tracheae (RP3), laying of eggs in clutches (RT1), construction of rigid tubes (TC3), preferences for fine detritus (FT1), debris less than 1 mm (FT2), plant debris greater than 1 mm (FT3) and dead animals (FT6), and preferences for litter and fine organic matter (SP6) and gravel, sand, silt and mud substrates (SP2) were positively associated with Cluster B sites (Figure 4.17). Chironomid species possessing traits representative of cluster B include *Chironomus* spp, *Microchironomus* spp, *Einfeldia* spp, *Dicrotendipes* spp, *Kiefferulus sp*., *Glyptotendipes* spp, *Microtendipes pedellus* and *Stictochironomus sp*. (Figure 4.17). The response of chironomid traits in Cluster B corresponded with the *a priori* predictions to increasing urban stressors except for very large body size (> 20–40 mm) and possession of 3 tracheae (RP3) (Table 4.1).

Collector-filterer (FM1) and shredder (FM3) feeding modes, small and medium body sizes (BS2 and BS3), production of more than 3 generations per year (V4), possession of body armour (BA1), fixed substrate relation (SR4), a preference for stone biotopes (SP1) and a preference for living microphytes (FT4) attributes were positively associated with Cluster C (DP, PP and AG sites of the Buffalo River) (Figure 4.16 and Figure 4.17). These traits followed *a priori* predictions to increase with increasing perturbation, except for FM3 (shredder feeding mode) (Table 4.1). Chironomid species representing Cluster C include *Virgatanytarsus sp*, *Euryhapsis sp*, *Rheotanytarsus* spp, *Eukiefferiella gracei*-type, *Paracricotopus niger*-type, *Polypedilum* spp and *Virgatanytarsus ardrennensis*-type (Figure 4.16).

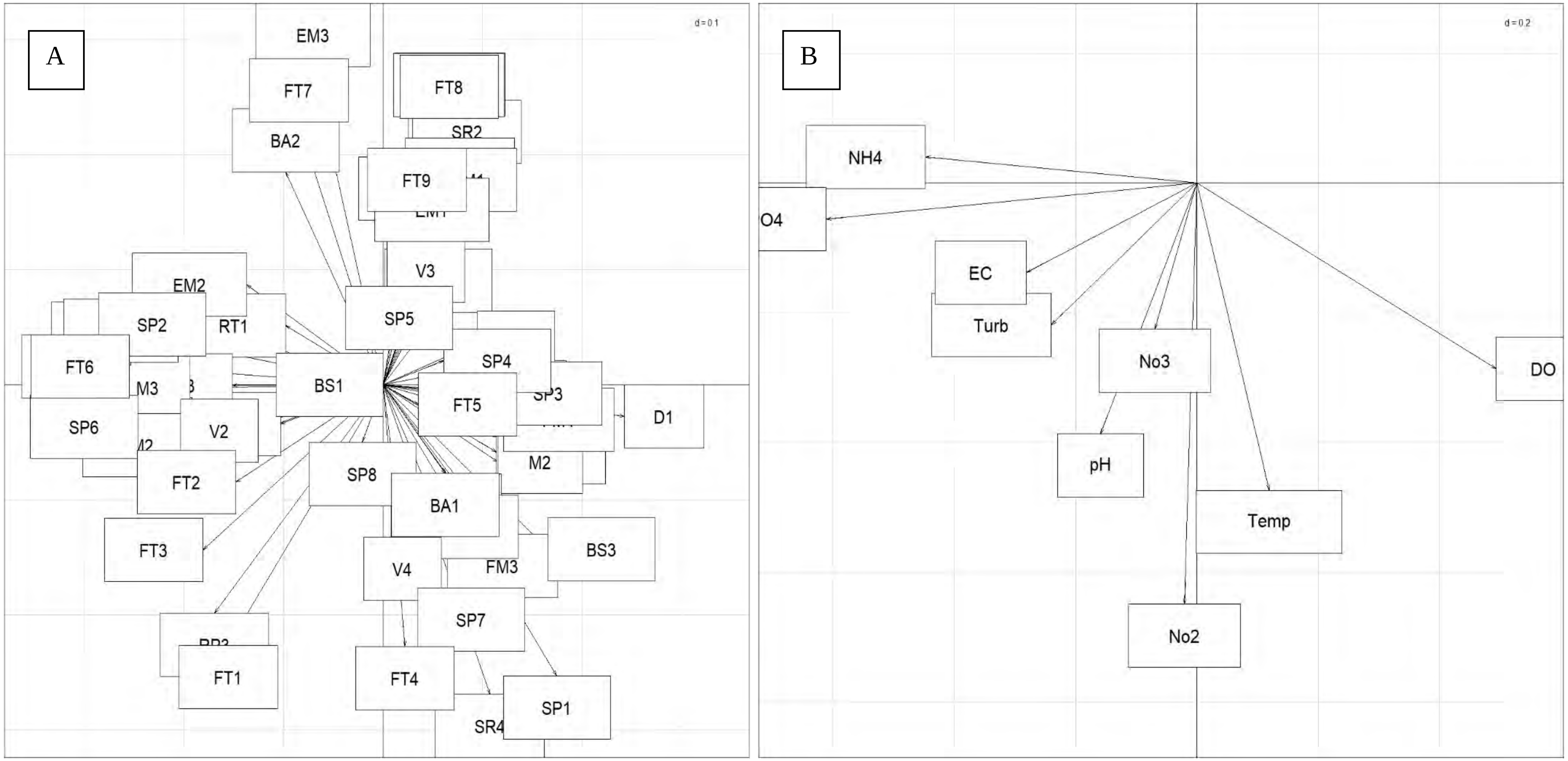
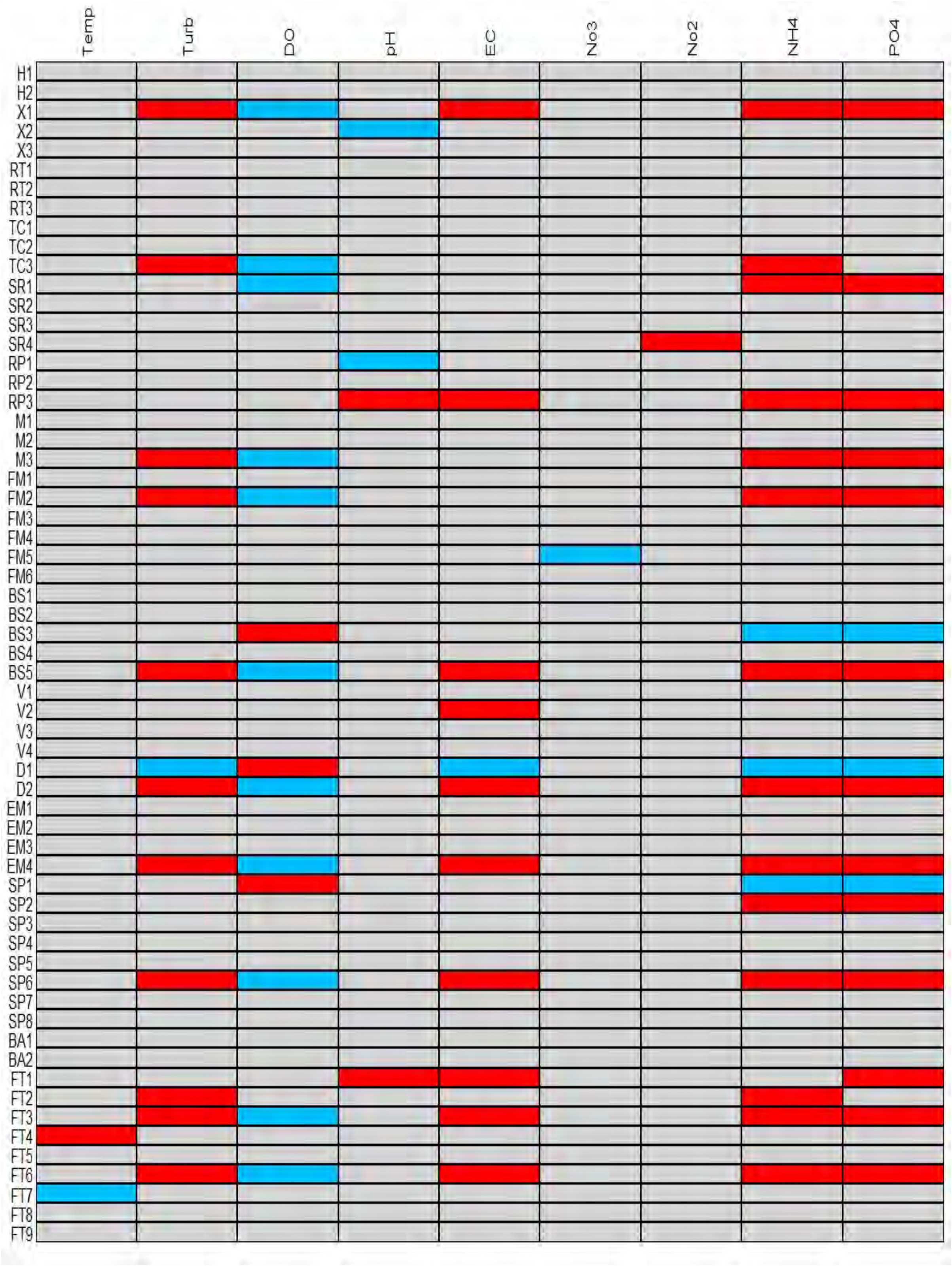


Figure 4.17 RLQ analysis showing the ordination of [A] trait attributes and [B] physicochemical variables. DO - dissolved oxygen, Temp - temperature, Turb - turbidity, EC - electrical conductivity, NO3 - Nitrate-Nitrogen, $NO_2$ - Nitrite-Nitrogen, NH4 - Ammonium, PO4 - Orthophosphate-phosphorus. See Table 4.1 for the interpretation of trait attribute abbreviations.

### 4.3.3 Identifying trait indicators of urban pollution

Of the sixty-one (61) chironomid ecological preferences and trait attributes analysed, 25 trait attributes showed significant correlations (positive or negative) with the physicochemical variables in the fourth-corner model ($p < 0.05$; Figure 4.18). Traits such as possession of tracheal gills (X1), burrowing (SR1), whole-body undulation (M3), construction of rigid tubes (TC3), possession of 3 tracheae (RP3), very large body size (BS5), collector-gathering (FM2), completion of lifecycle in more than one year (D2), production of more than 1000 eggs per egg mass (EM4), preferences for gravel, sand, silt and mud substrates (SP2) and litter and fine organic matter (SP6), and preferences for fine detritus (FT1), debris less than 1 mm (FT2), plant debris greater than 1 mm (FT3) and dead animals (FT6) were either negatively correlated with DO or positively correlated with increasing EC, pH, turbidity, $NH_4$-N and $PO_4$-P (Figure 4.18). These traits were mostly associated with Cluster B of the RLQ analysis (Figure 4.16), and therefore, were deemed tolerant of urban pollution.

Conversely, trait attributes including medium body size (BS3), cuticular respiration (X2), possession of 12 tracheae (RP1), completion of lifecycle in less than or equal to one year (D1), and a preference for stone biotopes (SP1) were either positively correlated with DO or negatively correlated with increasing EC, pH, turbidity, $NH_4$-N, and $PO_4$-P (Figure 4.18). These traits were deemed sensitive to urban pollution (Table 4.3).



| | Temp | Turb | DO | pH | EC | No3 | No2 | NH4 | PO4 |
|---|---|---|---|---|---|---|---|---|---|
| H1 | | | | | | | | | |
| H2 | | | | | | | | | |
| X1 | | 🟥 | 🟦 | | 🟥 | | | 🟥 | 🟥 |
| X2 | | | | 🟦 | | | | | |
| X3 | | | | | | | | | |
| RT1 | | | | | | | | | |
| RT2 | | | | | | | | | |
| RT3 | | | | | | | | | |
| TC1 | | | | | | | | | |
| TC2 | | | | | | | | | |
| TC3 | | 🟥 | 🟦 | | | | | 🟥 | |
| SR1 | | | 🟦 | | | | | 🟥 | 🟥 |
| SR2 | | | | | | | | | |
| SR3 | | | | | | | | | |
| SR4 | | | | | | | 🟥 | | |
| RP1 | | | | 🟦 | | | | | |
| RP2 | | | | | | | | | |
| RP3 | | | | 🟥 | 🟥 | | | 🟥 | 🟥 |
| M1 | | | | | | | | | |
| M2 | | | | | | | | | |
| M3 | | 🟥 | 🟦 | | | | | 🟥 | 🟥 |
| FM1 | | | | | | | | | |
| FM2 | | 🟥 | 🟦 | | | | | 🟥 | 🟥 |
| FM3 | | | | | | | | | |
| FM4 | | | | | | | | | |
| FM5 | | | | | | 🟦 | | | |
| FM6 | | | | | | | | | |
| BS1 | | | | | | | | | |
| BS2 | | | | | | | | | |
| BS3 | | | 🟥 | | | | | 🟦 | 🟦 |
| BS4 | | | | | | | | | |
| BS5 | | 🟥 | 🟦 | | 🟥 | | | 🟥 | 🟥 |
| V1 | | | | | | | | | |
| V2 | | | | | 🟥 | | | | |
| V3 | | | | | | | | | |
| V4 | | | | | | | | | |
| D1 | | 🟦 | 🟥 | | 🟦 | | | 🟦 | 🟦 |
| D2 | | 🟥 | 🟦 | | 🟥 | | | 🟥 | 🟥 |
| EM1 | | | | | | | | | |
| EM2 | | | | | | | | | |
| EM3 | | | | | | | | | |
| EM4 | | 🟥 | 🟦 | | 🟥 | | | 🟥 | 🟥 |
| SP1 | | | 🟥 | | | | | 🟦 | 🟦 |
| SP2 | | | | | | | | 🟥 | 🟥 |
| SP3 | | | | | | | | | |
| SP4 | | | | | | | | | |
| SP5 | | | | | | | | | |
| SP6 | | 🟥 | 🟦 | | 🟥 | | | 🟥 | 🟥 |
| SP7 | | | | | | | | | |
| SP8 | | | | | | | | | |
| BA1 | | | | | | | | | |
| BA2 | | | | | | | | | |
| FT1 | | | | 🟥 | 🟥 | | | | 🟥 |
| FT2 | | 🟥 | | | | | | 🟥 | |
| FT3 | | 🟥 | 🟦 | | 🟥 | | | 🟥 | 🟥 |
| FT4 | 🟥 | | | | | | | | |
| FT5 | | | | | | | | | |
| FT6 | | 🟥 | 🟦 | | 🟥 | | | 🟥 | 🟥 |
| FT7 | 🟦 | | | | | | | | |
| FT8 | | | | | | | | | |
| FT9 | | | | | | | | | |

Figure 4.18 Results of the fourth corner analysis showing the correlation between chironomid trait attributes and physicochemical variables. Red indicates a significant positive correlation, blue indicates a significant negative correlation ($p \leq 0.05$), while grey indicates no significant correlation.

Table 4.3 Traits and ecological preferences identified as sensitive or tolerant of urban pollution

| **Pollution sensitive traits** | **Pollution tolerant traits** |
|---|---|
| Medium body size | Tracheal gills |
| Cuticular respiration | Rigid tubes |
| Predator feeding mode | Burrowing |
| Preference for stone biotope | Whole-body undulation |
| Completion of lifecycle within 1 year | Very large body size |
|  | Completion of lifecycle within >1 year |
|  | Collector-gatherer |
|  | Laying more than 1000 eggs per egg mass |
|  | Bivoltinism |
|  | Preferences for fine detritus |

## 4.4 Discussion

### 4.4.1 Response patterns of chironomid traits and ecological preferences to pollution in the selected urban river systems

The spatial-temporal distribution of chironomid species traits and ecological preferences in response to urban stressors were analysed in this chapter. The results of this study show that, as with taxonomic approaches, the assemblage of traits at a site can be modified by anthropogenic factors (Usseglio-Polatera et al., 2000; Gayraud et al., 2003; Tomanova et al., 2008). Chironomid traits responded markedly to deteriorating water quality at the impacted study sites and were able to differentiate between least impacted sites (REF) and recovery sites (RECV). Trait attributes such as cuticular respiration, medium body size, completion of lifecycle in 1 year, predator feeding mode and a preference for stone biotopes decreased with increasing urban disturbance. In contrast, tracheal gills, collector-gatherer, laying more than 1000 eggs per egg mass, bivoltinism, burrowing, very large body size, construction of rigid tubes, completion of lifecycle within more than 1 year, and preference for fine detritus increased with urban perturbation in this study. Some of these traits and ecological preferences have been reported in impacted systems (Kuzmanovic et al., 2017; Edegbene et al., 2020a; Paz et al., 2022).

Abiotic conditions in aquatic systems can act as filters on aquatic organisms (Townsend & Hildrew, 1994; Poff et al., 2006; Statzner & Bêche, 2010). For example, rivers with depleted oxygen concentrations owing to increasing urban-sourced organic pollution are likely to contain organisms which have adaptive survival features. This may be a reasonable explanation for the high relative abundance of chironomids possessing tracheal gills at the highly impacted sites (DP, PP, and AG) compared to the least impacted (REF) and recovery sites (RECV) where DO concentrations were low and EC levels were high (see Chapter 3 Section 3.3.1). A similar pattern was observed for the haemoglobin trait, where chironomids which possessed the haemoglobin pigment dominated the abundance of chironomid assemblages at the DP, PP and AG sites during the study period. Haemoglobin in chironomids has a high affinity for oxygen and confers resilience or resistance to low oxygen concentrations in aquatic systems (Armitage et al., 1995; Porinchu & MacDonald, 2003; Jovem-Azevêdo et al., 2019). Notably, chironomid species which utilise solely cuticular respiration and do not possess haemoglobin are suited to water of good ecological quality such as that found at the REF and RECV sites. Moreover, improved water quality conditions (specifically increased oxygen levels) at some AG sites during autumn may be responsible for the transition from an assemblage of tolerant species (e.g., *Chironomus* sp1, *Polypedilum* spp, and *Dicrotendipes lobiger*-type) to the occurrence of species that are uncharacteristic of sites impacted by effluent discharge from upstream WWTWs, stormwater runoff from informal settlements and agricultural activities (e.g., *Corynoneura lobata*-type, *Eukiefferiella gracei*-type and *Abiskomyia sp*). These responses of chironomid traits to urban perturbation are consistent with studies which identified oxygen concentration as an important factor in determining the functional composition of aquatic communities (Rossaro et al., 2007; Rossaro et al., 2022).

The response of the body size trait to deteriorating stream water quality has been employed in numerous trait-based studies relating to anthropogenic stressors (Odume et al., 2014; Kuzmanovic et al., 2017; Paz et al., 2022). In this study, the relationship between chironomid body size distribution and physicochemical variables was explored. Chironomid species possessing very large body size dominated the assemblage structure at the highly impacted sites (DP, PP and AG), whereas species which possess the medium body size class were dominant at the least impacted (REF) and recovery (RECV) sites. This observation corresponds with the habitat template concept which postulates that sites with similar abiotic conditions will contain specific traits suited to that environment, regardless of taxonomic identities (Southwood, 1977; Townsend & Hildrew, 1994). Here, the body size trait attributes at the DP, PP and AG sites were significantly similar, but different from the least impacted sites. The logical explanation to the fact that chironomid species of very large body size (> 20–40mm) inhabited impacted water systems could be linked to adaptations suited to reducing the diffusion of dissolved solids associated with urban pollution (Odume et al., 2014). A large surface area to volume ratio presents aquatic organisms of small sizes with a large area for simple diffusion of salts to occur. Thus, the presence of small body-sized chironomids at the DP, PP and AG sites may have been impacted by the diffusion of pollutants, resulting in their elimination. On the other hand, large organisms are more likely to survive in water with elevated dissolved solids due to their small surface area to volume ratio, which reduces their exposure to pollutants through dermal contact (Statzner & Bêche, 2010). Similar observations of body size distribution in response to anthropogenic disturbances have been observed in the literature (Dolédec & Statzner, 2008; Statzner & Bêche, 2010; Odume et al., 2014).

Chironomids which take more than one year to complete their lifecycle were abundant at the DP, PP and AG sites, contrary to *a priori* predictions that impacted sites would contain chironomid species which exhibit a shorter lifecycle duration. Moreover, species with more than one generation per year and a short lifecycle duration were favoured at the least impacted sites in this study. Serra et al. (2017) noted a similar pattern while studying the impact of anthropogenic impacts on chironomid life strategies in Mediterranean streams. They attributed this occurrence to the lifecycle trait plasticity of species such as *Cricotopus*, which are known to occur in some disturbed sites (Serra et al., 2017b). In this study, however, the phenomenon proposed by Serra et al. (2017) may be attributed to the abundance of species of the genus *Chironomus* which inhabited the highly impacted sites, and exhibit plasticity of the lifecycle duration trait in the literature (see chironomid database by Serra et al., 2016 and associated references). In addition, as Postma et al. (1995), Serra et al. (2017) and Marziali et al. (2019) noted, changes in the timing of emergence to confer better species adaptation and survivability may be a possible reason for this occurrence. This is supported by the low relative abundance of *Chironomus* spp at the DP, PP and AG sites during the autumn season, when stream water quality appeared to be improving, stressing the importance of phenological data derived from chironomids in the locality of study.

Urban-impacted aquatic systems present chironomids with serious challenges in terms of reproductive success and the survival of offspring because chironomids are largely oviparous (Armitage et al., 1995). The production of numerous eggs in a single batch may be an adaptation for survival in severely impacted water. Hale et al. (2019) found that the hatching success of chironomid eggs decreased with increasing dissolved salts (indicated by electrical conductivity). In this study, the chironomid trait attribute of laying more than 1000 eggs per egg mass was negatively correlated with DO and positively correlated with EC, turbidity, and orthophosphate, associating the trait attribute with highly impacted sites in the RLQ ordination.

Although species which exhibit the collector-gatherer feeding mode were present at all the site categories in this study, they were strongly associated with the impacted sites. Collector-gatherers have been known to feed on fine particulate organic matter and detritus, which is usually abundant at sites impacted by elevated sediment and suspended solids (Palmer et al., 1996; Savić et al., 2017; Antczak-Orlewska et al., 2021). The abundance of fine detrital material at the impacted sites may be responsible for the relatively high abundance of collector-gatherers at the DP, PP and AG sites. Conversely, shredders were more abundant at the least impacted sites (REF) compared to the impacted sites, following the predictions in this study. The abundance of shredders may likely be related to canopy cover and riparian vegetation composition (i.e., food sources) that was present at the REF sites. Although the Kruskal-Wallis test indicated that the width of the site categories was not statistically significantly different, a considerable difference in the mean width between the RECV sites (average width of 23.17 m) and other site categories (REF - 6.90 m, DP - 10.67 m, PP - 9.09 m, and AG - 8.38 m) was recorded. Moreover, the RECV sites had a statistically significantly low percentage canopy cover compared to REF, PP, and AG sites (Table 3.2). However, shredders dominated the RECV sites downstream of the impacted sites in this study, indicating that there may be other factors involved in shaping the feeding assemblage structure at a site, a point which requires further exploration. Studies have recorded high predatory activities of macroinvertebrates in impacted systems (Kuzmanovic et al., 2017; Desrosiers et al., 2019). However, in this study, predatory feeding modes (piercer and engulfer) were significantly associated with the least impacted sites. Predators utilise visual cues (amongst other adaptive feeding strategies) in identifying and capturing prey (Akamagwuna, 2018). It is possible that their relatively low abundance in this study can be attributed to elevated suspended solids and turbidity at the impacted sites, which reduced the penetration of light through the water column and limited their ability to acquire food (Jones et al., 2012) or through biomagnification of pollutants across the food chain (Dolédec & Statzner, 2008; Kuzmanovic et al., 2017).

Substrate-related and motility traits such as burrowing and swimming are associated with species resilience, and have been predicted to confer resistance to urban perturbation (Statzner & Bêche, 2010; Mathers et al., 2017). Although burrowers were relatively abundant and significantly associated with the impacted sites (DP, PP and AG), the relative abundances of swimmers and crawlers were significantly low at the impacted sites and followed the predictions of this study. Burrowers prefer sediments, and may be favoured in highly disturbed sites, whereas crawlers lack the ability to immediately respond to depleted oxygen concentration or sediment input and may be easily eliminated at impacted sites (Wilkes et al., 2017). Swimmers, on the other hand, have been reported to actively respond to perturbation by escaping from impacted sites (Odume, 2020). This provides a plausible explanation for the low relative abundance of swimmers at the impacted sites in this study.

The results show that there exist relationships between substrate types and the distribution of chironomid substrate preferences in relation to urban perturbation. Chironomids that preferred macrophytes and filamentous algae dominated the relative abundances of the assemblages at the REF and RECV sites in all the seasons (Figure 4.13). Also, compared to the REF and RECV sites, the relative abundance of chironomid species that preferred litter and fine organic matter substrates increased at the DP, PP, and AG sites. Indeed, substrate preferences indicate significant associations between substrate size and traits. However, it is the relationship between organism preferences and the type of biotopes/substrate they prefer (rather than the size of the biotope/substrate) that was explored in this study. The quantification of streambed stability involves specific measurements along different hydraulic habitats (pool, riffle, run) for transect-divided stream reaches (Kusnierz & Holbrook, 2017; Kaufmann et al., 2022) which was not deemed necessary in this study and, therefore, not done taking the available resources into consideration. The relation of traits and biotope type (vegetation, stones, sediment) based on the sampling protocol employed in this study was deemed sufficient to provide an understanding of the impact of urbanisation on the distribution of chironomids and their vulnerability to impacts based on the traits they possess. In essence, the deteriorated water quality at urban-impacted sites imposes a constraint on the expression of organism traits in so much that even though there are surplus available substrates at a site, only organisms that are not eliminated by poor water quality can utilise the substrates based on the traits and ecological preference they possess. Nevertheless, it is recommended that future studies on the impact of urbanisation on chironomid assemblage structure take into account the quantification of relative streambed stability to determine if substrate size is natural or anthropogenic. It is also recommended that the analysis of food sources that are associated with the availability of substrates (for example, through stable isotope analysis) be put into consideration when designing research related to the impact of urbanisation on freshwater systems.

Overall, urban perturbation evidently constrained chironomid species assemblages, causing a selection of traits which respond to a combination of low dissolved oxygen concentrations, elevated electrical conductivity owing to increased dissolved solids and high sediment input. Chironomid species were able to respond to abiotic factors by possessing two or more trait attributes, including possession of haemoglobin, tracheal gills, burrowing, whole-body undulation, laying of more than 1000 eggs per egg mass, possessing collector-gatherer trait, very large body size, possession of 3 tracheae, construction of rigid tubes, and a preference for fine detritus, fine organic litter, and gravel, sand and mud substrates.

### 4.4.2 Identifying indicator traits and ecological preferences of urban pollution

In this chapter, the correlations of chironomid ecological preferences and traits with physicochemical variables were explored to identify indicator traits of urban pollution. Unlike taxonomic approaches, trait-based indicators can provide insight into the functional aspects of riverine community responses to urban disturbance. For example, traits such as possession of tracheal gills, rigid tube construction, long lifecycle duration, laying more than 1000 eggs per egg mass, very large body size, multivoltinism, burrowing, collector-gathering and a preference for fine detritus increased with urban perturbation and were deemed to be pollution-tolerant traits (Table 4.3). In contrast, a short lifecycle, medium body size (>5–10 mm), cuticular respiration, predator feeding mode and a preference for stone biotopes were deemed traits sensitive to urban disturbance because they were positively correlated with physicochemical indicators of good quality water (increasing DO levels) or negatively correlated with physicochemical indicators of deteriorating quality water (elevated EC, nutrients, turbidity).

The fourth-corner analysis also indicated traits which were linked to increasing temperature (e.g., preference for living microphytes), pH (e.g., possession of 3 trachea) and $NO_2$-N (fixed substrate relation). However, these were excluded from the final checklist of trait-based indicators of urban pollution because of their association with other selected traits or because they require further exploration to fully elucidate their association with the physicochemical variables in this study. For example, the possession of 3 tracheae is linked to the possession of tracheal gills and haemoglobin and would only provide redundant information regarding the selection of organisms with the ability to survive in low-oxygen environments. In another example, the preference for living microphytes was associated with increasing temperature and clustered with AG sites but was not associated with increasing nutrients, which are characteristic of biofilm production. This association could be due to changing water quality parameters at the AG sites during summer and autumn, which significantly shifted the trait assemblage structure at the sites, allowing for the presence of grazers. Notwithstanding, further investigation is required to fully uncover the reason for this association between grazers and temperature at sites with improving water quality conditions.

## 4.5 Conclusion

This study showed that chironomid species-level traits responded markedly to deteriorating water quality in the study rivers and were able to differentiate between the various site categories. Moreover, chironomid species traits enabled the identification of sensitive and tolerant traits linked to urban pollution. For example, traits such as the possession of tracheal gills, rigid tube construction, long lifecycle duration, laying more than 1000 eggs per egg mass, very large body size, multivoltinism, burrowing, collector-gathering and a preference for fine detritus which increased with urban perturbation were identified as pollution-tolerant traits. Sensitive chironomid traits included a short lifecycle, medium body size, cuticular respiration, predator feeding mode and a preference for stone biotopes. The results show that some of the traits confirmed the *a priori* predicted responses of chironomid traits to increasing urban disturbance. Therefore, the assemblage structure of chironomid species traits may solely be relied upon to provide accurate descriptions of the functional structure of urban streams.

However, as species do not respond to a disturbance using single traits, it is important to identify strategies by which chironomids are predisposed to the negative consequences of urban perturbation. One limitation of this study is the lack of sufficient autecological information generated specifically from Afrotropical chironomid species. Although a macroinvertebrate trait database for South Africa exists, an abundance of information on chironomid species ecology and biology (especially life-history traits) is yet to be collected.

# CHAPTER 5 – DEVELOPING A TRAIT-BASED APPROACH FOR PREDICTING THE RESILIENCE AND VULNERABILITY OF CHIRONOMIDS TO URBAN POLLUTION

## 5.1 Introduction

Urban pollution represents a critical ecological risk to riverine ecosystems (Walsh et al., 2005; Mackenzie et al., 2022; Roux & Clinton, 2023). The effects of urban pollution on aquatic biota can be direct, e.g. the burial of organisms due to sediment accretion, or indirect, through the modification of stream riparian zones or food quality (Finkenbine et al., 2000). For example, urban pollution alters the physical and chemical properties of aquatic systems through excessive urban stormwater return flow and direct discharges of effluents from households and industrial activities to streams (Brown et al., 2005; Masoner et al., 2019; Strokal et al., 2021). Taylor et al. (2005), Hayashi et al. (2013) and Mackenzie et al. (2022) noted elevated levels of organic pollutants, suspended solids, phosphorus and nitrogen in urban stormwater. Organic and inorganic pollutants have been reported to reduce dissolved oxygen concentrations and, consequently, the diversity and richness of aquatic communities (Stribling & Dressing, 2015; Voß & Schäfer, 2017; Odume, 2020).

The complex impacts of urban pollution on rivers can influence their dynamics and alter the structural and functional composition of biota (Segner et al., 2014; Edegbene et al., 2020b). Therefore, it is imperative to develop predictive tools which can predict the potential vulnerability and resilience of aquatic biota to urban pollution. In order to effectively tackle the challenge of predicting and managing the changes associated with the impacts of urban pollution on aquatic biota, Segner et al. (2014) suggested the use of “biological receptors” (used loosely to refer to organism traits) instead of stressor properties.

Trait-based approaches (TBA) are increasingly being used globally to assess the responses of biotic organisms to aquatic ecosystem stressors (Webb et al., 2010; Merritt et al., 2017; Benzina et al., 2021). For example, macroinvertebrate traits have been shown to respond markedly to agrichemicals (Liess & Von Der Ohe, 2005; Kuzmanovic et al., 2017; Collins & Fahrig, 2020), stream flow alteration (Lange et al., 2014; Scholl et al., 2016), surface water abstraction (Wooster et al., 2011) and urban pollution (Edegbene et al., 2020b; Paz et al., 2022). An important advantage of the trait-based approach is its predictive power, that is, when the environmental conditions are known, one can predict the likelihood of a species surviving in an environment based on the traits the species possesses. This comparative advantage of the trait-based approach is further explored in this chapter.

The traits of an organism mediate its relationship with the external environment (Verberk et al., 2008a; Menezes et al., 2010; Pyne & Poff, 2017). They influence the adaptive capacity of an organism to disturbances. The adaptive capacity of a species to its external environment is not influenced by a single trait, but a combination of traits, interacting among themselves in ways that allow the organism to adapt to its external environment (Verberk et al., 2008b). The implications are that predicting species which may become vulnerable or resilient to urban pollution requires three important considerations: 1) understanding the important urban pollution stressors which drive biological assemblage change; 2) understanding the combination of traits a species must possess to either become resilient or vulnerable to the diversity of stressors associated with urban pollution; and 3) the complex interactions between the stressors and traits which result in the filtering of species (Poff, 1997; Verberk et al., 2008b; Menezes et al., 2010). In this regard, traits which confer resilience on a species in the face of an environmental disturbance have been termed resilient traits, whereas those which predispose a species to the impact of the disturbance are termed vulnerable traits (Odume et al., 2018; Odume, 2022).

In this chapter, a trait-based approach for predicting the resilience and vulnerability of chironomids to urban pollution was elucidated by addressing the following specific sub-objectives: i) develop a trait-based approach for classifying chironomid species into vulnerability groups in relation to urban pollution and ii) use the developed trait-based approach to predict the assemblage responses of chironomid species to urban pollution. The approach followed in this chapter differs from that in Chapter 4 in that, instead of predicting the responses of individual traits, I predicted the species or groups of species based on the combination of traits they possess and the interactions of these traits with urban pollution. Because the outcome of the prediction is the species, instead of individual traits, this approach is useful for conservation and biotic management.

## 5.2 Materials and methods

### 5.2.1 Developing the trait-based approach for predicting the potential vulnerability of chironomid species to urban pollution

In predicting chironomid responses to urban pollution, a rule-based approach involving an understanding of the mechanistic role played by traits in species-environment interactions was adopted. This rule-based approach was developed by Odume et al. (2018) and applied by Libala et al. (2020) in investigating the effect of livestock grazing on hillslope seep wetland vegetation, and by Ntloko (2022) in assessing the impact of sediment stress on macroinvertebrate taxa. The approach involves six steps: i) reviewing the literature for modes of urban pollution impact on chironomids, ii) identifying traits that are mechanistically linked to each mode of urban impact, iii) identifying potentially vulnerable trait attributes per species; iv) identifying non-redundant vulnerable trait attributes per species, v) quantifying the diversity of trait expression per trait and per species; and, vi) calculating potential chironomid vulnerability based on the combination of trait attributes possessed, diversity of trait expression, and non-redundant vulnerable trait attributes.

#### *Reviewing the literature for modes of urban pollution impact on chironomids*

The literature was extensively reviewed to identify reported mechanisms by which urban pollution may impact chironomids. Urban pollution modes of stress could be through direct or indirect mechanisms, as summarised in Table 5.1.

Table 5.1 A summary of urban pollution modes of stress on chironomids

| Mode of stress | Notes |
|---|---|
| Dissolved oxygen depletion | Oxygen is required to perform various biological functions, including the production of energy (Kooijman, 2011). The influx of organic pollutants from urban catchments, coupled with elevated microbial organic matter processing, results in the depletion of dissolved oxygen levels (Delzer & Mckenzie, 2003). Dissolved oxygen concentrations below 8% saturation have been reported to result in chironomid mortality (Connolly et al., 2004). Low dissolved oxygen concentration can alter normal biological processes. For example, a reduction in the |

|  |  |
|---|---|
|  | emergence of chironomids was observed at low dissolved oxygen concentrations (Connolly et al., 2004). Moreover, Walshe (1950) observed that at dissolved oxygen saturation levels below 10%, *Chironomus sp* halted its feeding and tube-irrigation behaviours. |
| Sediment accretion | Sediment accretion due to urbanisation results in riparian modification, loss of refugia, physical damage to morphological structure and modification of food quality and acquisition (Alberti et al., 2007; Jones et al., 2012). Elevated sediment influx into streams may cover the surfaces of substrates, reducing the available food resources for shredders and grazers (Wilkes et al., 2017; Ntloko et al., 2021). Elevated suspended sediment levels may result in increased turbidity and impact the visibility of predators which require visual clarity for food acquisition (Wilkes et al., 2017). |
| Pollutants | Urban stormwater carries myriads of pollutants, including heavy metals, suspended solids, agrichemicals, nutrients, oil and grease, from urban catchments to rivers and streams (Masoner et al., 2019; Robertson et al., 2019; Kolbenschlag et al., 2023). Pollutants such as metals have been reported to delay the emergence of chironomids by causing them to invest more energy in dealing with toxic stress rather than in growth and development (Arambourou et al., 2020). Moreover, pollutants cause mentum deformities in chironomids, which has a direct effect on food acquisition (Odume et al., 2016; Deliberalli et al., 2018). |
| Flow and habitat alteration | Fluctuating flow regimes occasioned by urbanisation affect chironomids through direct and indirect means (Covich et al., 1978). The increase in the frequency and rate of storm water return flows can affect stream morphology, alter sediment regime and impact substrate composition (Finkenbine et al., 2000; Brown et al., 2005). Substrate composition has a direct influence on the provision of refugia for chironomids. Increased substrate scouring by high velocities may alter substrate dynamics, leading to coarse substrate (Growns et al., 2017). |

| | |
|---|---|
| | The drift and emergence patterns of some chironomid species are synchronised with seasonal fluctuations in streamflow (Serra et al., 2016). Thus, altered flow patterns can fluctuate the synchrony of chironomid life-history patterns and negatively affect their emergence and survival (Castro-Rebolledo & Donato-Rondon, 2015; Schulz et al., 2019). Flow alteration can also affect physicochemical properties (such as light penetration and temperature, oxygen levels and depth) which construct the habitat conditions within which organisms survive (Rolls et al., 2012). Moreover, a direct effect of flow alteration can be the washing away of chironomid communities which are not adapted to high flow velocities (Covich et al., 1978). |

### ***Trait selection***

Fifteen (15) categories of traits and ecological preferences were selected and resolved into 58 trait attributes (Table 5.2). Traits and ecological preferences related to chironomid life history (lifecycle duration, voltinism, reproduction type, and number of eggs per egg mass), morphology and physiology (haemoglobin, maximum body size, body armour, respiratory structure and number of tracheae), behaviour (motility, feeding mode, tube construction and substrate relation), and ecological preference (food type and substrate preference) were selected. Information on chironomid species-level traits and ecological preferences was obtained from the South African macroinvertebrate trait database (Odume et al., 2023), European chironomid trait database (Serra et al., 2016), freshwater ecology database (Schmidt-Kloiber & Hering, 2015, 2023; Brabec et al., 2023), literature on various studies (Walshe, 1950; Walshe, 1951; Forsyth & McCallum, 1978; Forsyth, 1979; Roback, 1981; Goddeeris, 1989; Nolte, 1992; Berg & Hellenthal, 1992; Dettinger-Klemm, 2003; Jacobsen & Bilyj, 2007; Moller Pillot, 2013; Rossaro et al., 2016; Imada, 2020) and laboratory measurements/observations.

Table 5.2 Selected chironomid traits and ecological preferences, urban stressors of interest, and vulnerable traits with the rationale and mechanistic link to urban stressors

| **Trait category** | **Trait attributes** | **Urban stressor** | **Vulnerable trait attributes** | **Rationale** |
|---|---|---|---|---|
| Haemoglobin | Present<br>Absent | Oxygen depletion | Absence of haemoglobin | Haemoglobin has been reported to confer resilience to species in low-oxygenated water bodies due to the high affinity of haem groups to oxygen in aquatic systems (Walshe, 1950). Thus, chironomids which do not possess haemoglobin are likely to be eliminated by low dissolved oxygen concentrations. |
| Respiration | Tracheal gills<br>Cuticular respiration | Elevated organic matter, sediment accretion | Cuticular respiration | Tracheal gills provide a high surface area to volume ratio for the diffusion of dissolved oxygen (Seymour & Matthews, 2013). Chironomids without tracheal gills are prone to impacts of depleted oxygen through abrasion and burial of their cuticle. |
| Tube construction | Absence of tube<br>Tube without shape | Sediment input, flow alteration | Absence of tube | Tube-dwelling protects a species from dissolved solids and contaminants (Halpern et al., 2002). Chironomids which do not construct tubes are prone |

| | | | | |
|---|---|---|---|---|
| | Tube rigid | | | to the impact of sedimentation on morphological and physiological features (Armitage et al., 1995; van Kleef et al., 2015; Serra et al., 2017b). |
| Reproduction type | Egg in clutches<br>Asexual reproduction | Organic pollution | Egg in clutches | Pollutants, including metals and endocrine disruptors, have been reported to modify gene expressions in chironomids and cause damage at the genetic level (Arambourou et al., 2020). Chironomid species which lay sexually produced eggs in impacted water are vulnerable to pollutants, including endocrine disruptors. |
| Substrate relation | Burrower<br>Free-living<br>Miner<br>Fixed | Flow alteration, organic pollution | Fixed<br>Free-living | Substrate relation describes an organism's use of aquatic substratum during its larval stages. Organisms which are permanently attached to the substrate (fixed), such as stones and macrophytes, are likely to be more affected by flow alteration because they lack the ability to escape during adverse environmental conditions (Armitage et al., 1995). |
| Number of tracheae | 12 tracheae | Pollutants | 12 tracheae | Cuticular respiration depends on the uptake efficiency of dissolved oxygen and its distribution |

|  |  |  |  |  |
|---|---|---|---|---|
|  | 6 tracheae<br>3 tracheae |  |  | within the body of the organism (Armitage et al., 1995; Serra et al., 2017a). Chironomids possessing a higher number of tracheae do not possess functional haemoglobin or tracheal gills and can be impacted at multiple cuticular sites by elevated dissolved solid concentrations. |
| Mobility | Swimmer/Sprawler<br>Crawler/climber<br>Whole-body undulator | Organic pollution, habitat modification, low dissolved oxygen | Crawler/climber | Mobility determines whether an organism can escape from impending danger, move into refugia during disturbance and re-establish when conditions become favourable (Townsend & Hildrew, 1994; Lamouroux et al., 2004). Urban pollutants modify river substratum and water chemistry and may cause the burial of organisms. Less motile species, such as crawlers, may be vulnerable to burial by sediment, oxygen depletion and substrate modification (Wilkes et al., 2017). |
| Feeding mode | Collector-filterer<br>Collector-gatherer | Organic pollution, sediment input, habitat modification | Collector-filterer | Elevated nutrient concentrations and sediment associated with organic pollution can alter the quality and availability of food resources. Collector-filterers and shredders may have their feeding |

| | | | | |
|---|---|---|---|---|
| | Shredder<br>Scraper/grazer<br>Predator-engulfer<br>Predator-piercer | | Shredder, predator-engulfer, predator-piercer | apparatus clogged by excess sediment accretion and poor food quality. Predators are likely to be vulnerable in such environments due to low visibility in turbid water (Akamagwuna, 2021). |
| Maximum body size (mm) | > 2.5–5<br>> 5–10<br>> 10–20<br>> 20–40 | Increased dissolved solids and contaminants | > 2.5–5<br>> 5–10 | Cuticular exposure to contaminants may predispose chironomids to severe impacts due to the influence of body surface area-to-volume ratio on contaminant uptake. Thus, individuals with small body sizes and a high body surface area-to-volume ratio may be vulnerable to contaminants (Paul & Meyer, 2001; Dolédec & Statzner, 2008). |
| Voltinism (number of generations per year) | 1<br>2<br>3<br>> 3 | Organic pollution, elevated metals | 1 | Voltinism determines the recovery rate of a species from adverse urban perturbations (Dolédec & Statzner, 2008). Disturbances in riverine systems may select individuals with an increased number of reproductive cycles per year and higher recovery rates (Groenendijk, 1999; Lamouroux et al., 2004; Serra et al., 2017b). In contrast, species which |

| | | | | |
|---|---|---|---|---|
| | | | | produce only one generation per year are vulnerable to adverse urban impacts. |
| Lifecycle duration (year) | ≤ 1<br>> 1 | Organic pollution, elevated metals | ≤ 1 | Lifecycle duration is critical because it determines the duration of exposure of a species to harsh environmental conditions. Species which are adapted to completing their lifecycle within one year may lack the ability to withstand urban impact, whereas those which complete their lifecycle in more than one year can hibernate and continue their development when conditions are favourable. |
| Number of eggs per egg mass | < 100<br>100–500<br>500–1000<br>> 1000 | Heavy metals, increased temperature from effluent discharge | < 100<br>100–500 | With increasing urban perturbation, eggs of species may be vulnerable to a highly toxic environment occasioned by metals (Edegbene et al., 2021). Thus, species which lay fewer eggs per egg mass are less likely to survive. |
| Substrate preference | Stone/cobble/boulders<br>Gravel, sand, mud, silt | Flow and habitat modification, sediment accretion | Stone/ cobble/ boulders, | The potential homogenisation of aquatic habitats can negatively impact macroinvertebrate assemblage structure. Gradual and sustained sediment accretion modifies the stability of substrates and fills up |

| | | | | |
|---|---|---|---|---|
| | Macrophyte & filamentous algae<br>Microphytes (microscopic algae)<br>Twigs, roots<br>Litter, fine organic matter<br>Wood<br>Moss | | Macrophyte & filamentous algae | interstitial spaces between stones, rendering taxa with a preference for more stable substrates such as stone and vegetation more likely to be vulnerable. The absence of stable substrates diminishes the availability of refugia (Serra et al., 2017b). |
| Body armour | Present<br>Absent | Elevated dissolved solids and pollutants | Absence of body armour | Body armour in the form of transportable cases or cases with feeding apparatus protects a species from direct contact with dissolved solids and metal. Organisms without body armour are more exposed to chemical uptake than those with body armour (Collins & Fahrig, 2020) |
| Food type | Fine sediment<br>Debris < 1mm | Sediment input, low dissolved oxygen, habitat modification | Living microphytes | An increase in turbidity can reduce the light penetration available for photosynthetic organisms such as living microphytes (periphyton) to function, |

| | | | | |
|---|---|---|---|---|
| | Plant debris > 1 mm<br>Living microphytes<br>Living macrophytes<br>Dead animals<br>Living microinvertebrates<br>Living macroinvertebrates<br>Wood | | Living macroinvertebrates | affecting the organic content of streams and the transfer of energy up the food chain. An impact on the trophic cascade by elevated sediment influx can reduce the availability of living macroinvertebrates preferred by predators. |

### *Identifying vulnerable trait attributes*

Potential trait attributes were identified per trait category (Table 5.2) following Odume et al. (2018), Akamagwuna (2018), Ruse et al. (1998) and Serra et al. (2017b).

### *Functionally redundant trait attributes*

Functional redundancy (FR) is a property of ecosystems where two or more species can fulfil similar roles in a way that the elimination of one species does not result in a discontinuation of the role performed by the ecosystem (Rosenfeld, 2002; Schmera et al., 2017). Thus, an ecosystem with a high functional redundancy can be considered more resilient to the impact of disturbances compared to those with low functional redundancy. A trait attribute is deemed functionally redundant if another trait can perform the same or similar function of that trait. For example, a species such as *Chironomus sp* possesses tracheal gills but also utilises cuticular respiration for oxygen intake in aquatic systems. In this case, tracheal gills and cuticular respiration may be considered functionally redundant, although the efficiency of oxygen uptake is higher for tracheal gills. According to Odume et al. (2018), “the impact of a stressor on an organism via a functionally redundant vulnerable trait attribute is likely to be moderated by the non-vulnerable trait attributes that can perform the same or similar function”. For example, where the respiratory type is resolved into tracheal gills and cuticular respiration, the latter is a functionally non-redundant trait attribute for species which do not possess tracheal gills, whereas it is functionally redundant for species which utilise it facultatively, such as *Glyptotendipes sp*.

### *Diversity of trait expression (Trait plasticity) (TP)*

The diversity of trait expression (trait plasticity) qualitatively describes the range of trait attributes belonging to a particular trait category expressed by a species (Hawlena et al., 2011; Levis & Pfennig, 2020). Thus, a species with higher trait plasticity (TP) is likely to be more resilient or adaptive to perturbation than one with lower trait plasticity for a particular trait category. A fuzzy-coding scale of 1–5 was assigned to species where 1 indicates a low TP for the specific trait category, 3 a moderate TP and 5 a high TP. For example, a species which prefers four or more food types as shown in Table 5.1 (FT1–9) would be assigned a TP score of 5 for the trait category food type, and a score of 3 would be awarded to a species which prefers two or three food types, whereas a species which prefers only one food type would be assigned a TP score of 1 for the same trait category. This logic was applied to all trait categories.

### *Classifying chironomid species into vulnerability groups*

Following Odume et al. (2018), vulnerability scores for chironomid species were calculated based on the vulnerable trait attributes possessed, non-redundant vulnerable trait attributes (NR) and TP per trait category using the equation:

Taxon vulnerability score (VS) = $N + \sum_{n_1}^{n^{th}} \left(\frac{x}{TP}\right) + 2NR$……………. Equation 1

Where $N$ = total number of vulnerable trait attributes possessed, $x$ = total number of vulnerable trait attributes per trait category, TP = species trait plasticity per trait category, $NR$ = total number of non-functionally redundant vulnerable trait attributes possessed and $n_1$ = trait category number (1...$n^{th}$ trait category) for a particular species.

The percentile distribution of the VS calculated for all the chironomids were used to designate potential distinct vulnerability groups (Table 5.3). The sectioning of vulnerability groups was done to identify chironomid species that may require more immediate attention in terms of their conservation status with regards to urban perturbation. In essence, species that possess a high number of vulnerable traits are likely to be more vulnerable to urban perturbation compared to species that possess fewer number of vulnerable traits. Thus, identifying different categories of vulnerability groups may indicate which species of chironomids may be eliminated first by urban disturbance based on the combination of traits they possess.

Table 5.3 Classification of chironomid species into vulnerability groups based on the percentile distribution of calculated vulnerability scores

| **Vulnerability Group** | **Percentile class** | **Description** |
|---|---|---|
| Group A | >75th percentile | Highly vulnerable |
| Group B | 50th–<75th percentile | Vulnerable |
| Group C | 25th–<50th percentile | Resilient/Tolerant |
| Group D | <25th percentile | Highly resilient/tolerant |

### 5.2.2 Predicting chironomid responses to urban pollution

Following the developed approach, the relative abundances of chironomid species in the vulnerable groups were predicted to i) decrease with increasing urban pollution, and ii) differentiate between the site categories. No *a priori* predictions were made concerning the relative abundances of the resilient groups (C and D) as they were expected to be present at all site categories regardless of urban impact.

### 5.2.3 Statistical and data analyses

The percent relative abundances of the vulnerable classes for each site category were computed using boxplots. The Kruskal-Wallis multiple comparison test was used to test for statistically significant differences between the relative abundances of the species belonging to the vulnerability groups (A–D) per site (Odume et al., 2018; Libala et al., 2019). The Conover-Iman post hoc test was used to check for multiple pairwise comparisons if the Kruskal-Wallis test indicated a significant difference.

Ordinary least squares linear regressions were used to model the relationship between the relative abundance of the species vulnerability groups and temperature, turbidity, DO, EC, nitrate-nitrogen, nitrite-nitrogen, ammonium-nitrogen and orthophosphate-phosphorous to evaluate the predicted responses of chironomids to urban pollution. The physicochemical variables were used as explanatory variables, while the relative abundances of the species groups were used as response variables (Libala et al., 2020). The assumptions of normality and homogeneity of variance were tested using the Shapiro-Wilks test and Levene's test, respectively. Where the assumptions were violated, data were log-transformed. Linear regression analyses were undertaken using R statistical software version 4.2.0 (R Core Team, 2022). Quantile regression (or Median regression) was also explored to check whether the associations between the response and predictor variables deviated markedly from the linear regression models. The quantile regression uses a general linear model of the median, rather than the mean, of the entire distribution to fit conditional quantiles of the response variable (Hallock, 2001; Koenker, 2005; Rodriguez & Yao, 2017). Although quantile regression is considered more robust to outliers than the ordinary least squares linear regression, the requirement for sufficient data (typically $n > 100$) resulted in its exclusion in this study. Given the small sample size in this study (n = 20), the ordinary least squares linear regression was employed in this study.

## 5.3 Results

### 5.3.1 Chironomid species vulnerability and resilience to urban pollution

Of the 104 chironomid species collected and identified, a total of 96 species were classified according to their potential vulnerability or resilience to urban pollution. Of the 96 species classified, twenty-four (24) were classified as highly vulnerable, twenty-five (25) as vulnerable, twenty-three (23) as resilient and twenty-four (24) as highly resilient (Table 5.4). The highly vulnerable species included mostly taxa which were predatory swimmers or shredding crawlers, whereas the highly resilient species included tube-making collector-gatherers and filterers (Table 5.4).

Table 5.4 Chironomid species classification into the vulnerability classes based on the developed TBA

| **Group A** | **Group B** | **Group C** | **Group D** |
|---|---|---|---|
| *Rheopelopia sp* | *Cricotopus bicinctus* | *Zavreliella marmotata* | *Dicrotendipes pulsus* |
| *Tanytarsus usmaensis* | *Cricotopus cylindraceus* | *Cladotanytarsus sp* | *Dicrotendipes lobiger* |
| *Ablabesmyia phatta* | *Cricotopus intersectus* | *Cladotanytarsus mancus* | *Glyptotendipes sp* |
| *Helopelopia cornuticaudata* | *Cricotopus* sp1 | *Rheotanytarsus curtistylus* | *Einfeldia pagana* |
| *Corynoneura scutellata* | *Cricotopus tremelus* | *Rheocricotopus sp* | *Einfeldia sp* |
| *Ablabesmyia monilis* | *Cricotopus trifasciata gr* | *Paratanytarsus austriacus* | *Microtendipes pedellus* |
| *Procladius sp* | *Nanocladius rectinervis* | *Virgatanytarsus ardrennensis* | *Dicrotendipes notatus* |
| *Thienemannimyia geijskesi* | *Nanocladius sp* | *Stictochironomus sp* | *Chironomus* sp1 |
| *Larsia sp* | *Orthocladius clarki* | *Tvetenia sp* | *Chironomus* sp2 |
| *Corynoneura lobata* | *Orthocladius dentifer* | *Eukiefferiella gracei* | *Microchironomus tener* |
| *Monopelopia tennicalcar* | *Orthocladius oliveri* | *Paratrichocladius skirwithensis* | *Trissocladius sp* |
| *Conchapelopia melanops* | *Orthocladius sp* | *Stempellinella sp* | *Glyptotendipes severini* |
| *Hydrobaenus johannseni* | *Parametriocnemus lundbecki* | *Metriocnemus fuscipes* | *Paracricotopus niger* |
| *Paralimnophyes hydrophilus* | *Paratrichocladius sp* | *Parakiefferiella sp* | *Dicrotendipes nervosus* |
| *Djalmabatista pulcher* | *Polypedilum convictum* | *Pseudorthocladius sp* | *Chaetocladius setosipennis* |
| *Conchapelopia sp* | *Polypedilum illinoense* | *Eukiefferiella sp* | *Virgatanytarsus sp* |
| *Procladius bellus* | *Polypedilum nubeculosum* | *Paratanytarsus sp* | *Pseudosmittia sp* |
| *Thienemannimyia senata* | *Polypedilum nubifer* | *Natarsia baltimoreus* | *Einfeldia carbonaria* |
| *Paralimnophyes sp* | *Polypedilum ophoides* | *Tvetenia paucunca* | *Paracladopelma nais* |
| *Paramerina anomalus* | *Polypedilum sp* | *Cryptochironomus sp* | *Cardiocladius sp* |
| *Pseudochironomus sp* | *Polypedilum tuberculum* | *Coelotanypus sp* | *Kiefferulus sp* |
| *Paramerina cingulata* | *Stenochironomus sp* | *Clinotanypus sp* | *Dicrotendipes sp* |
| *Ablabesmyia sp* | *Tanytarsus brundini* | *Rheotanytarsus sp* | *Chironomus* sp3 |
| *Tanypus sp* | *Tanytarsus sp* |  | *Microchironomus sp* |
|  | *Trissopelopia sp* |  |  |

### 5.3.2 Relationship between the species group and selected physicochemical variables

The results of the linear regressions indicate that the relative abundance of the highly vulnerable species (A) increased with temperature, dissolved oxygen and nitrate-nitrogen but decreased with increasing turbidity, electrical conductivity, nitrite-nitrogen, ammonium-nitrogen and orthophosphate-phosphorus (Figure 5.1). No statistically significant relationship was observed between the relative abundance of highly vulnerable species (A) and nitrate-nitrogen, orthophosphate-phosphorus, EC, nitrite-nitrogen, turbidity and temperature ($p > 0.05$). However, statistically significant relationships were observed between the relative abundance of highly vulnerable species (A) and dissolved oxygen ($R^2 = 0.2448$, $p = 0.02655$) and between the relative abundance of highly vulnerable species (A) and ammonium-nitrogen ($R^2 = 0.2093$, $p = 0.04252$).

The relative abundance of vulnerable species (B) increased with increasing dissolved oxygen but decreased as other physicochemical variables increased (Figure 5.2). Statistically significant relationships were observed between the relative abundance of vulnerable species (B) and dissolved oxygen ($R^2 = 0.3605$, $p = 0.005122$), turbidity ($R^2 = 0.2241$, $p = 0.03502$), electrical conductivity ($R^2 = 0.2327$, $p = 0.03124$), ammonium-nitrogen ($R^2 = 0.2939$, $p = 0.01353$) and orthophosphate-phosphorus ($R^2 = 0.5346$, $p = 0.0002498$). No statistically significant relationship was observed between the relative abundance of vulnerable species (B) and nitrate-nitrogen, nitrite-nitrogen and temperature ($p > 0.05$).

Although no *a priori* predictions were made concerning the relative abundance of resilient and highly resilient species, I deemed it important to explore how the species composition in these groups correlated with the selected physicochemical variables. The relative abundance of resilient species (C) decreased with increasing dissolved oxygen but increased with increasing temperature, turbidity, electrical conductivity, nitrate-nitrogen, nitrite-nitrogen and ammonium-nitrogen, and slightly increased with increasing orthophosphate-phosphorus (Figure 5.3). No statistically significant relationship was observed between the relative abundance of the resilient species (C) and the physicochemical variables ($p > 0.05$).

Moreover, the relative abundance of highly resilient species (D) decreased with increasing temperature and dissolved oxygen but increased as other physicochemical variables increased (Figure 5.4). Statistically significant relationships were observed between the relative abundance of highly resilient species (D) and dissolved oxygen ($R^2 = 0.3433$, $p = 0.006634$), turbidity ($R^2 = 0.2204$, $p = 0.03674$), electrical conductivity ($R^2 = 0.2445$, $p = 0.02666$), ammonium-nitrogen ($R^2 = 0.2522$, $p = 0.02405$) and orthophosphate-phosphorus ($R^2 = 0.486$, $p = 0.0006349$). No statistically significant relationship was observed between the relative abundance of highly resilient species (D) and nitrate-nitrogen, nitrite-nitrogen and temperature ($p > 0.05$). For the vulnerability groups, a cut-off point of 0.05 was found to sufficiently indicate significant results without a type-2 statistical error. The relationship between the vulnerability groups were mostly above a *p*-value of 0.1 for associations that were not significant at the 0.05 level.

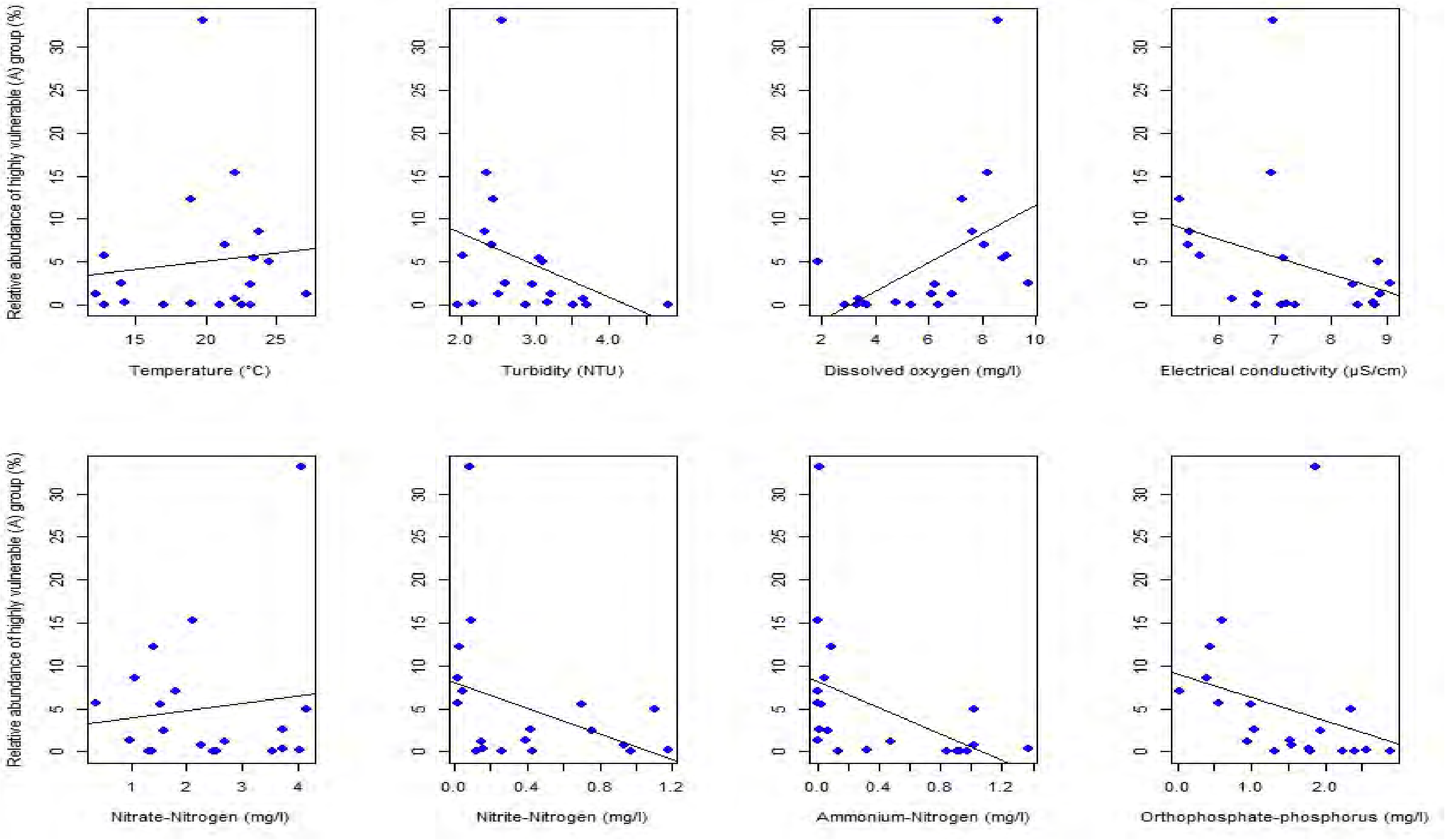


Figure 5.1 Linear regression of the relative abundance of the highly vulnerable species group (A) and physicochemical variables

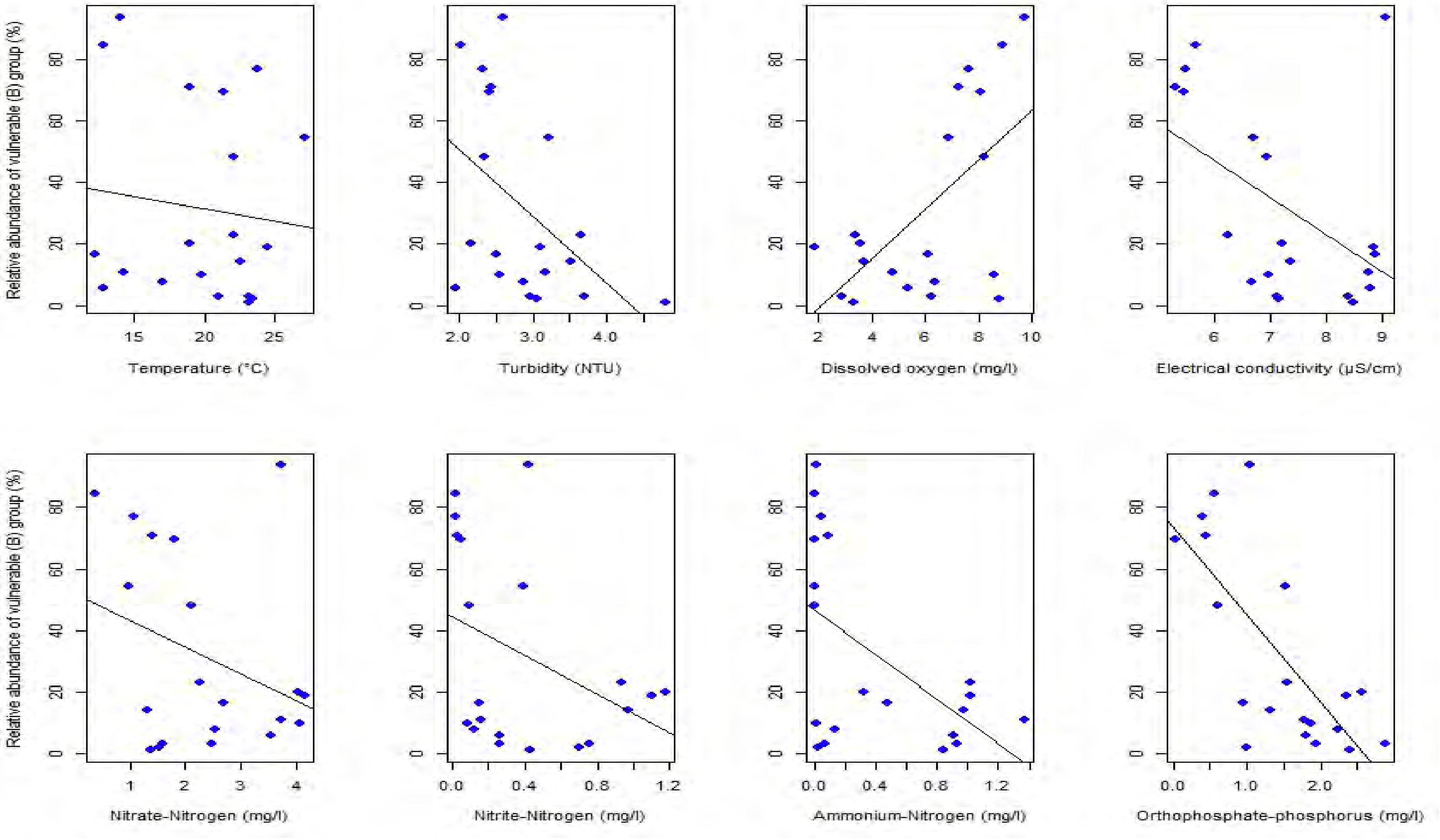


Figure 5.2 Linear regression of the relative abundance of the vulnerable species group (B) and physicochemical variables

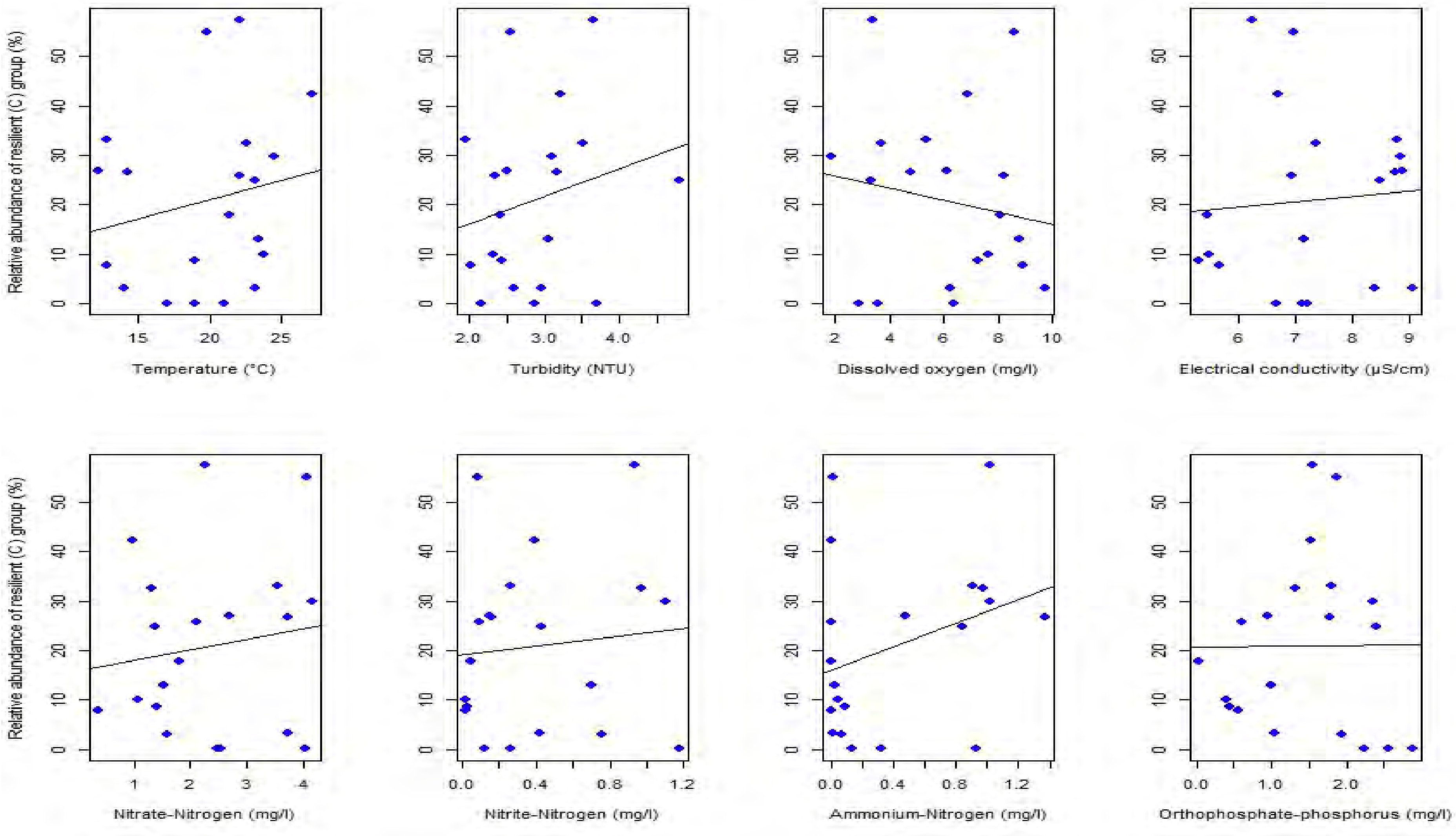


Figure 5.3 Linear regression of the relative abundance of the resilient species group (C) and physicochemical variables

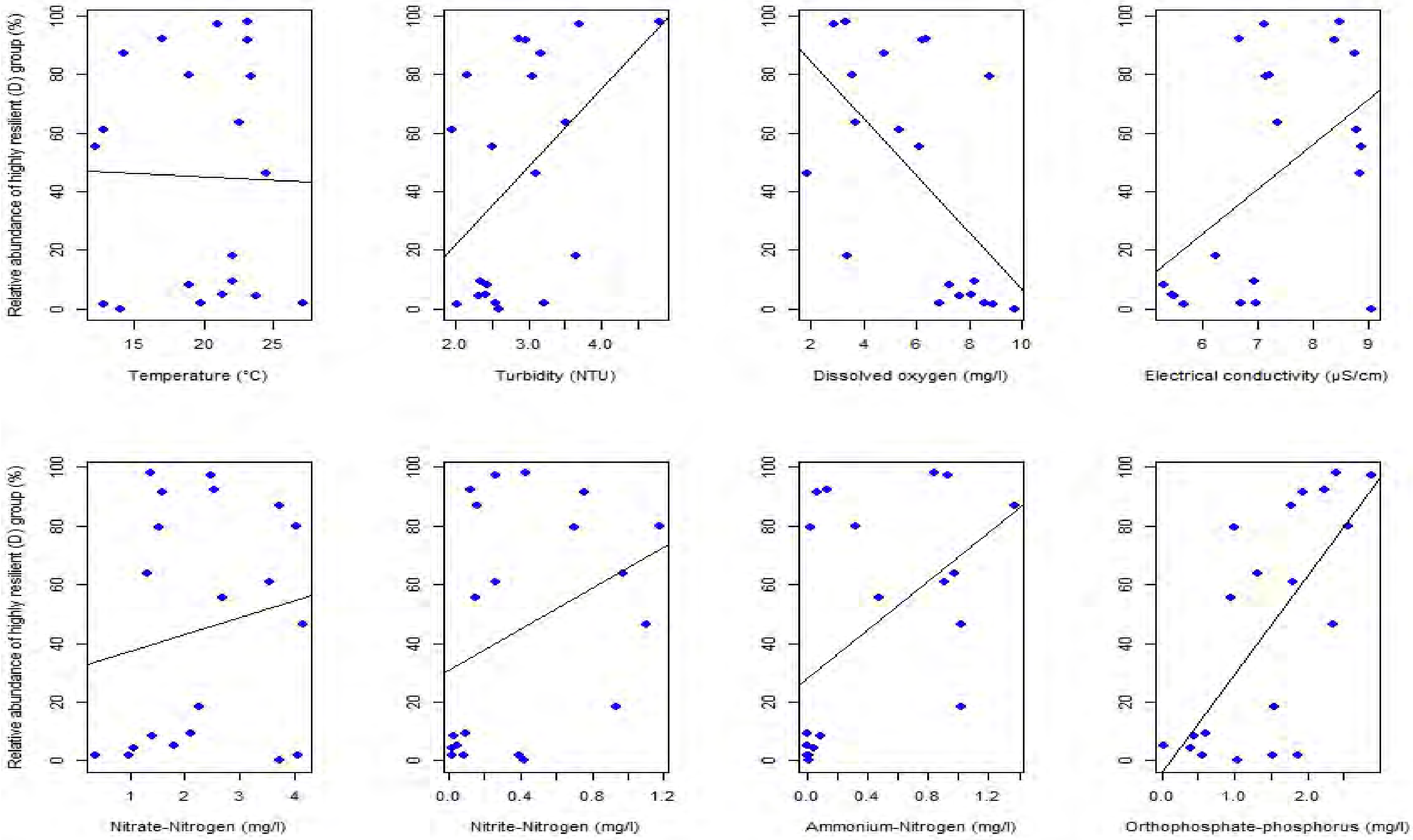


Figure 5.4 Linear regression of the relative abundance of the highly resilient species group (D) and physicochemical variables

### 5.3.3 Spatial distribution of the abundance and richness of vulnerable and resilient chironomid species groups

The relative abundances of chironomid species in the vulnerable groups (A and B) were predicted to decrease with increasing urban pollution and were also predicted to distinguish between highly impacted sites and moderately impacted sites. The results of the spatial distribution of the relative abundance of the highly vulnerable species groups are presented in Figure 5.5. The results indicate that the relative abundances of chironomid species group A were highest at the least impacted sites (REF), decreased markedly at the DP sites, and then recovered at the RECV sites. The relative abundances of the highly vulnerable species group were lowest at the PP and AG sites (Figure 5.5). The trend suggests that the highly vulnerable species group responded predictably to urban pollution. The Kruskal-Wallis test indicated that the relative abundances of this group of species were statistically significantly different between the site categories (KW-H = 12.923, $p = 0.01166$). The Conover-Iman post hoc test indicated that there was no significant difference between the REF and RECV sites, but that there were significant differences between these sites (REF and RECV) and the highly impacted sites (DP, PP and AG). Of the highly impacted sites, the only significant difference was found between the DP and PP sites.

Similar to the trend in the highly vulnerable species group (A), the relative abundances of the vulnerable species group (B) were highest at the REF and RECV sites compared to the highly impacted sites (Figure 5.5). However, amongst the highly impacted sites, vulnerable species (group B) were relatively more abundant at the AG sites. The Kruskal-Wallis test detected statistically significant differences between the relative abundances of vulnerable species in the site categories (KW-H = 13.0143, $p = 0.01121$). The Conover-Iman post hoc test indicated that the relative abundances of vulnerable species at the REF sites differed significantly from the relative abundances at the DP, PP, and AG sites. The relative abundances of vulnerable species at the RECV sites only differed significantly from the relative abundances at the DP and PP sites. There were no differences between the relative abundances of the highly impacted sites.

The relative abundances of the resilient species group (C) exhibited a steady increase across the site categories and were highest at the RECV sites (Figure 5.5). However, the Kruskal-Wallis test did not detect any statistically significant difference between the site categories.

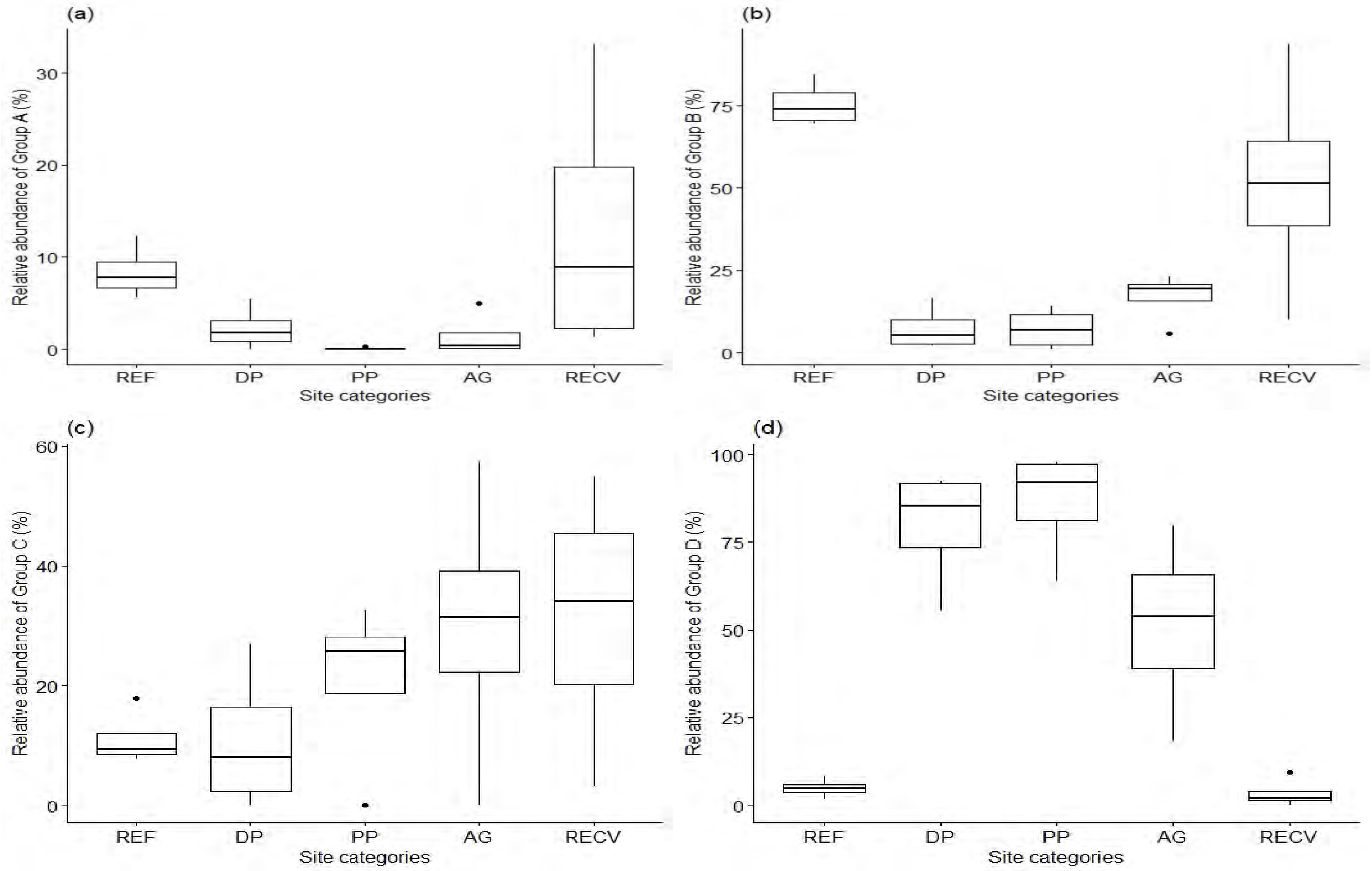


Figure 5.5 The relative abundance of chironomid species in the vulnerability groups (A, B, C, and D) across the site categories (REF, DP, PP, AG, and RECV) (a) Highly vulnerable species, (b) vulnerable species, (c) resilient species, and (d) Highly resilient species

The relative abundances of the highly resilient species group (D) were low at the least impacted sites (REF), sharply increased at the DP and PP sites and were low again at the RECV sites (Figure 5.5). Although no predictions were made for species belonging to group D, the trend suggests that the highly resilient species responded markedly to urban pollution. The Kruskal-Wallis test indicated that the relative abundances of this group of species were statistically significantly different between the site categories (KW-H = 15.543, $p = 0.003698$). The Conover-Iman post hoc test indicated that there was no significant difference between the REF and RECV sites, but there were significant differences between these sites (REF and RECV) and the highly impacted sites (DP, PP and AG). A significant difference was also indicated by the post hoc test between the relative abundances of highly resilient species at the PP sites and those at the AG sites.

The results of the spatial distribution of the species groups' richness per site are presented in Figure 5.6. The richness of the highly vulnerable species group was highest at the REF sites, declined steadily at the DP sites until it reached its lowest at the PP sites, and steadily increased at the AG sites before recovering at the RECV sites. A similar trend was observed for the richness of species groups B (Figure 5.6). The Kruskal-Wallis test showed significant differences between the site categories for the richness of the highly vulnerable species group A (KW-H = 13.745, $p = 0.008154$) and the vulnerable species group B (KW-H = 10.998, $p = 0.02658$). The Conover-Iman post hoc test showed that there was no significant difference between the richness of the highly vulnerable species group A at the REF and RECV sites, but there were significant differences between the REF and RECV sites and the highly impacted sites (DP, PP and AG). The Conover-Iman post hoc test only indicated differences between the richness of vulnerable species group B at the REF sites and that of the DP, PP and AG sites. The large interquartile range of the species richness of vulnerability group B compared to other vulnerability groups could be due to the seasonal variability in the occurrence of species belonging to group B.

The richness of the resilient species group C followed a similar trend like that of species groups A and B but was higher at the PP sites compared to the DP and AG sites. A similar pattern was observed for the richness of highly resilient species group D (Figure 5.6). No statistically significant difference was found by the Kruskal-Wallis test between the site categories for the richness of species groups C and D ($p > 0.05$).

Overall, the results show that the relative abundances and richness of the highly vulnerable group A and the vulnerable group B responded predictably to urban pollution. The results also show that although the richness of the resilient and highly resilient species groups was not different between the site categories, the relative abundance of the highly resilient group D responded markedly to urban pollution and was different between the site categories.

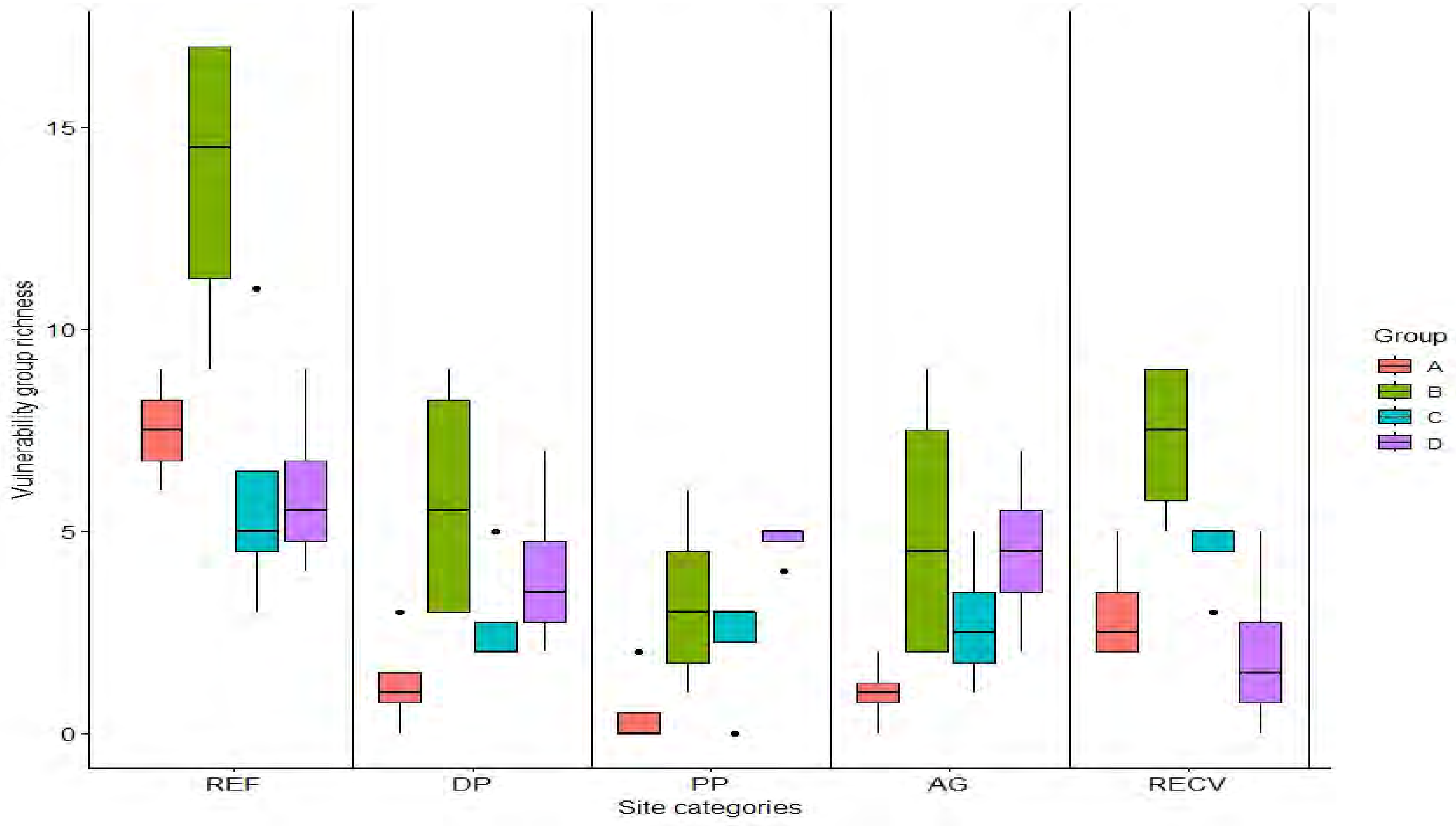


Figure 5.6 The richness of vulnerability groups across sites categories. REF (least impacted sites), DP (sites impacted by diffuse source pollution), PP (sites impacted by point source pollution), AG (Diffuse, point and agricultural impacted sites), RECV-Recovery sites, A-highly vulnerable species, B-vulnerable species, C-resilient species, D-highly resilient species

## 5.4 Discussion

Trait-based approaches have been used to predict the responses of macroinvertebrates to agricultural intensity (Lange et al., 2014), multiple environmental stressors (Pilière et al., 2016), and heavy metals and cargo ships (Dolédec & Statzner, 2008), amongst other responses. Indeed, traits are crucial to understanding the context-specific responses of organisms to anthropogenic pressures, as indicated by the results of this study. The response of chironomid species' trait combinations to urban pollution was predicted in this study and the developed trait-based approach was used to classify chironomid species into four distinct groups, namely, highly vulnerable (Group A), vulnerable (Group B), resilient (Group C) and highly resilient (Group D). Species possessing trait combinations, such as predators which possess 12 tracheae and are adapted for cuticular respiration, and neither construct tubes nor possess body armour, dominated the highly vulnerable species Group A, whereas shredders and grazers adapted to slow crawling movement which mine macrophytes or prefer living microphytes dominated the vulnerable species Group B. The trait combinations of chironomid species classified in groups A and B were mostly associated with the least impacted sites of this study, where the impacts of urban pollution stressors were low and dissolved oxygen could readily be obtained cutaneously without the need for additional respiratory structures such as tracheal gills. Thus, organisms possessing these trait combinations may be predisposed to elevated dissolved solids, sediment accretion, and low dissolved oxygen and will therefore become filtered out in urban-impacted systems (Wilkes et al., 2017; Collins & Fahrig, 2020). This could be the reason why species in Groups A and B were mostly negatively associated with increasing electrical conductivity, turbidity, ammonium-nitrogen, orthophosphate-phosphorous and nitrite-nitrogen but positively associated with increasing dissolved oxygen (Figure 5.1 and 5.2).

The majority of the species classified in groups C and D were collectors (gatherers and filterers), which possessed traits such as construction of tubes or transportable cases, a preference for fine detrital food materials and a fixed or sediment burrowing habit (Table 5.4). These species were adapted to abiotic conditions at urban-impacted sites and were protected from the effects of elevated stressors and low dissolved oxygen. For example, the resilient species *Rheotanytarsus curtistylus* dwells in tubes and projects arm-like structures to capture fine particulate matter from the water column. The tube-dwelling habit of this species, coupled with its preference for stone biotopes, protects it from the clogging and abrasive effects of sediment influx and from high exposure to elevated dissolved solids and pollutants such as metals (Hershey, 1987; Halpern et al., 2002; Podder et al., 2022). Moreover, the possession of haemoglobin, as seen in *Chironomus* spp., combined with traits such as whole-body undulation and the possession of tracheal gills, may offer an adaptive advantage to highly resilient species in highly impacted freshwater systems.

The results of this study largely corresponded with *a priori* predictions that the relative abundances (%) of the highly vulnerable and vulnerable species groups would decrease with increasing urban pollution and that the relative abundances of the highly vulnerable group A and vulnerable group B would differentiate between the site categories (Figure 5.5). The relative abundances of highly vulnerable species and vulnerable species groups were lowest at highly impacted sites and statistically significant differences were found between the site categories for each vulnerability group (A and B).

The richness of the highly vulnerable group A was highest at the least impacted REF sites compared to the impacted sites (DP, PP and AG) and recovery sites (Figure 5.6). However, the relative abundance of highly vulnerable species group A was low at the REF sites compared to that of species in vulnerable group B and resilient group C at the REF sites (Figure 5.5). A plausible explanation for this occurrence could be the effect of species rarity and diversity on their relative abundance at least impacted sites (Cao et al., 1998; Lenat & Resh, 2001). Chironomid species rarity was found to be more closely related to species richness rather than relative abundance (Lenat, 1983). Although rare species have been reported to have low relative abundances, the literature suggests that their occurrence at certain sites holds meaningful ecological significance with regard to their role in ecological processes and important conservation values (Cao et al., 1998; Lenat & Resh, 2001).

An interesting but not surprising observation was the high richness of highly resilient chironomids (Group D) at the REF sites compared to the DP, PP and AG sites, indicating that urban pollution greatly impacted the occurrence of highly resilient species, with more pronounced effects at sites receiving point source pollution (PP) (Figure 5.6). The relative abundances of highly resilient taxa were consistently higher at the impacted sites (DP, PP and AG) (Figure 5.5). In addition, the relative abundances of highly resilient species in group D were significantly different between the site categories and this is noteworthy, because the relative abundance of species belonging to this group can contribute substantially to biomonitoring efforts in predictive ecology. Thus, highly resilient species, although being able to survive in both impacted and unimpacted systems, may be expected to be relatively more abundant at impacted sites due to the filtering out of vulnerable species by adverse abiotic conditions, resulting in less interspecific competition for resources and reduced predation.

The trait-based approach applied in this study took account of chironomid species trait combinations and interactions by recognising the effects of trait diversity and functional redundancy on species' adaptive strategies and provided a mechanistic basis for explaining the potential vulnerability of chironomid species to urban pollution. The vulnerability groups responded predictably to urban stressors, as indicated by the linear regression models. Although some of the associations were not statistically significant, the real power of the TBA developed in this study may be more evident using data collected over a long study period and distinct sites from different urban-impacted streams rather than the short data collection period in this study. Moreover, the subsequent calibration of the models using data collected over a longer study period and from numerous urban stream sites may account for the natural variability observed in the vulnerability groups (especially in the richness of vulnerability group B). Overall, this study successfully provides evidence that species of the macroinvertebrate family Chironomidae can reliably be used to develop biomonitoring tools which are both predictive and diagnostic of urban pollution.

## 5.5 Conclusion

A novel trait-based approach was developed in this study and used to classify chironomids present in selected South African urban rivers into four vulnerability groups. The TBA proved promising in predicting the potential responses of chironomid species to urban pollution. The percent relative abundance of the highly vulnerable group decreased at the urban polluted site categories compared to the least impacted site groups. Although autecological information regarding chironomid species remains a limiting factor in the application of trait-based approaches in South Africa, this study makes an important contribution to the application and adoption of traits for freshwater biomonitoring in South Africa. As the outcome of the predictions were taxa-related rather than traits, the TBA developed here can contribute greatly to biodiversity conservation efforts in South African urban river systems. It is important to note that data used in this study were from a small geographical region. Therefore, the developed approach may require further refinement to better capture differences in the trait composition of chironomid communities as imposed by abiotic factors, to improve its predictive power. The refinements may include the sampling of multiple distinct sites across the Eastern Cape and the need for elaborate chironomid autecological studies to extract more information about the traits of chironomids (for example, dispersal, salinity preferences, temperature preferences, and other relevant trait information) that may impact on the survival, fitness and development of chironomids in response to urban disturbance.

# CHAPTER 6 – GENERAL DISCUSSION AND CONCLUSION

## 6.1 General introduction

Freshwater ecosystems such as rivers and streams in South Africa are threatened by a continuous decline in water quality and quantity, leading to adverse effects on aquatic biodiversity and the sustainability of ecosystem services (Gqomfa et al., 2022). Urbanisation occasioned by an increasing human population and rural-urban migration has contributed significantly to freshwater quality challenges in South Africa through effluent discharges from poorly managed wastewater treatment works, release of untreated effluent from industrial activities, conversion of natural landscapes to agricultural lands, sewage spills from vandalised sewer pipes, and stormwater run-off from formal and informal settlements (Lemley et al., 2014; Robertson et al., 2019; Agoro et al., 2020; Gqomfa et al., 2022). Effluent discharges from industrial activities and WWTWs, stormwater runoff from industrial facilities and human settlements, and runoff from agricultural lands carry pollutant mixtures including metals, oil and grease, nitrogen and phosphorus compounds and sediments into streams (Robertson et al., 2019; Lu et al., 2022). These pollutants have been known to cause decreased dissolved oxygen concentrations, elevated biological and chemical oxygen demand, increased electrical conductivity, turbidity, salinity, suspended solids and nutrient levels which bear adversely ecological consequences, including the modification of aquatic habitats, algal blooms and the loss of freshwater biota (Grobler et al., 1987; Dickens & Graham, 1998; Enns et al., 2023). To ensure the protection of freshwater biodiversity and the sustainable supply of ecosystem services, biomonitoring approaches that are sensitive, diagnostic and predictive of the responses of biota to various urban pollution stressors are needed. Therefore, this study employed an integrated water chemistry and chironomid-based taxonomic and trait-based approach to assess the impact of urban pollution on the community assemblage structure, ecological health and functioning of selected urban river systems in South Africa.

This chapter presents a synthesis of the approaches applied in this study and a summary of the key findings and their implications for water quality management. The limitations of this study and recommendations for future research are also discussed in this chapter.

### 6.1.1 Taxonomy-based biomonitoring of urban pollution in the study rivers

Taxonomy-based approaches combine an investigation of different aspects of macroinvertebrate assemblage structure including composition, abundance and richness in the rapid assessment of biotic changes along a gradient of disturbance (Lam-Gordillo et al., 2021; Edegbene et al., 2022). A taxonomy-based biomonitoring approach was introduced in Chapter 3, with the aim of developing and applying a chironomid species-based tool for assessing water quality deterioration in selected urban rivers in the Eastern Cape, South Africa. The major bioassessment tool used in South Africa is the SASS5 single biotic index method, which was developed using macroinvertebrate taxa at the family level as its highest taxonomic resolution (Dickens & Graham, 2002). This simple and rapid assessment tool contributes to freshwater resources management but does not account for the species-level responses of very diverse macroinvertebrate taxa such as the Chironomidae. Thus, the key question for this chapter was to understand whether water quality site classification based on family level as per SASS5 would be different to that achieved when a developed index based on chironomid species is applied. Therefore, a chironomid multimetric index for monitoring urban pollution was developed, used to classify study sites, and compared with results of the SASS5 method. In addition, physicochemical assessment of the study site categories and the spatial-temporal distribution of chironomids were explored. The results revealed that both the chironomid-based multimetric index and the SASS5 indicated similar ecological categories (E/F) for the sites immediately downstream of the WWTWs effluent discharge point (Figure 3.6). The SASS5 indicated that more than 60% of samples collected from the DP site of the Swartkops River were in the critically modified water quality class (ecological category E/F), whereas the chironomid-based index only classified 50% of the data points for the same site as having critically modified water quality, 25% as having moderately altered water quality (category C) and 25% as heavily altered (category D). The SASS5 scores classified the water quality condition at the AG site as critically/severely modified 100% of the time, whereas the CUMMI index classified the water quality condition at this site as critically/severely modified 90% of the time but moderately modified 10% of the time. Thus, the chironomid-based index was sensitive to water quality changes at the sites which received diffuse pollution but were upstream of the effluent discharge point of WWTWs (DP sites) and sites further downstream from the WWTWs which receive impacts from both point source and diffuse pollution (AG sites). Crucially, the results suggest that the output of the family-based assessment, and that of the species-level assessment were similar for heavily impacted and control or least impacted sites. The difference between the two levels of assessment was pronounced for sites which fall between these two extremes (heavily impacted and least impacted), and this is an important contribution to ecological science for several reasons. First, biological shifts are more likely to take place regularly in sites which fall between these extremes, as opposed to heavily impacted sites which may have already reached a regime shift state. Second, management interventions are often directed to sites which fall within these two extremes as the costs of restoring heavily impacted sites may be prohibitive, especially in the context of a developing country such as South Africa. It is thus critical that biomonitoring tools accurately place sites in the appropriate water quality category to avoid either under- or over-protection.

### 6.1.2 Trait-based responses to urban pollution in the study rivers

Chironomids are regarded as one of the most taxonomically and functionally diverse groups of aquatic macroinvertebrates, occupy a broad ecological range and possess varying degrees of sensitivity to anthropogenic disturbance (Cranston, 1990; Porinchu & MacDonald, 2003; Rodríguez Garay et al., 2020). As stated in Section 1.6.2 of Chapter 1, traits are inherent morphological, behavioural, physiological or life-history attributes of organisms that are measurable at the individual level without making reference to the external environment (McGill et al., 2006; Violle et al., 2007), whereas ecological preferences are the product of the direct interactions between an organism and its external environment (Odume, 2022). As organisms are only able to survive in an environment based on the traits they possess, a trait-based approach was used to explore the distribution pattern of chironomid species' trait-based responses to urban pollution, discussed in Chapter 4 of this study. The results of this study showed that the traits and ecological preferences of chironomids responded markedly to deteriorating water quality at the impacted study sites and clearly differentiated between the site categories. The results also show that chironomid trait attributes such as medium body size, cuticular respiration, completion of lifecycle in 1 year, predator feeding mode and a preference for stone biotopes were positively associated with the least impacted sites and decreased with increasing urban disturbance. Notably, chironomid species which utilise solely cuticular respiration and do not possess haemoglobin are suited to water of good ecological quality such as that found at the REF and RECV sites. In contrast, trait attributes such as burrowing, possession of tracheal gills, collector-gathering, laying more than 1000 eggs per egg mass, bivoltinism, construction of rigid tubes, very large body size, completion of lifecycle in more than 1 year and preference for fine detritus were positively associated with the highly impacted sites and increased with urban perturbation in this study. The results show that traits can provide a mechanistic link between organisms and their external environment (Townsend & Hildrew, 1994) and may be responsible for the occurrence of some chironomid species at the highly impacted sites. In addition, trait-based indicators of urban pollution, which can be used to develop bioassessment tools to complement taxonomic approaches and provide more information on the response of chironomids to pollution, were also identified in Chapter 4.

In Chapter 5, I developed a trait-based approach for predicting the potential vulnerability or resilience of chironomid species to urban pollution. The developed trait-based approach was used to classify chironomid species into four vulnerability groups (A–D). Group A comprised chironomid species which are highly vulnerable to urban pollution, chironomid species in Group B were deemed vulnerable, those in Group C were regarded as resilient, while species classified in Group D were highly resilient to increasing urban pollution. The abundance of species in Group A and Group B increased with increasing dissolved oxygen concentrations but decreased with increasing turbidity, electrical conductivity, nitrite-nitrogen, ammonium-nitrogen and orthophosphate-phosphorus. The vulnerability groups were assessed for their predictive potential to differentiate between the site categories. Species in groups A, B and D sufficiently distinguished between the least impacted and recovery sites and the highly impacted sites. Because the outcome of the predictions is taxa-related, the trait-based approach may be a useful tool to aid conservation efforts in South Africa.

## 6.2 Implications and recommendations for water quality management in the study rivers

Water is a natural resource which households, industries, and municipal facilities rely on for socioeconomic development. The South African National Water Act (NWA) No. 36 of 1998 recognises the scarcity of the nation’s water and emphasises the need to protect freshwater systems to ensure an ecologically sustainable development (RSA, 1998). Thus, the NWA No. 36 of 1998 actions the protection of South Africa’s natural water resources through the National Water Resource Strategy (NWRS) (DWAF, 2013). To protect water resources, the NWRS utilises the Resources Directed Measures (RDMs) which require the classification of water resources, determination of their ecological categories, assessment of the Reserve, and setting of resource quality objectives (RQOs) (DWAF, 2013). To facilitate the achievement of the RDMs, various biomonitoring approaches have been developed (Dickens & Graham, 2002; De La Rey et al., 2008; Mangadze et al., 2019). The focus of this study was partly to contribute to the objectives of the RDMs. Therefore, in this study, taxonomic and trait-based approaches were developed based on chironomid species assemblage and their response to urban pollution. The results of this study show that the chironomid-based multimetric index distinguished water quality conditions between the highly impacted PP sites and AG sites (Chapter 3). This may be useful for setting priority targets, for example selecting which sites to immediately focus restoration efforts on, which is an important consideration in water resources management. In addition, the trait-based approaches were able to provide a mechanistic understanding of the effects of urban pollution on chironomid species and enable the prediction of species' responses to deteriorating water quality (Chapters 4 and 5). These approaches may be useful in setting RQOs for water resources management. Overall, the approaches proved useful for revealing the effects of urban pollution on aquatic ecosystems and are highly recommended for inclusion in urban freshwater biomonitoring programmes in South Africa.

## 6.3 Strengths of the study

This study employed an integrated water chemistry and chironomid-based taxonomic and trait-based approach to assess the impact of urban pollution on the ecological health of selected urban river systems in South Africa. An index for urban pollution monitoring was developed using species-level information of chironomids. The developed index was sensitive to water quality changes, especially among sites that fall between the extremes of pollution (least impacted and heavily impacted). This is important because the accurate classification of sites may help to prevent the over-exploitation of aquatic resources and ensure that the right amount of protection is awarded in a way that does not hinder sustainable socioeconomic development. Moreover, the index can aid management decisions by highlighting which sites may best benefit from restoration efforts at minimal financial and time costs. The developed trait-based approach predicted the vulnerability or resilience of chironomid species to urban pollution in the study rivers. This approach can contribute towards setting the conservation priorities of chironomid species in South Africa, particularly rare and endemic species. For example, where an endemic species inhabits a river reach that is prone to urban impact, the trait combination of such a species can be used to predict its vulnerability or resilience and inform management decisions as to whether to maintain or increase the level of protection awarded to the reach. The approaches developed in this study highlighted the increased sensitivity of the species-level index compared to the family-level index in detecting differences in water quality conditions between moderately impacted sites and highly impacted sites. The results also show that the differential responses of chironomid species to urban pollution vary sufficiently to enable the development of predictive tools.

## 6.4 Limitations and recommendations for future studies

The results of this study have indicated some research gaps that need to be investigated further. Firstly, there is need for a targeted investment in chironomid species' identification in the Afrotropical region, especially in South Africa. This is because chironomids are one of the most diverse groups of macroinvertebrates which occupy a wide ecological range and have differential responses or sensitivity to water quality impairment. This group of macroinvertebrates can be explored further for the development of bioassessment tools. However, the approaches developed in this study would require a focused investment on chironomid species identification if they are to be adapted for use in urban river systems elsewhere. A comprehensive list, together with species-level identification keys which can aid the use of chironomid species in the development of bioassessment tools is lacking. Harrison (2004) provided a genus-level chironomid identification guide for South Africa. However, the guide did not aid the identification of chironomids to species as did the Wiederholm (1983) and Cranston (2014) identification keys. Unfortunately, these keys only enabled the identification of chironomid species to morphospecies level because of the absence of region-specific guides. Secondly, limited autecological information on chironomid species signifies a major drawback on the use of chironomid species traits for water quality monitoring. Although a trait database for Southern Africa macroinvertebrates was recently developed (Odume et al., 2023), the trait information on chironomid species within the database (especially those on chironomid life-history and ecological preferences) is scanty and requires additional research. Thirdly, chironomid data collected over a longer study period and a large number of distinctly different sites may provide additional supporting evidence for the bioindication potential of chironomids in biomonitoring urban pollution. Data collected once from multiple sites may enable the application of more robust statistical analyses to describe the impact of anthropogenic pressure on freshwater systems. A periodic seasonal collection of data from the same sites can contribute immensely to freshwater ecosystem management, in terms of the maintenance of water quality and riparian health status in accordance with the specific objectives (for example, RQOs) set by freshwater managers. Despite the fact that seasonality had little or no effect on the distribution of chironomids, it is recommended that sampling be conducted during the spring season to capture the spring synchronisation of many chironomid species. Also, this recommendation is suggested in order to incorporate the dilution effect of rainfall on stream pollutants during high base flows. The logic is that if developed indices can detect changes in ecosystem health during high flow when there is dilution and flushing of pollutants, then such indices may be able to accurately detect a difference between sites when there is low baseflows and higher impact of urban pollution. Finally, increased stream flow can provide habitat for the establishment of chironomid on marginal vegetation and more stone surfaces. Although this study focuses on urban pollution, agriculture-related impacts may impair more stream miles and constitute a major challenge in South Africa and the world at large.

## 6.5 Conclusion

The results of this study demonstrate the importance of a combined water chemistry and chironomid-based taxonomy- and trait-based approach to biomonitoring the effects of urban pollution on the ecological health and functioning of selected urban rivers in South Africa. This study highlighted, in addition to physicochemical measures, the comparative advantage of species-level taxonomic approaches over family-level macroinvertebrate-based tools in assessing water quality deterioration (Chapters 1 and 3). This study also provided evidence of the value of chironomid species traits-based approaches in freshwater biomonitoring (Chapters 4 and 5), indicating the complementary nature of trait-based approaches to taxonomy-based approaches. Chironomid species' traits aided the identification of sensitive and tolerant traits linked to urban pollution. The developed trait-based approach enabled the classification of chironomid species based on their resilience or vulnerability to urban pollution, and significantly differentiated the site categories. The approaches presented in this study provide significant advantages for freshwater biomonitoring in South Africa. However, for the developed approaches of this study to be widely applied, more refinement and testing are needed to capture differences in the taxonomic and trait composition of chironomid species in freshwater systems in other provinces within the country.

# REFERENCES

Adedeji, O. H., & Olayinka, O. O. (2013). Heavy Metal Concentrations in Urban Stormwater Runoff and Receiving Stream . *Journal of Environment and Earth Science, 3*(7), 141–151.

Adeyinka, G. C., Moodley, B., Birungi, G., & Ndungu, P. (2018). Quantitative analyses of selected polychlorinated biphenyl (PCB) congeners in water, soil, and sediment during winter and spring seasons from Msunduzi River, South Africa. *Environmental Monitoring and Assessment*, *190*(10). https://doi.org/10.1007/s10661-018-6993-8

Agboola, O. A. (2017). *Monitoring and assessment of macroinvertebrate communities in support of river health management in Kwazulu-Natal, South Africa*. University of KwaZulu-Natal.

Agoro, M. A., Adeniji, A. O., Adefisoye, M. A., & Okoh, O. O. (2020). Heavy metals in wastewater and sewage sludge from selected municipal treatment plants in eastern cape province, south africa. *Water (Switzerland), 12*(10). https://doi.org/10.3390/w12102746

Akamagwuna, F. C. (2018). *Taxonomic and Trait-Based Responses of the Orders Ephemeroptera, Plecoptera, Odonata, and Trichoptera (Epot) To Sediment Stress in the Tsitsa River and Its Tributaries, Eastern Cape, South Africa*. Rhodes University.

Akamagwuna, F. C. (2021). Application of macroinvertebrate-based biomonitoring and stable isotopes for assessing the effects of agricultural land-use on river ecosystem structure and function in the Kat River , Eastern Cape , South Africa A thesis submitted in fulfilment of the re. *Rhodes University, April*.

Akamagwuna, F. C., Mensah, P. K., Nnadozie, C. F., & Odume, O. N. (2019a). Evaluating the responses of taxa in the orders Ephemeroptera, Plecoptera and Trichoptera (EPT) to sediment stress in the Tsitsa River and its tributaries, Eastern Cape, South Africa. *Environmental Monitoring and Assessment*, *191*(11), 664. https://doi.org/10.1007/s10661-019-7846-9

Akamagwuna, F. C., Mensah, P. K., Nnadozie, C. F., & Odume, O. N. (2019b). Trait-based responses of Ephemeroptera, Plecoptera, and Trichoptera to sediment stress in the Tsitsa River and its tributaries, Eastern Cape, South Africa. *River Research and Applications*, *35*(7), 999–1012. https://doi.org/10.1002/rra.3458

Akamagwuna, F. C., Odume, O. N., & Richoux, N. B. (2022). Exploring the community structure of Afrotropical macroinvertebrate traits and ecological preferences along an agricultural pollution gradient in the Kat River, Eastern Cape, South Africa. *Ecological Indicators*, *135*(January), 108570. https://doi.org/10.1016/j.ecolind.2022.108570

Akyildiz, G. K., Bakir, R., Polat, S., & Duran, M. (2018). Mentum Deformities of Chironomid Larvae as an Indicator of Environmental Stress in Büyük Menderes River, Turkey. *Inland Water Biology*, *11*(4), 515–522. https://doi.org/10.1134/S1995082918040028

Al-Shami, S. A., Rawi, C. S. M., HassanAhmad, A., & Nor, S. A. M. (2010). Distribution of Chironomidae (Insecta: Diptera) in polluted rivers of the Juru River Basin, Penang, Malaysia. *Journal of Environmental Sciences*, *22*(11), 1718–1727. https://doi.org/10.1016/S1001-0742(09)60311-9

Alberti, M., Booth, D., Hill, K., Coburn, B., Avolio, C., Coe, S., & Spirandelli, D. (2007). The impact of urban patterns on aquatic ecosystems: An empirical analysis in Puget lowland sub-basins. *Landscape and Urban Planning*, *80*(4), 345–361. https://doi.org/10.1016/j.landurbplan.2006.08.001

Alves, A. T., Petsch, D. K., & Barros, F. (2020). Drivers of benthic metacommunity structure along tropical estuaries. *Scientific Reports*, *10*(1), 1739. https://doi.org/10.1038/s41598-020-58631-1

Antczak-Orlewska, O., Płóciennik, M., Sobczyk, R., Okupny, D., Stachowicz-Rybka, R., Rzodkiewicz, M., Siciński, J., Mroczkowska, A., Krąpiec, M., Słowiński, M., & Kittel, P. (2021). Chironomidae Morphological Types and Functional Feeding Groups as a Habitat Complexity Vestige. *Frontiers in Ecology and Evolution*, *8*(January), 1–16. https://doi.org/10.3389/fevo.2020.583831

APHA. (2005). *Standard Methods for the Examination of Water and Wastewater.* (21st Editi). Washington DC.

Arambourou, H., Llorente, L., Moreno-Ocio, I., Herrero, Ó., Barata, C., Fuertes, I., Delorme, N., Méndez-Fernández, L., & Planelló, R. (2020). Exposure to heavy metal-contaminated sediments disrupts gene expression, lipid profile, and life history traits in the midge

Chironomus riparius. *Water Research*, *168*. https://doi.org/10.1016/j.watres.2019.115165

Arimoro, F. O., Auta, Y. I., Odume, O. N., Keke, U. N., & Mohammed, A. Z. (2018). Mouthpart deformities in Chironomidae (Diptera) as bioindicators of heavy metals pollution in Shiroro Lake, Niger State, Nigeria. *Ecotoxicology and Environmental Safety*, *149*(June 2017), 96–100. https://doi.org/10.1016/j.ecoenv.2017.10.074

Arimoro, F. O., & Keke, U. N. (2021). Stream biodiversity and monitoring in North Central, Nigeria: the use of macroinvertebrate indicator species as surrogates. *Environmental Science and Pollution Research*, *28*(24), 31003–31012. https://doi.org/10.1007/s11356-021-12922-w

Armitage, P. D., Cranston, P. S., & Pinder, L. C. V. (1995). *The Chironomidae. The Biology and Ecology of Non-biting Midges*.

Ashford, O. S., Guan, S., Capone, D., Rigney, K., Rowley, K., Cordes, E. E., Cortés, J., Rouse, G. W., Mendoza, G. F., Sweetman, A. K., & Levin, L. A. (2021). Relationships between biodiversity and ecosystem functioning proxies strengthen when approaching chemosynthetic deep-sea methane seeps. *Proceedings of the Royal Society B: Biological Sciences*, *288*(1957), 4–6. https://doi.org/10.1098/rspb.2021.0950

Azrina, M. Z., Yap, C. K., Rahim Ismail, A., Ismail, A., & Tan, S. G. (2006). Anthropogenic impacts on the distribution and biodiversity of benthic macroinvertebrates and water quality of the Langat River, Peninsular Malaysia. *Ecotoxicology and Environmental Safety*, *64*(3), 337–347. https://doi.org/10.1016/j.ecoenv.2005.04.003

Baba, A., & Gündüz, O. (2017). Effect of geogenic factors on water quality and its relation to human health around Mount Ida, Turkey. *Water (Switzerland)*, *9*(1), 1–21. https://doi.org/10.3390/w9010066

Baptista, D. F., Buss, D. F., Egler, M., Giovanelli, A., Silveira, M. P., & Nessimian, J. L. (2007). A multimetric index based on benthic macroinvertebrates for evaluation of Atlantic Forest streams at Rio de Janeiro State, Brazil. *Hydrobiologia*, *575*(1), 83–94. https://doi.org/10.1007/s10750-006-0286-x

Baptista, D. F., de Souza, R. S. G., Vieira, C. A., Mugnai, R., Souza, A. S., & de Oliveira, R. B. S. (2011). Multimetric index for assessing ecological condition of running waters in the upper reaches of the Piabanha-Paquequer-Preto basin, Rio De Janeiro, Brazil. *Zoologia*, *28*(5), 619–628. https://doi.org/10.1590/S1984-46702011000500010

Basson, M. S. (2011). Water development in South Africa. *Water in the Green Economy in Practice: Towards Rio+20*, 12. http://www.un.org/waterforlifedecade/green_economy_2011/pdf/session_1_economic_instruments_south_africa.pdf

BCMM. (2020). *Buffalo City Metropolitan Municipality: Integrated Development Plan* (Issue June). https://www.ecsecc.org/documentrepository/informationcentre/BCM-INDICATOR_REPORT.pdf

BCMM. (2021). *Integrated Waste Management Plan 2021 - 2025 Draft*. https://www.buffalocity.gov.za/CM/uploads/documents/20210311111635944238O2Aug2021RevisedDraftIWMP_BCMv2.pdf

Beck, M., Billoir, E., Vincent, F., Albin, M., Usseglio-Polatera, P., & Danger, M. (2022). A database of West European headwater macroinvertebrate stoichiometric traits. *Global Ecology and Biogeography, 31*, 4–9.

Belmar, O., Bruno, D., Guareschi, S., Mellado-Díaz, A., Millán, A., & Velasco, J. (2019). Functional responses of aquatic macroinvertebrates to flow regulation are shaped by natural flow intermittence in Mediterranean streams. *Freshwater Biology*, *64*(5), 1064–1077. https://doi.org/10.1111/fwb.13289

Beneberu, G., Mengistou, S., Eggermont, H., & Verschuren, D. (2014). Chironomid distribution along a pollution gradient in Ethiopian rivers, and their potential for biological water quality monitoring. *African Journal of Aquatic Science*, *39*(1), 45–56. https://doi.org/10.2989/16085914.2013.870525

Benzina, I., Si Bachir, A., Santoul, F., & Céréghino, R. (2021). Macroinvertebrate functional trait responses to environmental gradients and anthropogenic disturbance in arid-land streams of North Africa. *Journal of Arid Environments, 195*(September). https://doi.org/10.1016/j.jaridenv.2021.104626

Berg, M. B., & Hellenthal, R. A. (1992). Life Histories and Growth of Lotic Chironomids (Diptera: Chironomidae). *Annals of the Entomological Society of America*, *85*(5), 578–

589. https://doi.org/10.1093/aesa/85.5.578

Bohus, A., Gál, B., Barta, B., & Szivák, I. (2023). Effects of urbanization - induced local alterations on the diversity and assemblage structure of macroinvertebrates in low - order streams. *Hydrobiologia*, *850*(4), 881–899. https://doi.org/10.1007/s10750-022-05130-1

Bonada, N., Dallas, H., Rieradevall, M., Prat, N., & Day, J. (2006). A comparison of rapid bioassessment protocols used in 2 regions with Mediterranean climates, the Iberian Peninsula and South Africa. *Journal of the North American Benthological Society, 25*(2), 487–500. https://doi.org/10.1899/0887-3593(2006)25[487:ACORBP]2.0.CO;2

Booth, D. B., & Bledsoe, B. P. (2009). Streams and Urbanization. In L. A. Baker (Ed.), *The Water Environment of Cities* (pp. 93–123). Springer Science+Business Media, LLC 2009. https://doi.org/10.1007/978-0-387-84891-4

Boretti, A., & Rosa, L. (2019). Reassessing the projections of the World Water Development Report. *Npj Clean Water*, *2*(1). https://doi.org/10.1038/s41545-019-0039-9

Bouchard, R. W. J., Huggins, D., & Kriz, J. (2005). A Review of the Issues Related to Taxonomic Resolution in Biological Monitoring of Aquatic Ecosystems with an Emphasis on Macroinvertebrates. In *USEPA* (Vol. 7, Issue 2). http://dx.doi.org/10.1016/j.intell.2008.09.007%0Ahttp://dx.doi.org/10.1016/S0010-9452(58)80010-6%0Ahttp://pss.sagepub.com/content/17/1/67.short%0Ahttp://dx.doi.org/10.1016/j.cogdev.2013.06.002%0Ahttp://www.chabris.com/Hooven2008.pdf%0Ahttp://www.ncbi.nlm.

Bournaud, M., Richoux, P., & Usseglio-polatera, P. (1992). An approach to the synthesis of qualitative ecological information from aquatic coleoptera communities. *Regulated Rivers: Resaerch and Management*, *7*, 165–180. https://doi.org/https://doi.org/10.1002/rrr.3450070205

Brabec, K., Janecek, B. F. U., Rossaro, B., Spies, M., Bitusik, P., Syrovatka, V., & Schmidt-Kloiber, A. (2023). *Dataset "Chironomidae". www.freshwaterecology.info - the taxa and autecology database for freshwater organisms, version 8.0 (accessed on 20.11.2023).* https://www.freshwaterecology.info/index.php

Brown, L., Gray, R. H., Hughes, R. M., & Meadors, M. R. (2005). Introduction to Effects of

Urbanization on Stream Ecosystems. In *Centennial History of the Carnegie Institution of Washington* (Issue January 2005, pp. 1–8). Cambridge University Press. https://doi.org/10.1017/CBO9780511535611.003

Bruno, D., Belmar, O., Maire, A., Morel, A., Dumont, B., & Datry, T. (2019). Structural and functional responses of invertebrate communities to climate change and flow regulation in alpine catchments. *Global Change Biology*, *25*(5), 1612–1628. https://doi.org/10.1111/gcb.14581

Burger, W., & Burge, M. (2007). *Digital Image Processing: An Algorithmic Approach Using Java*. Springer International Publishing.

Bwapwa, J. K. (2018). Review on Main Issues Causing Deterioration of Water Quality and Water Scarcity: Case Study of South Africa. *Environmental Management and Sustainable Development*, *7*(3), 14. https://doi.org/10.5296/emsd.v7i3.13156

Cairns, J., & Pratt, J. R. (1993). A history of biological monitoring using benthic macroinvertebrates. *Freshwater Biomonitoring and Benthic Macroinvertebrates*, 10–27.

Camara, M., Jamil, N. R., & Abdullah, A. F. Bin. (2019). Impact of land uses on water quality in Malaysia: a review. *Ecological Processes*, *8*(1), 10. https://doi.org/10.1186/s13717-019-0164-x

Cañedo-Argüelles, M., Bogan, M. T., Lytle, D. A., & Prat, N. (2016). Are Chironomidae (Diptera) good indicators of water scarcity? Dryland streams as a case study. *Ecological Indicators*, *71*, 155–162. https://doi.org/10.1016/j.ecolind.2016.07.002

Cao, Y., Williams, D., & Williams, N. E. (1998). How important are rare species in aquatic ecology and bioassessment? Limnology and Oceanography 43(7):1403-1409. *Limnology and Oceanography*, *43*(7), 0.

Carmignani, J. R., & Roy, A. H. (2017). Ecological impacts of winter water level drawdowns on lake littoral zones: a review. *Aquatic Sciences*, *79*(4), 803–824. https://doi.org/10.1007/s00027-017-0549-9

Caro-Borrero, A., Carmona-Jiménez, J., Rivera-Ramírez, K., & Bieber, K. (2021). The effects of urbanization on aquatic ecosystems in peri-urban protected areas of Mexico City: The contradictory discourse of conservation amid expansion of informal settlements. *Land Use Policy*, *102*(October 2020). https://doi.org/10.1016/j.landusepol.2020.105226

Castro-Rebolledo, M. I., & Donato-Rondon, J. C. (2015). Emergence patterns in tropical insects: The role of water discharge frequency in an Andean Stream. *Annales de Limnologie*, *51*(2), 147–155. https://doi.org/10.1051/limn/2015011

Chadwick, M. A., Thiele, J. E., Huryn, A. D., Benke, A. C., & Dobberfuhl, D. R. (2012). Effects of urbanization on macroinvertebrates in tributaries of the St. Johns River, Florida, USA. *Urban Ecosystems*, *15*(2), 347–365. https://doi.org/10.1007/s11252-011-0217-0

Chen, K., Rajper, A. R., Hughes, R. M., Olson, J. R., Wei, H., & Wang, B. (2019). Incorporating functional traits to enhance multimetric index performance and assess land use gradients. *Science of the Total Environment*, *691*, 1005–1015. https://doi.org/10.1016/j.scitotenv.2019.07.047

Chessman, B. C. (2021). What’s wrong with the Australian River Assessment System (AUSRIVAS)? *Marine and Freshwater Research*, *72*(8), 1110–1117. https://doi.org/10.1071/MF20361

Chevenet, F., Dolédec, S., & Chessel, D. (1994). A fuzzy coding approach for the analysis of long-term ecological data. *Freshwater Biology*, *31*(3), 295–309. https://doi.org/10.1111/j.1365-2427.1994.tb01742.x

Chutter, F. (1998). Division of Water Technology , CSIR ( now Environmentek , CSIR ) ( Current affiliation : AfriDev Consultants , Pretoria ). *Water Research*, *422*.

Clarke, K. R., & Warwick, R. M. (1994). Similarity-based testing for community pattern: the two-way layout with no replication. *Marine Biology*, *118*(1), 167–176. https://doi.org/10.1007/BF00699231

Clarke, K. R., & Warwick, R. M. (2001). Change in marine communities: an approach to statistical analysis and interpretation. 2nd edition. Primer-E, Plymouth. *Plymouth, United Kingsom: PRIMER-E*, 172. http://plymsea.ac.uk/7656/%250Ahttp://owasptop10.googlecode.com/files/OWASP Top 10 - 2013.pdf%0Ahttp://plymsea.ac.uk/7656/%0Ahttp://owasptop10.googlecode.com/files/O

WASP Top 10 - 2013.pdf

Clarke, R. T., Wright, J. F., & Furse, M. T. (2003). RIVPACS models for predicting the expected macroinvertebrate fauna and assessing the ecological quality of rivers. *Ecological Modelling*, *160*(3), 219–233. https://doi.org/10.1016/S0304-3800(02)00255-7

COGTA. (2018). *Sarah Baartman District Municipality Profile* (Issue 1). https://medium.com/@arifwicaksanaa/pengertian-use-case-a7e576e1b6bf

COGTA. (2020a). *Buffalo City profile and analysis*. https://www.cogta.gov.za/ddm/wp-content/uploads/2020/08/DistrictProfile_BUFFALOCITY07072020-1.pdf

COGTA, C. G. and T. A. (2020b). *Nelson Mandela Bay Metro EC Profile*. https://www.cogta.gov.za/ddm/wp-content/uploads/2020/11/Nelson-Mandela-Bay-Metro-October-2020-1.pdf

Collins, S. J., & Fahrig, L. (2020). Are macroinvertebrate traits reliable indicators of specific agrichemicals? *Ecological Indicators*, *111*(November 2019), 105965. https://doi.org/10.1016/j.ecolind.2019.105965

Connolly, N. M., Crossland, M. R., & Pearson, R. G. (2004). Effect of low dissolved oxygen on survival, emergence, and drift of tropical stream macroinvertebrates. *Journal of the North American Benthological Society*, *23*(2), 251–270. https://doi.org/10.1899/0887-3593(2004)023<0251:EOLDOO>2.0.CO;2

Corbi, J. J., Bernegossi, A. C., Moura, L., Felipe, M. C., Issa, C. G., Silva, M. R. L., & Gorni, G. R. (2019). Chironomus sancticaroli (Diptera, Chironomidae) as a Sensitive Test Species: Can We Rely on Its Use After Repeated Generations, Under Laboratory Conditions? *Bulletin of Environmental Contamination and Toxicology*, *103*(2), 213–217. https://doi.org/10.1007/s00128-019-02644-8

Covich, A. P., W.D. Sheparhd, E. A. B., & Carpenter, C. S. (1978). *Effects of Fluctuating Flow Rates and Water Levels on Chironomids: Direct and Indirect Alterations of Habitat Stability*. *January*, 141–156.

Cranston, P. S. (1990). Biomonitoring and invertebrate taxonomy. *Environmental Monitoring and Assessment*, *14*, 265–273.

Cranston, P. S. (2014). Insecta : Diptera , Chironomidae. *Freshwater Invertebrates of the Malaysian Region*, *May*, 711–735.

Cuffney, T. F., Kennen, J. G., & Waite, I. R. (2013). 1.9 Aquatic Ecosystems as Indicators of Status and Trends in Water Quality. In *Comprehensive Water Quality and Purification* (Vol. 1). Elsevier Ltd. https://doi.org/10.1016/B978-0-12-382182-9.00008-6

Culp, J. M., Armanini, D. G., Dunbar, M. J., Orlofske, J. M., LeRoy Poff, N., Pollard, A. I., Yates, A. G., & Hose, G. C. (2011). Incorporating traits in aquatic biomonitoring to enhance causal diagnosis and prediction. *Integrated Environmental Assessment and Management*, *7*(2), 187–197. https://doi.org/10.1002/ieam.128

Cummins, K. W. (2019). Functional Analysis of Stream Macroinvertebrates. In *Limnology - Some New Aspects of Inland Water Ecology* (Vol. 11, Issue tourism, p. 13). IntechOpen. https://doi.org/10.5772/intechopen.79913

Dallas, H. F. (1995). *An evaluation of SASS as a tool for the rapid bioassessment of water quality*. University of Cape Town.

Dallas, H. F. (1997). A preliminary evaluation of aspects of sass (south african scoring system) for the rapid bioassessment of water quality in rivers, with particular reference to the incorporation of sass in a national biomonitoring programme. *Southern African Journal of Aquatic Sciences*, *23*(1), 79–94. https://doi.org/10.1080/10183469.1997.9631389

Dallas, H. F. (2000). Ecological reference conditions for riverine macroinvertebrates and the River Health Programme, South Africa. *1st WARFSA/WaterNet Symposium: Sustainable Use of Water Resourc*, *November 2000*, 1–10.

Dallas, H. F. (2007). River Health Programme: South African Scoring System (SASS) Data Interpretation Guidelines. *Group*, *September*, 85. http://www.dwa.gov.za/iwqs/rhp/methods/SASS Interpretation Guidelines.pdf

Dallas, H. F. (2021). Rapid Bioassessment Protocols Using Aquatic Macroinvertebrates in Africa–Considerations for Regional Adaptation of Existing Biotic Indices. *Frontiers in Water*, *3*(February), 1–12. https://doi.org/10.3389/frwa.2021.628227

Dallas, H. F., & Day, J. A. (2007). Natural variation in macroinvertebrate assemblages and the development of a biological banding system for interpreting bioassessment data - A preliminary evaluation using data from upland sites in the south-western Cape, South Africa. *Hydrobiologia*, *575*(1), 231–244. https://doi.org/10.1007/s10750-006-0374-y

De La Rey, P. A., Van Rensburg, L., & Vosloo, A. (2008). On the use of diatom-based biological monitoring Part 1: A comparison of the response of diversity and aut-ecological diatom indices to water quality variables in the Marico-Molopo River catchment. *Water SA*, *34*(1), 53–60. https://doi.org/10.4314/wsa.v34i1.180762

Deliberalli, W., Cansian, R. L., Pereira, A. A. M. M., Loureiro, R. C., Hepp, L. U., & Restello, R. M. (2018). The effects of heavy metals on the incidence of morphological deformities in chironomidae (Diptera). *Zoologia*, *35*(Tüzen 2009), 1–7. https://doi.org/10.3897/zoologia.35.e12947

Delzer, G. C., & Mckenzie, S. W. (2003). Five-Day Biochemical Oxygen Demand. In *USGS TWRI Book* (Vol. 7, pp. 1–21).

Department of Human Settlements. (2021). *Baseline Evaluation of informal settlements targeted for upgrading in the 2019-2024 MTSF*. Department of Human Settlements, Pretoria.

Desrosiers, M., Usseglio-polatera, P., Archaimbault, V., Larras, F., Méthot, G., & Pinel-alloul, B. (2019). Science of the Total Environment Assessing anthropogenic pressure in the St . Lawrence River using traits of benthic macroinvertebrates. *Science of the Total Environment*, *649*, 233–246. https://doi.org/10.1016/j.scitotenv.2018.08.267

Dettinger-Klemm, P. M. A. (2003). *Chironomids (Diptera, Nematocera) of Temporary Pools* [der Phillips-Universität Marburg vorgelegt von]. https://core.ac.uk/reader/144302780

DFFE, D. of E. A. (2012). Chapter 8. Inland water. In *2nd South Africa Environment Outlook. A report on the state of the environment*. https://www.dffe.gov.za/sites/default/files/reports/environmentoutlook_chapter8.pdf

Dickens, C. W. S., & Graham, P. M. (1998). Biomonitoring for effective management of wastewater discharges and the health of the river environment. *Aquatic Ecosystem Health and Management*, *1*(2), 199–217. https://doi.org/10.1080/14634989808656914

Dickens, C. W. S., & Graham, P. M. (2002). The south african scoring system (SASS) version 5 rapid bioassessment method for rivers. *African Journal of Aquatic Science*, *27*(1), 1–10. https://doi.org/10.2989/16085914.2002.9626569

Dolédec, S., Chessel, D., ter Braak, C. J. F., & Champely, S. (1996). Matching species traits to environmental variables: a new three-table ordination method. *Environmental and Ecological Statistics*, *3*(2), 143–166. https://doi.org/10.1007/BF02427859

Dolédec, S., & Statzner, B. (2008). Invertebrate traits for the biomonitoring of large European rivers: An assessment of specific types of human impact. *Freshwater Biology*, *53*(3), 617–634. https://doi.org/10.1111/j.1365-2427.2007.01924.x

Dray, S., Choler, P., Dolédec, S., Peres-Neto, P. R., Thuiller, W., Pavoine, S., & Ter Braak, C. J. F. (2014). Combining the fourth-corner and the RLQ methods for assessing trait responses to environmental variation. *Ecology*, *95*(1), 14–21. https://doi.org/10.1890/13-0196.1

Dray, S., & Dufour, A. (2007). The ade4 Package: Implementing the Duality Diagram for Ecologists. *Journal of Statistical Software*, *22*(4), 1–20. https://doi.org/10.18637/jss.v022.i04

Drimie, S., Faber, M., Vearey, J., & Nunez, L. (2013). Dietary diversity of formal and informal residents in Johannesburg, South Africa. *BMC Public Health*, *13*(1). https://doi.org/10.1186/1471-2458-13-911

Dudgeon, D., Arthington, A. H., Gessner, M. O., Kawabata, Z. I., Knowler, D. J., Lévêque, C., Naiman, R. J., Prieur-Richard, A. H., Soto, D., Stiassny, M. L. J., & Sullivan, C. A. (2006). Freshwater biodiversity: Importance, threats, status and conservation challenges. *Biological Reviews of the Cambridge Philosophical Society*, *81*(2), 163–182. https://doi.org/10.1017/S1464793105006950

DWAF, D. of W. A. and F. (2004). National Water Resource Strategy, First Edition, September 2004. *Water Resources, September*, 55–135.

DWAF, D. of W. A. and F. (2008). *National Aquatic Ecosystem Health Monitoring Programme (NAEHMP): River Health Programme (RHP) Implementation Manual. Version 2*. Department of Water Affairs and Forestry.

DWAF, D. of W. A. and F. (2010). Regulations for the Establishment of a Water Resource classification system. In *Government gazette No. 33541*. https://cer.org.za/wp-content/uploads/2011/10/Regulations-for-the-Establishment-of-the-Classification-System-2010.pdf

DWAF, D. of W. A. and F. (2013). National Water Resource Strategy - 2nd edition 2013. In *Water Resources*. http://www.dwa.gov.za/documents/Other/Strategic Plan/NWRS2-Final-email-version.pdf.

DWS. (2022a). *Yearbook 2021/22 Water and Sanitation*. https://www.gcis.gov.za/sites/default/files/docs/gcis/21. Water and Sanitation.pdf

DWS, D. of W. and S. (2017a). *Water Quality Management Policies and Strategies for South Africa. Report No. 1.2.1: A Review of Water Quality Management Policies and Strategies for South Africa and International Experience.: Vol. 000/00/217*. http://www6.dwa.gov.za/iwqms/Documents/Report 1.2.1_Review of South African Policy and Strategy_Inaugural Report.pdf

DWS, D. of W. and S. (2017b). *Water Quality Management Policies and Strategies for South Africa. Report No. 3.2. Intergrated water quality management: policies and strategies for South Africa: Vol. 000/00/217*.

DWS, D. of W. and S. (2019). *River Ecostatus Monitoring Programme State of Rivers Report 2017-2018*. https://www.dws.gov.za/iwqs/rhp/state_of_rivers/annual/State_of_Water_2017_2018_hydro_year_updated_FINAL_2019_10_11_web.pdf

DWS, D. of W. and S. (2022b). *8 Water Resource Protection*. https://mtshastaca.gov/wp-content/uploads/2021/04/Water-Resource-Elementv1.pdf

DWS, D. of W. and S. (2022c). *Green Drop: Eastern Cape, 2022*. https://ws.dws.gov.za/iris/releases/Report_Eastern_Cape_Rev05_27Mar22_MN_web.pdf

ECSECC, E. C. S. economic consultative council. (2020). *Economic Review of the Eastern Cape*. https://www.ecsecc.org.za/datarepository/documents/ecsecc-gdp-report2020q3-1_FnB82.pdf

Edegbene, A. O. (2020). *Developing macroinvertebrate trait- and taxonomically-based approaches for biomonitoring wadeable riverine systems in the Niger Delta, Nigeria* (Issue March). Rhodes University.

Edegbene, A. O., Akamagwuna, F. C., Odume, O. N., Arimoro, F. O., Edegbene Ovie, T. T., Akumabor, E. C., Ogidiaka, E., Kaine, E. A., & Nwaka, K. H. (2022). A Macroinvertebrate-Based Multimetric Index for Assessing Ecological Condition of Forested Stream Sites Draining Nigerian Urbanizing Landscapes. *Sustainability (Switzerland)*, *14*(18), 1–21. https://doi.org/10.3390/su141811289

Edegbene, A. O., Arimoro, F. O., & Odume, O. N. (2019). Developing and applying a macroinvertebrate-based multimetric index for urban rivers in the Niger Delta, Nigeria. *Ecology and Evolution*, *9*(22), 12869–12885. https://doi.org/10.1002/ece3.5769

Edegbene, A. O., Arimoro, F. O., & Odume, O. N. (2020a). Exploring the distribution patterns of macroinvertebrate signature traits and ecological preferences and their responses to urban and agricultural pollution in selected rivers in the Niger Delta ecoregion, Nigeria. *Aquatic Ecology*, *54*(2), 553–573. https://doi.org/10.1007/s10452-020-09759-9

Edegbene, A. O., Arimoro, F. O., & Odume, O. N. (2020b). How does Urban Pollution Influence Macroinvertebrate Traits in Forested Riverine Systems? *Water*, *12*(11), 3111. https://doi.org/10.3390/w12113111

Edegbene, A. O., Arimoro, F. O., Odume, O. N., Ogidiaka, E., & Keke, U. N. (2021). Can Macroinvertebrate Traits Be Explored and Applied in Biomonitoring Riverine Systems Draining Forested Catchments? *Frontiers in Water*, *3*(March). https://doi.org/10.3389/frwa.2021.607556

El Yaagoubi, S., El Alami, M., Harrak, R., Azmizem, A., Ikssi, M., & Mansour, M. R. A. (2023). Assessment of functional feeding groups (FFG) structure of aquatic insects in North-western Rif - Morocco. *Biodiversity Data Journal*, *11*. https://doi.org/10.3897/BDJ.11.E104218

Elias, J. D. (2021). Simple and Cost-Effective Biomonitoring Method for Assessing Pollution in Tropical African Rivers. *Open Journal of Ecology*, *11*(04), 407–436. https://doi.org/10.4236/oje.2021.114027

Enns, D., Cunze, S., Baker, N. J., Oehlmann, J., & Jourdan, J. (2023). Flushing away the future: The effects of wastewater treatment plants on aquatic invertebrates. *Water Research*, 33. https://doi.org/10.1016/j.watres.2023.120388

Erasmus, J. H., Lorenz, A. W., Zimmermann, S., Wepener, V., Sures, B., Smit, N. J., & Malherbe, W. (2021). A diversity and functional approach to evaluate the macroinvertebrate responses to multiple stressors in a small subtropical austral river. *Ecological Indicators*, *131*(July), 108206. https://doi.org/10.1016/j.ecolind.2021.108206

Feris, L., & Kotze, L. (2015). The regulation of acid mine drainage in South Africa: law and governance perspectives. *Potchefstroom Electronic Law Journal/Potchefstroomse Elektroniese Regsblad*, *17*(5), 2104. https://doi.org/10.4314/pelj.v17i5.07

Ferreira, W., Paiva, L., & Callisto, M. (2011). Development of a benthic multimetric index for biomonitoring of a neotropical watershed. *Brazilian Journal of Biology, 71*(1), 15–25. https://doi.org/10.1590/s1519-69842011000100005

Finkenbine, J. K., Atwater, J. W., & Mavinic, D. S. (2000). Stream health after urbanization. *Journal of the American Water Resources Association*, *36*(5), 1149–1160. https://doi.org/10.1111/j.1752-1688.2000.tb05717.x

Forsyth, D. J. (1979). Life stages and taxonomic affinities of xenochironomus canterburyensis (Chironomidae: Diptera). *New Zealand Journal of Zoology*, *6*(3), 467–472. https://doi.org/10.1080/03014223.1979.10428387

Forsyth, D. J., & McCallum, I. D. (1978). Xenochironomus canterburyensis (Diptera: Chironomidae), a commensal of hyridella menziesi (lamellibranchia) in lake taupo; features of pre-adult life history. *New Zealand Journal of Zoology*, *5*(4), 795–800. https://doi.org/10.1080/03014223.1978.10423821

França, J. S., Solar, R., Hughes, R. M., & Callisto, M. (2019). Student monitoring of the ecological quality of neotropical urban streams. *Ambio*, *48*(8), 867–878. https://doi.org/10.1007/s13280-018-1122-z

Frost & Sullivan. (2010). *Eutrophication research impact assessment*. Water Research Commission.

Gadawski, P., Rossaro, B., Giłka, W., Montagna, M., Zawal, A., & Grabowski, M. (2021). First insights into the diversity and ecology of non-biting midges (Diptera: Chironomidae) of the unique ancient Skadar Lake basin (Montenegro/Albania). *Journal of Great Lakes Research, xxxx*. https://doi.org/10.1016/j.jglr.2021.02.003

Gao, J., Huang, Y., Zhi, Y., Yao, J., Wang, F., Yang, W., Han, L., Lin, D., He, Q., Wei, B., & Grieger, K. (2022). Assessing the impacts of urbanization on stream ecosystem functioning through investigating litter decomposition and nutrient uptake in a forest and a hyper-eutrophic urban stream. *Ecological Indicators*, *138*(January), 108859. https://doi.org/10.1016/j.ecolind.2022.108859

Gayraud, S., Statzner, B., Bady, P., Haybachp, A., Schöll, F., Usseglio-Polatera, P., & Bacchi, M. (2003). Invertebrate traits for the biomonitoring of large European rivers: An initial assessment of alternative metrics. *Freshwater Biology*, *48*(11), 2045–2064. https://doi.org/10.1046/j.1365-2427.2003.01139.x

Geldenhuys, C. J. (1989). *Environmental and biogeographic influences on the distribution and composition of the southern Cape forests (veld type 4)* [University of Cape Town]. http://hdl.handle.net/11427/23520

Gerber, A., & Gabriel, M. J. M. (2002). *Aquatic Inverterbrates of South African Rivers: Illustrations*. https://www.dws.gov.za/iwqs/biomon/aquabugsa/Aquatic_invertebrates_300dpi.pdf

Gininda, S. L. (2016). The ecological impacts of pollution on a river ecosystem: a community index and stable isotope approach. In *Rhodes University* (Vol. 147, Issue March).

Ginsburg, A. E., Crafford, J. G., Harris, K. R., Wilkinson, M., & Mashimbye, D. (2010). *Framework and manual for the evaluation of aquatic ecosystems services for the resource directed measures : report to the Water Research Commission*. Water Research Commission.

Goddeeris, B. R. (1989). Life cycle characteristics in Pseudochironomus prasinatus (Staeger, 1839) (Diptera: Chironomidae). *Bulletin de l'Institut Royal Des Sciences Naturelles de Belgique Entomologie*, *59*, 165–171.

Gordon, A. K., Griffin, N. J., & Palmer, C. G. (2015). The relationship between concurrently measured sass (South African scoring system) and turbidity data archived in the South African river health programme’s rivers database. *Water SA*, *41*(1), 21–26. https://doi.org/10.4314/wsa.v41i1.4

Gouissi, F. M., Samon, O., Abahi, K. S., Adje, D. D., Tchaou, C. M., Piami, Z. O., Okoya, J. G. A., & Gnohossou, M. P. (2019). Relationship between Macroinvertebrates and Physico-Chemical Parameters to Access Water Quality of the Affon River in B&#233;nin. *Advances in Entomology*, *07*(04), 92–104. https://doi.org/10.4236/ae.2019.74008

Gqomfa, B., Maphanga, T., & Shale, K. (2022). The impact of informal settlement on water quality of Diep River in Dunoon. *Sustainable Water Resources Management*, *8*(1), 1–18. https://doi.org/10.1007/s40899-022-00629-w

Gray, C. (2016). *Towards a networks based approach to biomonitoring* (Issue February) [Queen Mary University of London]. http://qmro.qmul.ac.uk/xmlui/handle/123456789/12820%0AInformation

Green, S. J., Brookson, C. B., Hardy, N. A., & Crowder, L. B. (2022). Trait-based approaches to global change ecology: Moving from description to prediction. *Proceedings of the Royal Society B: Biological Sciences*, *289*(1971). https://doi.org/10.1098/rspb.2022.0071

Greenland, S., Senn, S. J., Rothman, K. J., Carlin, J. B., Poole, C., Goodman, S. N., & Altman, D. G. (2016). Statistical tests, P values, confidence intervals, and power: a guide to misinterpretations. *European Journal of Epidemiology*, *31*(4), 337–350. https://doi.org/10.1007/s10654-016-0149-3

Gresens, S. E., Belt, K. T., Tang, J. A., Gwinn, D. C., & Banks, P. A. (2007). Temporal and spatial responses of Chironomidae (Diptera) and other benthic invertebrates to urban stormwater runoff. *Hydrobiologia*, *575*(1), 173–190. https://doi.org/10.1007/s10750-006-0366-y

Griffin, N. J., Palmer, C. G., & Scherman, P.-A. (2014). *Critical Analysis of Environmental Water Quality in South Africa: Historic and Current Trends Report to the WATER RESEARCH COMMISSION: Vol. WRC Report* (Issue 2184).

Grizzetti, B., Liquete, C., Pistocchi, A., Vigiak, O., Zulian, G., Bouraoui, F., De Roo, A., & Cardoso, A. C. (2019). Relationship between ecological condition and ecosystem services in European rivers, lakes and coastal waters. *Science of the Total Environment*, *671*, 452–465. https://doi.org/10.1016/j.scitotenv.2019.03.155

Grobler, D. C., & Toerien, D. F. (1986). The need to consider water quality in the planning of new urban development: A simulation study. *Water SA*, *12*(1), 27–30.

Grobler, D. C., Toerien, D. F., & Rossouw, J. N. (1987). A review of sediment/water quality interaction with particular reference to the Vaal River system. *Water SA*, *13*, 15–22.

Groenendijk, D. (1999). *Dynamics of metal adaptation in riverine chironomids* (Vol. 117, Issue 1) [University of Amsterdam]. https://doi.org/10.1016/S0269-7491(01)00154-3

Growns, I., Murphy, J. F., & Jones, J. I. (2017). The effects of altered flow and bed sediment on macroinvertebrates in stream mesocosms. *Marine and Freshwater Research*, *68*(3), 496. https://doi.org/10.1071/MF15160

Guerrero-Aguilar, A., Rodríguez-Castrejón, U. E., Serafín-Muñoz, A. H., Schüth, C., & Noriega-Luna, B. (2022). Bioindicators and biomonitoring: Review of methodologies applied in water bodies and use during the Covid-19 pandemic. *Acta Universitaria, 32,* 1–26. https://doi.org/10.15174/au.2022.3388

Hale, R., Colombo, V., Hoak, M., Pettigrove, V., & Swearer, S. E. (2019). The influence of potential stressors on oviposition site selection and subsequent growth , survival and emergence of the non-biting midge ( Chironomus tepperi ). *Ecology and Evolution*, *March*, 5512–5522. https://doi.org/10.1002/ece3.5148

Hallock, R. K. and K. F. (2001). Quantile Regression. *Journal OfEconomic Perspectives*, *15*(4), 143–156. https://doi.org/10.1164/rccm.201012-2095OC

Halpern, M., Gasith, A., Bresler, V. M., & Broza, M. (2002). The protective nature of Chironomus luridus larval tubes against copper sulfate. *Journal of Insect Science, 2*. https://doi.org/10.1093/jis/2.1.8

Hamache, C., Płóciennik, M., Saal, I., & Arab, A. (2021). The natural factors and anthropogenic stressors influence on Chironomidae communities of two north-African wadis. *Knowledge & Management of Aquatic Ecosystems, 422,* 35. https://doi.org/10.1051/kmae/2021034

Hamilton, A. T., Schäfer, R. B., Pyne, M. I., Chessman, B., Kakouei, K., Boersma, K. S., Verdonschot, P. F. M., Verdonschot, R. C. M., Mims, M., Khamis, K., Bierwagen, B., & Stamp, J. (2020). Limitations of trait-based approaches for stressor assessment: The case of freshwater invertebrates and climate drivers. *Global Change Biology*, *26*(2), 364–379. https://doi.org/10.1111/gcb.14846

Harding, W. R. (2015). Living with eutrophication in South Africa: A review of realities and challenges. *Transactions of the Royal Society of South Africa*, *70*(2), 155–171. https://doi.org/10.1080/0035919X.2015.1014878

Harrison, A. D. (2004). *The Non-biting Midges ( Diptera : Chironomidae ) of South Africa Family : CHIRONOMIDAE*. 1–41.

Harrison, P. (1992). The policies and politics of informal settlement in South Africa: A historical perspective. *Africa Insight*, *22*(1), 323–350. http://reference.sabinet.co.za/webx/access/journal_archive/02562804/553.pdf

Hawlena, D., Hughes, K. M., & Schmitz, O. J. (2011). Trophic trait plasticity in response to changes in resource availability and predation risk. *Functional Ecology*, *25*(6), 1223–1231. https://doi.org/10.1111/j.1365-2435.2011.01891.x

Hayashi, H., Tasaki, M., Uchiyama, N., & Masaru, M. (2013). Water quality and pollution load during flood and non-flood periods in an urban tidal river. *Journal of Japan Society of Civil Engineers, Ser. B1 (Hydraulic Engineering)*, *69*(4), I_1723-I_1728. https://doi.org/10.2208/jscejhe.69.I_1723

Heino, J. (2008). Patterns of functional biodiversity and function-environment relationships in lake littoral macroinvertebrates. *American Society of Limnology and Oceanography*, *53*(4), 1446–1455.

Heino, J., Soininen, J., Alahuhta, J., Lappalainen, J., & Virtanen, R. (2017). Metacommunity ecology meets biogeography: effects of geographical region, spatial dynamics and environmental filtering on community structure in aquatic organisms. *Oecologia*, *183*(1), 121–137. https://doi.org/10.1007/s00442-016-3750-y

Hershey, A. E. (1987). *Tubes and Foraging Behavior in Larval Chironomidae : Implications for Predator Avoidance Published by : Springer in cooperation with International*

*Association for Ecology Stable URL : https://www.jstor.org/stable/4218357 Tubes and foraging behavior in lar*. *73*(2), 236–241.

Hilsenhoff, W. L. (1987). An improved biotic index of organic stream pollution. *Great Lakes Entomologist*, *20*(1), 31–40.

Hilsenhoff, W. L. (1988). Rapid Field Assessment of Organic Pollution with a Family-Level Biotic Index Author ( s ): William L . Hilsenhoff Source : Journal of the North American Benthological Society , Vol . 7 , No . 1 ( Mar ., 1988 ), Published by : The University of Chicago Pre. *Journal of the North American Benthological Society*, *7*(1), 65–68.

Houri, P. H., Yousefi, S. S., & Kheradpir, N. (2020). Diversity of chironomidae (Insecta: Diptera) genera in Taleghan River, Alborz province, Iran. *Iranian Journal of Fisheries Sciences*, *19*(2), 974–985. https://doi.org/10.22092/ijfs.2019.120646

Hughes, R. M., Karr, J. R., Vadas, R. L., DellaSala, D. A., Callisto, M., João Feio, M., Ferreira, T., Kleynhans, N., Ruaro, R., Yoder, C. O., & Michael, J. H. (2023). Global concerns related to water biology and security: The need for language and policies that safeguard living resources versus those that dilute scientific knowledge. *Water Biology and Security*, *2*(4). https://doi.org/10.1016/j.watbs.2023.100191

Huizenga, J. M., Silberbauer, M., Dennis, R., & Dennis, I. (2013). An inorganic water chemistry dataset (1972-2011) of rivers, dams and lakes in South Africa. *Water SA*, *39*(2), 335–339. https://doi.org/10.4314/wsa.v39i2.18

Imada, Y. (2020). A novel leaf-rolling chironomid, eukiefferiella endobryonia sp. Nov. (diptera, chironomidae, orthocladiinae), highlights the diversity of underwater chironomid tube structures. *ZooKeys*, *2020*(906), 73–111. https://doi.org/10.3897/zookeys.906.47834

Isidoro, P. (2014). *Using species traits to assess ecological integrity of urban streams*. Universidade De Coimbra.

Jacobsen, R. E., & Bilyj, B. (2007). An unusal new Cladotanytarsus from oligotrophic Florida Everglades marshes (Diptera: Chironomidae). *Contributions to the Systematics and Ecology of Aquatic Diptera: A Tribute to Ole A. Sæther*, *January*, 145–154.

Jaureguiberry, P., Titeux, N., Wiemers, M., Bowler, D. E., Coscieme, L., Golden, A. S., Guerra,

C. A., Jacob, U., & Takahashi, Y. (2022). The direct drivers of recent global anthropogenic biodiversity loss. *Science Advances*, *9982*(November), 1–12.

Jiang, W., Pan, B., Jiang, X., Shi, P., Zhu, P., Zhang, L., Chen, J., & Wu, N. (2021). A comparative study on the indicative function of species and traits structure of stream macroinvertebrates to human disturbances. *Ecological Indicators*, *129*, 107939. https://doi.org/10.1016/j.ecolind.2021.107939

Jiang, X., Pan, B., Song, Z., & Xie, Z. (2019). Do functional traits of chironomid assemblages respond more readily to eutrophication than taxonomic composition in Chinese floodplain lakes? *Ecological Indicators*, *103*(February), 355–362. https://doi.org/10.1016/j.ecolind.2019.04.029

Jones, F. C. (2008). Taxonomic sufficiency: The influence of taxonomic resolution on freshwater bioassessments using benthic macroinvertebrates. *Environmental Reviews*, *16*, 45–69. https://doi.org/10.1139/A07-010

Jones, J. I., Murphy, J. F., Collins, A. L., Sear, D. A., Naden, P. S., & Armitage, P. D. (2012). THE IMPACT OF FINE SEDIMENT ON MACRO-INVERTEBRATES. *River Research and Applications*, *28*(8), 1055–1071. https://doi.org/10.1002/rra.1516

Jonsson, L. T. (2019). *Mouthpart deformities of Chironomid larvae as an indicator of heavy metal polluted water*. https://www.diva-portal.org/smash/get/diva2:1341779/FULLTEXT01.pdf

Jovem-Azevêdo, D., Bezerra-Neto, J. F., Azevêdo, E. L., Gomes, W. I. A., Molozzi, J., & Feio, M. J. (2019). Dipteran assemblages as functional indicators of extreme droughts. *Journal of Arid Environments*, *164*(April 2018), 12–22. https://doi.org/10.1016/j.jaridenv.2019.01.014

Jun, Y. C., Won, D. H., Lee, S. H., Kong, D. S., & Hwang, S. J. (2012). A multimetric benthic macroinvertebrate index for the assessment of stream biotic integrity in Korea. *International Journal of Environmental Research and Public Health*, *9*(10), 3599–3628. https://doi.org/10.3390/ijerph9103599

Juvigny-Khenafou, N. P. D., Piggott, J. J., Atkinson, D., Zhang, Y., Macaulay, S. J., Wu, N., & Matthaei, C. D. (2021). Impacts of multiple anthropogenic stressors on stream macroinvertebrate community composition and functional diversity. *Ecology and Evolution*, *11*(1), 133–152. https://doi.org/10.1002/ece3.6979

Karima, Z. (2010). Chironomidae: Biology, Ecology and Systematics. In *IntechOpen* (Vol. 34, Issue 8, pp. 57–67). https://doi.org/10.1007/s12559-021-09926-6%0Ahttps://www.intechopen.com/books/advanced-biometric-technologies/liveness-detection-in-biometrics%0Ahttp://dx.doi.org/10.1016/j.compmedimag.2010.07.003

Karr, J. R. (1981). Assessment of Biotic Integrity Using Fish Communities. *Fisheries*, *6*(6), 21–27. https://doi.org/10.1577/1548-8446(1981)006<0021:aobiuf>2.0.co;2

Kaufmann, P. R., Hughes, R. M., Paulsen, S. G., Peck, D. V., Seeliger, C. W., Weber, M. H., & Mitchell, R. M. (2022). Physical habitat in conterminous US streams and rivers, Part 1: Geoclimatic controls and anthropogenic alteration. *Ecological Indicators*, *141*(January), 109046. https://doi.org/10.1016/j.ecolind.2022.109046

Kefford, B. J., Reddy-Lopata, K., Clay, C., Hagen, T., Parkanyi, O., & Nugegoda, D. (2011). Size of anal papillae in chironomids: Does it indicate their salinity stress? *Limnologica*, *41*(2), 96–106. https://doi.org/10.1016/j.limno.2010.09.004

Kerans, B. L., & Karr, J. R. (1994). A Benthic Index of Biotic Integrity (B-IBI) for Rivers of the Tennessee Valley. *Ecological Applications*, *4*(4), 768–785. https://doi.org/10.2307/1942007

Khumalo, J. (2021). *Adaptation strategies of smallholder maize farmers to drought in the buffalo city metropolitan municipality, Eastern Cape, South Africa* (Issue February). University of Free State, Bloemfontein.

Kleynhans, C. J., & Louw, M. D. (2008). River EcoClassifictaion: Manual for EcoStatus Determination (version 2) Module A: EcoClassification and EcoStatus determination. In *WRC Report TT 329/08*.

Kleynhans, C., & Louw, M. (2007). River Ecoclassification Module a : Ecoclassification and Ecostatus. *Joint Water Research Commission and Department of Water Affairs and Forestry Report*, *Version 2*, Aii-Avii. https://www.dwa.gov.za/iwqs/rhp/eco/EcoStatus/ModuleA_EcoStatus/ModuleA_EcoClassification.pdf

Kleynhans, C., Thirion, C., & Moolman, J. (2005). A Level I River Ecoregion classification System for South Africa, Lesotho and Swaziland. In *Bulletin des Sociétés Chimiques Belges* (Vol. 53, Issue September). https://www.dws.gov.za/iwqs/gis_data/ecoregions/LEVEL_I_ECOREGIONSsigned_small2.pdf

Ko, N. T., Suter, P., Conallin, J., Rutten, M., & Bogaard, T. (2020). The urgent need for river health biomonitoring tools for large tropical rivers in developing countries: Preliminary development of a river health monitoring tool for Myanmar rivers. *Water (Switzerland)*, *12*(5), 1–15. https://doi.org/10.3390/w12051408

Koenker, R. (2005). Fundamentals of Quantile Regression. In *Quantile Regression*. https://doi.org/10.1017/ccol0521845734.002

Kok, P., & Collinson, M. (2006). Migration and urbanisation in South Africa. In *Statistics South Africa*. https://repository.hsrc.ac.za/bitstream/handle/20.500.11910/6798/3823(1).pdf?sequence=1

Kolbenschlag, S., Gerstle, V., Eberhardt, J., Bollinger, E., Schulz, R., Brühl, C. A., & Bundschuh, M. (2023). A temporal perspective on aquatic subsidy: Bti affects emergence of Chironomidae. *Ecotoxicology and Environmental Safety*, *250*(August 2022), 114503. https://doi.org/10.1016/j.ecoenv.2023.114503

Kolkwitz, R., & Marsson, M. (1909). Ökologie der tierischen Saprobien. Beiträge zur Lehre von der biologischen Gewässerbeurteilung. *Internationale Revue Der Gesamten Hydrobiologie Und Hydrographie*, *2*, 126–52.

Kooijman, S. A. L. M. (2011). Energy budgets. In A. Hasting & L. Gross (Eds.), *Sourcebook in Theoretical Ecology* (pp. 370–378). University of California Press. https://doi.org/10.1525/9780520951785-048

Kunz, S., Kefford, B. J., Usseglio-Polatera, P., Hawkins, C. P., Poff, N. L. R., Akamagwuna, F., Odume, N., Schmidt-Kloiber, A., Graf, W., Metzeling, L., Matthaei, C. D., Phillips, N., & Schäfer, R. B. (2024). Similarity of stream insect trait profiles across biogeographic regions. *Diversity and Distributions*, *December 2023*, 1–17.

https://doi.org/10.1111/ddi.13812

Kusnierz, P. C., & Holbrook, C. M. (2017). Relative performance of three stream bed stability indices as indicators of stream health. *Environmental Monitoring and Assessment*, *189*(11), 563. https://doi.org/10.1007/s10661-017-6291-x

Kutor, S. K. (2014). Development and Underdevelopment of African Continent: The Blame Game and the Way Forward. *Research on Humanities and Social Sciences*, *4*(7), 14–20. www.iiste.org

Kuzmanovic, M., Dolédec, S., de Castro-Catala, N., Ginebreda, A., Sabater, S., Muñoz, I., & Barceló, D. (2017). Environmental stressors as a driver of the trait composition of benthic macroinvertebrate assemblages in polluted Iberian rivers. *Environmental Research*, *156*(April), 485–493. https://doi.org/10.1016/j.envres.2017.03.054

Kwak, S. (2023). Are Only p-Values Less Than 0.05 Significant? A p-Value Greater Than 0.05 Is Also Significant! *Journal of Lipid and Atherosclerosis*, *12*(2), 89–95. https://doi.org/10.12997/jla.2023.12.2.89

Lam-Gordillo, O., Baring, R., & Dittmann, S. (2021). Taxonomic and Functional Patterns of Benthic Communities in Southern Temperate Tidal Flats. *Frontiers in Marine Science*, *8*(September), 1–19. https://doi.org/10.3389/fmars.2021.723749

Lamouroux, N., Dolédec, S., & Gayraud, S. (2004). Biological traits of stream macroinvertebrate communities: Effects of microhabitat, reach, and basin filters. *Journal of the North American Benthological Society*, *23*(3), 449–466. https://doi.org/10.1899/0887-3593(2004)023<0449:BTOSMC>2.0.CO;2

Lange, K., Townsend, C. R., & Matthaei, C. D. (2014). Can biological traits of stream invertebrates help disentangle the effects of multiple stressors in an agricultural catchment? *Freshwater Biology*, *59*(12), 2431–2446. https://doi.org/10.1111/fwb.12437

Lau, J. K., & Lauer, T. E. (2015). Nationwide Benthic Macroinvertebrate Assemblage Multimetric Indices: Identifying Inconsistencies and Limitations in Reporting Stream Impairment Status, USA. *Environmental Management*, *56*(1), 11–23. https://doi.org/10.1007/s00267-015-0478-0

Legendre, P., & Legendre, L. (2012). Numerical ecology. Developments in environmental modeling. In *Developments in Environmental Modelling* (Vol. 24). https://books.google.com/books?id=6ZBOA-iDviQC&printsec=frontcover&source=gbs_ge_summary_r&cad=0#v=onepage&q&f=false

Lemley, D., Taljaard, S., Adams, J., & Strydom, N. (2014). Nutrient characterisation of river inflow into the estuaries of the Gouritz Water Management Area, South Africa. *Water SA*, *40*(4), 687. https://doi.org/10.4314/wsa.v40i4.14

Lenat, D. R. . (1983). Chironomid Taxa Richness : Natural Variation and Use in Pollution Assessment. *Freshwater Invertebrate Biology*, *2*(4), 192–198.

Lenat, D. R., & Resh, V. H. (2001). Taxonomy and stream ecology - The benefits of genus- and species-level identifications. *Journal of the North American Benthological Society*, *20*(2), 287–298. https://doi.org/10.2307/1468323

Lencioni, V., Marziali, L., & Rossaro, B. (2012). Chironomids as bioindicators of environmental quality in mountain springs. *Freshwater Science*, *31*(2), 525–541. https://doi.org/10.1899/11-038.1

Leszczyńska, J., Głowacki, Grzybkowska, M., & Przybylski, M. (2021). Chironomid riverine assemblages at the regional temperate scale–compositional distance and species diversity. *European Zoological Journal*, *88*(1), 731–748. https://doi.org/10.1080/24750263.2021.1926565

Lévesque, D., Hudon, C., James, P. M. A., & Legendre, P. (2017). Environmental factors structuring benthic primary producers at different spatial scales in the St. Lawrence River (Canada). *Aquatic Sciences*, *79*(2), 345–356. https://doi.org/10.1007/s00027-016-0501-4

Levis, N. A., & Pfennig, D. W. (2020). Phenotypic plasticity and the origins of novelty. In *Phenotypic Switching: Implications in Biology and Medicine*. INC. https://doi.org/10.1016/B978-0-12-817996-3.00017-7

Li, L., Zheng, B., & Liu, L. (2010). Biomonitoring and bioindicators used for river ecosystems: Definitions, approaches and trends. *Procedia Environmental Sciences*, *2*, 1510–1524. https://doi.org/10.1016/j.proenv.2010.10.164

Libala, N., Palmer, C. G., & Nelson, O. (2020). Using a trait-based approach for assessing the vulnerability and resilience of hillslope seep wetland vegetation cover to disturbances in the Tsitsa River catchment , Eastern Cape , South Africa. *Ecology and Evolution, October 2019*, 277–291. https://doi.org/10.1002/ece3.5893

Liess, M., & Von Der Ohe, P. C. (2005). Analyzing effects of pesticides on invertebrate communities in streams. *Environmental Toxicology and Chemistry, 24*(4), 954–965. https://doi.org/10.1897/03-652.1

Lin, L., Yang, H., & Xu, X. (2022). Effects of Water Pollution on Human Health and Disease Heterogeneity: A Review. *Frontiers in Environmental Science, 10*(June). https://doi.org/10.3389/fenvs.2022.880246

Lourenço, J., Gutiérrez-Cánovas, C., Carvalho, F., Cássio, F., Pascoal, C., & Pace, G. (2023). Non-interactive effects drive multiple stressor impacts on the taxonomic and functional diversity of atlantic stream macroinvertebrates. *Environmental Research, 229*(April). https://doi.org/10.1016/j.envres.2023.115965

Lu, A., Li, J., Zheng, B., & Yin, X. (2022). Effect of Different Land Use Types on the Taxonomic and Functional Diversity of Macroinvertebrates in an Urban Area of Northern China. *Water, 14*(23), 3793. https://doi.org/10.3390/w14233793

Mabidi, A., Bird, M. S., & Perissinotto, R. (2018). Increasing salinity drastically reduces hatching success of crustaceans from depression wetlands of the semi-arid Eastern Cape Karoo region, South Africa. *Scientific Reports, 8*(1), 1–9. https://doi.org/10.1038/s41598-018-24137-0

Mackenzie, K. M., Singh, K., Binns, A. D., Whiteley, H. R., & Gharabaghi, B. (2022). Effects of urbanization on stream flow , sediment , and phosphorous regime. *Journal of Hydrology, 612*(PC), 128283. https://doi.org/10.1016/j.jhydrol.2022.128283

Maclear, L. G. A. (1996). *The geohydrology of the Swartkops River Basin: Uitenhage Region, Eastern Cape.* University of Cape Town.

Mafuta, C., Formo, R. K., & Nellemann, C. (2011). *Green Hills , Blue Cities: an Ecosystems Approach to Water Resources management for African Cities.A Rapid Response Assessment. United Nations Environment Programme, GRID-Arendal.*

Makana Local Municipality. (2017). *Final Makana Municipality Integrated Development Plan 2017-2022*. http://www.makana.gov.za/wp-content/uploads/2013/06/IDP-2017-2022-Final.pdf

Makana Local Municipality. (2020). *Draft Annual Report 2020* (Vol. 21, Issue 1). https://doi.org/10.1016/j.tmaid.2020.101607%0Ahttps://doi.org/10.1016/j.ijsu.2020.02.034%0Ahttps://onlinelibrary.wiley.com/doi/abs/10.1111/cjag.12228%0Ahttps://doi.org/10.1016/j.ssci.2020.104773%0Ahttps://doi.org/10.1016/j.jinf.2020.04.011%0Ahttps://doi.o

Makana Local Municipality. (2021). *Draft Review Makana Municipality Integrated Development Plan*. https://lg.treasury.gov.za/supportingdocs/EC104/EC104_IDP Draft_2021_Y_20200605T125604Z_gerardg.pdf

Mangadze, T. (2019). *Ecological Assessment of a Temperate River System Using Biomonitoring Techniques : a Case Study of the Bloukrans River System , South Africa* (Issue November). Rhodes University.

Mangadze, T., Dalu, T., & William Froneman, P. (2019). Biological monitoring in southern Africa: A review of the current status, challenges and future prospects. *Science of the Total Environment*, *648*, 1492–1499. https://doi.org/10.1016/j.scitotenv.2018.08.252

Mantel, S. K., Hughes, D. A., & Slaughter, A. S. (2015). Water resources management in the context of future climate and development changes: A South African case study. *Journal of Water and Climate Change*, *6*(4), 772–786. https://doi.org/10.2166/wcc.2015.098

Maree, J. P., Greben, H. A., & De Beer, M. (2004). Treatment of acid and sulphate-rich effluents in an integrated biological/chemical process. *Water SA*, *30*(2), 183–189. https://doi.org/10.4314/wsa.v30i2.5062

Martini, S., Larras, F., Boyé, A., Faure, E., Aberle, N., Archambault, P., Bacouillard, L., Beisner, B. E., Bittner, L., Castella, E., Danger, M., Gauthier, O., Karp-Boss, L., Lombard, F., Maps, F., Stemmann, L., Thiébaut, E., Usseglio-Polatera, P., Vogt, M., … Ayata, S. D. (2021). Functional trait-based approaches as a common framework for aquatic ecologists. *Limnology and Oceanography*, *66*(3), 965–994. https://doi.org/10.1002/lno.11655

Martins, I., Castro, D. M. P., Macedo, D. R., Hughes, R. M., & Callisto, M. (2021). Anthropogenic impacts influence the functional traits of Chironomidae (Diptera) assemblages in a neotropical savanna river basin. *Aquatic Ecology*, *55*(3), 1081–1095. https://doi.org/10.1007/s10452-021-09884-z

Martins, I., Macedo, D. R., Hughes, R. M., & Callisto, M. (2020). Are multiple multimetric indices effective for assessing ecological condition in tropical basins? *Ecological Indicators*, *110*(November 2019), 105953. https://doi.org/10.1016/j.ecolind.2019.105953

Marziali, L., Rosignoli, F., Valsecchi, S., Polesello, S., & Stefani, F. (2019). Effects of perfluoralkyl substances (PFASs) on a multigenerational scale: a case study with Chironomus riparius (Diptera, Chironomidae). *Environmental Toxicology and Chemistry*, *38*(5). https://doi.org/10.1002/etc.4392

Masese, F. O., & Mcclain, M. E. (2012). Trophic resources and emergent food web attributes in rivers of the Lake Victoria Basin: A review with reference to anthropogenic influences. *Ecohydrology*, *5*(6), 685–707. https://doi.org/10.1002/eco.1285

Masese, F. O., Omukoto, J. O., & Nyakeya, K. (2013). Biomonitoring as a prerequisite for sustainable water resources: A review of current status, opportunities and challenges to scaling up in East Africa. *Ecohydrology and Hydrobiology*, *13*(3), 173–191. https://doi.org/10.1016/j.ecohyd.2013.06.004

Masese, F. O., Wanderi, E. W., Nyakeya, K., Achieng, A. O., Fouchy, K., & Mcclain, M. E. (2023). *Bioassessment of multiple stressors in Afrotropical rivers : Evaluating the performance of a macroinvertebrate-based index of biotic integrity , diversity , and regional biotic indices*. *March*, 1–27. https://doi.org/10.3389/fenvs.2023.1015623

Maseti, P. P. (2005). *Biomonitoing in two contrasting catchments* (Vol. 7, Issue 2) [Rhodes University]. http://dx.doi.org/10.1016/j.intell.2008.09.007%0Ahttp://dx.doi.org/10.1016/S0010-9452(58)80010-6%0Ahttp://pss.sagepub.com/content/17/1/67.short%0Ahttp://dx.doi.org/10.1016/j.cogdev.2013.06.002%0Ahttp://www.chabris.com/Hooven2008.pdf%0Ahttp://www.ncbi.nlm.

Masoner, J. R., Kolpin, D. W., Cozzarelli, I. M., Barber, L. B., Burden, D. S., Foreman, W. T., Forshay, K. J., Furlong, E. T., Groves, J. F., Hladik, M. L., Hopton, M. E., Jaeschke, J. B., Keefe, S. H., Krabbenhoft, D. P., Lowrance, R., Romanok, K. M., Rus, D. L., Selbig, W. R., Williams, B. H., & Bradley, P. M. (2019). Urban Stormwater: An Overlooked Pathway of Extensive Mixed Contaminants to Surface and Groundwaters in the United States. *Environmental Science and Technology*, *53*(17), 10070–10081. https://doi.org/10.1021/acs.est.9b02867

Mathers, K. L., Rice, S. P., & Wood, P. J. (2017). Temporal effects of enhanced fine sediment loading on macroinvertebrate community structure and functional traits. *Science of the Total Environment*, *599–600*, 513–522. https://doi.org/10.1016/j.scitotenv.2017.04.096

Mathers, K. L., Stubbington, R., Leeming, D., Westwood, C., & England, J. (2019). Structural and functional responses of macroinvertebrate assemblages to long-term flow variability at perennial and nonperennial sites. *Ecohydrology*, *12*(6), 1–14. https://doi.org/10.1002/eco.2112

Matthews, M. W., & Bernard, S. (2015). Eutrophication and cyanobacteria in South Africa’s standing water bodies: A view from space. *South African Journal of Science*, *111*(5–6), 1–8. https://doi.org/10.17159/sajs.2015/20140193

McDowell, R. W., Snelder, T. H., Cox, N., Booker, D. J., & Wilcock, R. J. (2013). Establishment of reference or baseline conditions of chemical indicators in New Zealand streams and rivers relative to present conditions. *Marine and Freshwater Research*, *64*(5), 387–400. https://doi.org/10.1071/MF12153

McGill, B. J., Enquist, B. J., Weiher, E., & Westoby, M. (2006). Rebuilding community ecology from functional traits. *Trends in Ecology and Evolution*, *21*(4), 178–185. https://doi.org/10.1016/j.tree.2006.02.002

McGrane, S. J. (2016). Impacts of urbanisation on hydrological and water quality dynamics, and urban water management: a review. *Hydrological Sciences Journal*, *61*(13), 2295–2311. https://doi.org/10.1080/02626667.2015.1128084

MDB, M. D. B. (2021). *Buffalo City Metropolitan Municipality*. https://municipalities.co.za/sources

Menezes, S., Baird, D. J., & Soares, A. M. V. M. (2010). Beyond taxonomy: A review of macroinvertebrate trait-based community descriptors as tools for freshwater biomonitoring. *Journal of Applied Ecology*, *47*(4), 711–719. https://doi.org/10.1111/j.1365-2664.2010.01819.x

Merritt, R. W., Fenoglio, S., & Cummins, K. W. (2017). Promoting a functional macroinvertebrate approach in the biomonitoring of Italian lotic systems. *Journal of Limnology*, *76*(1S), 5–8. https://doi.org/10.4081/jlimnol.2016.1502

Mezgebu, A. (2022). A review on freshwater biomonitoring with benthic invertebrates in Ethiopia. *Environmental and Sustainability Indicators*, *14*(December 2021), 100174. https://doi.org/10.1016/j.indic.2022.100174

Mezgebu, A., Lakew, A., Lemma, B., & Beneberu, G. (2019). The potential use of chironomids (Insecta: Diptera) as bioindicators in streams and rivers around Sebeta, Ethiopia. *African Journal of Aquatic Science*, *44*(4), 369–376. https://doi.org/10.2989/16085914.2019.1650711

Mgaba, N. (2014). *Macroinvertebrate-Based Biomonitoring of the Bloukrans River , Eastern Cape , South Africa* (Issue July). Rhodes University.

Mgaba, N. (2018). *Macroinvertebrate and diatom assemblage responses to pollution , with emphasis on salinity , in the Kat River, Eastern Cape, South Africa* (Issue January). Rhodes University.

Miliša, M., Stubbington, R., Datry, T., Cid, N., Bonada, N., Šumanović, M., & Milošević, D. (2022). Taxon-specific sensitivities to flow intermittence reveal macroinvertebrates as potential bioindicators of intermittent rivers and streams. *Science of The Total Environment*, *804*, 150022. https://doi.org/10.1016/j.scitotenv.2021.150022

Milošević, D., Mančev, D., Čerba, D., Stojković Piperac, M., Popović, N., Atanacković, A., Đuknić, J., Simić, V., & Paunović, M. (2018a). The potential of chironomid larvae-based metrics in the bioassessment of non-wadeable rivers. *Science of the Total Environment*, *616–617*, 472–479. https://doi.org/10.1016/j.scitotenv.2017.10.262

Milošević, D., Stojanović, K., Djurdjević, A., Marković, Z., Stojković Piperac, M., Živić, M., & Živić, I. (2018b). The response of chironomid taxonomy- and functional trait-based metrics to fish farm effluent pollution in lotic systems. *Environmental Pollution*, *242*, 1058–1066. https://doi.org/10.1016/j.envpol.2018.07.100

Mmachaka, T. (2022). *Smart Catchment Management and application in the Swartkops River and Estuary*. Nelson Mandela University.

Molineri, C., Tejerina, E. G., Torrejón, S. E., Pero, E. J. I., & Hankel, G. E. (2020). Indicative value of different taxonomic levels of Chironomidae for assessing the water quality. *Ecological Indicators*, *108*(February 2019), 105703. https://doi.org/10.1016/j.ecolind.2019.105703

Moller Pillot, H. K. M. (2013). Chironomidae Larvae, Vol. 2: Chironomini. In *Chironomidae Larvae, Vol. 2: Chironomini* (pp. 1–5). KNNV Publishing. https://doi.org/10.1163/9789004278042_001

Mouillot, D., Villéger, S., Scherer-Lorenzen, M., & Mason, N. W. H. (2011). Functional Structure of Biological Communities Predicts Ecosystem Multifunctionality. *PLoS ONE*, *6*(3), 17476. https://doi.org/10.1371/journal.pone.0017476

Moya, N., Hughes, R. M., Domínguez, E., Gibon, F. M., Goitia, E., & Oberdorff, T. (2011). Macroinvertebrate-based multimetric predictive models for evaluating the human impact on biotic condition of Bolivian streams. *Ecological Indicators*, *11*(3), 840–847. https://doi.org/10.1016/j.ecolind.2010.10.012

Muñiz-González, A. B., Silva, C. J. M., Patricio Silva, A. L., Campos, D., Pestana, J. L. T., & Martínez-Guitarte, J. L. (2021). Suborganismal responses of the aquatic midge Chironomus riparius to polyethylene microplastics. *Science of the Total Environment*, *783*, 146981. https://doi.org/10.1016/j.scitotenv.2021.146981

Namugize, J. N. (2017). *Effects of Land Use and Land Cover Changes on Water Quality of the Upper uMngeni River, KwaZulu-Natal Province, South Africa* (Issue April) [University of KwaZulu-Natal]. https://ukzn-dspace.ukzn.ac.za/bitstream/handle/10413/16271/Namugize_Jean_Nepomuscene_2017.pdf?sequence=1&isAllowed=y

Nel, H. A., Dalu, T., & Wasserman, R. J. (2018). Sinks and sources: Assessing microplastic abundance in river sediment and deposit feeders in an Austral temperate urban river system. *Science of the Total Environment*, *612*, 950–956.

https://doi.org/10.1016/j.scitotenv.2017.08.298

Nelson Mandela Bay Municipality. (2022). *Nelson Mandela Bay Metropolitan Municipality: 2022/23-2026/27 Integrated Development Plan*. https://www.nelsonmandelabay.gov.za/DataRepository/Documents/draft-2022-23-idp-noted_CvOpv.pdf

Nguyen, H., & Donini, A. (2010). Larvae of the midge Chironomus riparius possess two distinct mechanisms for ionoregulation in response to ion-poor conditions. *American Journal of Physiology - Regulatory Integrative and Comparative Physiology, 299*(3), 5–9. https://doi.org/10.1152/ajpregu.00745.2009

Nicacio, G., & Juen, L. (2015). Chironomids as indicators in freshwater ecosystems: An assessment of the literature. *Insect Conservation and Diversity*, *8*(5), 393–403. https://doi.org/10.1111/icad.12123

Nkoane, A. (2019). *Understanding informal settlements in South Africa: The Waterworks informal settlement profile and responses* (Issue October). University of the Witwatersrand.

Nolte, U. (1992). The 11th International Symposium. *Newsletter of Chrironomid Research, August 1991*.

Ntloko, P. (2022). *Developing taxonomic and trait-based approaches for assessing and predicting macroinvertebrate responses to elevated fine sediments in the Tsitsa River and its tributaries, South Africa*. Rhodes University.

Ntloko, P., Palmer, C. G., Akamagwuna, F. C., & Odume, O. N. (2021). Exploring macroinvertebrates ecological preferences and trait-based indicators of suspended fine sediment effects in the tsitsa river and its tributaries, eastern cape, south africa. *Water (Switzerland), 13*(6). https://doi.org/10.3390/w13060798

O’Driscoll, M., Clinton, S., Jefferson, A., Manda, A., & McMillan, S. (2010). Urbanization Effects on Watershed Hydrology and In-Stream Processes in the Southern United States. *Water*, *2*(3), 605–648. https://doi.org/10.3390/w2030605

O’Keeffe, J. H., van Ginkel, C. E., Hughes, D. A., Hill, T. R., & Ashton, P. J. (1996). *A* *situation analysis of water quality in the catchment of the Buffalo River, Eastern Cape, with special emphasis on the impacts of low cost, high-density urban development on water quality* (WRC 405/1/96; Vol. 1, Issue 40).

Obubu, J. P., Mengistou, S., Fetahi, T., Graf, W., & Odong, R. (2021). A critical review of macroinvertebrate-based bioassessment approaches in Africa's lotic systems: developments, challenges, and legal requirements. *African Journal of Aquatic Science,* 1–13. https://doi.org/10.2989/16085914.2021.1924609

Odume, O. N. (2014). *An evaluation of macroinvertebrate-based biomonitoring and ecotoxicological assessments of deteriorating environmental water quality in the Swartkops River, South Africa* (Issue January). https://hsgm.saglik.gov.tr/depo/birimler/saglikli-beslenme-hareketli-hayat-db/Yayinlar/kitaplar/diger-kitaplar/TBSA-Beslenme-Yayini.pdf

Odume, O. N. (2020). Searching for urban pollution signature and sensitive macroinvertebrate traits and ecological preferences in a river in the Eastern Cape of South Africa. *Ecological Indicators*, *108*(April 2019), 105759. https://doi.org/10.1016/j.ecolind.2019.105759

Odume, O. N. (2022). The contribution and value of the trait-based approach to water resource protection. *African Journal of Aquatic Science*, *May*. https://doi.org/10.2989/16085914.2022.2047590

Odume, O. N., Akamagwuna, F. C., Ntloko, P., Dallas, H. F., Nnadozie, C. F., & Barber-James, H. M. (2023). A trait database for southern African freshwater invertebrates. *African Journal of Aquatic Science*, *48*(1), 64–70. https://doi.org/10.2989/16085914.2022.2142505

Odume, O. N., & Muller, W. J. (2011). Diversity and structure of Chironomidae communities in relation to water quality differences in the Swartkops River. *Physics and Chemistry of the Earth*, *36*(14–15), 929–938. https://doi.org/10.1016/j.pce.2011.07.063

Odume, O. N., Muller, W. J., Arimoro, F. O., & 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. https://doi.org/10.2989/16085914.2012.670613

Odume, O. N., Ntloko, P., Akamagwuna, F. C., Dallas, H. M., & Barber-James, H. (2018). *Development of macroinvertebrate trait-based approach for assessing and managing ecosystem health in south african rivers – incorporating a case study in the Tsitsa River and its tributaries, Eastern Cape* (Vol. 7, Issue 2).

Odume, O. N., Onyima, B. N., Nnadozie, C. F., Omovoh, G. O., Mmachaka, T., Omovoh, B. O., Uku, J. E., Akamagwuna, F. C., & Arimoro, F. O. (2022). Governance and Institutional Drivers of Ecological Degradation in Urban River Ecosystems: Insights from Case Studies in African Cities. *Sustainability (Switzerland)*, *14*(21). https://doi.org/10.3390/su142114147

Odume, O. N., Palmer, C. G., Arimoro, F. O., & Mensah, P. K. (2014). Patterns of chironomid body-size distribution in an effluent-impacted river in the Eastern Cape, South Africa. *African Journal of Aquatic Science*, *39*(4), 377–386. https://doi.org/10.2989/16085914.2014.982498

Odume, O. N., Palmer, C. G., Arimoro, F. O., & Mensah, P. K. (2016). Chironomid assemblage structure and morphological response to pollution in an effluent-impacted river, Eastern Cape, South Africa. *Ecological Indicators*, *67*, 391–402. https://doi.org/10.1016/j.ecolind.2016.03.001

Olisah, C., Adams, J. B., & Rubidge, G. (2021). The state of persistent organic pollutants in South African estuaries: A review of environmental exposure and sources. *Ecotoxicology and Environmental Safety*, *219*, 112316. https://doi.org/10.1016/j.ecoenv.2021.112316

Olisah, C., Okoh, O. O., & Okoh, A. I. (2020). Spatial, seasonal and ecological risk assessment of organohalogenated contaminants in sediments of Swartkops and Sundays Estuaries, Eastern Cape province, South Africa. *Journal of Soils and Sediments*, *20*(2), 1046–1059. https://doi.org/10.1007/s11368-019-02487-0

Olujimi, O. O., Fatoki, O. S., Odendaal, J. P., & Okonkwo, J. O. (2010). Endocrine disrupting chemicals (phenol and phthalates) in the South African environment: A need for more monitoring. *Water SA*, *36*(5), 671–682. https://doi.org/10.4314/wsa.v36i5.62001

Owolabi, S. T., Madi, K., Kalumba, A. M., & Alemaw, B. F. (2020). Assessment of recession flow variability and the surficial lithology impact: a case study of Buffalo River catchment, Eastern Cape, South Africa. *Environmental Earth Sciences, 79*(8), 187. https://doi.org/10.1007/s12665-020-08925-4

Özkan, N., Moubayed-breil, J., & Çamur-elipek, B. (2010). *Ecological Analysis of Chironomid Larvae ( Diptera , Chironomidae ) in Ergene River Basin ( Turkish Thrace ). 99*, 93–99. https://doi.org/10.4194/trjfas.2010.0114

Palmer, C. G. (1991a). *Benthic assemblage structure, and the feeding biology of sixteen macroinvertebrate taxa from the Buffalo River, Eastern Cape, South Africa* (Issue September) [Rhodes University]. http://hdl.handle.net/10962/d1005328

Palmer, C. G., Maart, B., Palmer, A. R., & O'Keeffe, J. H. (1996). An assessment of macroinvertebrate functional feeding groups as water quality indicators in the Buffalo River, eastern Cape Province, South Africa. *Hydrobiologia, 318*(3), 153–164. https://doi.org/10.1007/BF00016677

Palmer, R. W. (1991b). *Ecological effects of impoundments in the Buffalo River , eastern Cape , with particular reference to the distribution of blackflies ( Diptera : Simuliidae ).* Rhodes University.

Palmer, T., Berold, R., & Muller, N. (2004). Integrated Water Resources Management : From Research to Implementation. In *Water* (Vol. 25, Issue 1).

Pauchard, A., Aguayo, M., Peña, E., & Urrutia, R. (2006). Multiple effects of urbanization on the biodiversity of developing countries: The case of a fast-growing metropolitan area (Concepción, Chile). *Biological Conservation, 127*(3), 272–281. https://doi.org/10.1016/j.biocon.2005.05.015

Paul, M. J., & Meyer, J. L. (2001). Streams in the urban landscape. *Annual Review of Ecology, Evolution, and Systematics, 32,* 333–365.

Paz, L. E. E., Rodriguez, M., Gullo, B., & Rodrigues Capítulo, A. (2022). Impacts of urban and industrial pollution on functional traits of benthic macroinvertebrates: Are some traits advantageous for survival? *Science of the Total Environment, 807*, 150650. https://doi.org/10.1016/j.scitotenv.2021.150650

Pelizzo, R., Kinyondo, A., & Nwokora, Z. (2018). Development in Africa. In *World Affairs*

(Vol. 181, Issue 3). https://doi.org/10.1177/0043820018805307

Pilière, A. F. H., Verberk, W. C. E. P., Gräwe, M., Breure, A. M., Dyer, S. D., Posthuma, L., de Zwart, D., Huijbregts, M. A. J., & Schipper, A. M. (2016). On the importance of trait interrelationships for understanding environmental responses of stream macroinvertebrates. *Freshwater Biology*, *61*(2), 181–194. https://doi.org/10.1111/fwb.12690

Plafkin, J. L., Barbour, M. T., Porter, K. D., Gross, S. K., & Hughes, R. M. (1989). Rapid bioassessment protocols for use in stream and rivers: benthic macroinvertebrates and fish. EPA/444/4-89-011. In *U.S. Environmental Protection Agency, Assessment and Watershed Protection Division* (p. 150).

Płóciennik, M., Dmitrović, D., Pešić, V., & Gadawski, P. (2016). Ecological patterns of Chironomidae assemblages in Dynaric karst springs. *Knowledge and Management of Aquatic Ecosystems*, *2016-Janua*(417). https://doi.org/10.1051/kmae/2015044

Podder, R., Nath, S., Modak, B. K., Weltje, L., & Malakar, B. (2022). Tube length of chironomid larvae as an indicator for dissolved oxygen in water bodies. *Scientific Reports*, *12*(1), 1–8. https://doi.org/10.1038/s41598-022-23953-9

Poff, N. L. (1997). Landscape Filters and Species Traits : Towards Mechanistic Understanding and Prediction in Stream Ecology Author ( s ): N . LeRoy Poff Source : Journal of the North American Benthological Society , Vol . 16 , No . 2 , New Concepts in Stream Ecology : Proc. *North American Benthological Society*, *16*(2), 391–409.

Poff, N. L., Olden, J. D., Vieira, N. K. M., Finn, D. S., Simmons, M. P., & Kondratieff, B. C. (2006). Functional trait niches of North American lotic insects: Traits-based ecological applications in light of phylogenetic relationships. *Journal of the North American Benthological Society*, *25*(4), 730–755. https://doi.org/10.1899/0887-3593(2006)025[0730:FTNONA]2.0.CO;2

Porinchu, D. F., & MacDonald, G. M. (2003). The use and application of freshwater midges (Chironomidae: Insecta: Diptera) in geographical research. *Progress in Physical Geography*, *27*(3), 378–422. https://doi.org/10.1191/030913303767888491

Postma, J. F., van Kleunen, A., & Admiraal, W. (1995). Alterations in life-history traits of

Chironomus riparius (Diptera) obtained from metal contaminated rivers. *Archives of Environmental Contamination and Toxicology*, *29*, 469–475. https://doi.org/10.1007/BF00208376

Pyne, M. I., & Poff, N. L. R. (2017). Vulnerability of stream community composition and function to projected thermal warming and hydrologic change across ecoregions in the western United States. *Global Change Biology*, *23*(1), 77–93. https://doi.org/10.1111/gcb.13437

QGIS development Team. (2022). *QGIS Geographic Information System* (3.28.15). http://qgis.osgeo.org

R Core Team. (2022). *R: A language and environment for statistical computing* (4.2.0). R Foundation for Statistical Computing. https://www.r-project.org/

Rabeni, C. F., & Wang, N. (2001). Bioassessment of Streams Using Macroinvertebrates : Are the chironomids necessary? *Environmental Monitoring and Assessment*, *71*, 177–185.

Rahman, A., Jahanara, I., & Jolly, Y. N. (2021). Assessment of physicochemical properties of water and their seasonal variation in an urban river in Bangladesh. *Water Science and Engineering*, *14*(2), 139–148. https://doi.org/10.1016/j.wse.2021.06.006

Ramulifho, P., Rivers-Moore, N., & Foord, S. (2023). Relationships between reference site quality and baetid mayfly assemblages in mountainous streams of the Luvuvhu catchment, South Africa. *Water SA*, *49*(3), 251–259. https://doi.org/10.17159/wsa/2023.v49.i3.4023

Rebelo, T., Boucher, C., Helme, N., Mucina, L., Rutherford, M., Smit, W., Powrie, L., Ellis, F., Lambrechts, J., Scott, L., Radloff, G., Johnson, S., Richardson, D., Ward, D., Proches, S., Oliver, E., Manning, J., Juergens, N., McDonald, D., … Hoare, D. (2006). Fynbos Biome. In *The vegetation of South Africa, Lesotho and Swaziland*.

Resh, V. H., & Unzicker, J. D. (1975). Water quality monitoring and aquatic organisms: the importance of species identification. *Journal of the Water Pollution Control Federation*, *47*(1), 9–19.

Reyes-Maldonado, R., Marie, B., & Ramírez, A. (2021). Rearing methods and life cycle characteristics of Chironomus sp. Florida (Chironomidae: Diptera): A rapid-developing species for laboratory studies. *PLoS ONE*, *16*(2 February), 1–16. https://doi.org/10.1371/journal.pone.0247382

Reynolds, S. K., & Ferrington, L. C. (2002). Differential Morphological Responses of Chironomid Larvae to Severe Heavy Metal Exposure ( Diptera : Chironomidae ). *Journal of the Kansas Entomological Society*, *75*(3), 172–184. http://www.jstor.org/stable/25086066

Reynoldson, T., Bailey, R., Day, K., & Norris, R. (1995). Biological guidelines for freshwater sediment based on BEnthic Assessment of SedimenT (the BEAST) using a multivariate approach for predicting biological state. *Austral Ecology*, *20*(1), 198–219. https://doi.org/10.1111/j.1442-9993.1995.tb00532.x

Rezapour, S., Siavash Moghaddam, S., Nouri, A., & Khosravi Aqdam, K. (2022). Urbanization influences the distribution, enrichment, and ecological health risk of heavy metals in croplands. *Scientific Reports*, *12*(1), 1–16. https://doi.org/10.1038/s41598-022-07789-x

Richards, C., Haro, R., Johnson, L., & Host, G. (1997). Catchment and reach-scale properties as indicators of macroinvertebrate species traits. *Freshwater Biology*, *37*(1), 219–230. https://doi.org/10.1046/j.1365-2427.1997.d01-540.x

Roback, S. S. (1981). The Immature Chironomids of the Eastern United States V . Pentaneurini- Thienemannimyia Group. *Proceedings of the Academy of Natural Sciences of Philadelphia*, *133*, 73–128.

Robertson, A., Armitage, N., & Zuidgeest, M. H. P. (2019). Stormwater runoff quality on an urban highway in South Africa. *Journal of the South African Institution of Civil Engineering*, *61*(2), 51–56. https://doi.org/10.17159/2309-8775/2019/v61n2a5

Rodríguez-Lozano, P., Verkaik, I., Rieradevall, M., & Prat, N. (2015). Small but powerful: Top predator local extinction affects ecosystem structure and function in an intermittent stream. *PLoS ONE*, *10*(2), 1–16. https://doi.org/10.1371/journal.pone.0117630

Rodríguez Garay, G. N., Paggi, A. C., & Scheibler, E. E. (2020). Chironomidae assemblages at different altitudes in northwest Argentina: The role of local factors. *Anais Da Academia Brasileira de Ciencias*, *92*, 1–18. https://doi.org/10.1590/0001-3765202020190953

Rodriguez, R. N., & Yao, Y. (2017). Five Things You Should Know about Quantile Regression. *Paper SAS525-2017*, 2–6. https://support.sas.com/resources/papers/proceedings17/SAS0525-2017.pdf

Rolls, R. J., Leigh, C., & Sheldon, F. (2012). Mechanistic effects of low-flow hydrology on riverine ecosystems: Ecological principles and consequences of alteration. *Freshwater Science*, *31*(4), 1163–1186. https://doi.org/10.1899/12-002.1

Rosenfeld, J. S. (2002). Functional redundancy in ecology and conservation. *Oikos*, *98*(1), 156–162. https://doi.org/10.1034/j.1600-0706.2002.980116.x

Rossaro, B., Marziali, L., & Boggero, A. (2022). Response of Chironomids to Key Environmental Factors: Perspective for Biomonitoring. *Insects*, *13*(10). https://doi.org/10.3390/insects13100911

Rossaro, B., Montagna, M., & Lencioni, V. (2016). Environmental traits affect chironomid communities in glacial areas of the Southern Alps: Evidence from a long-lasting case study. *Insect Conservation and Diversity*, *9*(3), 192–201. https://doi.org/10.1111/icad.12157

Rossaro, B., Solimini, A., Lencioni, V., Marziali, L., Giacchini, R., & Parenti, P. (2007). The relationship between body size , pupal thoracic horn development and dissolved oxygen in Chironomini ( Diptera : Chironomidae ). *Fundamental and Applied Limnology*. https://doi.org/10.1127/1863-9135/2007/0169-0331

Roux, A. J., & Clinton, S. M. (2023). Evaluation of the Relationship between Stream Habitat Quality and Taxa and Trait Richness and Diversity in Piedmont Streams in North Carolina. *Hydrobiology*, *2*(2), 363–381. https://doi.org/10.3390/hydrobiology2020024

Rowntree, K. M., Wadeson, R. A., & O'keeffe, J. H. (2000). The development of a geomorphological classification system for the longitudinal zonation of south african rivers. *South African Geographical Journal*, *82*(3), 163–172. https://doi.org/10.1080/03736245.2000.9713710

RSA, N. W. A. (1998). *South Africa National Water Act* (36; Issue 36). https://doi.org/10.1109/BEIAC.2013.6560231

Ruaro, R., Gubiani, É. A., Padial, A. A., Karr, J. R., Hughes, R. M., & Mormul, R. P. (2024). Responses of multimetric indices to disturbance are affected by index construction features. *Environmental Reviews*, *32*(2), 278–293. https://doi.org/10.1139/er-2023-0098

Ruiz-Picos, R. A., Sedeño-Díaz, J. E., & López-López, E. (2017). Calibrating and Validating the Biomonitoring Working Party (BMWP) Index for the Bioassessment of Water Quality in Neotropical Streams. *Water Quality*. https://doi.org/10.5772/66221

Ruse, L. P., Herrmann, S. J., & Sublette, J. E. (1998). Chironomidae ( diptera ) species distribution related to environmental characteristics of the metal-polluted Arkansas River , Colorado. *Western North American Naturalist*, *60*(1), 34–56.

Saghir, J., & Santoro, J. (2018). Urbanization in Sub-Saharan Africa- Meeting Challenges by Bridging Stakeholders. *Center for Strategic & International Studies*, *April*, 1–7. http://thegreentimes.co.za/wp-content/uploads/2019/03/Urbanization-in-Sub-Saharan-Africa.pdf%0Ahttps://www.csis.org/analysis/urbanization-sub-saharan-africa

Savić, A., Đorđević, M., Jušković, M., & Pešić, V. (2017). Ecological analysis of macroinvertebrate communities based on functional feeding groups: a case study in southeastern Serbia. *Biologica Nyssana*, *8*(2), 159–166. https://doi.org/10.5281/zenodo.1135973

Schmera, D., Heino, J., Podani, J., Erős, T., & Dolédec, S. (2017). Functional diversity: a review of methodology and current knowledge in freshwater macroinvertebrate research. *Hydrobiologia*, *787*(1), 27–44. https://doi.org/10.1007/s10750-016-2974-5

Schmera, D., Podani, J., Heino, J., Erös, T., & Poff, N. L. R. (2015). A proposed unified terminology of species traits in stream ecology. *Freshwater Science*, *34*(3), 823–830. https://doi.org/10.1086/681623

Schmidt-Kloiber, A., & Hering, D. (2015). Www.freshwaterecology.info - An online tool that unifies, standardises and codifies more than 20,000 European freshwater organisms and their ecological preferences. *Ecological Indicators*, *53*, 271–282. https://doi.org/10.1016/j.ecolind.2015.02.007

Schmidt-Kloiber, A., & Hering, D. (2023). *www.freshwaterecology.info - the taxa and autecology database for freshwater organisms, version 8.0 (accessed on 20.11.2023).*

Schmidt-Kloiber, A., & Nijboer, R. C. (2004). The effect of taxonomic resolution on the assessment of ecological water quality classes. *Hydrobiologia*, *516*(1–3), 269–283. https://doi.org/10.1023/B:HYDR.0000025270.10807.10

Scholl, E. A., Rantala, H. M., Whiles, M. R., & Wilkerson, G. V. (2016). Influence of Flow on Community Structure and Production of Snag-Dwelling Macroinvertebrates in an Impaired Low-Gradient River. *River Research and Applications*, *32*(4), 677–688. https://doi.org/10.1002/rra.2882

Schreiner, B. (2013). Viewpoint - Why has the South African national water act been so difficult to implement? *Water Alternatives*, *6*(2), 239–245.

Schulz, U. H., da Rosa, M. F., & Klein-Stolz, G. F. (2019). Drift and emergence patterns of nonbiting midges (Chironomidae, Diptera, Insecta) in a subtropical river. *Ecological Research*, *34*(6), 835–841. https://doi.org/10.1111/1440-1703.12061

Schwarzenbach, R. P., Egli, T., Hofstetter, T. B., Von Gunten, U., & Wehrli, B. (2010). Global water pollution and human health. *Annual Review of Environment and Resources*, *35*, 109–136. https://doi.org/10.1146/annurev-environ-100809-125342

Segner, H., Schmitt-Jansen, M., & Sabater, S. (2014). Assessing the impact of multiple stressors on aquatic biota: The receptor's side matters. *Environmental Science and Technology*, *48*(14), 7690–7696. https://doi.org/10.1021/es405082t

Serra, S. R. Q., Cobo, F., Graça, M. A. S., Dolédec, S., & Feio, M. J. (2016). Synthesising the trait information of European Chironomidae (Insecta: Diptera): Towards a new database. *Ecological Indicators*, *61*, 282–292. https://doi.org/10.1016/j.ecolind.2015.09.028

Serra, S. R. Q., Graca, M. A. S., Dolédec, S., & Feio, M. J. (2017a). Discriminating permanent from temporary rivers with traits of chironomid genera. *Annales de Limnologie*, *53*, 161–174. https://doi.org/10.1051/limn/2017004

Serra, S. R. Q., Graça, M. A. S., Dolédec, S., & Feio, M. J. (2017b). Chironomidae traits and life history strategies as indicators of anthropogenic disturbance. *Environmental Monitoring and Assessment*, *189*(7). https://doi.org/10.1007/s10661-017-6027-y

Seymour, R. S., & Matthews, P. G. D. (2013). Physical gills in diving insects and spiders:

Theory and experiment. *Journal of Experimental Biology*, *216*(2), 164–170. https://doi.org/10.1242/jeb.070276

Simpson, K. W., & Bode, R. W. (1980). Common larvae of Chironomidae (Diptera) from New York State streams and rivers : with particular reference to the fauna of artificial substrates. *Bulletin No. 439*, 105 pp.

Sinchembe, M., & Ellery, W. N. (2010). *Human impacts on hydrological health and the provision of ecosystem services : a case study of the eMthonjeni – Fairview Spring Wetland , Grahamstown , South Africa*. *35*(3), 227–239. https://doi.org/10.2989/16085914.2010.538508

Sivaruban, T., Barathy, S., Venkataraman, K., Arunachalam, M., & Srinivasan, P. (2022). Diversity of the EPT complex (Ephemeroptera, Plecoptera and Trichoptera) in the Western and Eastern Ghats (South India) caused by the variations of landscape elements and mesohabitats. *Ege Journal of Fisheries and Aquatic Sciences*, *39*(1), 24–31. https://doi.org/10.12714/egejfas.39.1.04

Slaughter, A. R., Mantel, S. K., & Hughes, D. A. (2014). Investigating possible climate change and development effects on water quality within an arid catchment in South Africa: A comparison of two models. *Proceedings - 7th International Congress on Environmental Modelling and Software: Bold Visions for Environmental Modeling, IEMSs 2014*, *3*, 1568–1575.

Southwood, T. R. . (1977). Habitat , the Templet for Ecological Strategies? *Journal of Animal Ecology*, *46*(2), 336–365.

Southwood, T. R. . (1988). Tactics , Strategies and Templets. *Oikos*, *52*(1), 3–18. http://www.jstor.org/stable/3565974

Stats SA, S. S. A. (2014). *General Household Survey* (Issue May). https://www.statssa.gov.za/publications/P0318/P03182014.pdf

Stats SA, S. S. A. (2016). *Provincial profile: Eastern Cape Community Survey 2016*. Statistics South Africa. http://www.statssa.gov.za/publications/Report 03-01-08/Report 03-01-082016.pdf

Statzner, B. (2008). How views about flow adaptations of benthic stream invertebrates changed over the last century. *International Review of Hydrobiology*, *93*(4–5), 593–605. https://doi.org/10.1002/iroh.200711018

Statzner, B., & Bêche, L. A. (2010). Can biological invertebrate traits resolve effects of multiple stressors on running water ecosystems? *Freshwater Biology*, *55*(SUPPL. 1), 80–119. https://doi.org/10.1111/j.1365-2427.2009.02369.x

Stoddard, J. L., Herlihy, A. T., Peck, D. V., Hughes, R. M., Whittier, T. R., & Tarquinio, E. (2008). A process for creating multimetric indices for large-scale aquatic surveys. *Journal of the North American Benthological Society*, *27*(4), 878–891. https://doi.org/10.1899/08-053.1

Storey, R. G., & Quinn, J. M. (2011). Life histories and life history strategies of invertebrates inhabiting intermittent streams in Hawke's Bay, New Zealand. *New Zealand Journal of Marine and Freshwater Research*, *45*(2), 213–230. https://doi.org/10.1080/00288330.2011.554988

Strahler, A. (1957). Quantitative Analysis of Watershed Geomorphology, Transactions of the American Geophysical Union. *Transactions, American Geophysical Union*, *38*(6), 913–920.

Stribling, J. B., & Dressing, S. A. (2015). *Applying Benthic Macroinvertebrate Multimetric Indexes to Stream Condition Assessments Introduction* (Issue Figure 1). https://www.epa.gov/polluted-runoff- nonpoint-source-pollution/watershed-approach-technical- resources

Strokal, M., Bai, Z., Franssen, W., Hofstra, N., Koelmans, A. A., Ludwig, F., Ma, L., van Puijenbroek, P., Spanier, J. E., Vermeulen, L. C., van Vliet, M. T. H., van Wijnen, J., & Kroeze, C. (2021). Urbanization: an increasing source of multiple pollutants to rivers in the 21st century. *Npj Urban Sustainability*, *1*(1). https://doi.org/10.1038/s42949-021-00026-w

Sudaryanti, S., Trihadiningrum, Y., Hart, B. T., Davies, P. E., Humphrey, C., Norris, R., Simpson, J., & Thurtell, L. (2001). Assessment of the biological health of the Brantas River, East Java, Indonesia using the Australian River Assessment System (AUSRIVAS) methodology. *Aquatic Ecology*, *35*(2), 135–146. https://doi.org/10.1023/A:1011458520966

Suhaila, A. H., & Che Salmah, M. R. (2017). Application of aquatic insects (Ephemeroptera, plecoptera and trichoptera) in water quality assessment of Malaysian headwater. *Tropical Life Sciences Research*, *28*(2), 143–162. https://doi.org/10.21315/tlsr2017.28.2.11

Suriano, M. T., Fonseca-Gessner, A. A., Roque, F. O., & Froehlich, C. G. (2011). Choice of macroinvertebrate metrics to evaluate stream conditions in Atlantic Forest, Brazil. *Environmental Monitoring and Assessment*, *175*(1–4), 87–101. https://doi.org/10.1007/s10661-010-1495-3

Suzzi, A. L., Gaston, T. F., McKenzie, L., Mazumder, D., & Huggett, M. J. (2022). Tracking the impacts of nutrient inputs on estuary ecosystem function. *Science of the Total Environment*, *811*, 152405. https://doi.org/10.1016/j.scitotenv.2021.152405

Szabolcs, M., Kapusi, F., Carrizo, S., Markovic, D., Freyhof, J., Cid, N., Cardoso, A. C., Scholz, M., Kasperidus, H. D., Darwall, W. R. T., & Lengyel, S. (2022). Spatial priorities for freshwater biodiversity conservation in light of catchment protection and connectivity in Europe. *PLoS ONE*, *17*(5 May), 1–15. https://doi.org/10.1371/journal.pone.0267801

Tampo, L., Kaboré, I., Alhassan, E. H., Ouéda, A., Bawa, L. M., & Djaneye-Boundjou, G. (2021). Benthic Macroinvertebrates as Ecological Indicators: Their Sensitivity to the Water Quality and Human Disturbances in a Tropical River. *Frontiers in Water*, *3*(September), 1–17. https://doi.org/10.3389/frwa.2021.662765

Taylor, G. D., Fletcher, T. D., Wong, T. H. F., Breen, P. F., & Duncan, H. P. (2005). Nitrogen composition in urban runoff - Implications for stormwater management. *Water Research*, *39*(10), 1982–1989. https://doi.org/10.1016/j.watres.2005.03.022

Tchounwou, P. B., Yedjou, C. G., Patlolla, A. K., & Sutton, D. J. (2012). Heavy Metals Toxicity and the Environment. *Molecular, Clinical and Environmental Toxicology*, *101*, 133–164. https://doi.org/10.1007/978-3-7643-8340-4_6

Thioulouse, J., Dray, S., Dufour, A., Siberchicot, A., Jombart, T., & Pavoine, S. (2018). Multivariate Analysis of Ecological Data with ade4. *Springer*. https://doi.org/https://doi.org/10.1007/978-1-4939-8850-1

Thirion, C. (2008). River EcoClassification: Mannual for EcoStatus determination Module E: Volume 1: Macro-Invertebrate Response Assessment Index (MIRAI). In *WRC Report TT 332/08*.

Thirion, C. (2016). *The determination of flow and habitat requirements for selected riverine macroinvertebrates* (Issue May). North-West University.

Tomanova, S., Goitia, E., & Helešic, J. (2006). Trophic levels and functional feeding groups of macroinvertebrates in neotropical streams. *Hydrobiologia*, *556*(1), 251–264. https://doi.org/10.1007/s10750-005-1255-5

Tomanova, S., Moya, N., & Oberdorff, T. (2008). Using macroinvertebrate biological traits for assessing biotic integrity of neotropical streams. *River Research and Applications, 24*(9), 1230–1239. https://doi.org/10.1002/rra.1148

Townsend, C. R., & Hildrew, A. G. (1994). Species traits in relation to a habitat templet for river systems. *Freshwater Biology*, *31*(3), 265–275. https://doi.org/10.1111/j.1365-2427.1994.tb01740.x

Trajkovic, V. K. (2016). Application of the Canadian Aquatic Biomonitoring Network ( CABIN ). In *The Miramichi River Environmental Assessment Committee Synopsis*.

Tubić, B., Andjus, S., Zorić, K., Vasiljević, B., Jovičić, K., Čanak Atlagić, J., & Paunović, M. (2024). Aquatic Insects (Ephemeroptera, Plecoptera and Trichoptera) Metric as an Important Tool in Water Quality Assessment in Hilly and Mountain Streams. *Water (Switzerland)*, *16*(6). https://doi.org/10.3390/w16060849

UNEP-GEMS, U. N. E. P. G. E. M. S. W. P. (2008). *Water Quality for Ecosystem and Human Health* (Vol. 69, Issue 4).

Usseglio-Polatera, P., Bournaud, M., Richoux, P., & Tachet, H. (2000). Biomonitoring through biological traits of benthic macroinvertebrates: How to use species trait databases? *Hydrobiologia*, *422–423*, 153–162. https://doi.org/10.1007/978-94-011-4164-2_12

Valente-Neto, F., Martínez, B. T., Hughes, R. M., Ferreira, A., Severo-Neto, F., Souza, F. L. de, Souza, R. P., Escarpinati, S. C., & Roque, F. de O. (2021). Incorporating costs, thresholds and spatial extents for selecting stream bioindicators in an ecotone between two Brazilian biodiversity hotspots. *Ecological Indicators*, *127*(August). https://doi.org/10.1016/j.ecolind.2021.107761

van der Meer, J. R. (2016). Towards improved biomonitoring tools for an intensified sustainable multi-use environment. In *Microbial Biotechnology* (Vol. 9, Issue 5, pp. 658–665). https://doi.org/10.1111/1751-7915.12395

van der Meer, T. V., Verdonschot, P. F. M., Dokter, L., Absalah, S., & Kraak, M. H. S. (2022). Organic matter degradation and redistribution of sediment associated contaminants by benthic invertebrate activities. *Environmental Pollution*, *306*(December 2021), 119455. https://doi.org/10.1016/j.envpol.2022.119455

van Ginkel, C. E. (2011). Eutrophication: Present reality and future challenges for South Africa. *Water SA*, *37*(5), 693–702. https://doi.org/10.4314/wsa.v37i5.6

van Kleef, H., Verberk, W. C. E. P., Kimenai, F. F. P., van der Velde, G., & Leuven, R. S. E. W. (2015). Natural recovery and restoration of acidified shallow soft-water lakes: Successes and bottlenecks revealed by assessing life-history strategies of chironomid larvae. *Basic and Applied Ecology*, *16*(4), 325–334. https://doi.org/10.1016/j.baae.2015.02.007

Veevers, J. J., Cole, D. I., & Cowan, E. J. (1994). Southern Africa: Karoo Basin and Cape Fold Belt. *Memoir of the Geological Society of America*, *184*(1), 223–279. https://doi.org/10.1130/MEM184-p223

Vega, R., Brooks, S. J., Hockaday, W., Lee, S., & Vane-Wright, R. I. (2021). Diversity of Chironomidae (Diptera) breeding in the Great Stour, Kent: baseline results from the Westgate Parks non-biting midge project. *Journal of Natural History*, *55*(11–12), 665–682. https://doi.org/10.1080/00222933.2021.1919776

Verberk, W. C. E. P., Siepel, H., & Esselink, H. (2008a). Applying life-history strategies for freshwater macroinvertebrates to lentic waters. *Freshwater Biology*, *53*(9), 1739–1753. https://doi.org/10.1111/j.1365-2427.2008.02036.x

Verberk, W. C. E. P., Siepel, H., & Esselink, H. (2008b). Life-history strategies in freshwater macroinvertebrates. *Freshwater Biology*, *53*(9), 1722–1738. https://doi.org/10.1111/j.1365-2427.2008.02035.x

Verberk, W. C. E. P., Van Noordwijk, C. G. E., & Hildrew, A. G. (2013). Delivering on a promise: Integrating species traits to transform descriptive community ecology into a predictive science. *Freshwater Science*, *32*(2), 531–547. https://doi.org/10.1899/12-092.1

Villamarín, C., Villamarín-cortez, S., & Salcido, D. M. (2021). *Drivers of diversity and altitudinal distribution of chironomids ( Diptera : Chironomidae ) in the Ecuadorian Andes*. *69*(March), 113–126. https://doi.org/10.15517/rbt.v69i1.40964

Violle, C., Navas, M.-L., Vile, D., Kazakou, E., Fortunel, C., Hummel, I., & Garnier, E. (2007). Let the concept of trait be functional! *Oikos*, *116*(5), 882–892. https://doi.org/10.1111/j.2007.0030-1299.15559.x

Von Der Meden, C. E. O., Palmer, C. G., Davies-Coleman, H. D., & Gola, N. P. (2004). Environmental water quality in makana municipality, Eastern Cape, South Africa: a case study of local government responsibility for water resource management. *CWL Publishing Enterprises, Inc., Madison*, *2004*(1), 352. http://onlinelibrary.wiley.com/doi/10.1002/cbdv.200490137/abstract

Vosloo, R., & Bouwman, H. (2005). Survey of certain persistent organic pollutants in major South African waters: Report to the Water Research Commission. In *Water Research Commission* (Issue 1213/1/05.).

Voß, K., & Schäfer, R. B. (2017). Taxonomic and functional diversity of stream invertebrates along an environmental stress gradient. *Ecological Indicators*, *81*(May), 235–242. https://doi.org/10.1016/j.ecolind.2017.05.072

Walsh, C. J., Roy, A. H., Feminella, J. W., Cottingham, P. D., Groffman, P. M., & Morgan, R. P. (2005). The urban stream syndrome: current knowledge and the search for a cure. *Journal of the North American Benthological Society*, *24*(3), 706–723. https://doi.org/10.1899/04-028.1

Walshe, B. M. (1950). The Function of Haemoglobin in Chironomus Plumosus Under Natural Conditions. *Journal of Experimental Biology*, *27*(1), 73–95. https://doi.org/10.1242/jeb.27.1.73

Walshe, B. M. (1951). The feeding habits of certain chironomid larvae (subfamily Tendipedinae). *Proceedings of the Zoological Society of London*, *121*(1), 63–79.

https://doi.org/10.1111/j.1096-3642.1951.tb00738.x

Wang, C., Wang, D., Wang, H., & Dong, R. (2011). Impacts of urbanisation on river systems and their functions in Yanggong River watershed of Lijiang City. *International Journal of Sustainable Development and World Ecology*, *18*(6), 498–502. https://doi.org/10.1080/13504509.2011.603759

Wasserstein, R. L., & Lazar, N. A. (2016). The ASA's Statement on p-Values: Context, Process, and Purpose. *American Statistician*, *70*(2), 129–133. https://doi.org/10.1080/00031305.2016.1154108

Waterhouse, J. C., & Farrell, M. P. (1985). Identifying pollution related changes in chironomid communities as a function of taxonomic rank. *Canadian Journal of Fisheries and Aquatic Sciences*, *42*(3), 406–413. https://doi.org/10.1139/f85-056

Webb, C. T., Hoeting, J. A., Ames, G. M., Pyne, M. I., & LeRoy Poff, N. (2010). A structured and dynamic framework to advance traits-based theory and prediction in ecology. *Ecology Letters*, *13*(3), 267–283. https://doi.org/10.1111/j.1461-0248.2010.01444.x

Whittier, T. R., & Van Sickle, J. (2010). Macroinvertebrate tolerance values and an assemblage tolerance index (ATI) for western USA streams and rivers. *Journal of the North American Benthological Society*, *29*(3), 852–866. https://doi.org/10.1899/09-160.1

Wickham, H. (2016). *ggplot2: Elegant Graphics for Data Analysis* (978-3-319-24277–4). Springer-Verlag. https://ggplot2.tidyverse.org

Wiederholm, T. (1983). *Chironomidae of the Holarctic region. Keys and diagnoses. Part 1. Larvae.* Entomologica Scandinavica.

Wilkes, M. A., Edwards, F., Jones, J. I., Murphy, J. F., England, J., Friberg, N., Hering, D., Poff, N. L. R., Usseglio-Polatera, P., Verberk, W. C. E. P., Webb, J., & Brown, L. E. (2020). Trait-based ecology at large scales: Assessing functional trait correlations, phylogenetic constraints and spatial variability using open data. *Global Change Biology*, *26*(12), 7255–7267. https://doi.org/10.1111/gcb.15344

Wilkes, M. A., Mckenzie, M., Murphy, J. F., & Chadd, R. P. (2017). Assessing the Mechanistic Basis for Fine Sediment Biomonitoring: Inconsistencies among the Literature, Traits and

Indices. *River Research and Applications*, *33*(10), 1618–1629. https://doi.org/10.1002/rra.3139

Wooster, D. E., Miller, S. W., & DeBano, S. J. (2011). An examination of the impact of multiple disturbances on a river system: taxonomic metrics versus biological traits. *River Research and Applications*, *28*(10), 1630–1643. https://doi.org/10.1002/rra.1555

World Economic Forum. (2016). Inspiring Future Cities & Urban Services Shaping the Future of Urban Development & Services Initiative. *World Economic Forum Industry Agenda Report, April*, 1–60. http://www3.weforum.org/docs/WEF_Urban-Services.pdf

Xia, W., Wang, R., Zhu, B., Rudstam, L. G., Liu, Y., Xu, Y., Xin, W., & Chen, Y. (2020). Heavy metal gradients from rural to urban lakes in central China. *Ecological Processes*, *9*(1). https://doi.org/10.1186/s13717-020-00251-8

Zhao, Q., Guo, F., Zhang, Y., Yang, Z., & Ma, S. (2018). Effects of secondary salinisation on macroinvertebrate functional traits in surface mining-contaminated streams, and recovery potential. *Science of the Total Environment*, *640–641*, 1088–1097. https://doi.org/10.1016/j.scitotenv.2018.05.347

Ziajahromi, S., Kumar, A., Neale, P. A., & Leusch, F. D. L. (2019). Effects of polyethylene microplastics on the acute toxicity of a synthetic pyrethroid to midge larvae (Chironomus tepperi) in synthetic and river water. *Science of the Total Environment*, *671*, 971–975. https://doi.org/10.1016/j.scitotenv.2019.03.425

# APPENDICES

## APPENDIX A

Table 1: Description of selected metrics and their predicted response to increasing urban pollution

| S/N | Metric description | Code | Predicted response |
|---|---|---|---|
| *Composition and abundance measures* | | | |
| 1 | Relative abundance of species in the Chironomus genera | Cmus. | + |
| 2 | Relative abundance of species in the *Dicrotendipes* genera | Dicro. | + |
| 3 | Sum of relative abundances of species in the *Chironomus* and *Dicrotendipes* genera | Cms_Dicr. | + |
| 4 | Total *Chironomus* spp abundance | Cmus_Abun | + |
| 5 | Total *Dicrotendipes* spp abundance | Dicro_Abun | + |
| 6 | Total *Cricotopus* spp abundance | Cric_Abun | + |
| 7 | Total *Rheotanytarsus* spp abundance | Rheotan_Abun | + |
| 8 | Total *Conchapelopia* spp abundance | Conc_Abun | - |
| 9 | Total *Ablabesmyia* spp abundance | Abla_Abun | - |
| 10 | Total *Eukiefferiella* spp abundance | Eukie_Abun | + |
| 11 | Total *Orthocladius* spp abundance | Orth_Abun | - |
| 12 | Total *Rheocricotopus* spp abundance | Rheocr_Abun | - |

| | | | |
|---|---|---|---|
| 13 | Total *Tanytarsus* spp abundance | Tsus_Abun | - |
| 14 | Total *Cladotanytarsus* spp abundance | Clado_Abun | - |
| 15 | Total *Corynoneura* spp abundance | Cory_Abun | - |
| 16 | Total *Polypedilum* spp abundance | Poly_Abun | + |
| 17 | Total *Tvetenia* spp abundance | Tvet_Abun | - |
| 18 | Ratio of abundance of species in tribe Chironomini and total abundance of *Chironomus* spp | Chrni_ratio | - |
| 19 | Ratio of *Chironomus* spp abundance to abundance of other chironomid taxa | Cmus_ratio | + |
| 20 | Ratio of total abundance of *Chironomus* and *Cricotopus* species to sum of *Orthocladius* and *Rheocricotopus* species abundances | CmusRheo_ratio | + |
| 21 | Ratio of total abundance of *Chironomus, Polypedilum*, and *Cricotopus* species to sum of *Orthocladius, Tvetenia*, and *Rheocricotopus* species abundances | PolyOrtho_ratio | + |
| 22 | Ratio of total abundance of *Chironomus* and *Polypedilum* species to *Orthocladius* species abundance | CP_Orth_ratio | + |
| 23 | Ratio of total abundance of species in Chironominae to sum of total abundance of species in Orthocladiinae and Tanypodinae | CHR_ORTTAP ratio | + |
| 24 | Ratio of total abundance of species in Chironominae and total abundance of species in Orthocladiinae | CHRORT_ratio | + |
| 25 | Ratio of total abundance of species in Tanypodinae Chironominae to total abundance of species in other Chironomidae subfamilies | TAPCHR_ratio | - |

| | | | |
|---|---|---|---|
| 26 | Total abundance of *Chironomus*, *Polypedilum* and *Cricotopus* species | CPC_Abun | + |
| 27 | Total abundance of *Conchapelopia*, *Ablabesmyia*, *Polypedilum* and *Rheotanytarsus* species | CAPR | - |
| 28 | Ratio of *Ablabesmyia*, *Conchapelopia*, and *Polypedilum* species total abundance to abundance of *Rheotanytarsus* species | ACP_R | - |
| 29 | Ratio of *Cricotopus, Orthocladius, Corynoneura* species total abundance to sum of abundance of *Chironomus* and *Dicrotendipes* species | COC_CD | - |
| 30 | Ratio of *Tanytarsus* and *Rheotanytarsus* species abundance to sum of abundance of *Chironomus* and *Dicrotendipes* | TR_CD | - |
| 31 | Ratio of *Cladotanytarsus, Tanytarsus, Ablabesmyia, Orthocladius, Polypedilum* and *Rheotanytarsus* species total abundance to sum of abundance of *Chironomus* and *Dicrotendipes* species | CTAOPR_CD | - |
| 32 | Ratio of *Cricotopus, Corynoneura, Ablabesmyia, Orthocladius* and *Conchapelopia* species total abundance to sum of abundance of *Chironomus* and *Dicrotendipes* species | CCOAC_CD | - |
| 33 | Total abundance of *Cladotanytarsus, Tanytarsus, Ablabesmyia, Rheotanytarsus Polypedilum* and *Orthocladius* species | CTAOPR | - |
| 34 | Total abundance of *Cladotanytarsus, Tanytarsus* and *Rheotanytarsus* species | TRC | + |
| *Richness measures* | | | |

|  |  |  |  |
|---|---|---|---|
| 35 | Total number of chironomid genera | TT | - |
| 36 | Total number of chironomid species | Spe_rich | - |
| 37 | Total number of chironomid genera in tribe Tanypodinae | Tap_taxa | - |
| 38 | Total number of chironomid genera in tribe Orthocladiinae | Ort_taxa | - |
| 39 | Total number of chironomid genera in tribe Chironomini | Chi_taxa…46 | + |
| 40 | Total number of chironomid genera in tribe Tanytarsini | Tan_taxa | - |
| 41 | Total number of chironomid species in tribe Tanypodinae | Tap_sp | - |
| 42 | Total number of chironomid species in tribe Orthocladiinae | Ort_sp | - |
| 43 | Total number of chironomid species in tribe Chironomini | Chi_sp | + |
| 44 | Total number of chironomid species in tribe Tanytarsini | Tan_sp | - |
| 45 | Total number of *Chironomus* species | Chi_rich | + |
| 46 | Total number of *Dicrotendipes* species | Dic_rich | + |
| 47 | Total number of *Polypedilum* species | Pol_rich | + |
| 48 | Total number of *Cricotopus* species | Cri_rich | + |
| 49 | Total number of *Einfeldia* species | Ein_rich | + |
| 50 | Total number of *Orthocladius* species | Ort_rich | - |
| 51 | Total number of *Corynoneura* species | Cor_rich | - |

| | | | |
|---|---|---|---|
| 52 | Total number of *Tvetenia* species | Tve_rich | - |
| 53 | Total number of *Rheocricotopus* species | Rhe_rich | - |
| 54 | Ratio of total *Chironomus* and *Cricotopus* species richness to sum of *Orthocladius* and *Rheocricotopus* species richness | X.Ch_Rheo.61 | + |
| 55 | Ratio of total *Chironomus*, *Polypedilum*, and *Cricotopus* species richness to sum of *Orthocladius*, *Tvetenia*, and *Rheocricotopus* species richness | X.Ch_Rheo.62 | + |
| 56 | Ratio of total *Chironomus* and *Polypedilum* species richness to *Orthocladius* species richness | X.Ch_Orth | + |
| 57 | Ratio of total number of species in Chironominae to sum of total number of species in Orthocladiinae and Tanypodinae | Chi_Tany…64 | + |
| 58 | Ratio of total number of species in Chironominae to total number of species in Orthocladiinae | Chi_Orth | + |
| 59 | Ratio of total number of species in Tanypodinae to total number of species in other Chironomidae subfamilies | Tan_Chir | - |
| 60 | Sum of *Chironomus* and *Polypedilum* species richness | Chi_Poly | + |
| 61 | Ratio of total *Cricotopus* species richness to *Orthocladius* species richness | Cri_Orth | + |
| 62 | Ratio of total number of species in tribe Chironominii to total number of species in tribe Tanytarsinii | Chi_Tany…69 | + |
| 63 | Ratio of total *Chironomus* and *Cricotopus* species richness to total chironomid species richness | Chi_Cric.spe | + |

|  |  |  |  |
|---|---|---|---|
| 64 | Ratio of total number of species in tribe Chironominii to total number of other chironomid species | Chi_Chir | + |
| 65 | Ratio of total number of species in Orthocladiinae to total number of species in tribe Chironominii | Ort_Chir…72 | - |
| *Tolerance measures (degree of sensitivity to disturbance)* |  |  |  |
| 66 | Ratio of abundance of species in Tanypodinae to abundance of species in tribe Chironomini | Tan_Chir | - |
| 67 | Ratio of abundance of species in Orthocladiinae to abundance of species in tribe Chironomini | Ort_Chir…74 | - |
| 68 | Ratio of total abundance of *Corynoneura* and *Rheocricotopus* species to *Chironomus* species abundance | Cor_Chir | - |
| 69 | Ratio of abundance of species in tribe Chironomini to abundance of other chironomid species | Chi_taxa…77 | + |
| *Diversity measures* |  |  |  |
| 70 | Shannon-Weiner diversity index | Sha_inde | - |
| 71 | Simpson diversity | Sim_dive | - |
| 72 | Margalef index | Mar_inde | - |
| 73 | Evenness index | Eve_inde | - |
| 74 | first Hill’s effective number of taxa (H1) | fir_(H1) | - |
| 75 | Berger–Parker dominance index | Ber_inde | - |
| 76 | Pielou index | Pie_inde | - |
| 77 | Menhinick’s diversity index | Men_inde | - |

Table 2: Tribe, species name, and code names of chironomids used in this study.

| Subfamily/Tribe | *Species* | Code |
|---|---|---|
| Chironomini | Chironomini Genus B | CGENUSB |
|  | *Chironomus* sp3 | CHIRO3 |
|  | *Chironomus* sp1 | CHIRO1 |
|  | *Chironomus* sp2 | CHIRO2 |
|  | *Cryptochironomus sp* | CRYPTCHIR |
|  | *Dicrotendipes lobiger* | DICRL |
|  | *Dicrotendipes nervosus* | DICRNERV |
|  | *Dicrotendipes notatus* | DICRN |
|  | *Dicrotendipes pulsus* | DICRP |
|  | *Dicrotendipes sp* | DICRPESS |
|  | *Einfeldia carbonaria* | EINCARB |
|  | *Einfeldia pagana* | EINP |
|  | *Einfeldia sp* | EINS |
|  | *Gillotia alboviridis* | GILA |
|  | *Glyptotendipes severini* | GLSEVE |
|  | *Glyptotendipes sp* | GLYPS |
|  | *Kiefferulus sp* | KIEFFU |
|  | *Microchironomus sp* | MUSSP |
|  | *Microchironomus tener* | MICHIRO |
|  | *Microtendipes pedellus* | MICP |
|  | *Paracladopelma nais* | PELMANA |
|  | *Polypedilum convictum* | PLPC |
|  | *Polypedilum illinoense* | PLPI |

| | | |
|---|---|---|
| | *Polypedilum nubeculosum* | PLPN |
| | *Polypedilum nubifer* | PLPNR |
| | *Polypedilum ophoides* | PLPO |
| | *Polypedilum sp* | PLPS |
| | *Polypedilum tuberculum* | PLPT |
| | *Pseudochironomus sp* | PCHIRO |
| | *Stempellinella sp* | STEMPELL |
| | *Stenochironomus sp* | STENO |
| | *Stictochironomus sp* | STICTO |
| | *Zavreliella marmotata* | ZAVM |
| Chironomini Total | 33 | |
| | | |
| Orthocladiinae | *Abiskomyia sp* | ABISK |
| | *Chaetocladius setosipennis* | CHAESET |
| | *Cardiocladius sp* | CARDIO |
| | *Corynoneura lobata* | CORYLOB |
| | *Corynoneura scutellata* | CORYSCU |
| | *Cricotopus bicinctus* | CRICBICIN |
| | *Cricotopus cylindraceus* | CCYLIN |
| | *Cricotopus intersectus* | CRICI |
| | *Cricotopus tremelus* | CRICT |
| | *Cricotopus* sp1 | CRIC1 |
| | *Cricotopus trifasciata gr* | CRICTRIF |
| | *Eukiefferiella gracei* | EUKIG |
| | *Eukiefferiella sp* | EUKIES |
| | *Euryhapsis cilium* | EURYCIL |

| | | |
|---|---|---|
| | *Euryhapsis sp* | EURYHS |
| | *Hydrobaenus johannseni* | HYDROJOH |
| | *Krenosmittia sp* | KRENOSM |
| | *Metriocnemus fuscipes* | METRIFUS |
| | *Nanocladius rectinervis* | NANO |
| | *Nanocladius sp* | NANOCLAD |
| | *Orthocladius clarki* | ORTC |
| | *Orthocladius dentifer* | ORTDENT |
| | *Orthocladius oliveri* | ORTO |
| | *Orthocladius sp* | ORTSS |
| | *Paracricotopus niger* | PARANIGER |
| | *Parakiefferiella sp* | PARAKIE |
| | *Paralimnophyes hydrophilus* | PARAHYDRO |
| | *Paralimnophyes sp* | PARALIMS |
| | *Parametriocnemus lundbecki* | PARALUND |
| | *Paratrichocladius skirwithensis* | PSKIR |
| | *Paratrichocladius sp* | TRICHOCLAD |
| | *Pseudorthocladius sp* | PSEUDORT |
| | *Pseudosmittia sp* | PSMITTIA |
| | *Rheocricotopus sp* | RHEOCRIC |
| | *Trissocladius sp* | TRISSO |
| | *Tvetenia paucunca* | TVETPAUC |
| | *Tvetenia sp* | TVETS |
| Orthocladiinae Total | 37 | |
| | | |
| Tanypodinae | *Ablabesmyia monilis* | ABLM |

| | | |
|---|---|---|
| | *Ablabesmyia phatta* | ABLP |
| | *Ablabesmyia sp* | ABLASP |
| | *Arctopelopia sp* | ARCT |
| | *Clinotanypus sp* | CLINOTANY |
| | *Coelotanypus sp* | COELOTAN |
| | *Conchapelopia melanops* | CONCMEL |
| | *Conchapelopia sp* | CONCHS |
| | *Djalmabatista pulcher* | DJALP |
| | *Helopelopia cornuticaudata* | HELOP |
| | *Larsia sp* | LARSIA |
| | *Meropelopia americana* | MERO |
| | *Monopelopia tennicalcar* | MONOTEN |
| | *Natarsia baltimoreus* | NATARB |
| | *Paramerina anomalus* | PAMERANO |
| | *Paramerina cingulata* | PAMECING |
| | *Procladius bellus* | PROBELL |
| | *Procladius sp* | PROCLAD |
| | *Rheopelopia sp* | RHP |
| | *Tanypus sp* | TANYPS |
| | *Thienemannimyia geijskesi* | THIEN |
| | *Thienemannimyia senata* | THIENSEN |
| | *Trissopelopia sp* | TRISSOPEL |
| Tanypodinae Total | 23 | |
| | | |
| Tanytarsini | *Cladotanytarsus mancus* | CLAM |
| | *Cladotanytarsus sp* | CLAS |

| | | |
|---|---|---|
| | *Paratanytarsus austriacus* | PARAA |
| | *Paratanytarsus sp* | PARATANS |
| | *Rheotanytarsus curtistylus* | RHEOTC |
| | *Rheotanytarsus sp* | RHEOTANS |
| | *Tanytarsus brundini* | TBRUND |
| | *Tanytarsus sp* | TARSSP |
| | *Tanytarsus usmaensis* | TUSM |
| | *Virgatanytarsus ardrennensis* | VIRGA |
| | *Virgatanytarsus sp* | VIRGS |
| Tanytarsini Total | 11 species | |
| Grand Total | 104 species | |

Table 3: Relative abundances of chironomid species collected from the Buffalo and Bloukrans Rivers (November 2021 – June 2022) and the Swartkops River (2009-2012) pooled based on the site categories per season. REF-least impacted sites, DP-sites that are upstream from wastewater treatment works (WWTWs) that receive diffuse pollution, PP-sites that receive point source pollution from WWTWs effluent discharge points, AG-sites downstream PP sites that receive impact from point and diffuse sources, RECV-recovery sites. Au-Autumn, Sp-Spring, Su-Summer, Wi-Winter.

| **Species** | **REF** | | | | **DP** | | | | **PP** | | | | **AG** | | | | **RECV** | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Au | Sp | Su | Wi | Au | Sp | Su | Wi | Au | Sp | Su | Wi | Au | Sp | Su | Wi | Au | Sp | Su | Wi |
| *Rheopelopia sp* | 1.05 | 0.63 | 0.73 | 0.30 | | | | | | | | | | | | | | 0.66 | | |
| *Polypedilum tuberculum* | 6.32 | 0.63 | 27.38 | 7.16 | | | 0.52 | 10.57 | | | | 6.53 | | | | 5.70 | 3.53 | 1.32 | 14.74 | 19.62 |
| *Polypedilum convictum* | | 0.21 | | 0.60 | | | 0.20 | | | | | | | | 0.97 | | 1.18 | 1.32 | 14.10 | 2.53 |
| *Tanytarsus usmaensis* | 2.81 | 3.35 | 1.22 | | | | | | | | | | | | | | 12.94 | 27.15 | 0.64 | 1.27 |
| *Polypedilum nubeculosum* | 1.40 | 4.39 | | | | 3.65 | | | | | | 0.30 | | 13.75 | | | | | | 0.63 |
| *Polypedilum illinoense* | 1.05 | 6.07 | 8.56 | 2.69 | 1.09 | | 1.15 | | 0.95 | | 0.93 | | | | | | 5.88 | 1.32 | 4.49 | |

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|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *Ablabesmyia phatta* | 0.70 | 0.84 | | | | | | | | | | | | | | | | | | |
| *Polypedilum nubifer* | 0.70 | 0.42 | | | 0.73 | | 0.44 | | 10.10 | | 0.12 | | 22.38 | 5.82 | 7.41 | | 14.12 | 1.99 | 19.87 | 48.73 |
| *Zavreliella marmotata* | | 0.21 | | | | | | | | | | | | | | | | | | |
| *Gillotia alboviridis* | | 1.05 | | 0.30 | | | | | | | | | | | | | | | | |
| *Cladotanytarsus sp* | | 0.63 | | 0.90 | | | 0.08 | | | | | 0.15 | | 0.04 | | | | | | |
| *Cladotanytarsus mancus* | | 3.35 | 0.49 | | | | | | | | | | | | | | | 5.30 | 0.64 | |
| *Dicrotendipes pulsus* | | 0.21 | | | | | | | | | | | | | 1.36 | | | 1.99 | | |
| *Dicrotendipes lobiger* | | 1.46 | | | | | | | | 6.56 | | | | 3.82 | | | | | | |
| *Glyptotendipes sp* | | 1.26 | | | | | | | | | | | | | 0.82 | | 2.35 | | | |

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|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *Einfeldia pagana* | | 1.05 | | | | | | | | | | | | | | | 3.53 | | | |
| *Polypedilum sp* | 1.40 | 10.46 | | 2.39 | | 0.01 | | | | | | | | 0.12 | | | | | | 10.13 |
| *Polypedilum ophoides* | 2.46 | 1.26 | 2.44 | | | 0.01 | | | | | | | | | 1.94 | | 14.12 | | | |
| *Einfeldia sp* | | 0.21 | | | | | | | | | | | | | | | | | | |
| *Rheotanytarsus curtistylus* | 16.14 | 0.84 | 8.07 | 5.67 | 12.89 | | 2.55 | 23.81 | 21.02 | | 0.51 | 1.34 | 31.47 | | 17.72 | 25.37 | 7.06 | 43.05 | 35.90 | 1.27 |
| *Rheocricotopus sp* | 1.05 | 0.42 | 0.98 | 0.60 | 0.15 | | 0.35 | 3.13 | 0.36 | | 0.47 | 0.30 | | | 6.59 | 0.95 | 1.18 | 3.97 | 0.64 | |
| *Orthocladius clarki* | 4.56 | 0.42 | | 4.18 | | | | | | | | 0.74 | | | 4.75 | | 1.18 | | | |
| *Microtendipes pedellus* | | 0.42 | | | | | | | | | | | | | | | | | 1.28 | |
| *Dicrotendipes notatus* | | 0.21 | | | | | | | | | | | | | | | | | | |
| *Chironomus* sp1 | 0.70 | | 1.22 | 0.30 | 73.20 | 75.18 | 84.17 | 54.91 | 54.51 | 75.96 | 81.86 | 80.56 | 17.48 | 68.88 | 37.14 | 55.09 | | | | |

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|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *Chironomus* sp2 | | | | | 3.13 | 16.72 | 3.82 | 0.45 | 3.68 | 14.46 | 14.94 | 5.93 | | 6.94 | 4.16 | 2.99 | | | | |
| *Cricotopus intersectus* | | | | | | | | | | 2.97 | | | | | | | | | | |
| *Paratanytarsus austriacus* | | | | | | | | | | | | | | | | | | 1.32 | | |
| *Virgatanytarsus ardrennensis* | | | | | | | | | | | | | | | | | 2.35 | 1.32 | | |
| *Helopelopia cornuticaudata* | | | | 0.90 | | | | | | | | | | | | | 2.35 | 2.65 | | |
| *Cricotopus tremelus* | 0.35 | 0.21 | | | 0.29 | | 0.20 | 2.68 | 2.26 | | | 2.08 | | | 1.65 | | 4.71 | 0.66 | | 0.63 |
| *Parametriocnemus lundbecki* | 4.21 | 0.42 | 5.87 | | | | | | | | | | | | | | | | | 0.63 |
| *Stenochironomus sp* | 1.40 | | 0.49 | 0.90 | | | | | | | | | | | | | 2.35 | | | |
| *Corynoneura scutellata* | | | 1.71 | | | | | | | | | | | | | | | | | |

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|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *Stictochironomus sp* | | | 0.24 | | | | | | | | | | | | | | | | | |
| *Ablabesmyia monilis* | | | 0.49 | 0.30 | | | 0.08 | | | | | | | | | | | | | |
| *Procladius sp* | | 0.21 | 0.24 | | | | | | | | | | | | | | | | | |
| *Microchironomus tener* | | | 0.24 | | | | | | | | | | | | | | | | | |
| *Arctopelopia sp* | | | | | | | 0.08 | | | | | | | | | | | | | |
| *Tvetenia sp* | 0.70 | | | | | | 0.08 | | | | | | | | 4.75 | | | | | |
| *Nanocladius rectinervis* | 1.40 | | | | | | 0.08 | 3.27 | 0.95 | | | 1.04 | | | 0.05 | | 1.18 | | | |
| *Trissocladius sp* | | | | 0.30 | 0.07 | | | | 4.39 | | 0.24 | | | | | | 1.18 | | 0.64 | |
| *Eukiefferiella gracei* | | | | | | | | | 0.71 | | 0.12 | | 25.87 | | 0.05 | 6.78 | 14.12 | | 4.49 | 1.27 |
| *Orthocladius oliveri* | | | | 0.30 | | | | | | | | | 0.70 | | 0.05 | | | | 1.28 | |
| *Paratrichocladius skirwithensis* | | | | | | | | | | | | | | | | | | | 0.64 | |

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|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *Meropelopia americana* | 0.35 | | | | | | | | | | | | | | | | | | | |
| *Cricotopus cylindraceus* | 0.35 | | | | | | | | | | | 0.15 | | | 1.50 | | | 3.31 | | |
| *Thienemannimyia geijskesi* | 0.35 | | | | | | | | | | | | | | | | | | | 0.63 |
| Chironomini Genus B | 0.35 | | | | | | | | | | | | | | | | | | | |
| *Larsia sp* | 0.35 | | | | | | | | | | | | | | | | | | | |
| *Glyptotendipes severini* | 1.05 | | | | | | | | | | | | | | | | | | | |
| *Corynoneura lobata* | | | | | | | | | | | | | 0.70 | | 4.50 | | | | 0.64 | |
| *Abiskomyia sp* | | | | | | | | | | | | | 0.70 | | | | | | | |
| *Paracricotopus niger* | 2.46 | | | 0.30 | | | | | | | | | | | | | | | | |
| *Monopelopia tennicalcar* | | | | 0.90 | | | | | | | | | | | | | | | | |

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|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *Stempellinella sp* | | | | 0.30 | | | | | | | | | | | | | | | | |
| *Conchapelopia melanops* | | | | 0.60 | | | | | | | | | | | | | | | | 0.63 |
| *Hydrobaenus johannseni* | | | | | | | | 1.19 | | | | 0.15 | | | | | | | | |
| *Paralimnophyes hydrophilus* | | | | | | | | | | | | 0.15 | | 0.17 | | | | | | |
| *Dicrotendipes nervosus* | | | | | | | | | | | | 0.15 | | | | | 1.18 | | | |
| *Euryhapsis cilium* | | | | | | | | | | | | 0.15 | | | | | | | | |
| *Chaetocladius setosipennis* | | | | | | | | | | | | | | | | 0.81 | | | | |
| *Metriocnemus fuscipes* | | | | | | | | | | | | | | | | | | | | 0.63 |
| *Krenosmittia sp* | | | | | | | | | | | | | | | | | | | | 0.63 |
| *Cricotopus bicinctus* | | | | | | | | | | | | | | | | | | | | 5.70 |

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|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *Parakiefferiella sp* | | 0.42 | | | | 0.01 | | | | | | | | | | | | | | |
| *Pseudorthocladius sp* | | 0.42 | | | | | | | | | | | | | | | | | | |
| *Djalmabatista pulcher* | | 0.63 | | | | | | | | | | | | | | | | | | |
| *Conchapelopia sp* | | 0.42 | | | | | | | | | | | | | | | | | | |
| *Procladius bellus* | | 0.42 | | | | | | | | | | | | | | | | | | |
| *Eukiefferiella sp* | | 1.46 | | | | 0.03 | | | | | | | | | | | | | | |
| *Thienemannimyia senata* | | 0.21 | | | | | | | | | | | | | | | | | | |
| *Paralimnophyes sp* | | | | | | | | | | | | | | | | | | 1.32 | | |
| *Paramerina anomalus* | | | | | | | | | | | | | | | | | | 1.32 | | |

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|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *Virgatanytarsus sp* | | | 1.47 | | | | | | | | | | | | | | | | | |
| *Pseudosmittia sp* | | | 0.49 | | | | | | | | | | | | | | | | | |
| *Pseudochironomus sp* | | | 1.47 | | | | | | | | | | | | | | | | | |
| *Orthocladius sp* | 3.86 | 9.41 | 3.91 | 3.58 | | 0.31 | 0.13 | | | | | | | | 0.68 | | | | | |
| *Paratanytarsus sp* | | | | | | | 0.03 | | | | | | | | 0.68 | | | | | |
| *Einfeldia carbonaria* | | | | | | | | | | | | | | | 2.47 | | | | | |
| *Tanytarsus brundini* | 0.35 | | | | | | | | | | | | | | | | | | | |
| *Natarsia baltimoreus* | | | | | | | | | | | | | | | | | 1.18 | | | |
| *Paracladopelma nais* | | | | | | | | | | | | | | | | | 1.18 | | | |
| *Euryhapsis sp* | | | | | | | | | | | | | | | | | 1.18 | | | |

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|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *Tvetenia paucunca* | | | | 0.30 | | | | | | | | | | | | | | | | |
| *Paramerina cingulata* | | | | 0.30 | | | | | | | | | | | | | | | | |
| *Orthocladius dentifer* | | | | | | | | | | | | | | | | | | | | 5.06 |
| *Cricotopus* sp1 | 6.32 | 23.43 | 13.20 | 2.09 | | 1.14 | 0.05 | | | | | | | 0.04 | | | | | | |
| *Cricotopus trifasciata gr* | 25.96 | 6.69 | 11.74 | 25.37 | | 2.36 | 0.22 | | | | | | | 0.17 | | | | | | |
| *Paratrichocladius sp* | | 1.46 | | 0.30 | | 0.20 | | | | | | | | 0.04 | | | | | | |
| *Nanocladius sp* | | 0.84 | | | | 0.03 | | | | | | | | | | | | | | |
| *Cardiocladius sp* | | 3.14 | 0.73 | 0.90 | | | | | | | | | | | | | | | | |
| *Kiefferulus sp* | | 0.21 | | | 0.58 | | 1.06 | | | 0.04 | | | | | | | | | | |
| *Cryptochironomus sp* | | 0.21 | | | | | | | | | | | | | | | | | | |

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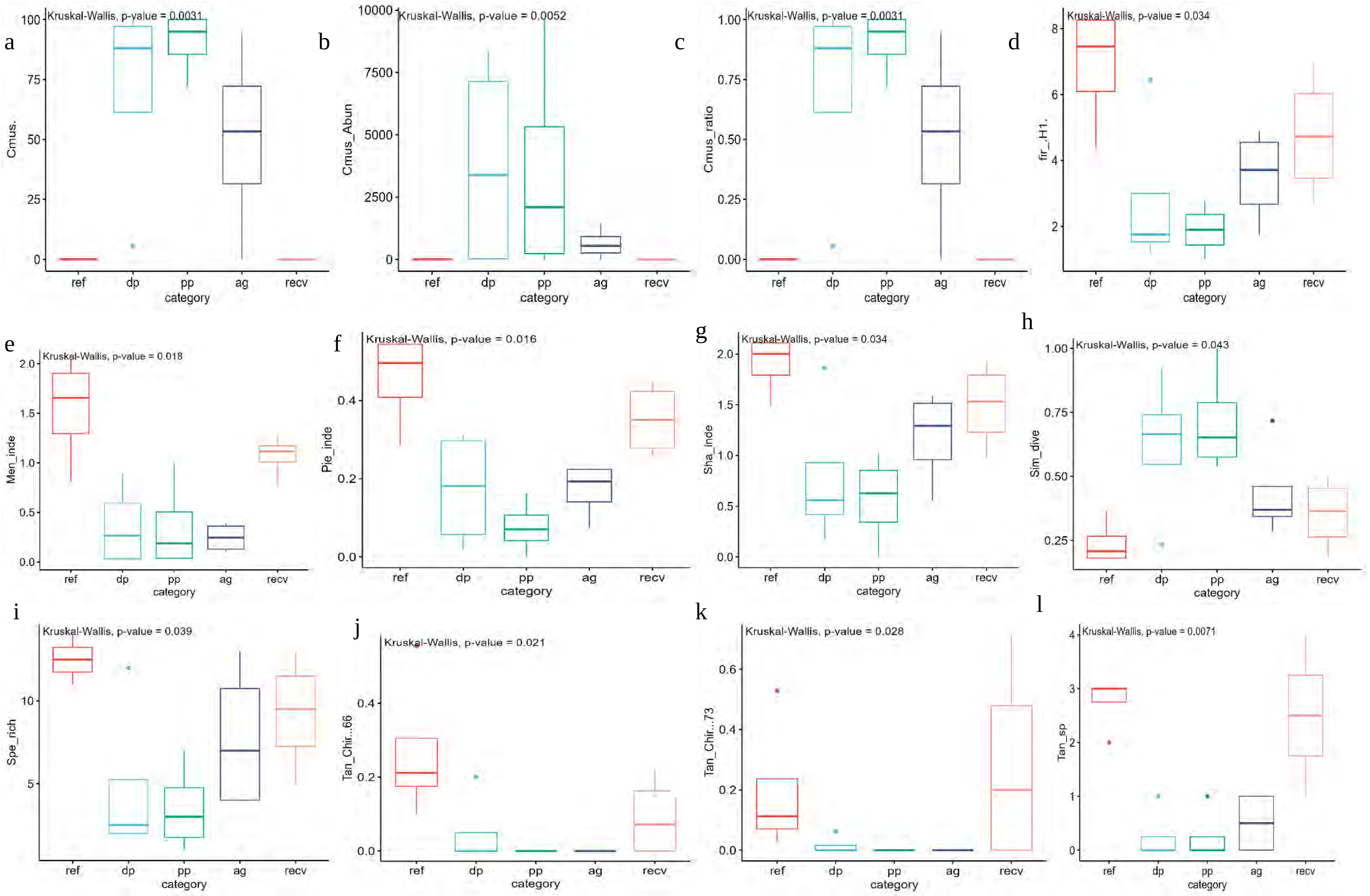
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *Tanytarsus sp* | 7.37 | 3.14 | 3.42 | 34.93 | | | | | | | | | | 0.12 | | 0.14 | | | | |
| *Ablabesmyia sp* | | 5.44 | 2.69 | 1.49 | | | 0.01 | | | | | | | | 0.48 | | | | | |
| *Coelotanypus sp* | | 0.21 | | | | | | | | | | | | | | | | | | |
| *Trissopelopia sp* | | 0.63 | | | | | | | | | | | | | | | | | | |
| *Clinotanypus sp* | | 0.42 | | | | | | | | | | | | | | | | | | |
| *Dicrotendipes sp* | | | 0.24 | | 1.46 | 0.33 | 2.41 | | 0.12 | 0.01 | 0.14 | 0.30 | | 0.08 | 0.10 | 0.68 | | | | |
| *Rheotanytarsus sp* | | | 0.24 | | | | | | | | | | | | | | | | | |
| *Tanypus sp* | 1.75 | | | 0.90 | 5.46 | | 2.28 | | | | | | | | | | | | | |
| *Chironomus* sp3 | 0.35 | | | | 0.44 | | | | 0.95 | | 0.67 | | | | 0.19 | 1.49 | | | | |
| *Microchironomus sp* | 0.35 | | | | 0.51 | | | | | | | | 0.70 | | | | | | | |


a
Kruskal-Wallis, p-value = 0.0031
Cmus.
100
75
50
25
0
ref
dp
pp
ag
recv
category
b
Kruskal-Wallis, p-value = 0.0052
Cmus_Abun
10000
7500
5000
2500
0
ref
dp
pp
ag
recv
category
c
Kruskal-Wallis, p-value = 0.0031
Cmus_ratio
1.00
0.75
0.50
0.25
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ref
dp
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ag
recv
category
d
Kruskal-Wallis, p-value = 0.034
fir_H1.
8
6
4
2
ref
dp
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ag
recv
category
e
Kruskal-Wallis, p-value = 0.018
Men_inde
2.0
1.5
1.0
0.5
0.0
ref
dp
pp
ag
recv
category
f
Kruskal-Wallis, p-value = 0.016
Pie_inde
0.4
0.2
0.0
ref
dp
pp
ag
recv
category
g
Kruskal-Wallis, p-value = 0.034
Sha_inde
2.0
1.5
1.0
0.5
0.0
ref
dp
pp
ag
recv
category
h
Kruskal-Wallis, p-value = 0.043
Sim_dive
1.00
0.75
0.50
0.25
ref
dp
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i
Kruskal-Wallis, p-value = 0.039
Spe_rich
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5
ref
dp
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ag
recv
category
j
Kruskal-Wallis, p-value = 0.021
Tan_Chir...66
0.4
0.2
0.0
ref
dp
pp
ag
recv
category
k
Kruskal-Wallis, p-value = 0.028
Tan_Chir...73
0.6
0.4
0.2
0.0
ref
dp
pp
ag
recv
category
l
Kruskal-Wallis, p-value = 0.0071
Tan_sp
4
3
2
1
0
ref
dp
pp
ag
recv
category

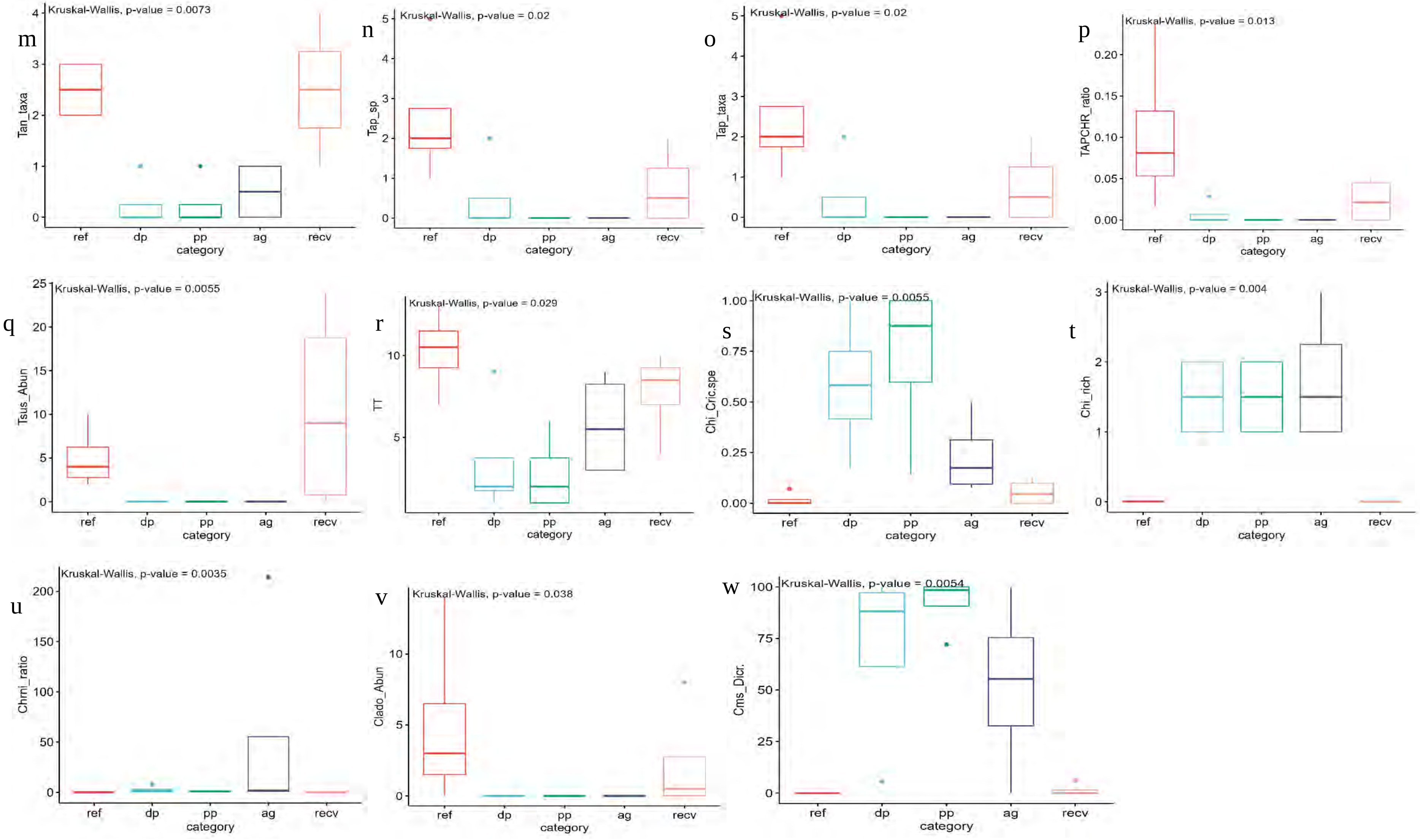

Figure 1: Metrics (a-w) that discriminated the site categories in the Bloukrans and Buffalo Rivers. ref-REF, dp-DP, ag-AG, pp-PP, recv-RECV

APPENDIX B1: Fuzzy coding of chironomid traits in the study rivers. Code: H1- haemoglobin present, H2- haemoglobin absent, X1- tracheal gills, X2-cuticular respiration, X3-aerial respiration, RT1-lay eggs in clutches, RT2-asexual reproduction, RT3-lay free isolated eggs, TC1-tubes absent, TC2-irregularly shaped tubes, TC3-rigid tubes, SR1-burrowers, SR2-free-living, SR3-miners, SR4-fixed to the substratum, RP1-12 tracheae, RP2- 6 tracheae, RP3-3 tracheae, M1-swimmers, M2-those that are crawlers, M3- whole-body undulator, BS1-very small body size (<2.5 mm), BS2- small body size (= >2.5-5 mm), BS3-medium body size (>5-10 mm), BS4-large body size (>10-20 mm), BS5-very large body size (>20-40 mm), BA1-body armour present, BA2-body armour absent, D1-complete their lifecycle in less than one year, D2-complete their lifecycle in more than one year, V1-produce one generation per year , V2-produce two generations per year , V3-produce three generations per year , V4-produce more than three generations per year, EM1-lay less than 100 eggs per egg mass , EM2-lay 100-500 eggs per egg mass , EM3-lay 500-100 eggs per egg mass, EM4-lay more than 1000 eggs per egg mass, SP1-prefer stones/cobbles/pebbles , SP2-gravel, sand, silt, mud , SP3-macrophytes and filamentous algae, SP4-microphytes, SP5-twigs and roots, SP6-litter, fine organic matter , SP7-wood , SP8-Mosses , FM1-collector-filterer, FM2- collector-gatherers , FM3- shredders , FM4- grazers/scrapers, FM5- engulfers , FM6-piercers , that prefer FT1-fine detritus, prefer FT2-debris <1mm, FT3-plant debriss >1mm, FT4-living microphytes, FT5-living macrophytes, FT6-dead animals, FT7-living microinvertebrates, FT8-living macroinvertebrates, FT9-wood

|  | Taxon | H1 | H2 | X1 | X2 | X3 | RT1 | RT2 | RT3 | TC1 | TC2 | TC3 | SR1 | SR2 | SR3 | SR4 | RP1 | RP2 | RP3 | M1 | M2 | M3 | FM1 | FM2 | FM3 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *Rheopelopia sp* | *RHP* | 0 | 5 | 0 | 5 | 0 | 0 | 0 | 0 | 5 | 0 | 0 | 0 | 5 | 0 | 0 | 0 | 0 | 0 | 5 | 0 | 0 | 0 | 0 | 0 |
| *Polypedilum tuberculum* | *PLPT* | 5 | 0 | 0 | 3 | 0 | 5 | 0 | 0 | 0 | 5 | 0 | 3 | 0 | 5 | 1 | 0 | 0 | 5 | 0 | 5 | 0 | 0 | 1 | 5 |
| *Polypedilum convictum* | *PLPC* | 5 | 0 | 0 | 3 | 0 | 5 | 0 | 0 | 0 | 5 | 0 | 3 | 0 | 5 | 1 | 0 | 0 | 5 | 0 | 5 | 0 | 0 | 1 | 5 |
| *Tanytarsus usmaensis* | *TUSM* | 5 | 0 | 0 | 5 | 0 | 5 | 3 | 0 | 0 | 3 | 5 | 5 | 0 | 3 | 1 | 0 | 5 | 0 | 0 | 5 | 0 | 0 | 3 | 3 |

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| *Polypedilum nubeculosum* | *PLPN* | 5 | 0 | 0 | 3 | 0 | 5 | 0 | 0 | 0 | 5 | 0 | 3 | 0 | 5 | 1 | 0 | 0 | 5 | 0 | 5 | 0 | 0 | 1 | 5 |
| *Polypedilum illinoense* | *PLPI* | 5 | 0 | 0 | 3 | 0 | 5 | 0 | 0 | 0 | 5 | 0 | 3 | 0 | 5 | 1 | 0 | 0 | 5 | 0 | 5 | 0 | 0 | 1 | 5 |
| *Ablabesmyia phatta* | *ABLP* | 0 | 5 | 0 | 5 | 0 | 5 | 0 | 0 | 5 | 0 | 0 | 0 | 5 | 0 | 0 | 5 | 0 | 0 | 5 | 0 | 0 | 0 | 0 | 0 |
| *Polypedilum nubifer* | *PLPNR* | 5 | 0 | 0 | 3 | 0 | 5 | 0 | 0 | 0 | 5 | 0 | 3 | 0 | 5 | 1 | 0 | 0 | 5 | 0 | 5 | 0 | 0 | 1 | 5 |
| *Zavreliella marmotata* | *ZAVM* | 5 | 0 | 5 | 3 | 0 | 0 | 5 | 0 | 0 | 0 | 5 | 0 | 5 | 0 | 0 | 0 | 0 | 0 | 5 | 0 | 0 | 0 | 5 | 0 |
| *Gillotia alboviridis* | *GILA* | 0 | 0 | 0 | 5 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 5 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 5 | 0 |
| *Cladotanytarsus sp* | *CLAS* | 5 | 0 | 0 | 5 | 0 | 5 | 0 | 0 | 0 | 0 | 5 | 5 | 0 | 0 | 3 | 0 | 5 | 0 | 0 | 0 | 5 | 5 | 0 | 0 |
| *Cladotanytarsus mancus* | *CLAM* | 5 | 0 | 0 | 5 | 0 | 5 | 0 | 0 | 0 | 0 | 5 | 5 | 0 | 0 | 3 | 0 | 5 | 0 | 0 | 0 | 5 | 5 | 0 | 0 |
| *Dicrotendipes pulsus* | *DICRP* | 5 | 0 | 0 | 5 | 0 | 5 | 0 | 0 | 0 | 5 | 0 | 5 | 0 | 0 | 0 | 0 | 0 | 5 | 0 | 0 | 5 | 0 | 5 | 0 |
| *Dicrotendipes lobiger* | *DICRL* | 5 | 0 | 0 | 5 | 0 | 5 | 0 | 0 | 0 | 5 | 0 | 5 | 0 | 0 | 0 | 0 | 0 | 5 | 0 | 0 | 5 | 0 | 5 | 0 |
| *Glyptotendipes sp* | *GLYPS* | 5 | 0 | 5 | 3 | 0 | 5 | 0 | 0 | 0 | 0 | 5 | 5 | 0 | 5 | 0 | 0 | 0 | 5 | 0 | 0 | 5 | 0 | 0 | 5 |
| *Einfeldia pagana* | *EINP* | 5 | 0 | 5 | 0 | 0 | 5 | 0 | 0 | 0 | 3 | 3 | 5 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 5 | 0 | 0 | 0 |
| *Polypedilum sp* | *PLPS* | 5 | 0 | 0 | 3 | 0 | 5 | 0 | 0 | 0 | 5 | 0 | 3 | 0 | 5 | 1 | 0 | 0 | 5 | 0 | 5 | 0 | 0 | 1 | 5 |
| *Polypedilum ophoides* | *PLPO* | 5 | 0 | 0 | 3 | 0 | 5 | 0 | 0 | 0 | 5 | 0 | 3 | 0 | 5 | 1 | 0 | 0 | 5 | 0 | 5 | 0 | 0 | 1 | 5 |
| *Einfeldia sp* | *EINS* | 5 | 0 | 5 | 0 | 0 | 5 | 0 | 0 | 0 | 3 | 3 | 5 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 5 | 0 | 0 | 0 |
| *Rheotanytarsus curtistylus* | *RHEOTC* | 5 | 0 | 5 | 3 | 0 | 5 | 0 | 0 | 0 | 1 | 5 | 0 | 0 | 0 | 5 | 0 | 5 | 0 | 0 | 0 | 5 | 5 | 0 | 0 |

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| *Rheocricotopus sp* | *RHEOCRIC* | 0 | 5 | 0 | 5 | 0 | 5 | 0 | 0 | 0 | 0 | 5 | 0 | 0 | 0 | 5 | 5 | 0 | 0 | 5 | 0 | 0 | 0 | 5 | 3 |
| *Orthocladius clarki* | *ORTC* | 0 | 5 | 0 | 5 | 0 | 5 | 0 | 0 | 0 | 5 | 3 | 0 | 5 | 0 | 0 | 5 | 0 | 0 | 5 | 0 | 0 | 0 | 0 | 0 |
| *Microtendipes pedellus* | *MICP* | 5 | 0 | 5 | 3 | 0 | 0 | 0 | 0 | 0 | 3 | 3 | 5 | 0 | 0 | 3 | 0 | 0 | 5 | 0 | 0 | 5 | 1 | 5 | 0 |
| *Dicrotendipes notatus* | *DICRN* | 5 | 0 | 0 | 5 | 0 | 5 | 0 | 0 | 0 | 5 | 0 | 5 | 0 | 0 | 0 | 0 | 0 | 5 | 0 | 0 | 5 | 0 | 5 | 0 |
| *Chironomus sp1* | *CHIRO1* | 5 | 0 | 5 | 3 | 0 | 5 | 0 | 0 | 0 | 0 | 5 | 5 | 0 | 0 | 0 | 0 | 0 | 5 | 0 | 0 | 5 | 0 | 5 | 0 |
| *Chironomus sp2* | *CHIRO2* | 5 | 0 | 5 | 3 | 0 | 5 | 0 | 0 | 0 | 0 | 5 | 5 | 0 | 0 | 0 | 0 | 0 | 5 | 0 | 0 | 5 | 0 | 5 | 0 |
| *Cricotopus intersectus* | *CRICI* | 5 | 0 | 0 | 5 | 0 | 5 | 0 | 0 | 0 | 5 | 0 | 0 | 0 | 5 | 0 | 5 | 0 | 0 | 0 | 5 | 0 | 0 | 1 | 5 |
| *Paratanytarsus austriacus* | *PARAA* | 5 | 0 | 0 | 5 | 0 | 3 | 5 | 0 | 0 | 3 | 5 | 0 | 0 | 0 | 5 | 0 | 5 | 0 | 0 | 0 | 5 | 5 | 0 | 0 |
| *Virgatanytarsus ardrennensis* | *VIRGA* | 0 | 0 | 0 | 5 | 0 | 0 | 0 | 0 | 0 | 3 | 3 | 0 | 0 | 3 | 3 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 5 | 0 |
| *Helopelopia cornuticaudata* | *HELOP* | 0 | 5 | 0 | 5 | 0 | 0 | 0 | 0 | 5 | 0 | 0 | 0 | 5 | 0 | 0 | 5 | 0 | 0 | 5 | 0 | 0 | 0 | 0 | 0 |
| *Cricotopus tremelus* | *CRICT* | 5 | 0 | 0 | 5 | 0 | 5 | 0 | 0 | 0 | 5 | 0 | 0 | 0 | 5 | 0 | 5 | 0 | 0 | 0 | 5 | 0 | 0 | 1 | 5 |
| *Parametriocnemus lundbecki* | *PARALUND* | 0 | 5 | 0 | 5 | 0 | 0 | 0 | 0 | 5 | 0 | 0 | 0 | 5 | 0 | 0 | 0 | 0 | 0 | 5 | 0 | 0 | 0 | 5 | 0 |
| *Stenochironomus sp* | *STENO* | 5 | 0 | 0 | 5 | 0 | 5 | 0 | 0 | 5 | 0 | 0 | 0 | 0 | 5 | 0 | 0 | 0 | 0 | 0 | 5 | 0 | 0 | 3 | 5 |
| *Corynoneura scutellata* | *CORYSCU* | 0 | 5 | 0 | 5 | 0 | 5 | 3 | 0 | 5 | 0 | 0 | 0 | 5 | 0 | 0 | 0 | 0 | 0 | 0 | 5 | 0 | 0 | 0 | 0 |

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| *Stictochironomus sp* | *STICTO* | 5 | 0 | 0 | 5 | 0 | 5 | 0 | 0 | 0 | 3 | 5 | 5 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 5 | 0 | 5 | 0 | 3 |
| *Ablabesmyia monilis* | *ABLM* | 0 | 5 | 0 | 5 | 0 | 5 | 0 | 0 | 5 | 0 | 0 | 0 | 5 | 0 | 0 | 5 | 0 | 0 | 5 | 0 | 0 | 0 | 0 | 0 |
| *Procladius sp* | *PROCLAD* | 0 | 5 | 0 | 5 | 0 | 5 | 0 | 0 | 5 | 0 | 0 | 0 | 5 | 0 | 0 | 5 | 0 | 0 | 5 | 0 | 0 | 0 | 0 | 0 |
| *Microchironomus tener* | *MICHIRO* | 5 | 0 | 5 | 3 | 0 | 0 | 0 | 0 | 0 | 5 | 3 | 5 | 0 | 0 | 0 | 0 | 0 | 5 | 0 | 0 | 5 | 0 | 5 | 1 |
| *Arctopelopia sp* | *ARCT* | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| *Tvetenia sp* | *TVETS* | 0 | 5 | 0 | 5 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 5 | 0 | 0 | 5 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| *Nanocladius rectinervis* | *NANO* | 0 | 5 | 0 | 5 | 0 | 0 | 0 | 0 | 5 | 0 | 0 | 0 | 5 | 0 | 0 | 0 | 0 | 0 | 5 | 0 | 0 | 0 | 5 | 0 |
| *Trissocladius sp* | *TRISSO* | 5 | 0 | 0 | 5 | 0 | 0 | 0 | 0 | 0 | 5 | 0 | 5 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 5 | 0 | 5 | 0 |
| *Eukiefferiella gracei* | *EUKIG* | 0 | 5 | 0 | 5 | 0 | 0 | 0 | 0 | 0 | 5 | 0 | 0 | 0 | 0 | 5 | 5 | 0 | 0 | 0 | 0 | 5 | 0 | 5 | 0 |
| *Orthocladius oliveri* | *ORTO* | 0 | 5 | 0 | 5 | 0 | 5 | 0 | 0 | 0 | 5 | 3 | 0 | 5 | 0 | 0 | 5 | 0 | 0 | 5 | 0 | 0 | 0 | 0 | 0 |
| *Paratrichocladius skirwithensis* | *PSKIR* | 0 | 5 | 0 | 5 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 5 | 0 | 0 | 0 | 0 | 0 | 5 | 0 | 0 | 0 | 0 | 0 |
| *Meropelopia americana* | *MERO* | 0 | 0 | 0 | 5 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| *Cricotopus cylindraceus* | *CCYLIN* | 5 | 0 | 0 | 5 | 0 | 5 | 0 | 0 | 0 | 5 | 0 | 0 | 0 | 5 | 0 | 5 | 0 | 0 | 0 | 5 | 0 | 0 | 1 | 5 |
| *Thienemannimyia geijskesi* | *THIEN* | 0 | 5 | 0 | 5 | 0 | 0 | 0 | 0 | 5 | 0 | 0 | 0 | 5 | 0 | 0 | 0 | 0 | 0 | 5 | 0 | 0 | 0 | 0 | 0 |
| Chironomini Genus B | *CGENUSB* | 5 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |

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| *Larsia sp* | *LARSIA* | 0 | 5 | 0 | 5 | 0 | 0 | 0 | 0 | 5 | 0 | 0 | 0 | 5 | 0 | 0 | 0 | 0 | 0 | 5 | 0 | 0 | 0 | 0 | 0 |
| *Glyptotendipes severini* | *GLSEVE* | 5 | 0 | 5 | 3 | 0 | 5 | 0 | 0 | 0 | 0 | 5 | 5 | 0 | 5 | 0 | 0 | 0 | 5 | 0 | 0 | 5 | 0 | 0 | 5 |
| *Corynoneura lobata* | *CORYLOB* | 0 | 5 | 0 | 5 | 0 | 5 | 3 | 0 | 5 | 0 | 0 | 0 | 5 | 0 | 0 | 0 | 0 | 0 | 0 | 5 | 0 | 0 | 0 | 0 |
| *Abiskomyia sp* | *ABISK* | 0 | 0 | 0 | 5 | 0 | 0 | 5 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| *Paracricotopus niger* | *PARANIGER* | 0 | 5 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| *Monopelopia tennicalcar* | *MONOTEN* | 0 | 5 | 0 | 5 | 0 | 0 | 0 | 0 | 5 | 0 | 0 | 0 | 5 | 0 | 0 | 0 | 0 | 0 | 5 | 0 | 0 | 0 | 0 | 0 |
| *Stempellinella sp* | *STEMPELL* | 0 | 0 | 0 | 5 | 0 | 0 | 0 | 0 | 0 | 0 | 5 | 0 | 5 | 0 | 0 | 0 | 0 | 0 | 5 | 0 | 0 | 5 | 3 | 0 |
| *Conchapelopia melanops* | *CONCMEL* | 0 | 5 | 0 | 5 | 0 | 0 | 0 | 0 | 5 | 0 | 0 | 0 | 5 | 0 | 0 | 5 | 0 | 0 | 5 | 0 | 0 | 0 | 0 | 0 |
| *Hydrobaenus johannseni* | *HYDROJOH* | 0 | 5 | 0 | 5 | 0 | 5 | 0 | 0 | 5 | 0 | 0 | 0 | 5 | 0 | 0 | 0 | 0 | 0 | 5 | 0 | 0 | 0 | 3 | 0 |
| *Paralimnophyes hydrophilus* | *PARAHYDRO* | 0 | 5 | 0 | 5 | 0 | 5 | 0 | 0 | 5 | 0 | 0 | 0 | 5 | 0 | 0 | 0 | 0 | 0 | 5 | 0 | 0 | 0 | 5 | 0 |
| *Dicrotendipes nervosus* | *DICRNERV* | 5 | 0 | 0 | 5 | 0 | 5 | 0 | 0 | 0 | 5 | 0 | 5 | 0 | 0 | 0 | 0 | 0 | 5 | 0 | 0 | 5 | 0 | 5 | 0 |
| *Euryhapsis cilium* | *EURYCIL* | 0 | 0 | 0 | 5 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 3 | 3 |
| *Chaetocladius setosipennis* | *CHAESET* | 0 | 0 | 0 | 5 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 5 | 0 | 0 | 0 | 0 | 0 | 5 | 0 | 0 | 0 | 5 | 0 |
| *Metriocnemus fuscipes* | *METRIFUS* | 0 | 5 | 0 | 5 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 3 | 3 | 0 | 0 | 0 | 0 | 0 | 3 | 0 | 0 | 0 | 0 | 3 |

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| *Krenosmittia sp* | *KRENOSM* | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 5 | 0 |
| *Cricotopus bicinctus* | *CRICBICIN* | 5 | 0 | 0 | 5 | 0 | 5 | 0 | 0 | 0 | 5 | 0 | 0 | 0 | 5 | 0 | 5 | 0 | 0 | 0 | 5 | 0 | 0 | 1 | 5 |
| *Parakiefferiella sp* | *PARAKIE* | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 5 | 0 | 0 | 0 | 5 | 0 | 0 | 0 | 0 | 0 | 5 | 0 | 0 | 0 | 5 | 0 |
| *Pseudorthocladius sp* | *PSEUDORT* | 0 | 5 | 0 | 5 | 0 | 5 | 0 | 0 | 5 | 0 | 0 | 0 | 5 | 0 | 0 | 0 | 0 | 0 | 5 | 0 | 0 | 0 | 5 | 0 |
| *Djalmabatista pulcher* | *DJALP* | 0 | 5 | 0 | 5 | 0 | 0 | 0 | 0 | 5 | 0 | 0 | 0 | 5 | 0 | 0 | 0 | 0 | 0 | 5 | 0 | 0 | 0 | 0 | 0 |
| *Conchapelopia sp* | *CONCHS* | 0 | 5 | 0 | 5 | 0 | 0 | 0 | 0 | 5 | 0 | 0 | 0 | 5 | 0 | 0 | 5 | 0 | 0 | 5 | 0 | 0 | 0 | 0 | 0 |
| *Procladius bellus* | *PROBELL* | 0 | 5 | 0 | 5 | 0 | 5 | 0 | 0 | 5 | 0 | 0 | 0 | 5 | 0 | 0 | 5 | 0 | 0 | 5 | 0 | 0 | 0 | 0 | 0 |
| *Eukiefferiella sp* | *EUKIES* | 0 | 5 | 0 | 5 | 0 | 0 | 0 | 0 | 0 | 5 | 0 | 0 | 0 | 0 | 5 | 5 | 0 | 0 | 0 | 0 | 5 | 0 | 5 | 0 |
| *Thienemannimyia senata* | *THIENSEN* | 0 | 5 | 0 | 5 | 0 | 0 | 0 | 0 | 5 | 0 | 0 | 0 | 5 | 0 | 0 | 0 | 0 | 0 | 5 | 0 | 0 | 0 | 0 | 0 |
| *Paralimnophyes sp* | *PARALIMS* | 0 | 5 | 0 | 5 | 0 | 5 | 0 | 0 | 5 | 0 | 0 | 0 | 5 | 0 | 0 | 0 | 0 | 0 | 5 | 0 | 0 | 0 | 5 | 0 |
| *Paramerina anomalus* | *PAMERANO* | 0 | 5 | 0 | 5 | 0 | 0 | 0 | 0 | 5 | 0 | 0 | 0 | 5 | 0 | 0 | 0 | 0 | 0 | 5 | 0 | 0 | 0 | 0 | 0 |
| *Virgatanytarsus sp* | *VIRGS* | 0 | 0 | 0 | 5 | 0 | 0 | 0 | 0 | 0 | 3 | 3 | 0 | 0 | 3 | 3 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 5 | 0 |
| *Pseudosmittia sp* | *PSMITTIA* | 0 | 0 | 0 | 5 | 0 | 0 | 3 | 0 | 5 | 0 | 0 | 0 | 5 | 0 | 0 | 0 | 0 | 0 | 5 | 0 | 0 | 0 | 5 | 0 |
| *Pseudochironomus sp* | *PCHIRO* | 5 | 0 | 0 | 5 | 0 | 5 | 0 | 0 | 5 | 0 | 0 | 5 | 0 | 0 | 0 | 0 | 0 | 5 | 0 | 5 | 0 | 0 | 5 | 0 |
| *Orthocladius sp* | *ORTSS* | 0 | 5 | 0 | 5 | 0 | 5 | 0 | 0 | 0 | 5 | 3 | 0 | 5 | 0 | 0 | 5 | 0 | 0 | 5 | 0 | 0 | 0 | 0 | 0 |

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| *Paratanytarsus sp* | *PARATANS* | 5 | 0 | 0 | 5 | 0 | 3 | 5 | 0 | 0 | 3 | 5 | 0 | 0 | 0 | 5 | 0 | 5 | 0 | 0 | 0 | 5 | 5 | 0 | 0 |
| *Einfeldia carbonaria* | *EINCARB* | 5 | 0 | 5 | 0 | 0 | 5 | 0 | 0 | 0 | 3 | 3 | 5 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 5 | 0 | 0 | 0 |
| *Tanytarsus brundini* | *TBRUND* | 5 | 0 | 0 | 5 | 0 | 5 | 3 | 0 | 0 | 3 | 5 | 5 | 0 | 3 | 0 | 0 | 5 | 0 | 0 | 5 | 0 | 0 | 3 | 3 |
| *Natarsia baltimoreus* | *NATARB* | 5 | 0 | 0 | 5 | 0 | 0 | 0 | 0 | 5 | 0 | 0 | 0 | 5 | 0 | 0 | 0 | 0 | 0 | 5 | 0 | 0 | 0 | 0 | 0 |
| *Paracladopelma nais* | *PELMANA* | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 5 | 0 | 0 | 0 | 0 | 0 | 0 | 5 | 0 | 0 | 0 | 5 | 0 |
| *Euryhapsis sp* | *EURYHS* | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 3 | 3 |
| *Tvetenia paucunca* | *TVETPAUC* | 0 | 5 | 0 | 5 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 5 | 0 | 0 | 5 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| *Paramerina cingulata* | *PAMECING* | 0 | 5 | 0 | 5 | 0 | 0 | 0 | 0 | 5 | 0 | 0 | 0 | 5 | 0 | 0 | 0 | 0 | 0 | 5 | 0 | 0 | 0 | 0 | 0 |
| *Orthocladius dentifer* | *ORTDENT* | 0 | 5 | 0 | 5 | 0 | 5 | 0 | 0 | 0 | 5 | 3 | 0 | 5 | 0 | 0 | 5 | 0 | 0 | 5 | 0 | 0 | 0 | 0 | 0 |
| *Cricotopus sp1* | *CRIC1* | 5 | 0 | 0 | 5 | 0 | 5 | 0 | 0 | 0 | 5 | 0 | 0 | 0 | 5 | 0 | 5 | 0 | 0 | 0 | 5 | 0 | 0 | 1 | 5 |
| *Cricotopus trifasciata gr* | *CRICTRIF* | 5 | 0 | 0 | 5 | 0 | 5 | 0 | 0 | 0 | 5 | 0 | 0 | 0 | 5 | 0 | 5 | 0 | 0 | 0 | 5 | 0 | 0 | 1 | 5 |
| *Paratrichocladius sp* | *TRICHOCLAD* | 0 | 5 | 0 | 5 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 5 | 0 | 0 | 0 | 0 | 0 | 5 | 0 | 0 | 0 | 0 | 0 |
| *Nanocladius sp* | *NANOCLAD* | 0 | 5 | 0 | 5 | 0 | 0 | 0 | 0 | 5 | 0 | 0 | 0 | 5 | 0 | 0 | 0 | 0 | 0 | 5 | 0 | 0 | 0 | 5 | 0 |
| *Cardiocladius sp* | *CARDIO* | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 5 | 0 | 5 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 5 | 0 | 0 | 0 |

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| *Kiefferulus sp* | *KIEFFU* | 5 | 0 | 5 | 3 | 0 | 5 | 0 | 0 | 0 | 5 | 0 | 0 | 0 | 5 | 0 | 0 | 0 | 0 | 0 | 0 | 5 | 0 | 5 | 0 |
| *Cryptochironomus sp* | *CRYPTCHIR* | 5 | 0 | 0 | 5 | 0 | 0 | 0 | 0 | 5 | 0 | 0 | 0 | 5 | 0 | 0 | 0 | 0 | 5 | 5 | 0 | 0 | 0 | 0 | 0 |
| *Tanytarsus sp* | *TARSSP* | 5 | 0 | 0 | 5 | 0 | 5 | 3 | 0 | 0 | 3 | 5 | 5 | 0 | 3 | 0 | 0 | 5 | 0 | 0 | 5 | 0 | 0 | 3 | 3 |
| *Ablabesmyia sp* | *ABLASP* | 0 | 5 | 0 | 5 | 0 | 5 | 0 | 0 | 5 | 0 | 0 | 0 | 5 | 0 | 0 | 5 | 0 | 0 | 5 | 0 | 0 | 0 | 0 | 0 |
| *Coelotanypus sp* | *COELOTAN* | 5 | 0 | 5 | 3 | 0 | 0 | 0 | 0 | 5 | 0 | 0 | 3 | 5 | 0 | 0 | 0 | 0 | 0 | 5 | 0 | 0 | 0 | 0 | 0 |
| *Trissopelopia sp* | *TRISSOPEL* | 0 | 5 | 0 | 5 | 0 | 5 | 0 | 0 | 5 | 0 | 0 | 0 | 5 | 0 | 0 | 0 | 0 | 0 | 5 | 0 | 0 | 0 | 0 | 0 |
| *Clinotanypus sp* | *CLINOTANY* | 5 | 0 | 0 | 0 | 0 | 5 | 0 | 0 | 0 | 0 | 5 | 0 | 5 | 0 | 0 | 0 | 0 | 0 | 5 | 0 | 0 | 0 | 0 | 0 |
| *Dicrotendipes sp* | *DICRPESS* | 5 | 0 | 0 | 5 | 0 | 5 | 0 | 0 | 0 | 5 | 0 | 5 | 0 | 0 | 0 | 0 | 0 | 5 | 0 | 0 | 5 | 0 | 5 | 0 |
| *Rheotanytarsus sp* | *RHEOTANS* | 5 | 0 | 5 | 3 | 0 | 5 | 0 | 0 | 0 | 1 | 5 | 0 | 0 | 0 | 5 | 0 | 5 | 0 | 0 | 0 | 5 | 5 | 0 | 0 |
| *Tanypus sp* | *TANYPS* | 5 | 0 | 0 | 5 | 0 | 5 | 0 | 0 | 5 | 0 | 0 | 0 | 5 | 0 | 0 | 5 | 0 | 0 | 5 | 0 | 0 | 0 | 0 | 0 |
| *Chironomus sp3* | *CHIRO3* | 5 | 0 | 5 | 3 | 0 | 5 | 0 | 0 | 0 | 0 | 5 | 5 | 0 | 0 | 0 | 0 | 0 | 5 | 0 | 0 | 5 | 0 | 5 | 0 |
| *Microchironomus sp* | *MUSSP* | 5 | 0 | 5 | 3 | 0 | 0 | 0 | 0 | 0 | 5 | 3 | 5 | 0 | 0 | 0 | 0 | 0 | 5 | 0 | 0 | 5 | 0 | 5 | 1 |

CONT'D

|  | Taxon | FM 4 | FM 5 | FM 6 | BS 1 | BS 2 | BS 3 | BS 4 | BS 5 | V 1 | V 2 | V 3 | V 4 | D 1 | D 2 | EM 1 | EM 2 | EM 3 | EM 4 | SP 1 | SP 2 | SP 3 | SP 4 | SP 5 | SP 6 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *Rheopelopia sp* | RHP | 0 | 5 | 5 | 0 | 0 | 5 | 0 | 0 | 3 | 3 | 0 | 0 | 5 | 0 | 0 | 0 | 0 | 0 | 5 | 1 | 0 | 0 | 0 | 0 |
| *Polypedilum tuberculum* | PLPT | 3 | 0 | 0 | 0 | 0 | 5 | 3 | 0 | 3 | 5 | 1 | 0 | 5 | 0 | 0 | 5 | 0 | 0 | 3 | 3 | 5 | 0 | 0 | 1 |
| *Polypedilum convictum* | PLPC | 3 | 0 | 0 | 0 | 0 | 5 | 3 | 0 | 3 | 5 | 1 | 0 | 5 | 0 | 0 | 5 | 0 | 0 | 3 | 3 | 5 | 0 | 0 | 1 |
| *Tanytarsus usmaensis* | TUSM | 0 | 0 | 0 | 0 | 0 | 5 | 0 | 0 | 3 | 3 | 0 | 0 | 5 | 0 | 5 | 0 | 0 | 0 | 1 | 5 | 5 | 0 | 0 | 0 |
| *Polypedilum nubeculosum* | PLPN | 3 | 0 | 0 | 0 | 0 | 5 | 3 | 0 | 3 | 5 | 1 | 0 | 5 | 0 | 0 | 5 | 0 | 0 | 3 | 3 | 5 | 0 | 0 | 1 |
| *Polypedilum illinoense* | PLPI | 3 | 0 | 0 | 0 | 0 | 5 | 3 | 0 | 3 | 5 | 1 | 0 | 5 | 0 | 0 | 5 | 0 | 0 | 3 | 3 | 5 | 0 | 0 | 1 |
| *Ablabesmyia phatta* | ABLP | 0 | 5 | 5 | 0 | 0 | 0 | 5 | 0 | 3 | 3 | 1 | 0 | 5 | 0 | 0 | 5 | 0 | 0 | 0 | 0 | 5 | 0 | 0 | 0 |
| *Polypedilum nubifer* | PLPNR | 3 | 0 | 0 | 0 | 0 | 5 | 3 | 0 | 3 | 5 | 1 | 0 | 5 | 0 | 0 | 5 | 0 | 0 | 3 | 3 | 5 | 0 | 0 | 1 |
| *Zavreliella marmotata* | ZAVM | 0 | 0 | 0 | 0 | 5 | 0 | 0 | 0 | 0 | 5 | 0 | 0 | 5 | 0 | 0 | 5 | 0 | 0 | 3 | 3 | 5 | 0 | 0 | 1 |
| *Gillotia alboviridis* | GILA | 0 | 0 | 0 | 0 | 0 | 5 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 5 | 0 | 0 | 0 | 0 |
| *Cladotanytarsus sp* | CLAS | 0 | 0 | 0 | 0 | 5 | 0 | 0 | 0 | 0 | 5 | 3 | 0 | 5 | 0 | 0 | 5 | 0 | 0 | 0 | 5 | 5 | 3 | 0 | 0 |
| *Cladotanytarsus mancus* | CLAM | 0 | 0 | 0 | 0 | 5 | 0 | 0 | 0 | 0 | 5 | 3 | 0 | 5 | 0 | 0 | 5 | 0 | 0 | 0 | 5 | 5 | 3 | 0 | 0 |
| *Dicrotendipes pulsus* | DICRP | 0 | 0 | 0 | 0 | 0 | 0 | 5 | 0 | 0 | 5 | 0 | 0 | 5 | 0 | 0 | 5 | 0 | 0 | 0 | 5 | 1 | 0 | 0 | 0 |
| *Dicrotendipes lobiger* | DICRL | 0 | 0 | 0 | 0 | 0 | 0 | 5 | 0 | 0 | 5 | 0 | 0 | 5 | 0 | 0 | 5 | 0 | 0 | 0 | 5 | 1 | 0 | 0 | 0 |

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| *Glyptotendipes sp* | GLYPS | 3 | 0 | 0 | 0 | 0 | 3 | 5 | 0 | 0 | 5 | 0 | 0 | 5 | 0 | 0 | 0 | 5 | 3 | 0 | 3 | 5 | 0 | 0 | 0 |
| *Einfeldia pagana* | EINP | 5 | 0 | 0 | 0 | 0 | 0 | 5 | 0 | 3 | 3 | 0 | 0 | 5 | 0 | 0 | 3 | 3 | 3 | 0 | 5 | 1 | 0 | 0 | 0 |
| *Polypedilum sp* | PLPS | 3 | 0 | 0 | 0 | 0 | 5 | 3 | 0 | 3 | 5 | 1 | 0 | 5 | 0 | 0 | 5 | 0 | 0 | 3 | 3 | 5 | 0 | 0 | 1 |
| *Polypedilum ophoides* | PLPO | 3 | 0 | 0 | 0 | 0 | 5 | 3 | 0 | 3 | 5 | 1 | 0 | 5 | 0 | 0 | 5 | 0 | 0 | 3 | 3 | 5 | 0 | 0 | 1 |
| *Einfeldia sp* | EINS | 5 | 0 | 0 | 0 | 0 | 0 | 5 | 0 | 3 | 3 | 0 | 0 | 5 | 0 | 0 | 3 | 3 | 3 | 0 | 5 | 1 | 0 | 0 | 0 |
| *Rheotanytarsus curtistylus* | RHEOTC | 0 | 0 | 0 | 0 | 5 | 0 | 0 | 0 | 0 | 3 | 0 | 5 | 5 | 0 | 0 | 0 | 0 | 0 | 5 | 1 | 3 | 1 | 0 | 0 |
| *Rheocricotopus sp* | RHEOCRIC | 0 | 0 | 0 | 0 | 0 | 5 | 0 | 0 | 0 | 0 | 5 | 3 | 5 | 0 | 0 | 0 | 0 | 0 | 5 | 0 | 3 | 0 | 0 | 0 |
| *Orthocladius clarki* | ORTC | 5 | 0 | 0 | 0 | 0 | 0 | 5 | 0 | 3 | 5 | 1 | 0 | 5 | 0 | 0 | 5 | 3 | 0 | 5 | 0 | 5 | 0 | 0 | 0 |
| *Microtendipes pedellus* | MICP | 1 | 0 | 0 | 0 | 0 | 0 | 5 | 0 | 0 | 3 | 3 | 0 | 5 | 0 | 0 | 3 | 3 | 0 | 5 | 3 | 5 | 1 | 0 | 0 |
| *Dicrotendipes notatus* | DICRN | 0 | 0 | 0 | 0 | 0 | 0 | 5 | 0 | 0 | 5 | 0 | 0 | 5 | 0 | 0 | 5 | 0 | 0 | 0 | 5 | 1 | 0 | 0 | 0 |
| *Chironomus sp1* | CHIRO1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 5 | 1 | 5 | 1 | 1 | 0 | 5 | 0 | 5 | 1 | 1 | 0 | 5 | 3 | 0 | 0 | 5 |
| *Chironomus sp2* | CHIRO2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 5 | 1 | 5 | 1 | 1 | 0 | 5 | 0 | 5 | 1 | 1 | 0 | 5 | 3 | 0 | 0 | 5 |
| *Cricotopus intersectus* | CRICI | 3 | 0 | 0 | 0 | 0 | 5 | 0 | 0 | 1 | 1 | 5 | 3 | 5 | 1 | 1 | 5 | 1 | 0 | 0 | 0 | 5 | 0 | 0 | 0 |
| *Paratanytarsus austriacus* | PARAA | 0 | 0 | 0 | 0 | 0 | 5 | 0 | 0 | 5 | 3 | 1 | 0 | 5 | 0 | 5 | 0 | 0 | 0 | 3 | 3 | 5 | 1 | 0 | 1 |
| *Virgatanytarsus ardrennensis* | VIRGA | 3 | 0 | 0 | 0 | 5 | 0 | 0 | 0 | 5 | 0 | 0 | 0 | 5 | 0 | 0 | 0 | 0 | 0 | 3 | 0 | 5 | 0 | 0 | 0 |

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| *Helopelopia cornuticaudata* | HELOP | 0 | 5 | 5 | 0 | 0 | 5 | 0 | 0 | 5 | 1 | 0 | 0 | 5 | 0 | 0 | 5 | 0 | 0 | 1 | 0 | 5 | 0 | 0 | 0 |
| *Cricotopus tremelus* | CRICT | 3 | 0 | 0 | 0 | 0 | 5 | 0 | 0 | 1 | 1 | 5 | 3 | 5 | 1 | 1 | 5 | 1 | 0 | 0 | 0 | 5 | 0 | 0 | 0 |
| *Parametriocnemus lundbecki* | PARALUND | 3 | 0 | 0 | 0 | 0 | 5 | 0 | 0 | 0 | 5 | 3 | 0 | 5 | 0 | 0 | 0 | 0 | 0 | 3 | 0 | 5 | 0 | 0 | 0 |
| *Stenochironomus sp* | STENO | 0 | 0 | 0 | 0 | 0 | 0 | 5 | 0 | 5 | 0 | 0 | 0 | 5 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 3 | 0 | 0 | 0 |
| *Corynoneura scutellata* | CORYSCU | 5 | 0 | 0 | 0 | 5 | 0 | 0 | 0 | 0 | 0 | 5 | 0 | 5 | 0 | 5 | 0 | 0 | 0 | 5 | 0 | 3 | 0 | 0 | 0 |
| *Stictochironomus sp* | STICTO | 0 | 0 | 0 | 0 | 0 | 0 | 5 | 0 | 5 | 0 | 0 | 0 | 0 | 5 | 5 | 0 | 0 | 0 | 0 | 5 | 0 | 0 | 0 | 0 |
| *Ablabesmyia monilis* | ABLM | 0 | 5 | 5 | 0 | 0 | 0 | 5 | 0 | 3 | 3 | 1 | 0 | 5 | 0 | 0 | 5 | 0 | 0 | 0 | 0 | 5 | 0 | 0 | 0 |
| *Procladius sp* | PROCLAD | 0 | 5 | 5 | 0 | 0 | 5 | 0 | 0 | 0 | 5 | 0 | 0 | 5 | 0 | 0 | 5 | 0 | 0 | 5 | 3 | 0 | 0 | 0 | 0 |
| *Microchironomus tener* | MICHIRO | 1 | 1 | 0 | 0 | 0 | 5 | 0 | 0 | 1 | 5 | 1 | 0 | 5 | 0 | 0 | 0 | 5 | 0 | 3 | 5 | 3 | 3 | 0 | 0 |
| *Arctopelopia sp* | ARCT | 0 | 5 | 5 | 0 | 0 | 5 | 0 | 0 | 5 | 0 | 0 | 0 | 5 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| *Tvetenia sp* | TVETS | 5 | 0 | 0 | 0 | 0 | 5 | 0 | 0 | 0 | 0 | 0 | 5 | 5 | 0 | 0 | 0 | 0 | 0 | 3 | 0 | 5 | 0 | 0 | 0 |
| *Nanocladius rectinervis* | NANO | 0 | 0 | 0 | 0 | 5 | 0 | 0 | 0 | 0 | 0 | 5 | 0 | 5 | 0 | 0 | 0 | 0 | 0 | 3 | 0 | 5 | 1 | 0 | 0 |
| *Trissocladius sp* | TRISSO | 0 | 0 | 0 | 0 | 0 | 5 | 0 | 0 | 5 | 0 | 0 | 0 | 5 | 0 | 0 | 0 | 0 | 0 | 0 | 5 | 0 | 0 | 0 | 0 |
| *Eukiefferiella gracei* | EUKIG | 3 | 0 | 0 | 0 | 3 | 5 | 0 | 0 | 1 | 3 | 5 | 1 | 5 | 0 | 5 | 0 | 0 | 0 | 5 | 0 | 5 | 0 | 0 | 0 |

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| *Orthocladius oliveri* | ORTO | 5 | 0 | 0 | 0 | 0 | 0 | 5 | 0 | 3 | 5 | 1 | 0 | 5 | 0 | 0 | 5 | 3 | 0 | 5 | 0 | 5 | 0 | 0 | 0 |
| *Paratrichocladius skirwithensis* | PSKIR | 5 | 0 | 0 | 0 | 0 | 5 | 0 | 0 | 3 | 3 | 3 | 0 | 5 | 0 | 0 | 0 | 0 | 0 | 3 | 1 | 5 | 3 | 0 | 0 |
| *Meropelopia americana* | MERO | 0 | 0 | 0 | 0 | 0 | 5 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| *Cricotopus cylindraceus* | CCYLIN | 3 | 0 | 0 | 0 | 0 | 5 | 0 | 0 | 1 | 1 | 5 | 3 | 5 | 1 | 1 | 5 | 1 | 0 | 0 | 0 | 5 | 0 | 0 | 0 |
| *Thienemannimyia geijskesi* | THIEN | 0 | 5 | 5 | 0 | 0 | 5 | 0 | 0 | 5 | 0 | 0 | 0 | 5 | 0 | 0 | 0 | 0 | 0 | 1 | 5 | 0 | 0 | 1 | 0 |
| Chironomini Genus B | CGENUSB | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 5 | 0 | 0 | 0 | 0 |
| *Larsia sp* | LARSIA | 0 | 5 | 0 | 0 | 5 | 0 | 0 | 0 | 5 | 0 | 0 | 0 | 5 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| *Glyptotendipes severini* | GLSEVE | 3 | 0 | 0 | 0 | 0 | 3 | 5 | 0 | 0 | 5 | 0 | 0 | 5 | 0 | 0 | 0 | 5 | 3 | 0 | 3 | 5 | 0 | 0 | 0 |
| *Corynoneura lobata* | CORYLOB | 5 | 0 | 0 | 0 | 5 | 0 | 0 | 0 | 0 | 0 | 5 | 0 | 5 | 0 | 5 | 0 | 0 | 0 | 5 | 0 | 3 | 0 | 0 | 0 |
| *Abiskomyia sp* | ABISK | 0 | 0 | 0 | 0 | 5 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 5 | 3 | 0 | 0 | 0 |
| *Paracricotopus niger* | PARANIGER | 5 | 0 | 0 | 0 | 5 | 0 | 0 | 0 | 0 | 3 | 3 | 0 | 5 | 0 | 0 | 0 | 0 | 0 | 3 | 0 | 5 | 0 | 0 | 0 |
| *Monopelopia tennicalcar* | MONOTEN | 0 | 5 | 3 | 0 | 5 | 0 | 0 | 0 | 0 | 5 | 3 | 0 | 5 | 0 | 0 | 0 | 0 | 0 | 0 | 3 | 5 | 0 | 0 | 0 |
| *Stempellinella sp* | STEMPELL | 0 | 0 | 0 | 0 | 5 | 0 | 0 | 0 | 5 | 0 | 0 | 0 | 5 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 5 | 0 | 0 | 0 |
| *Conchapelopia melanops* | CONCMEL | 0 | 5 | 5 | 0 | 0 | 5 | 0 | 0 | 5 | 1 | 0 | 0 | 5 | 0 | 0 | 5 | 0 | 0 | 1 | 0 | 5 | 0 | 0 | 0 |

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| *Hydrobaenus johannseni* | HYDROJOH | 3 | 0 | 0 | 0 | 0 | 5 | 0 | 0 | 5 | 0 | 0 | 0 | 5 | 0 | 0 | 5 | 3 | 0 | 0 | 0 | 5 | 0 | 0 | 0 |
| *Paralimnophyes hydrophilus* | PARAHYDRO | 0 | 0 | 0 | 0 | 5 | 0 | 0 | 0 | 0 | 0 | 0 | 5 | 5 | 0 | 5 | 0 | 0 | 0 | 0 | 0 | 5 | 0 | 0 | 0 |
| *Dicrotendipes nervosus* | DICRNERV | 0 | 0 | 0 | 0 | 0 | 0 | 5 | 0 | 0 | 5 | 0 | 0 | 5 | 0 | 0 | 5 | 0 | 0 | 0 | 5 | 1 | 0 | 0 | 0 |
| *Euryhapsis cilium* | EURYCIL | 0 | 0 | 0 | 0 | 0 | 5 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| *Chaetocladius setosipennis* | CHAESET | 3 | 0 | 0 | 0 | 0 | 5 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 5 | 3 | 0 | 3 | 1 | 5 | 0 | 0 | 0 |
| *Metriocnemus fuscipes* | METRIFUS | 0 | 3 | 3 | 0 | 0 | 5 | 0 | 0 | 0 | 0 | 5 | 0 | 3 | 3 | 5 | 0 | 0 | 0 | 0 | 0 | 5 | 3 | 0 | 0 |
| *Krenosmittia sp* | KRENOSM | 1 | 0 | 0 | 0 | 5 | 0 | 0 | 0 | 5 | 0 | 0 | 0 | 5 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| *Cricotopus bicinctus* | CRICBICIN | 3 | 0 | 0 | 0 | 0 | 5 | 0 | 0 | 1 | 1 | 5 | 3 | 5 | 1 | 1 | 5 | 1 | 0 | 0 | 0 | 5 | 0 | 0 | 0 |
| *Parakiefferiella sp* | PARAKIE | 0 | 0 | 0 | 0 | 5 | 0 | 0 | 0 | 0 | 5 | 0 | 0 | 5 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 5 | 0 | 0 | 0 |
| *Pseudorthocladius sp* | PSEUDORT | 0 | 0 | 0 | 0 | 0 | 5 | 0 | 0 | 0 | 5 | 0 | 0 | 5 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| *Djalmabatista pulcher* | DJALP | 0 | 5 | 5 | 0 | 5 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 5 | 0 | 0 | 0 | 0 | 0 | 5 | 3 | 0 | 0 | 0 | 0 |
| *Conchapelopia sp* | CONCHS | 0 | 5 | 5 | 0 | 0 | 5 | 0 | 0 | 5 | 1 | 0 | 0 | 5 | 0 | 0 | 5 | 0 | 0 | 1 | 0 | 5 | 0 | 0 | 0 |
| *Procladius bellus* | PROBELL | 0 | 5 | 5 | 0 | 0 | 5 | 0 | 0 | 0 | 5 | 0 | 0 | 5 | 0 | 0 | 5 | 0 | 0 | 5 | 3 | 0 | 0 | 0 | 0 |

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| *Eukiefferiella sp* | EUKIES | 3 | 0 | 0 | 0 | 3 | 5 | 0 | 0 | 1 | 3 | 5 | 1 | 5 | 0 | 5 | 0 | 0 | 0 | 5 | 0 | 5 | 0 | 0 | 0 |
| *Thienemannimyia senata* | THIENSEN | 0 | 5 | 5 | 0 | 0 | 5 | 0 | 0 | 5 | 0 | 0 | 0 | 5 | 0 | 0 | 0 | 0 | 0 | 1 | 5 | 0 | 0 | 1 | 0 |
| *Paralimnophyes sp* | PARALIMS | 0 | 0 | 0 | 0 | 5 | 0 | 0 | 0 | 0 | 0 | 0 | 5 | 5 | 0 | 5 | 0 | 0 | 0 | 0 | 0 | 5 | 0 | 0 | 0 |
| *Paramerina anomalus* | PAMERANO | 0 | 5 | 5 | 0 | 5 | 0 | 0 | 0 | 0 | 0 | 5 | 0 | 5 | 0 | 0 | 0 | 0 | 0 | 3 | 5 | 3 | 0 | 0 | 0 |
| *Virgatanytarsus sp* | VIRGS | 3 | 0 | 0 | 0 | 5 | 0 | 0 | 0 | 5 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 3 | 0 | 5 | 0 | 0 | 0 |
| *Pseudosmittia sp* | PSMITTIA | 0 | 0 | 0 | 0 | 5 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 3 | 0 | 5 | 0 | 0 | 0 |
| *Pseudochironomus sp* | PCHIRO | 0 | 0 | 0 | 0 | 0 | 5 | 0 | 0 | 5 | 3 | 0 | 0 | 5 | 0 | 5 | 0 | 0 | 0 | 1 | 5 | 1 | 1 | 0 | 0 |
| *Orthocladius sp* | ORTSS | 5 | 0 | 0 | 0 | 0 | 0 | 5 | 0 | 3 | 5 | 1 | 0 | 5 | 0 | 0 | 5 | 3 | 0 | 5 | 0 | 5 | 0 | 0 | 0 |
| *Paratanytarsus sp* | PARATANS | 0 | 0 | 0 | 0 | 0 | 5 | 0 | 0 | 5 | 3 | 1 | 0 | 5 | 0 | 5 | 0 | 0 | 0 | 3 | 3 | 5 | 1 | 0 | 1 |
| *Einfeldia carbonaria* | EINCARB | 5 | 0 | 0 | 0 | 0 | 0 | 5 | 0 | 3 | 3 | 0 | 0 | 5 | 0 | 0 | 3 | 3 | 3 | 0 | 5 | 1 | 0 | 0 | 0 |
| *Tanytarsus brundini* | TBRUND | 0 | 0 | 0 | 0 | 0 | 5 | 0 | 0 | 3 | 3 | 0 | 0 | 5 | 0 | 5 | 0 | 0 | 0 | 1 | 5 | 5 | 0 | 0 | 0 |
| *Natarsia baltimoreus* | NATARB | 0 | 5 | 5 | 0 | 0 | 5 | 0 | 0 | 0 | 5 | 0 | 0 | 5 | 0 | 0 | 0 | 0 | 0 | 0 | 5 | 0 | 0 | 0 | 0 |
| *Paracladopelma nais* | PELMANA | 0 | 0 | 0 | 0 | 0 | 5 | 0 | 0 | 0 | 0 | 0 | 5 | 5 | 0 | 0 | 0 | 0 | 0 | 0 | 5 | 0 | 0 | 0 | 0 |
| *Euryhapsis sp* | EURYHS | 0 | 0 | 0 | 0 | 0 | 5 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |

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| *Tvetenia paucunca* | TVETPAUC | 5 | 0 | 0 | 0 | 0 | 5 | 0 | 0 | 0 | 0 | 0 | 5 | 5 | 0 | 0 | 0 | 0 | 0 | 3 | 0 | 5 | 0 | 0 | 0 |
| *Paramerina cingulata* | PAMECING | 0 | 5 | 5 | 0 | 5 | 0 | 0 | 0 | 0 | 0 | 5 | 0 | 5 | 0 | 0 | 0 | 0 | 0 | 3 | 5 | 3 | 0 | 0 | 0 |
| *Orthocladius dentifer* | ORTDENT | 5 | 0 | 0 | 0 | 0 | 0 | 5 | 0 | 3 | 5 | 1 | 0 | 5 | 0 | 0 | 5 | 3 | 0 | 5 | 0 | 5 | 0 | 0 | 0 |
| *Cricotopus sp1* | CRIC1 | 3 | 0 | 0 | 0 | 0 | 5 | 0 | 0 | 1 | 1 | 5 | 3 | 5 | 1 | 1 | 5 | 1 | 0 | 0 | 0 | 5 | 0 | 0 | 0 |
| *Cricotopus trifasciata gr* | CRICTRIF | 3 | 0 | 0 | 0 | 0 | 5 | 0 | 0 | 1 | 1 | 5 | 3 | 5 | 1 | 1 | 5 | 1 | 0 | 0 | 0 | 5 | 0 | 0 | 0 |
| *Paratrichocladius sp* | TRICHOCLAD | 5 | 0 | 0 | 0 | 0 | 5 | 0 | 0 | 3 | 3 | 3 | 0 | 5 | 0 | 5 | 0 | 0 | 0 | 3 | 1 | 5 | 3 | 0 | 0 |
| *Nanocladius sp* | NANOCLAD | 0 | 0 | 0 | 0 | 5 | 0 | 0 | 0 | 0 | 0 | 5 | 0 | 5 | 0 | 0 | 0 | 0 | 0 | 3 | 0 | 5 | 1 | 0 | 0 |
| *Cardiocladius sp* | CARDIO | 3 | 5 | 5 | 0 | 0 | 0 | 5 | 0 | 0 | 3 | 3 | 5 | 5 | 0 | 0 | 0 | 0 | 0 | 0 | 5 | 0 | 0 | 0 | 0 |
| *Kiefferulus sp* | KIEFFU | 0 | 0 | 0 | 0 | 0 | 5 | 0 | 0 | 0 | 5 | 0 | 0 | 5 | 0 | 0 | 5 | 0 | 0 | 0 | 5 | 3 | 0 | 0 | 0 |
| *Cryptochironomus sp* | CRYPTCHIR | 0 | 5 | 5 | 0 | 0 | 0 | 5 | 0 | 1 | 5 | 1 | 0 | 5 | 0 | 0 | 5 | 0 | 0 | 5 | 5 | 3 | 0 | 0 | 0 |
| *Tanytarsus sp* | TARSSP | 0 | 0 | 0 | 0 | 0 | 5 | 0 | 0 | 3 | 3 | 0 | 0 | 5 | 0 | 5 | 0 | 0 | 0 | 1 | 5 | 5 | 0 | 0 | 0 |
| *Ablabesmyia sp* | ABLASP | 0 | 5 | 5 | 0 | 0 | 0 | 5 | 0 | 3 | 3 | 1 | 0 | 5 | 0 | 0 | 5 | 0 | 0 | 0 | 0 | 5 | 0 | 0 | 0 |

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| *Coelotanypus sp* | COELOTAN | 0 | 5 | 5 | 0 | 0 | 5 | 0 | 0 | 0 | 5 | 0 | 0 | 5 | 0 | 0 | 0 | 0 | 0 | 0 | 5 | 0 | 0 | 0 | 0 |
| *Trissopelopia sp* | TRISSOPEL | 0 | 5 | 5 | 0 | 0 | 5 | 0 | 0 | 0 | 5 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 3 | 5 | 3 | 0 | 0 | 0 |
| *Clinotanypus sp* | CLINOTANY | 0 | 5 | 5 | 0 | 0 | 0 | 5 | 0 | 5 | 0 | 0 | 0 | 5 | 0 | 0 | 0 | 0 | 0 | 0 | 5 | 1 | 0 | 0 | 0 |
| *Dicrotendipes sp* | DICRPESS | 0 | 0 | 0 | 0 | 0 | 0 | 5 | 0 | 0 | 5 | 0 | 0 | 5 | 0 | 0 | 5 | 0 | 0 | 0 | 5 | 1 | 0 | 0 | 0 |
| *Rheotanytarsus sp* | RHEOTANS | 0 | 0 | 0 | 0 | 5 | 0 | 0 | 0 | 0 | 3 | 0 | 5 | 5 | 0 | 0 | 0 | 0 | 0 | 5 | 1 | 3 | 1 | 0 | 0 |
| *Tanypus sp* | TANYPS | 0 | 5 | 5 | 0 | 0 | 0 | 5 | 0 | 5 | 0 | 0 | 0 | 5 | 0 | 0 | 0 | 5 | 0 | 3 | 5 | 3 | 0 | 0 | 0 |
| *Chironomus sp3* | CHIRO3 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 5 | 1 | 5 | 1 | 1 | 0 | 5 | 0 | 5 | 1 | 1 | 0 | 5 | 3 | 0 | 0 | 5 |
| *Microchironomus sp* | MUSSP | 1 | 1 | 0 | 0 | 0 | 5 | 0 | 0 | 1 | 5 | 1 | 0 | 5 | 0 | 0 | 0 | 5 | 0 | 3 | 5 | 3 | 3 | 0 | 0 |

CONT'D

| | Taxon | SP7 | SP8 | BA1 | BA2 | FT1 | FT2 | FT3 | FT4 | FT5 | FT6 | FT7 | FT8 | FT9 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *Rheopelopia sp* | RHP | 0 | 0 | 0 | 5 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 5 | 0 |

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|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *Polypedilum tuberculum* | PLPT | 1 | 1 | 0 | 5 | 5 | 3 | 3 | 5 | 3 | 0 | 0 | 0 | 0 |
| *Polypedilum convictum* | PLPC | 1 | 1 | 0 | 5 | 5 | 3 | 3 | 5 | 3 | 0 | 0 | 0 | 0 |
| *Tanytarsus usmaensis* | TUSM | 0 | 0 | 0 | 5 | 0 | 5 | 0 | 5 | 0 | 0 | 0 | 0 | 0 |
| *Polypedilum nubeculosum* | PLPN | 1 | 1 | 0 | 5 | 5 | 3 | 3 | 5 | 3 | 0 | 0 | 0 | 0 |
| *Polypedilum illinoense* | PLPI | 1 | 1 | 0 | 5 | 0 | 3 | 3 | 5 | 3 | 0 | 0 | 0 | 0 |
| *Ablabesmyia phatta* | ABLP | 0 | 0 | 0 | 5 | 0 | 0 | 0 | 0 | 0 | 0 | 5 | 5 | 0 |
| *Polypedilum nubifer* | PLPNR | 1 | 1 | 0 | 5 | 5 | 3 | 3 | 5 | 3 | 0 | 0 | 0 | 0 |
| *Zavreliella marmotata* | ZAVM | 0 | 0 | 0 | 5 | 5 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| *Gillotia alboviridis* | GILA | 0 | 0 | 0 | 0 | 5 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| *Cladotanytarsus sp* | CLAS | 0 | 0 | 5 | 0 | 0 | 5 | 5 | 0 | 0 | 0 | 0 | 0 | 0 |
| *Cladotanytarsus mancus* | CLAM | 0 | 0 | 5 | 0 | 0 | 5 | 5 | 0 | 0 | 0 | 0 | 0 | 0 |
| *Dicrotendipes pulsus* | DICRP | 0 | 0 | 0 | 5 | 3 | 5 | 1 | 5 | 0 | 0 | 0 | 0 | 0 |
| *Dicrotendipes lobiger* | DICRL | 0 | 0 | 0 | 5 | 3 | 5 | 1 | 5 | 0 | 0 | 0 | 0 | 0 |
| *Glyptotendipes sp* | GLYPS | 1 | 0 | 0 | 5 | 0 | 0 | 0 | 3 | 5 | 0 | 0 | 0 | 0 |
| *Einfeldia pagana* | EINP | 0 | 0 | 0 | 5 | 5 | 3 | 3 | 3 | 0 | 0 | 0 | 0 | 0 |
| *Polypedilum sp* | PLPS | 1 | 1 | 0 | 5 | 5 | 3 | 3 | 5 | 3 | 0 | 0 | 0 | 0 |

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|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *Polypedilum ophoides* | PLPO | 1 | 1 | 0 | 5 | 5 | 3 | 3 | 5 | 3 | 0 | 0 | 0 | 0 |
| *Einfeldia sp* | EINS | 0 | 0 | 0 | 5 | 5 | 3 | 3 | 3 | 0 | 0 | 0 | 0 | 0 |
| *Rheotanytarsus curtistylus* | RHEOTC | 0 | 0 | 5 | 0 | 0 | 5 | 3 | 3 | 0 | 0 | 0 | 0 | 0 |
| *Rheocricotopus sp* | RHEOCRIC | 0 | 0 | 0 | 0 | 3 | 3 | 3 | 5 | 0 | 0 | 0 | 0 | 0 |
| *Orthocladius clarki* | ORTC | 0 | 3 | 0 | 5 | 0 | 5 | 1 | 3 | 1 | 0 | 0 | 0 | 3 |
| *Microtendipes pedellus* | MICP | 0 | 0 | 0 | 0 | 5 | 3 | 3 | 3 | 0 | 0 | 0 | 0 | 0 |
| *Dicrotendipes notatus* | DICRN | 0 | 0 | 0 | 5 | 3 | 5 | 1 | 5 | 0 | 0 | 0 | 0 | 0 |
| *Chironomus sp1* | CHIRO1 | 0 | 1 | 0 | 5 | 5 | 5 | 5 | 3 | 1 | 1 | 1 | 0 | 0 |
| *Chironomus sp2* | CHIRO2 | 0 | 1 | 0 | 5 | 5 | 5 | 5 | 3 | 1 | 1 | 1 | 0 | 0 |
| *Cricotopus intersectus* | CRICI | 0 | 0 | 0 | 5 | 0 | 1 | 3 | 5 | 5 | 0 | 0 | 0 | 0 |
| *Paratanytarsus austriacus* | PARAA | 0 | 0 | 5 | 0 | 3 | 3 | 0 | 3 | 0 | 0 | 0 | 0 | 0 |
| *Virgatanytarsus ardrennensis* | VIRGA | 0 | 0 | 0 | 0 | 5 | 3 | 0 | 3 | 0 | 0 | 0 | 0 | 0 |
| *Helopelopia cornuticaudata* | HELOP | 0 | 3 | 0 | 5 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 5 | 0 |
| *Cricotopus tremelus* | CRICT | 0 | 0 | 0 | 5 | 0 | 1 | 3 | 5 | 5 | 0 | 0 | 0 | 0 |
| *Parametriocnemus lundbecki* | PARALUND | 0 | 3 | 0 | 5 | 5 | 3 | 0 | 1 | 0 | 0 | 0 | 0 | 0 |
| *Stenochironomus sp* | STENO | 5 | 0 | 0 | 5 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 5 |

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|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *Corynoneura scutellata* | CORYSCU | 0 | 0 | 0 | 5 | 0 | 0 | 0 | 5 | 0 | 0 | 0 | 0 | 0 |
| *Stictochironomus sp* | STICTO | 0 | 0 | 0 | 5 | 5 | 3 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| *Ablabesmyia monilis* | ABLM | 0 | 0 | 0 | 5 | 0 | 0 | 0 | 0 | 0 | 0 | 5 | 5 | 0 |
| *Procladius sp* | PROCLAD | 0 | 0 | 0 | 5 | 0 | 0 | 0 | 3 | 0 | 0 | 3 | 5 | 0 |
| *Microchironomus tener* | MICHIRO | 0 | 0 | 0 | 0 | 0 | 0 | 5 | 3 | 0 | 0 | 0 | 0 | 0 |
| *Arctopelopia sp* | ARCT | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 5 | 0 |
| *Tvetenia sp* | TVETS | 0 | 1 | 0 | 0 | 3 | 1 | 3 | 5 | 0 | 0 | 0 | 0 | 0 |
| *Nanocladius rectinervis* | NANO | 0 | 0 | 0 | 5 | 3 | 0 | 0 | 5 | 0 | 0 | 0 | 0 | 0 |
| *Trissocladius sp* | TRISSO | 0 | 0 | 0 | 5 | 5 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| *Eukiefferiella gracei* | EUKIG | 0 | 3 | 0 | 0 | 0 | 5 | 0 | 5 | 3 | 0 | 0 | 0 | 0 |
| *Orthocladius oliveri* | ORTO | 0 | 3 | 0 | 5 | 0 | 5 | 1 | 3 | 1 | 0 | 0 | 0 | 3 |
| *Paratrichocladius skirwithensis* | PSKIR | 0 | 0 | 0 | 5 | 0 | 3 | 3 | 5 | 0 | 0 | 0 | 0 | 0 |
| *Meropelopia americana* | MERO | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| *Cricotopus cylindraceus* | CCYLIN | 0 | 0 | 0 | 5 | 0 | 1 | 3 | 5 | 5 | 0 | 0 | 0 | 0 |
| *Thienemannimyia geijskesi* | THIEN | 3 | 0 | 0 | 5 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 5 | 0 |
| Chironomini Genus B | CGENUSB | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |

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|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *Larsia sp* | LARSIA | 0 | 0 | 0 | 5 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 5 | 0 |
| *Glyptotendipes severini* | GLSEVE | 1 | 0 | 0 | 5 | 0 | 0 | 0 | 3 | 5 | 0 | 0 | 0 | 0 |
| *Corynoneura lobata* | CORYLOB | 0 | 0 | 0 | 5 | 0 | 0 | 0 | 5 | 0 | 0 | 0 | 0 | 0 |
| *Abiskomyia sp* | ABISK | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| *Paracricotopus niger* | PARANIGER | 0 | 0 | 0 | 0 | 0 | 3 | 0 | 5 | 0 | 0 | 0 | 0 | 0 |
| *Monopelopia tennicalcar* | MONOTEN | 0 | 3 | 0 | 5 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 5 | 0 |
| *Stempellinella sp* | STEMPELL | 0 | 3 | 5 | 0 | 5 | 3 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| *Conchapelopia melanops* | CONCMEL | 0 | 3 | 0 | 5 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 5 | 0 |
| *Hydrobaenus johannseni* | HYDROJOH | 0 | 0 | 0 | 5 | 0 | 0 | 0 | 5 | 0 | 0 | 0 | 0 | 0 |
| *Paralimnophyes hydrophilus* | PARAHYDRO | 0 | 0 | 0 | 5 | 5 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| *Dicrotendipes nervosus* | DICRNERV | 0 | 0 | 0 | 5 | 3 | 5 | 1 | 5 | 0 | 0 | 0 | 0 | 0 |
| *Euryhapsis cilium* | EURYCIL | 0 | 0 | 0 | 0 | 3 | 0 | 3 | 3 | 0 | 0 | 0 | 0 | 0 |
| *Chaetocladius setosipennis* | CHAESET | 0 | 0 | 0 | 5 | 3 | 3 | 3 | 3 | 0 | 0 | 0 | 0 | 0 |
| *Metriocnemus fuscipesssss* | METRIFUS | 0 | 5 | 0 | 0 | 0 | 0 | 0 | 5 | 0 | 0 | 5 | 0 | 0 |
| *Krenosmittia sp* | KRENOSM | 0 | 0 | 0 | 0 | 5 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| *Cricotopus bicinctus* | CRICBICIN | 0 | 0 | 0 | 5 | 0 | 1 | 3 | 5 | 5 | 0 | 0 | 0 | 0 |

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|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *Parakiefferiella sp* | PARAKIE | 0 | 0 | 0 | 5 | 5 | 0 | 3 | 3 | 0 | 0 | 0 | 0 | 0 |
| *Pseudorthocladius sp* | PSEUDORT | 0 | 5 | 0 | 0 | 5 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| *Djalmabatista pulcher* | DJALP | 0 | 0 | 0 | 5 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 5 | 0 |
| *Conchapelopia sp* | CONCHS | 0 | 3 | 0 | 5 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 5 | 0 |
| *Procladius bellus* | PROBELL | 0 | 0 | 0 | 5 | 0 | 0 | 0 | 3 | 0 | 0 | 3 | 5 | 0 |
| *Eukiefferiella sp* | EUKIES | 0 | 3 | 0 | 0 | 0 | 5 | 0 | 5 | 3 | 0 | 0 | 0 | 0 |
| *Thienemannimyia senata* | THIENSEN | 3 | 0 | 0 | 5 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 5 | 0 |
| *Paralimnophyes sp* | PARALIMS | 0 | 0 | 0 | 5 | 5 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| *Paramerina anomalus* | PAMERANO | 0 | 0 | 0 | 5 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 5 | 0 |
| *Virgatanytarsus sp* | VIRGS | 0 | 0 | 0 | 0 | 5 | 3 | 0 | 3 | 0 | 0 | 0 | 0 | 0 |
| *Pseudosmittia sp* | PSMITTIA | 0 | 5 | 0 | 0 | 5 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| *Pseudochironomus sp* | PCHIRO | 0 | 0 | 0 | 5 | 5 | 0 | 0 | 3 | 0 | 0 | 1 | 0 | 0 |
| *Orthocladius sp* | ORTSS | 0 | 3 | 0 | 5 | 0 | 5 | 1 | 3 | 1 | 0 | 0 | 0 | 3 |
| *Paratanytarsus sp* | PARATANS | 0 | 0 | 5 | 0 | 3 | 3 | 0 | 3 | 0 | 0 | 0 | 0 | 0 |
| *Einfeldia carbonaria* | EINCARB | 0 | 0 | 0 | 5 | 5 | 3 | 3 | 3 | 0 | 0 | 0 | 0 | 0 |
| *Tanytarsus brundini* | TBRUND | 0 | 0 | 0 | 5 | 0 | 5 | 0 | 5 | 0 | 0 | 0 | 0 | 0 |

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|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *Natarsia baltimoreus* | NATARB | 0 | 0 | 0 | 5 | 0 | 0 | 0 | 0 | 0 | 0 | 3 | 5 | 0 |
| *Paracladopelma nais* | PELMANA | 0 | 0 | 0 | 0 | 5 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| *Euryhapsis sp* | EURYHS | 0 | 0 | 0 | 0 | 3 | 0 | 3 | 3 | 0 | 0 | 0 | 0 | 0 |
| *Tvetenia paucunca* | TVETPAUC | 0 | 1 | 0 | 0 | 3 | 1 | 3 | 5 | 0 | 0 | 0 | 0 | 0 |
| *Paramerina cingulata* | PAMECING | 0 | 0 | 0 | 5 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 5 | 0 |
| *Orthocladius dentifer* | ORTDENT | 0 | 3 | 0 | 5 | 0 | 5 | 1 | 3 | 1 | 0 | 0 | 0 | 3 |
| *Cricotopus sp1* | CRIC1 | 0 | 0 | 0 | 5 | 0 | 1 | 3 | 5 | 5 | 0 | 0 | 0 | 0 |
| *Cricotopus trifasciata gr* | CRICTRIF | 0 | 0 | 0 | 5 | 0 | 1 | 3 | 5 | 5 | 0 | 0 | 0 | 0 |
| *Paratrichocladius sp* | TRICHOCLAD | 0 | 0 | 0 | 5 | 3 | 3 | 3 | 5 | 0 | 0 | 0 | 0 | 0 |
| *Nanocladius sp* | NANOCLAD | 0 | 0 | 0 | 5 | 3 | 0 | 0 | 5 | 0 | 0 | 0 | 0 | 0 |
| *Cardiocladius sp* | CARDIO | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 3 | 5 | 0 |
| *Kiefferulus sp* | KIEFFU | 0 | 0 | 0 | 5 | 0 | 5 | 3 | 0 | 1 | 0 | 0 | 0 | 0 |
| *Cryptochironomus sp* | CRYPTCHIR | 0 | 0 | 0 | 0 | 0 | 3 | 0 | 0 | 0 | 0 | 0 | 5 | 0 |
| *Tanytarsus sp* | TARSSP | 0 | 0 | 0 | 5 | 0 | 5 | 0 | 5 | 0 | 0 | 0 | 0 | 0 |
| *Ablabesmyia sp* | ABLASP | 0 | 0 | 0 | 5 | 0 | 0 | 0 | 0 | 0 | 0 | 5 | 5 | 0 |
| *Coelotanypus sp* | COELOTAN | 0 | 0 | 0 | 5 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 5 | 0 |

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|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *Trissopelopia sp* | TRISSOPEL | 0 | 0 | 0 | 5 | 0 | 0 | 0 | 0 | 0 | 0 | 5 | 5 | 0 |
| *Clinotanypus sp* | CLINOTANY | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 3 | 5 | 0 |
| *Dicrotendipes sp* | DICRPESS | 0 | 0 | 0 | 5 | 3 | 5 | 1 | 5 | 0 | 0 | 0 | 0 | 0 |
| *Rheotanytarsus sp* | RHEOTANS | 0 | 0 | 5 | 0 | 0 | 5 | 3 | 3 | 0 | 0 | 0 | 0 | 0 |
| *Tanypus sp* | TANYPS | 0 | 0 | 0 | 5 | 1 | 0 | 0 | 0 | 0 | 0 | 3 | 5 | 0 |
| *Chironomus* sp3 | CHIRO3 | 0 | 1 | 0 | 5 | 5 | 5 | 5 | 3 | 1 | 1 | 1 | 0 | 0 |
| *Microchironomus sp* | MUSSP | 0 | 0 | 0 | 0 | 0 | 0 | 5 | 3 | 0 | 0 | 0 | 0 | 0 |

APPENDIX B2: Chironomid trait by site matrix for chironomid species collected from the Buffalo and Bloukrans Rivers (November 2021 – June 2022) and the Swartkops River (2009-2012) pooled based on the site categories per season. REF-least impacted sites, DP-sites that are upstream from wastewater treatment works (WWTWs) that receive diffuse pollution, PP-sites that receive point source pollution from WWTWs effluent discharge points, AG-sites downstream PP sites that receive impact from point and diffuse sources, RECV-recovery sites.

| Trait attribute | REF | REF | REF | REF | DP | DP | DP | DP | PP | PP | PP | PP | AG | AG | AG | AG | RECV | RECV | RECV | RECV |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Spring | Summer | Autumn | Winter | Spring | Summer | Autumn | Winter | Spring | Summer | Autumn | Winter | Spring | Summer | Autumn | Winter | Spring | Summer | Autumn | Winter |
| H1 | 1700 | 1650 | 1125 | 1410 | 36475 | 47860 | 6855 | 3105 | 56140 | 24585 | 4125 | 3285 | 12010 | 8065 | 515 | 3370 | 670 | 720 | 320 | 715 |
| H2 | 580 | 340 | 295 | 240 | 210 | 360 | 10 | 255 | 0 | 145 | 85 | 80 | 25 | 2260 | 195 | 285 | 75 | 60 | 85 | 70 |
| X1 | 105 | 200 | 265 | 100 | 33720 | 44205 | 6230 | 2660 | 50785 | 24230 | 3375 | 2960 | 9125 | 6455 | 355 | 3130 | 325 | 290 | 55 | 10 |
| X2 | 2011 | 1636 | 1203 | 1529 | 22657 | 30094 | 4323 | 2154 | 35826 | 14934 | 2674 | 2094 | 7437 | 7164 | 509 | 2349 | 607 | 498 | 318 | 523 |
| X3 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| RT1 | 2110 | 1850 | 1260 | 1580 | 36590 | 48134 | 6825 | 3250 | 56140 | 24640 | 3955 | 3330 | 12030 | 9797 | 520 | 3405 | 706 | 725 | 320 | 755 |

| | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| RT2 | 98 | 84 | 90 | 351 | 0 | 15 | 0 | 0 | 0 | 0 | 0 | 0 | 9 | 349 | 8 | 3 | 133 | 6 | 33 | 6 |
| RT3 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| TC1 | 285 | 290 | 160 | 110 | 15 | 1185 | 375 | 150 | 0 | 0 | 40 | 45 | 20 | 520 | 5 | 0 | 45 | 5 | 30 | 15 |
| TC2 | 1801 | 1512 | 921 | 1200 | 2880 | 3330 | 502 | 605 | 5375 | 410 | 957 | 354 | 2874 | 2671 | 400 | 675 | 290 | 530 | 314 | 753 |
| TC3 | 490 | 394 | 500 | 796 | 33789 | 43959 | 6186 | 2765 | 50765 | 24345 | 3390 | 2990 | 9145 | 7442 | 356 | 3170 | 616 | 307 | 118 | 44 |
| SR1 | 804 | 661 | 299 | 759 | 34650 | 44336 | 5485 | 2073 | 54455 | 24356 | 2959 | 3073 | 11037 | 5414 | 226 | 2351 | 287 | 274 | 194 | 400 |
| SR2 | 570 | 330 | 270 | 245 | 205 | 1290 | 375 | 150 | 0 | 0 | 40 | 70 | 25 | 1575 | 10 | 30 | 45 | 20 | 25 | 58 |
| SR3 | 1413 | 1380 | 785 | 1041 | 2635 | 1845 | 185 | 445 | 1685 | 260 | 560 | 305 | 2404 | 1475 | 160 | 213 | 204 | 418 | 244 | 701 |
| SR4 | 256 | 376 | 291 | 157 | 280 | 1661 | 920 | 976 | 0 | 322 | 1023 | 104 | 477 | 2798 | 442 | 1262 | 445 | 413 | 145 | 151 |
| RP1 | 1180 | 680 | 650 | 680 | 1410 | 1645 | 405 | 195 | 1665 | 145 | 140 | 110 | 25 | 2115 | 190 | 285 | 80 | 50 | 100 | 105 |
| RP2 | 270 | 275 | 380 | 695 | 0 | 1285 | 885 | 800 | 0 | 125 | 885 | 50 | 20 | 1900 | 225 | 940 | 580 | 290 | 85 | 20 |
| RP3 | 650 | 850 | 225 | 220 | 35190 | 44740 | 5530 | 2215 | 54455 | 24400 | 2960 | 3160 | 11965 | 5585 | 290 | 2430 | 60 | 425 | 180 | 645 |
| M1 | 580 | 315 | 275 | 250 | 205 | 1420 | 385 | 255 | 0 | 115 | 55 | 80 | 25 | 1300 | 5 | 65 | 75 | 20 | 35 | 58 |
| M2 | 1440 | 1470 | 830 | 1275 | 2635 | 1335 | 145 | 445 | 1665 | 260 | 560 | 305 | 2410 | 1855 | 165 | 215 | 280 | 425 | 250 | 705 |

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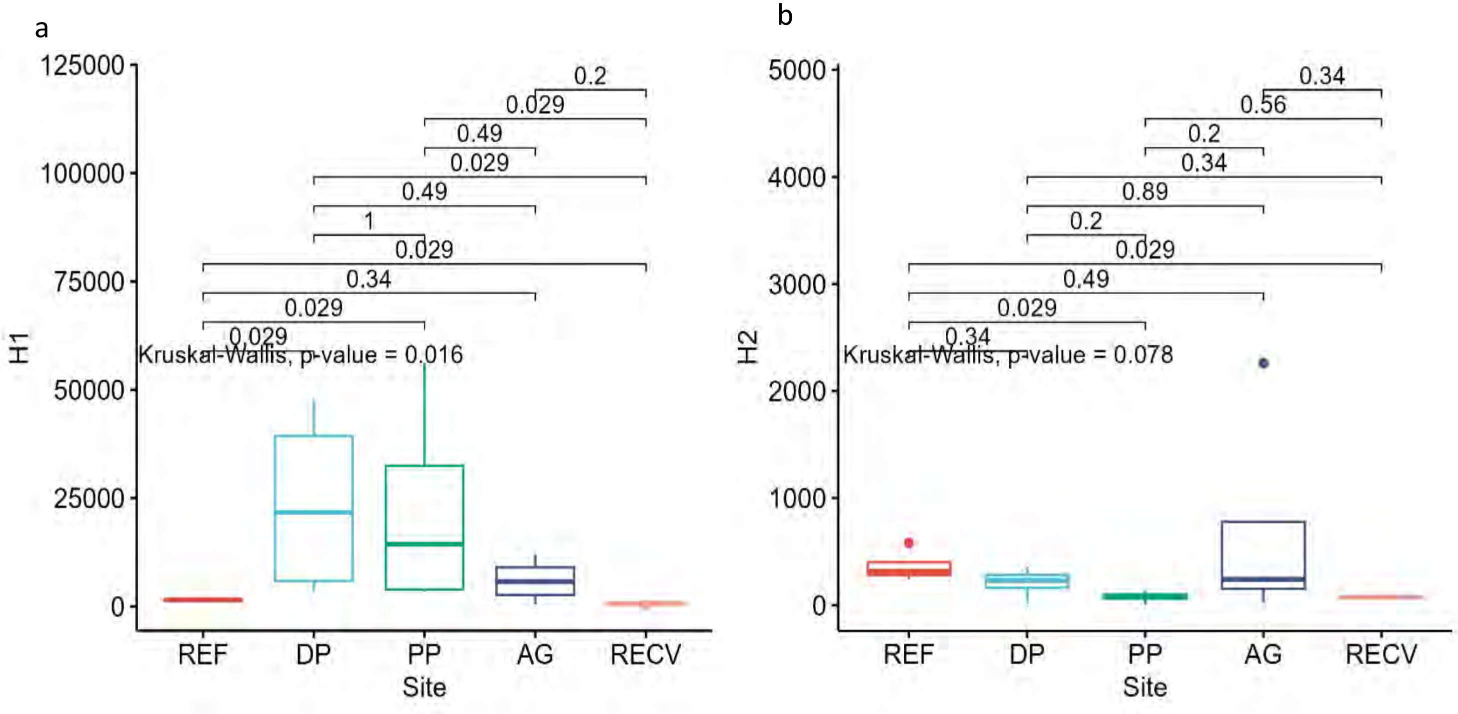
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| M3 | 345 | 230 | 265 | 135 | 33850 | 45425 | 6335 | 2660 | 54475 | 24355 | 3595 | 2980 | 9600 | 6680 | 540 | 3405 | 390 | 335 | 125 | 20 |
| FM1 | 117 | 185 | 230 | 115 | 0 | 1285 | 885 | 800 | 0 | 125 | 885 | 50 | 5 | 1900 | 225 | 935 | 375 | 287 | 30 | 10 |
| FM2 | 535 | 567 | 349 | 523 | 34392 | 44617 | 5489 | 2188 | 54808 | 24397 | 2877 | 3047 | 10103 | 5403 | 347 | 2580 | 203 | 141 | 174 | 165 |
| FM3 | 1414 | 1378 | 795 | 1047 | 2635 | 1437 | 158 | 508 | 1665 | 329 | 569 | 314 | 2404 | 1883 | 161 | 234 | 216 | 421 | 244 | 704 |
| FM4 | 1163 | 992 | 607 | 564 | 1777 | 906 | 94 | 291 | 999 | 174 | 354 | 211 | 1442 | 2663 | 218 | 294 | 51 | 292 | 179 | 467 |
| FM5 | 320 | 101 | 61 | 110 | 0 | 1185 | 382 | 0 | 0 | 0 | 0 | 0 | 0 | 50 | 1 | 0 | 35 | 0 | 15 | 13 |
| FM6 | 320 | 100 | 55 | 104 | 0 | 1185 | 375 | 0 | 0 | 0 | 0 | 0 | 0 | 50 | 0 | 0 | 35 | 0 | 15 | 13 |
| BS1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| BS2 | 186 | 255 | 290 | 140 | 21 | 1310 | 885 | 910 | 0 | 143 | 943 | 90 | 25 | 2303 | 346 | 1085 | 395 | 311 | 81 | 21 |
| BS3 | 1653 | 1585 | 934 | 1320 | 2720 | 2110 | 235 | 590 | 1685 | 465 | 790 | 325 | 2415 | 2686 | 350 | 530 | 345 | 470 | 341 | 735 |
| BS4 | 926 | 651 | 304 | 339 | 1045 | 3041 | 550 | 213 | 3690 | 191 | 284 | 178 | 1892 | 1744 | 101 | 151 | 42 | 269 | 144 | 427 |
| BS5 | 0 | 25 | 15 | 5 | 33720 | 42465 | 5270 | 1860 | 50765 | 24105 | 2490 | 2915 | 9125 | 4285 | 125 | 2195 | 0 | 0 | 0 | 0 |
| V1 | 886 | 807 | 444 | 743 | 7927 | 10425 | 1520 | 643 | 10486 | 5043 | 987 | 756 | 3264 | 2154 | 162 | 618 | 199 | 273 | 195 | 444 |

| | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| V2 | 1507 | 1371 | 763 | 926 | 35618 | 46174 | 6105 | 2713 | 54808 | 24493 | 3528 | 3232 | 11987 | 7541 | 541 | 3144 | 437 | 631 | 292 | 719 |
| V3 | 1115 | 844 | 630 | 585 | 8387 | 9199 | 1116 | 748 | 11818 | 5018 | 771 | 757 | 2330 | 2687 | 249 | 766 | 105 | 142 | 124 | 205 |
| V4 | 543 | 508 | 534 | 398 | 7517 | 10000 | 1957 | 1289 | 11152 | 5021 | 1455 | 684 | 1860 | 3781 | 287 | 1445 | 371 | 290 | 62 | 42 |
| D1 | 2350 | 1975 | 1400 | 1665 | 2970 | 5795 | 1595 | 1500 | 5375 | 625 | 1720 | 450 | 2910 | 6040 | 585 | 1460 | 755 | 780 | 420 | 788 |
| D2 | 145 | 132 | 109 | 97 | 33977 | 42510 | 5274 | 1878 | 51098 | 24105 | 2509 | 2930 | 9130 | 4350 | 125 | 2195 | 6 | 0 | 4 | 13 |
| EM1 | 370 | 267 | 244 | 682 | 342 | 60 | 4 | 18 | 333 | 30 | 49 | 20 | 45 | 605 | 190 | 255 | 231 | 45 | 119 | 35 |
| EM2 | 1874 | 1485 | 805 | 885 | 36590 | 45625 | 5555 | 2345 | 56140 | 24400 | 3055 | 3270 | 11995 | 6593 | 290 | 2460 | 150 | 436 | 214 | 740 |
| EM3 | 340 | 160 | 214 | 189 | 7070 | 9677 | 1468 | 414 | 10486 | 4821 | 517 | 616 | 1830 | 1499 | 33 | 457 | 6 | 12 | 26 | 34 |
| EM4 | 36 | 5 | 12 | 1 | 6744 | 8493 | 1054 | 372 | 10153 | 4821 | 498 | 583 | 1825 | 1061 | 25 | 439 | 0 | 0 | 15 | 0 |
| SP1 | 781 | 920 | 618 | 517 | 986 | 2848 | 1216 | 1184 | 0 | 426 | 1233 | 239 | 1428 | 4523 | 519 | 1365 | 450 | 598 | 221 | 454 |
| SP2 | 865 | 701 | 377 | 809 | 34665 | 46201 | 6077 | 2233 | 54475 | 24381 | 3136 | 3082 | 11038 | 5788 | 276 | 2544 | 369 | 327 | 201 | 404 |

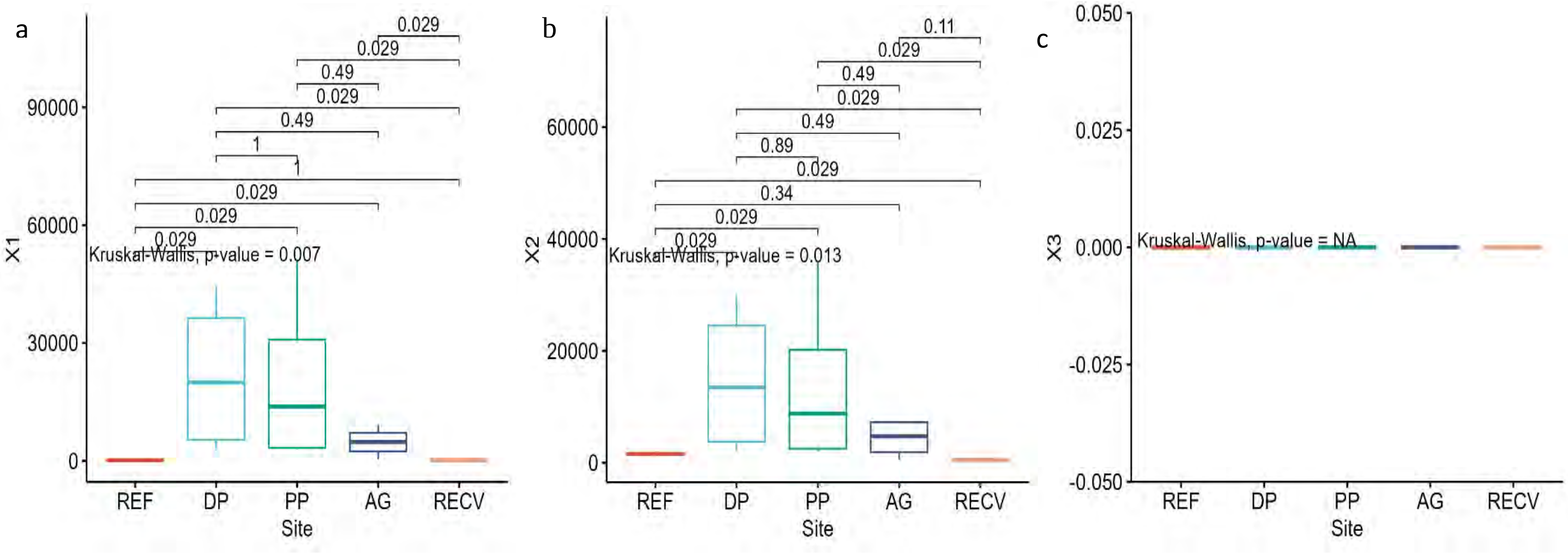
| | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| SP3 | 2135 | 1871 | 1266 | 1587 | 23106 | 29098 | 4134 | 2254 | 32874 | 14904 | 2665 | 2165 | 8009 | 7097 | 569 | 2399 | 592 | 659 | 371 | 776 |
| SP4 | 88 | 49 | 53 | 31 | 47 | 281 | 198 | 182 | 0 | 25 | 185 | 19 | 6 | 381 | 48 | 187 | 91 | 64 | 7 | 5 |
| SP5 | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 |
| SP6 | 113 | 182 | 53 | 48 | 33990 | 42690 | 5295 | 1931 | 50765 | 24157 | 2583 | 2961 | 9599 | 4512 | 157 | 2237 | 11 | 83 | 33 | 129 |
| SP7 | 121 | 167 | 64 | 58 | 270 | 222 | 25 | 71 | 0 | 52 | 93 | 46 | 474 | 230 | 32 | 42 | 9 | 83 | 45 | 132 |
| SP8 | 296 | 292 | 151 | 153 | 7089 | 8762 | 1079 | 443 | 10153 | 4891 | 609 | 644 | 2299 | 1510 | 171 | 631 | 21 | 110 | 78 | 170 |
| BA1 | 115 | 180 | 230 | 115 | 0 | 1285 | 885 | 800 | 0 | 125 | 885 | 50 | 5 | 1900 | 225 | 935 | 375 | 285 | 30 | 10 |
| BA2 | 2095 | 1785 | 1120 | 1520 | 36680 | 46725 | 5935 | 2455 | 56140 | 24460 | 3280 | 3305 | 12030 | 7250 | 295 | 2465 | 340 | 445 | 310 | 760 |
| FT1 | 581 | 847 | 286 | 208 | 35198 | 44098 | 5466 | 2344 | 52979 | 24285 | 3136 | 3189 | 11800 | 6442 | 285 | 2459 | 88 | 398 | 189 | 655 |
| FT2 | 1131 | 1063 | 791 | 1072 | 35077 | 46305 | 6380 | 2954 | 54808 | 24520 | 3717 | 3164 | 11045 | 8240 | 636 | 3575 | 648 | 596 | 276 | 471 |
| FT3 | 994 | 948 | 593 | 521 | 35396 | 44722 | 5973 | 2670 | 52514 | 24412 | 3367 | 3147 | 10664 | 7215 | 362 | 2926 | 301 | 436 | 146 | 431 |

| | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| FT4 | 1797 | 1681 | 1133 | 1426 | 23154 | 29015 | 3969 | 2296 | 35814 | 14978 | 2675 | 2164 | 8360 | 7439 | 566 | 2421 | 532 | 650 | 360 | 751 |
| FT5 | 1160 | 1002 | 626 | 617 | 8868 | 9499 | 1157 | 675 | 11822 | 4995 | 890 | 801 | 3272 | 2022 | 233 | 715 | 57 | 272 | 166 | 451 |
| FT6 | 0 | 5 | 3 | 1 | 6744 | 8493 | 1054 | 372 | 10153 | 4821 | 498 | 583 | 1825 | 857 | 25 | 439 | 0 | 0 | 0 | 0 |
| FT7 | 225 | 88 | 28 | 49 | 6744 | 9198 | 1279 | 372 | 10153 | 4821 | 498 | 583 | 1825 | 907 | 25 | 439 | 0 | 0 | 3 | 5 |
| FT8 | 320 | 100 | 60 | 110 | 0 | 1185 | 375 | 0 | 0 | 0 | 0 | 0 | 0 | 50 | 0 | 0 | 35 | 0 | 15 | 10 |
| FT9 | 141 | 58 | 92 | 96 | 69 | 39 | 0 | 0 | 0 | 0 | 0 | 15 | 0 | 339 | 3 | 0 | 0 | 6 | 13 | 24 |

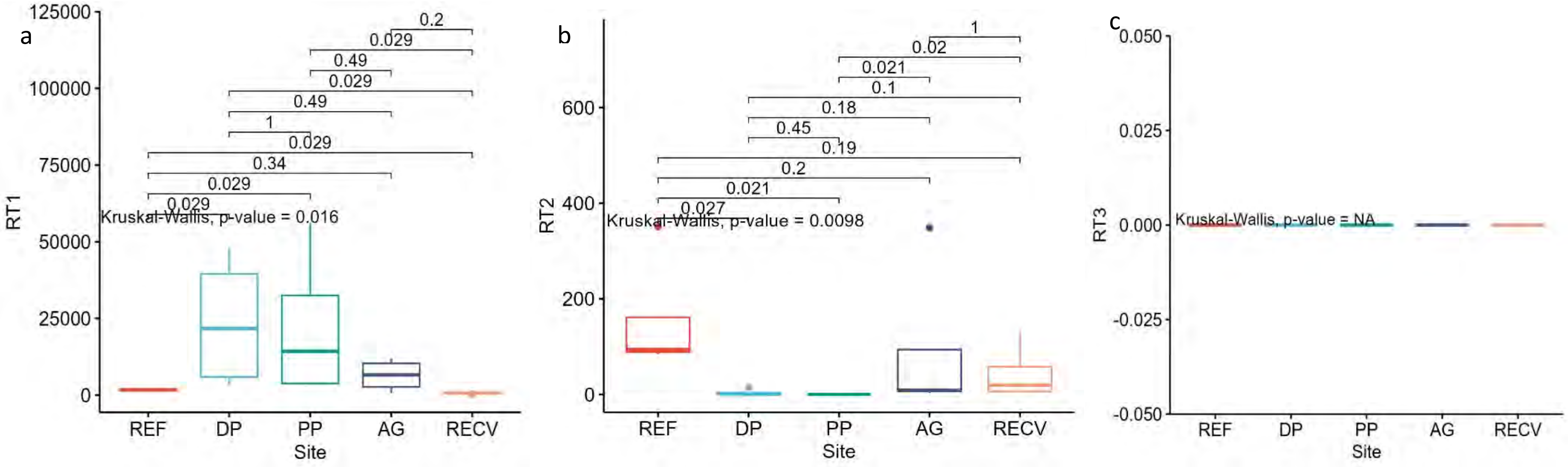
APPENDIX B3: Boxplots of chironomid species abundances showing differences between the site categories for each trait attribute per trait category.



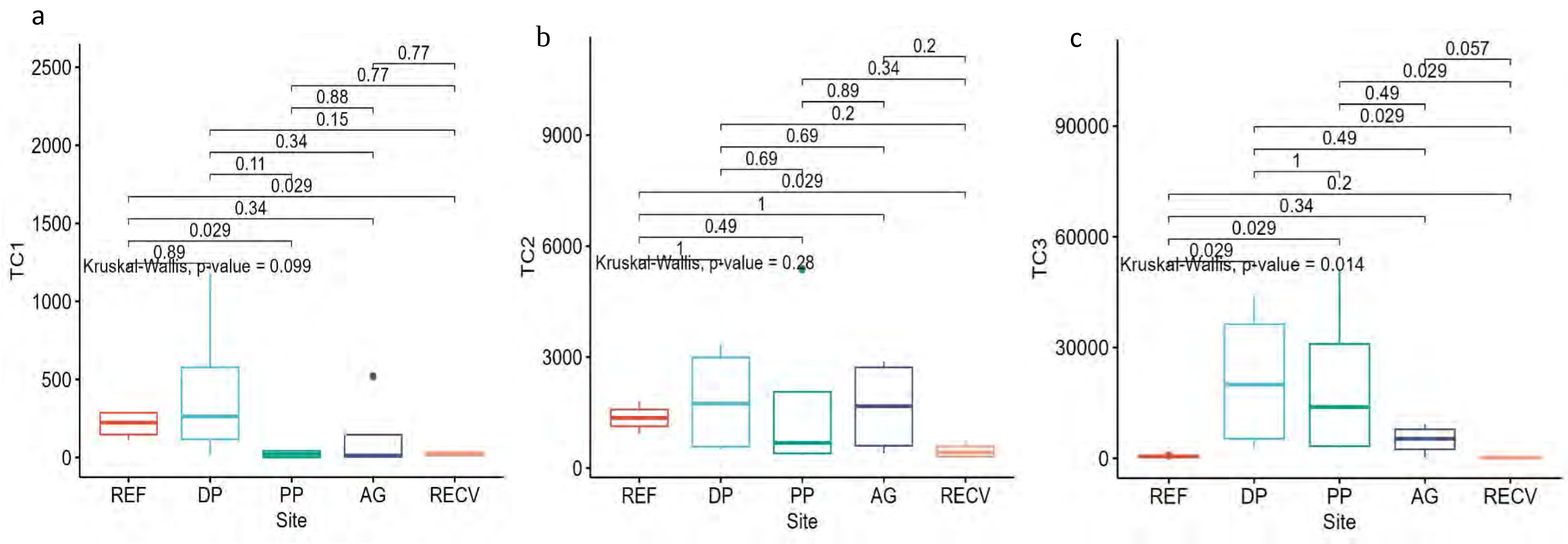
Appendix B3a: Boxplots of chironomid species abundances showing differences between the site categories for chironomids that (a) possess haemoglobin (H1) and (b) do not possess haemoglobin (H2)



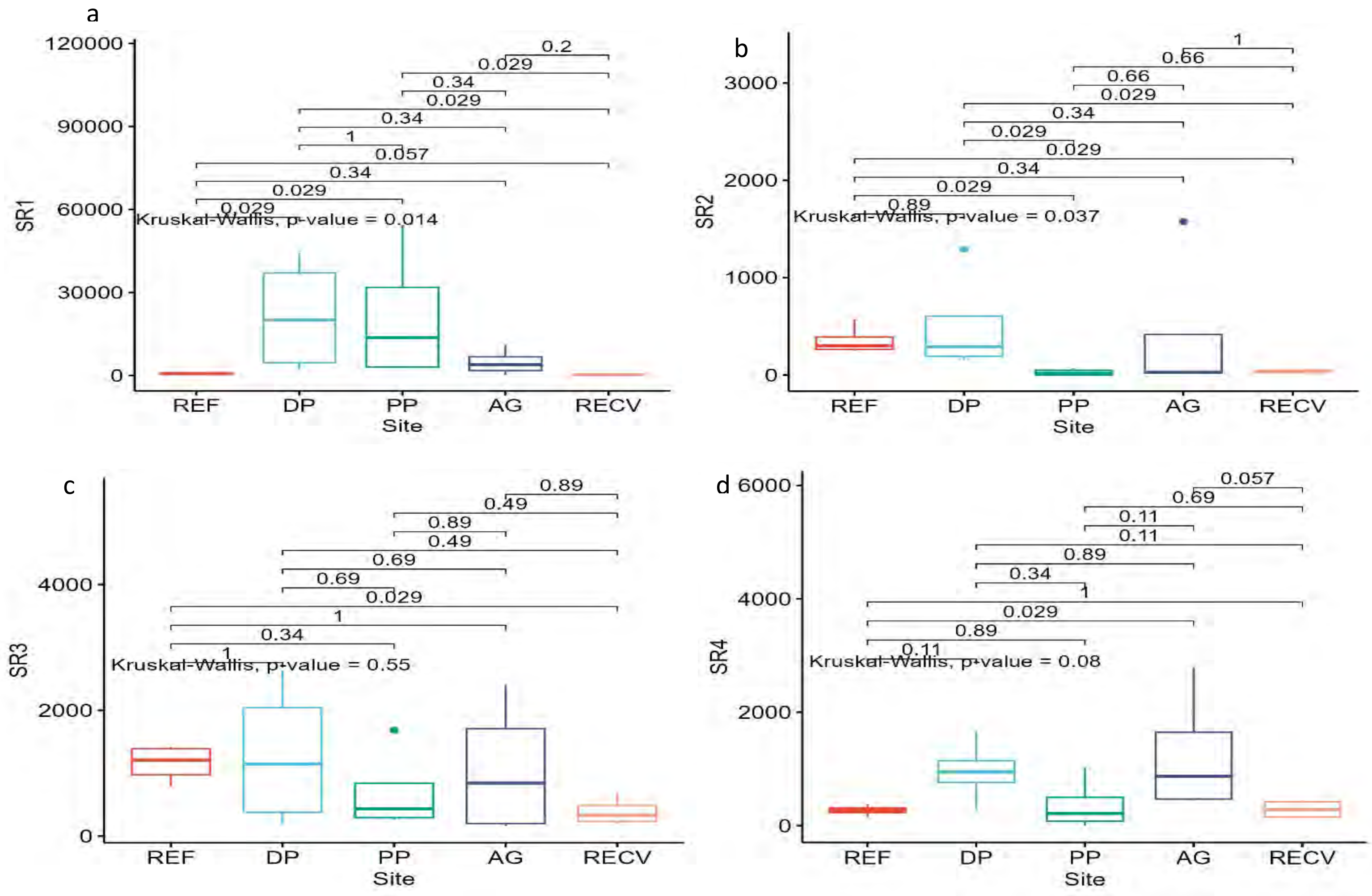
Appendix B3b: Boxplots of chironomid species abundances showing differences between the site categories for (a) chironomids species that possess tracheal gills (X1), (b) chironomids species that utilise cuticular respiration & (c) chironomid species utilising aerial respiration.



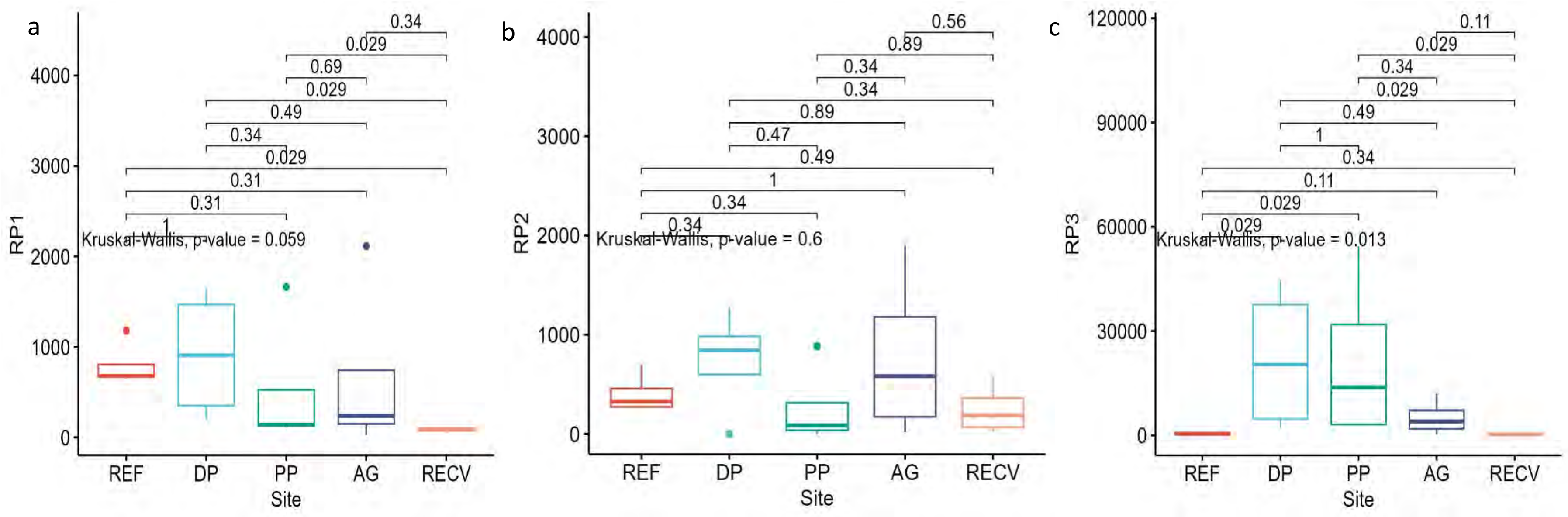
Appendix B3c: Boxplots of chironomid species abundances showing differences between the site categories for (a) chironomid species that lay eggs in clutches (RT1), (b) chironomids species that reproduce asexually by parthenogenesis (RT2) & (c) chironomids species that lay free isolated eggs (RT3)



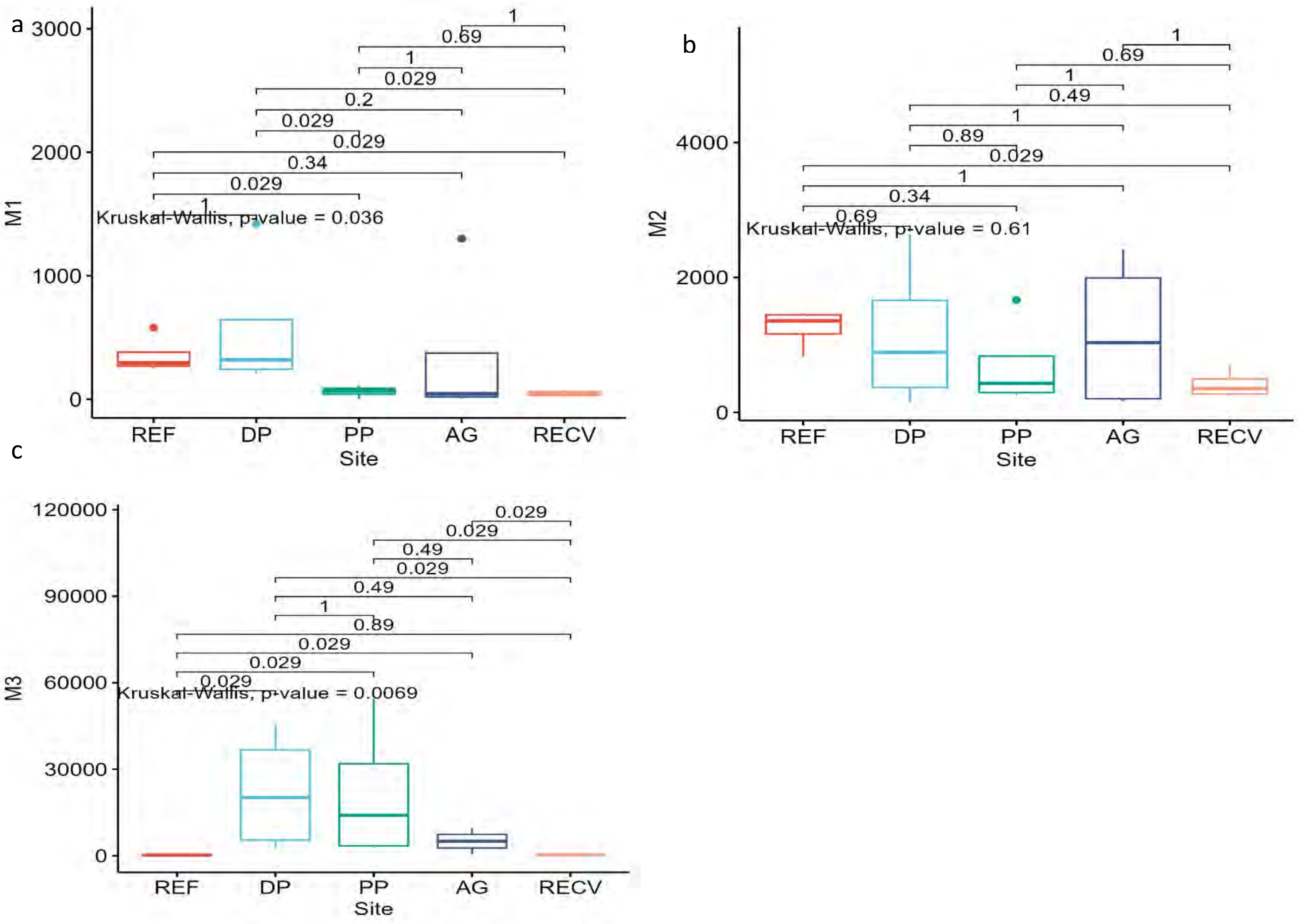
Appendix B3d: Boxplots of chironomid species abundances showing differences between the site categories for (a) chironomid species that do not construct tubes (TC1), (b) those that construct irregularly shaped tubes (TC2) and (c) those that construct rigid tubes (TC3)



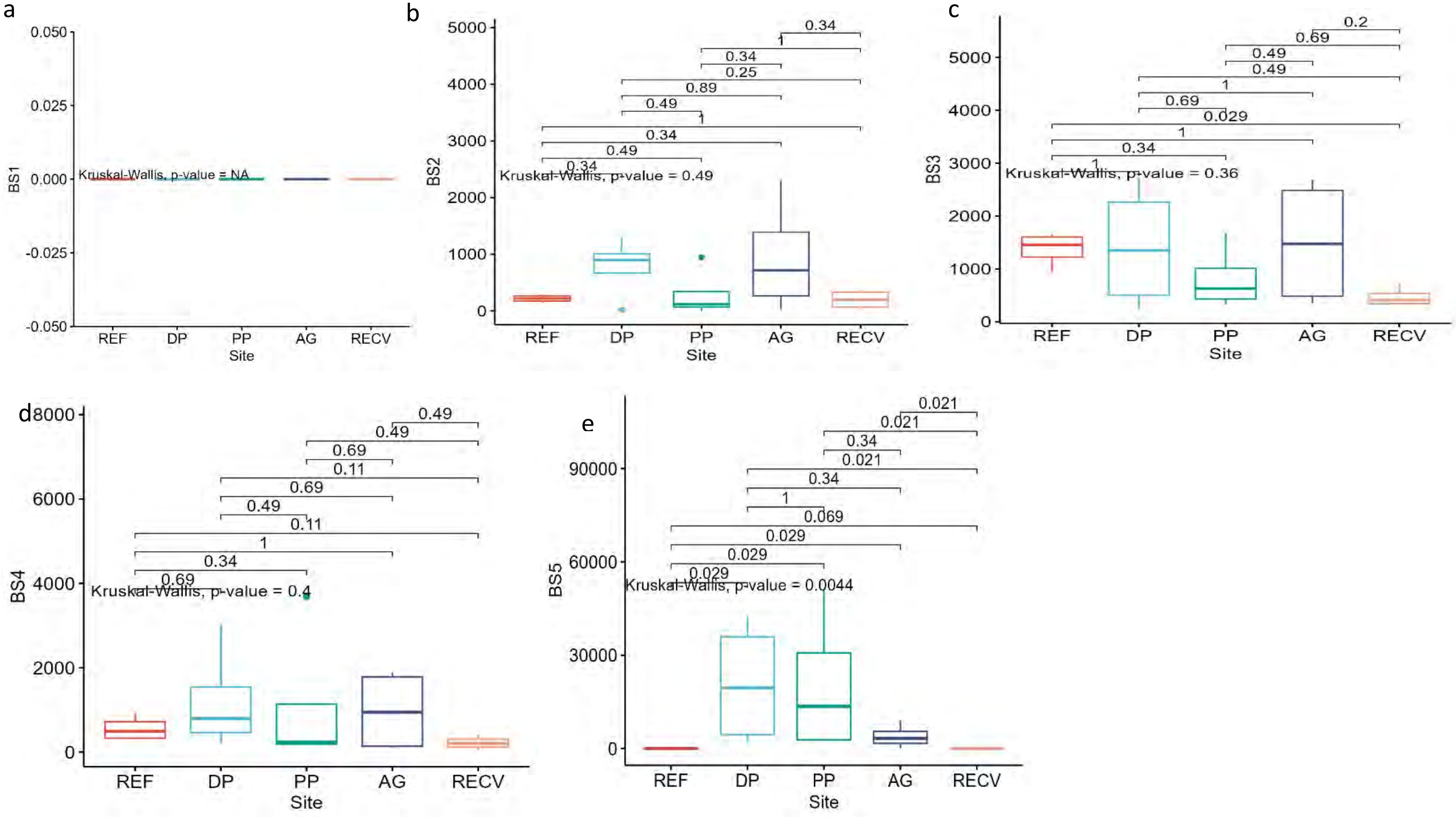
Appendix B3e: Boxplots of chironomid species abundances showing differences between the site categories for chironomid species that are (a) burrowers (SR1), (b) free-living (SR2), (c) miners (SR3), and (d) fixed/attached (SR4)



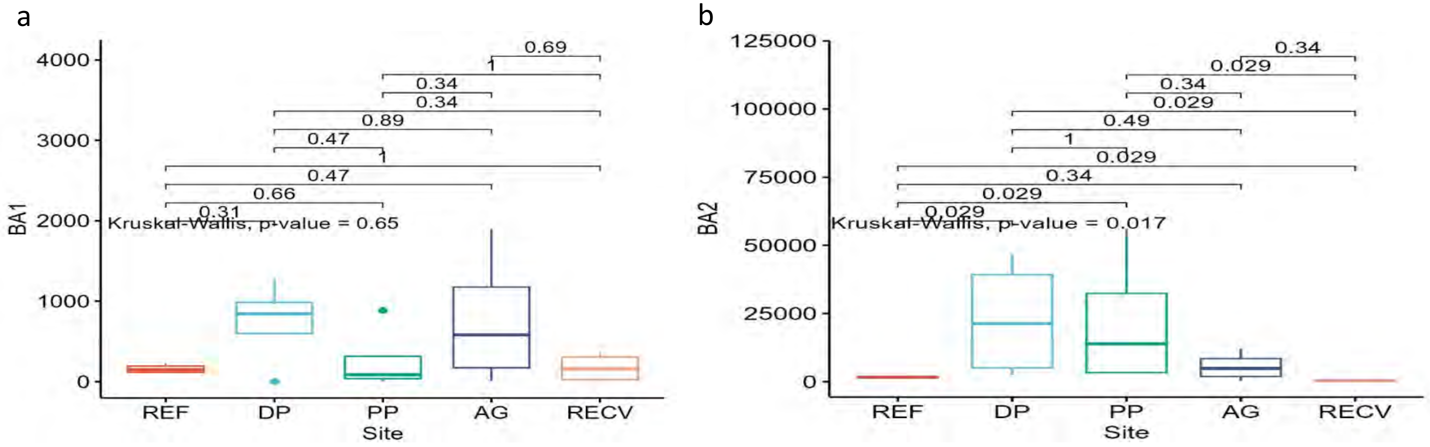
Appendix B3f: Boxplots of chironomid species abundances showing differences between the site categories for chironomid species that possess (a) 12 tracheae (RP1), (b) 6 tracheae (RP2), and (c) 3 tracheae (RP3)



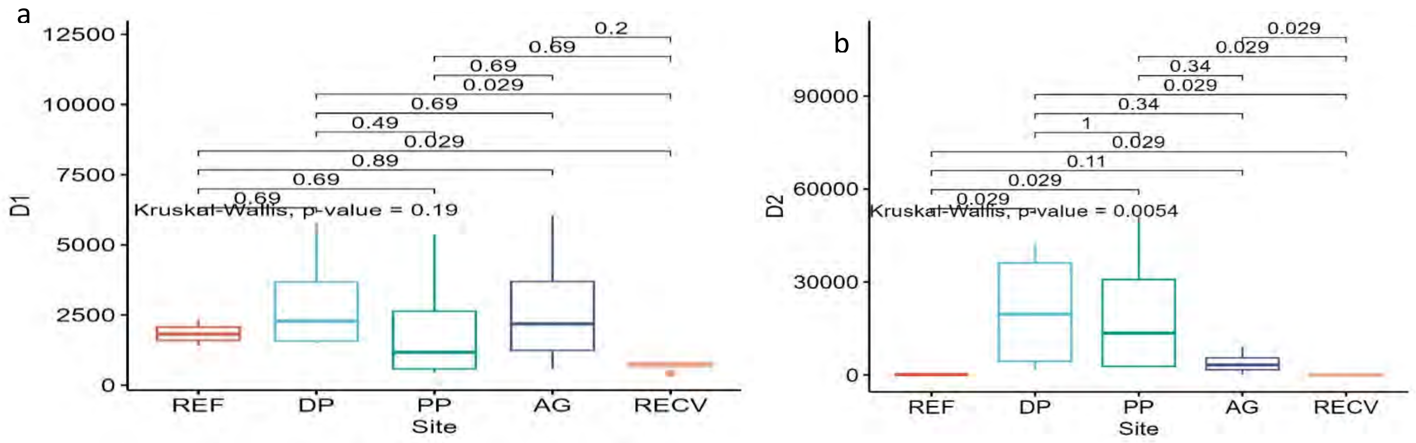
Appendix B3g: Boxplots of chironomid species abundances showing differences between the site categories for chironomid species that are (a) swimmers (M1), (b) crawlers (M2), and (c) Whole-body undulators (M3).



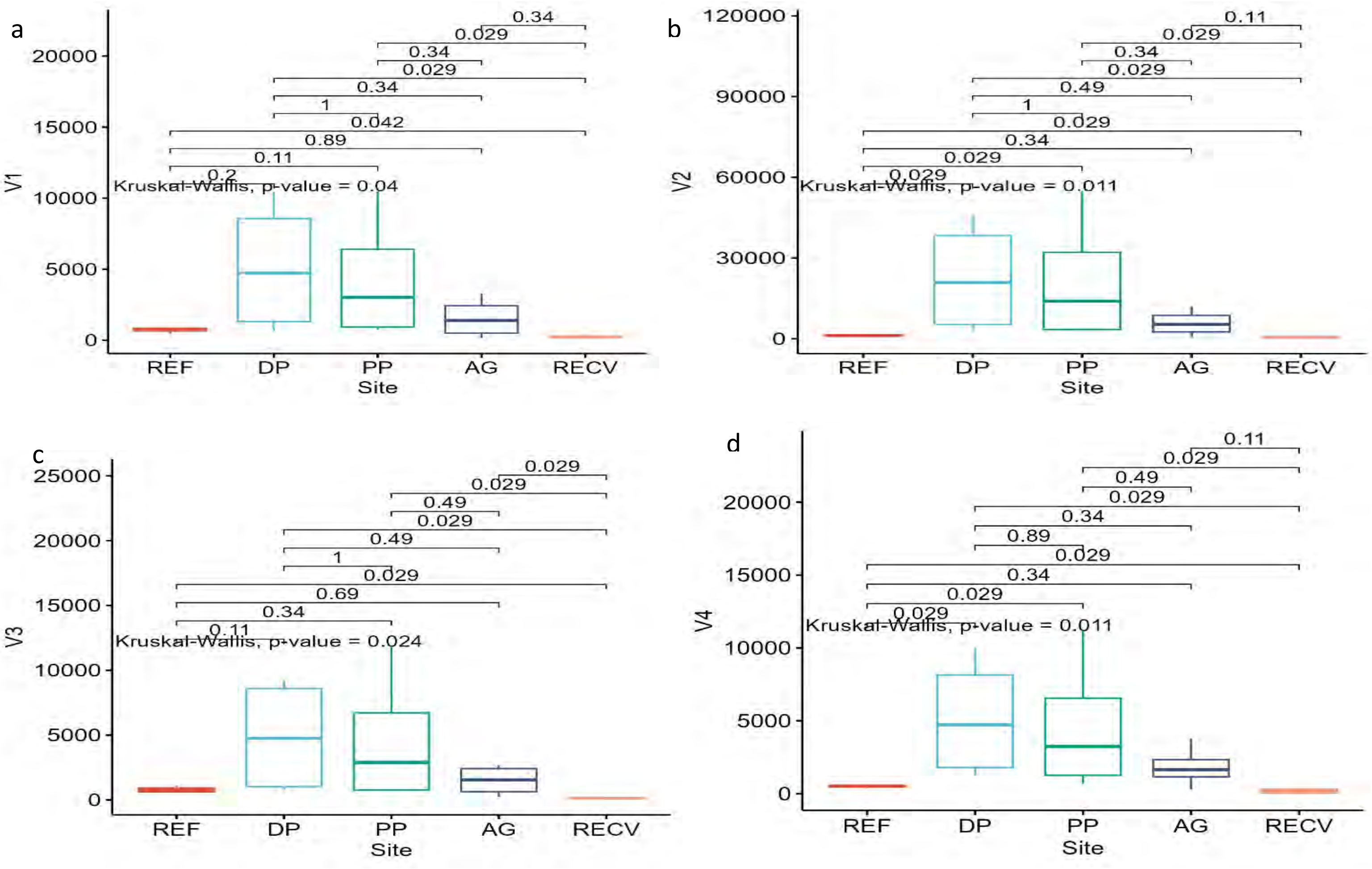
Appendix B3h: Boxplots of chironomid species abundances showing differences between the site categories for chironomid species that possess (a) very small (BS1), (b) small (BS2), (c) medium (BS3), (d) large (BS4), and (e) very large (BS5) body size.



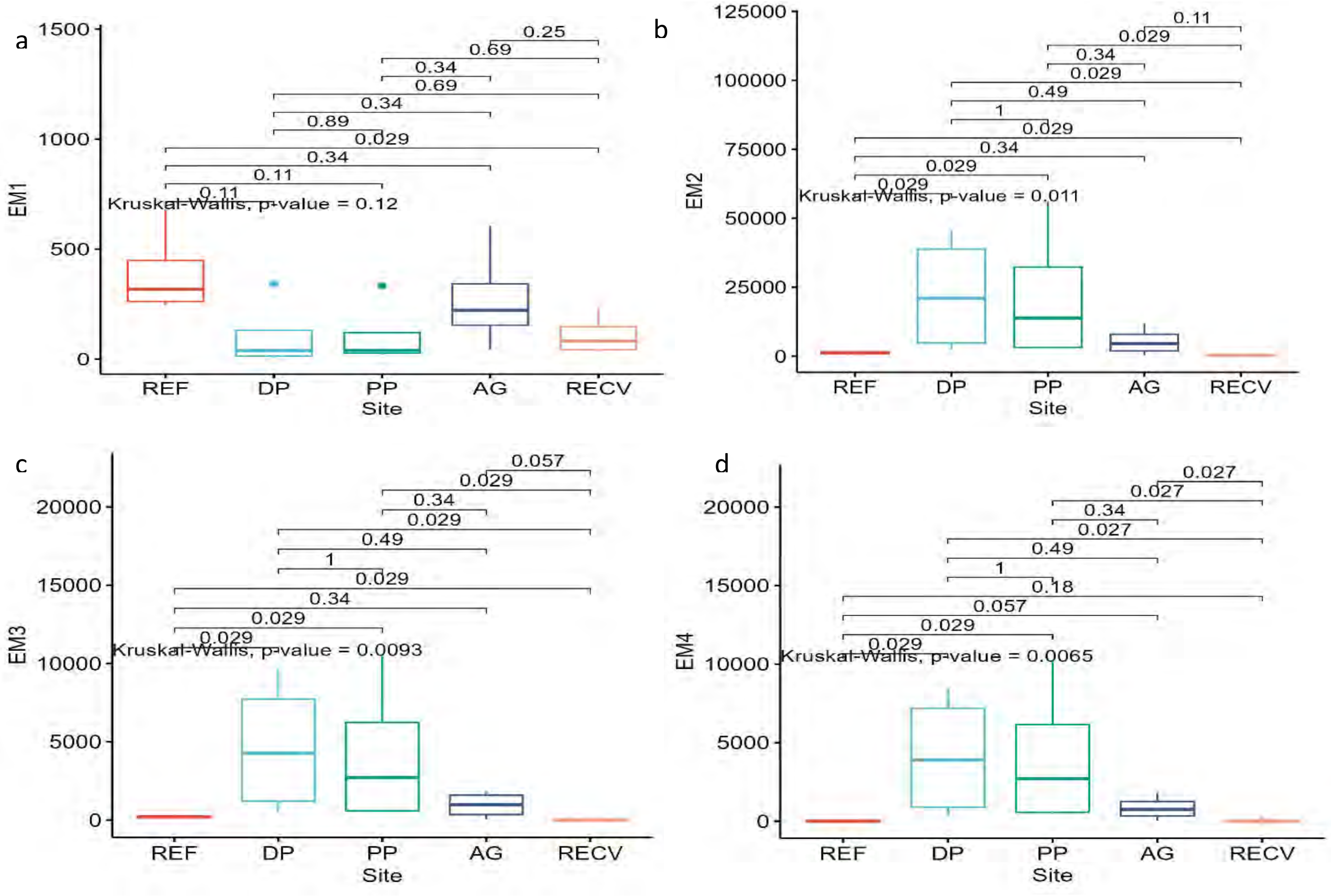
Appendix B3i: Boxplots of chironomid species abundances showing differences between the site categories for chironomid species that (a) possess body armour (BA1) and (b) do not possess body armour (BA2)



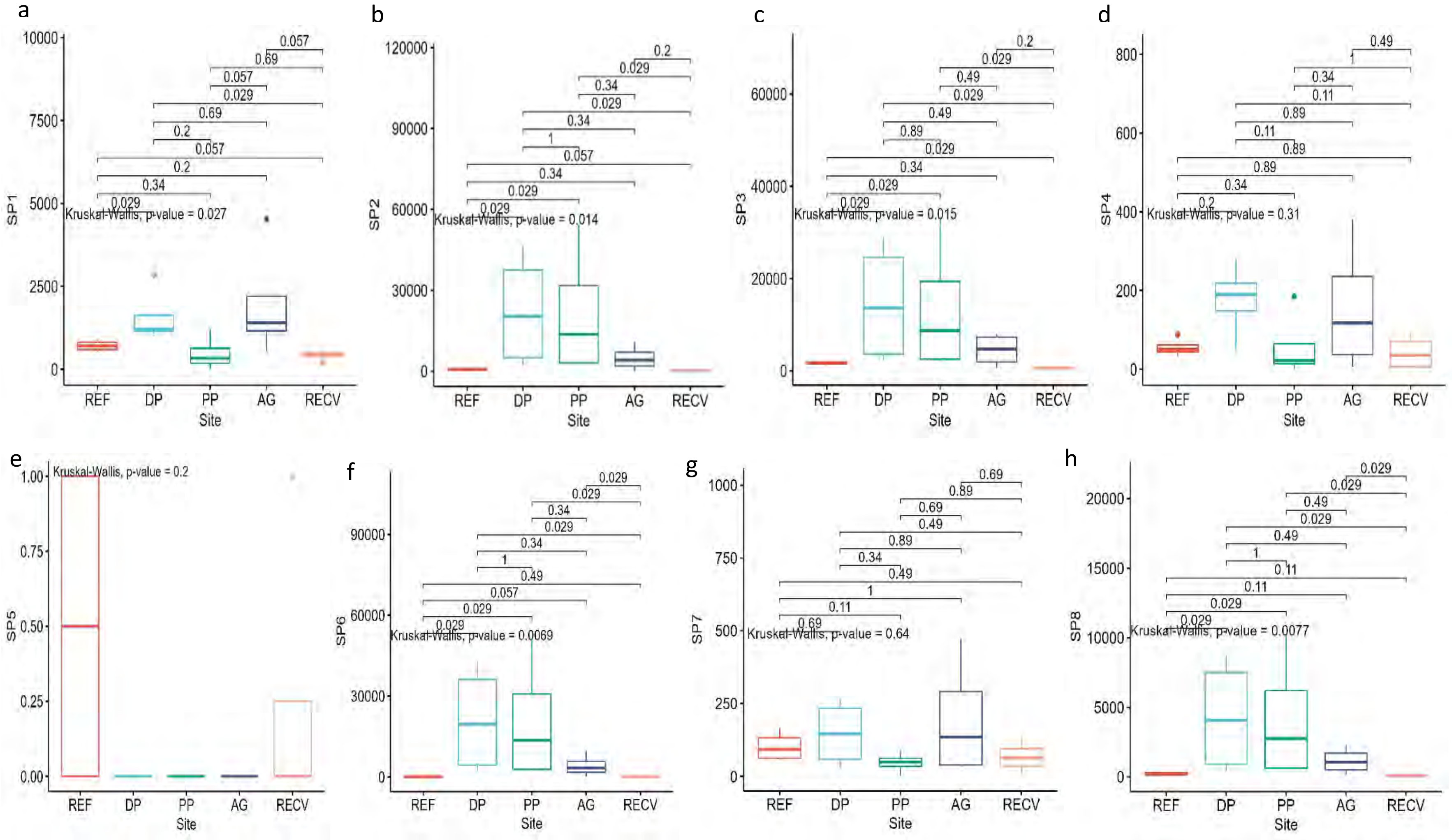
Appendix B3j: Boxplots of chironomid species abundances showing differences between the site categories for chironomid species that complete their lifecycle in (a) one year or less (D1) and (b) more than one year (D2).



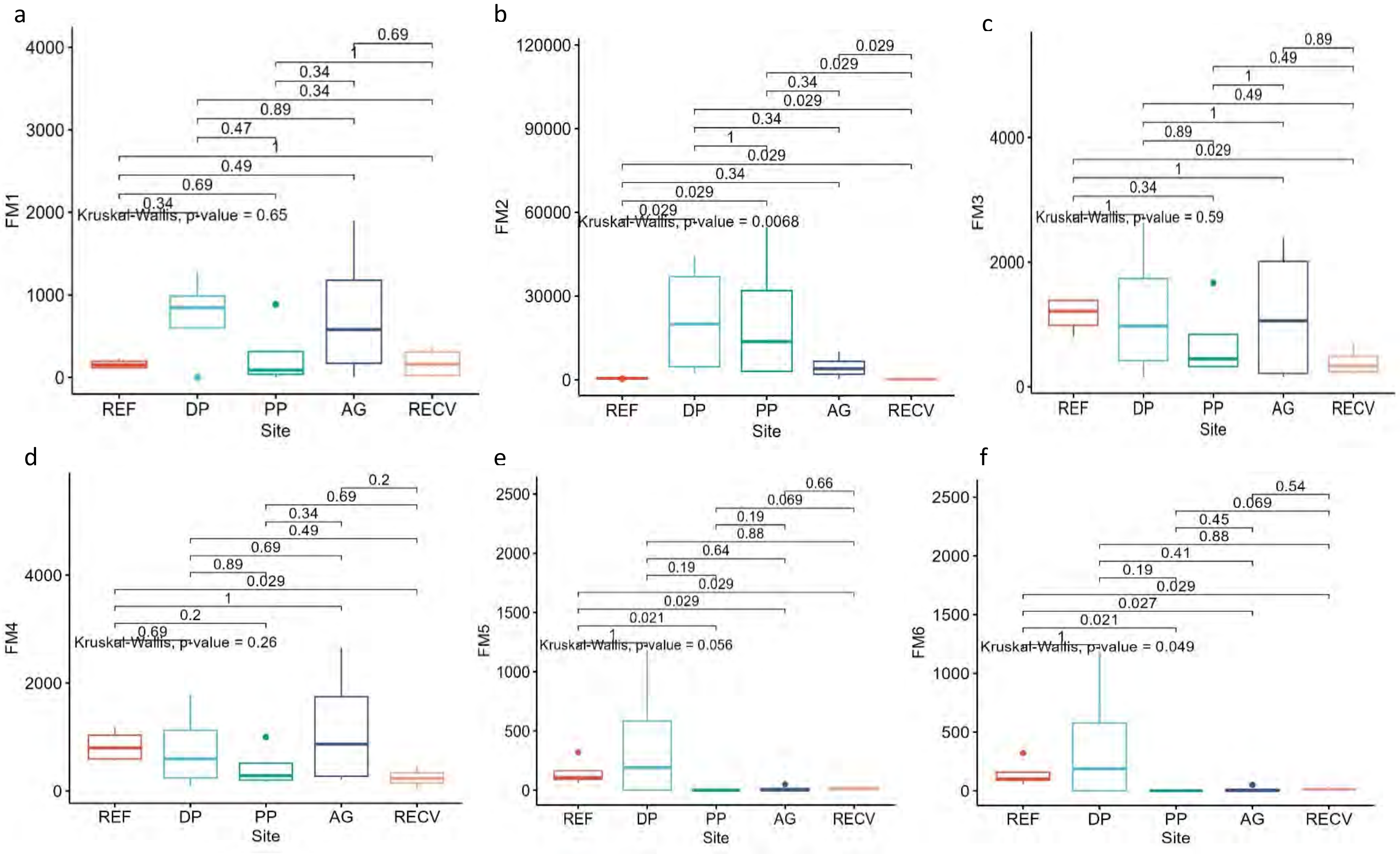
Appendix B3k: Boxplots of chironomid species abundances showing differences between the site categories for chironomid species that produce (a) one generation per year (V1), (b) two generations per year (V2), (c) three generations per year (V3), and (d) more than three generations per year (V4)



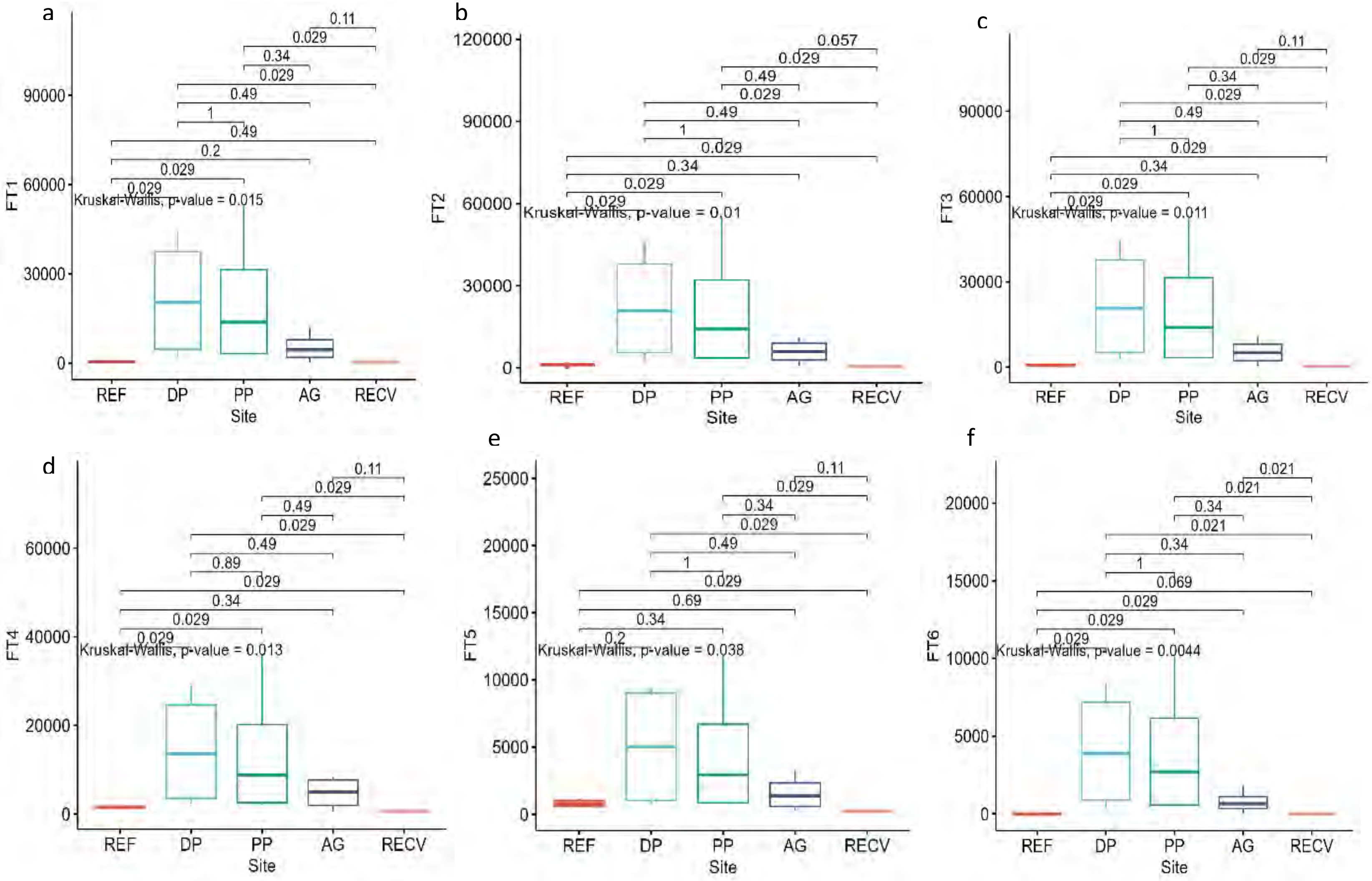
Appendix B3l: Boxplots of chironomid species abundances showing differences between the site categories for chironomids that lay (a) less than 100 eggs per egg mass (EM1), (b) 100-500 eggs per egg mass (EM2), (c) 500-100 eggs per egg mass (EM3) and (d) more than 1000 eggs per egg mass (EM4).

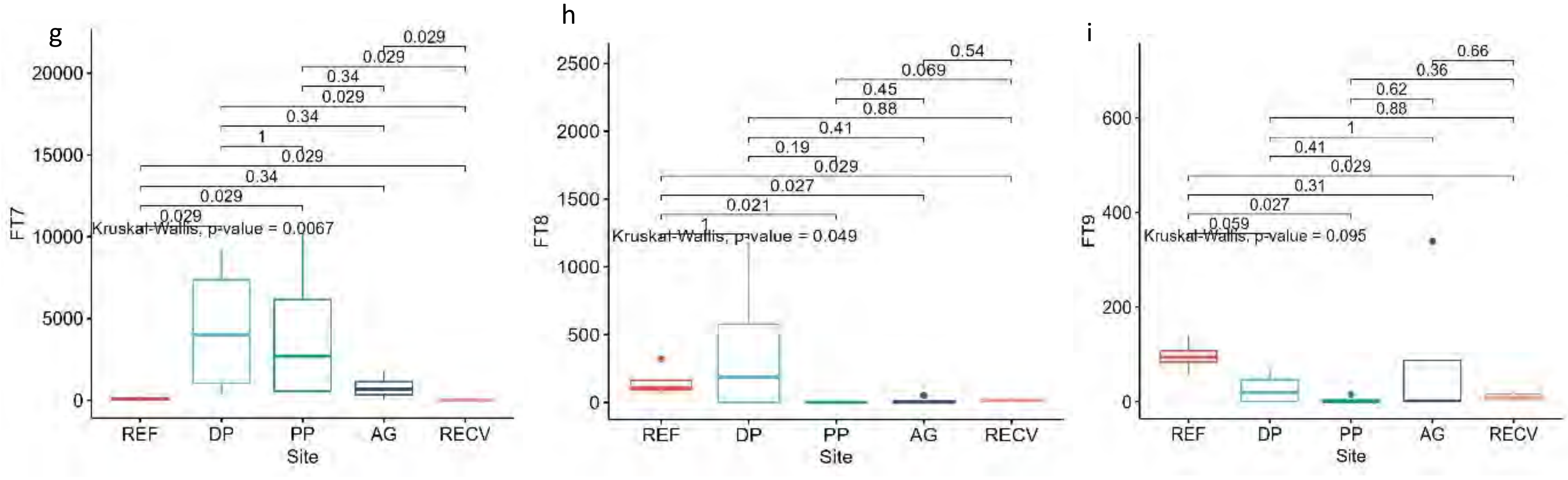


Appendix B3m: Boxplots of chironomid species abundances showing differences between the site categories for chironomids that prefer (a) stones/cobbles/pebbles (SP1) (b) gravel, sand, silt, mud (SP2) (c) macrophytes and filamentous algae (SP3), (d) microphytes (SP4) (e) twigs and roots (SP5) (f) litter, fine organic matter (SP6) (g) woods (SP7), & (h) mosses (SP8).



Appendix B3n: Boxplots of chironomid species abundances showing differences between the site categories for chironomids that are (a) collector-filterer (FM1), (b) collector-gatherers (FM2), (c) shredders (FM3), (d) grazers/scrapers (FM4) (e) engulfers (FM5) and (f) piercers (FM6).


a
FT1
0
30000
60000
90000
0.11
0.029
0.34
0.029
0.49
1
0.49
0.2
0.029
0.029
Kruskal-Wallis, p-value = 0.015
REF
DP
PP
AG
RECV
Site
b
FT2
0
30000
60000
90000
120000
0.057
0.029
0.49
0.029
0.49
1
0.029
0.34
0.029
0.029
Kruskal-Wallis, p-value = 0.01
REF
DP
PP
AG
RECV
Site
c
FT3
0
30000
60000
90000
0.11
0.029
0.34
0.029
0.49
1
0.029
0.34
0.029
0.029
Kruskal-Wallis, p-value = 0.011
REF
DP
PP
AG
RECV
Site
d
FT4
0
20000
40000
60000
0.11
0.029
0.49
0.029
0.49
0.89
0.029
0.34
0.029
0.029
Kruskal-Wallis, p-value = 0.013
REF
DP
PP
AG
RECV
Site
e
FT5
0
5000
10000
15000
20000
25000
0.11
0.029
0.34
0.029
0.49
1
0.029
0.69
0.34
0.2
Kruskal-Wallis, p-value = 0.038
REF
DP
PP
AG
RECV
Site
f
FT6
0
5000
10000
15000
20000
0.021
0.021
0.34
0.021
0.34
1
0.069
0.029
0.029
0.029
Kruskal-Wallis, p-value = 0.0044
REF
DP
PP
AG
RECV
Site



Appendix B3o: Boxplots of chironomid species abundances showing differences between the site categories for chironomids that prefer (a) fine detritus (FT1), (b) debris <1mm (FT2), (c) plant debris >1mm (FT3), (d) living microphytes (FT4), (e) living macrophytes (FT5), (f) Dead animals (FT6), (g) living microinvertebrates (FT7), (h) living macroinvertebrates (FT8), (i) Wood (FT9).