APPLICATION OF MACROINVERTEBRATE BASED BIOMONITORING APPROACHES TO ASSESS ANTHROPOGENIC IMPACTS IN THE SWARTKOPS RIVER, SOUTH AFRICA

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ABSTRACT

A growing human population accompanied by urbanisation and industrialisation have led to over exploitation and pollution of freshwater resources and have consequently impacted on aquatic ecosystem health. The Swartkops River in the Eastern Cape of South Africa is no exception. It drains a heavily industrialised catchment which has led to deterioration of its water quality due to pollution. Integrated water resources management (IWRM) requires the concurrent sustainable use of water resources and the protection of aquatic ecosystem health. Macroinvertebrates are well known for their ability to reflect the health of the environment in which they live, thus they were used to assess anthropogenic impacts in the Swartkops River for this study.

Macroinvertebrate based biomonitoring approaches, including the South African Scoring System version 5 (SASS5); a multimetric approach involving 19 metrics; Chironomidae community assessments and screening of morphological deformities in Chironomidae larvae, were applied at four selected sampling sites to assess environmental water quality in the Swartkops River. Macroinvertebrates were sampled using the SASS5 protocols. Chironomidae were mounted and identified as far as practically possible using available keys. Mentum, ligula, mandible, paraligula and antenna in Chironomidae larvae were screened for deformities. Physical and chemical water quality variables were measured at each of the selected sampling sites. All data were subjected to relevant statistical analyses.

Of the four sites sampled during the study period, results revealed that water quality at site 1 was the least impacted with highest SASS5 scores, average score per taxa (ASPT) values, richness, diversity, equitability and Ephemeroptera –Plecoptera-Trichoptera (EPT) richness, as well as least incidences of chironomid deformities. Water quality at site 2 was considered the next least impacted with higher SASS5 scores, ASPT values, richness, diversity and equitability, and lower incidences of deformities compared to sites 3 and 4. SASS5 scores and ASPT values revealed that both sites 3 and 4 were critically modified but the multimetric analysis, Chironomidae community assessment and incidences of deformities in Chironomidae larvae indicated that site 3 is the most impacted of the four sampling sites, with least species diversity, richness, equitability and highest incidences of deformities.

The study revealed the importance of multicriteria apporoach to environmental biomonitoring as an integrated water resources management tool, and based on the results, site 3, as the most impacted, could be prioritised for restoration intervention.

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ABBREVIATIONS AND ACRONYMS

AChE	Acetylcholinesterase
AMD	Acid Mine Drainage
ANOSIM	Analysis of similarity
ANOVA	Analysis of variance
APHA	American Public Health Association
ASPT	Average Score per Taxon
BMWP	Biological MonitoringWorking Party
BOD	Biochemical oxygen demand
CCA	Canonical Correspondence Analysis
DEAT	Department of Environmental Affairs and Tourism
DI	Deformity incidence
DO	Dissolved oxygen
DWA	Department of Water Affairs
DWAF	Department of Water Affairs and Forestry
EC	Electrical conductivity
EPA	Environmental Protection Agency
EPT	Ephemeroptera – Plecoptera – Trichoptera
ETOC	Ephemeroptera – Trichoptera – Odonata- Coleoptera
GSM	Gravel Sand and Mud
GST	Glutathion *S*-Transferase
HSD	Honestly Significant Difference
HSP	Heat Shock Protein
IBI 12	Index of Biotic Integrity 12
IHAS	Integrated Habitat Assessment System
IMR	Index of Morphological Response

ISAD	Index of Severity of Antennal Deformity
ISLD	Index of Severity of Ligula Deformation
IWRM	Integrated Water Resources Management
MTSI	Modified Toxic Score Index
NWA	National Water Act
NWP	National Water Policy
NWRS	National Water Resources Strategy
PCA	Principal Component Analysis
RDM	Resource Directed Measures
RHP	River Health Programme
RIVPACS	River Invertebrate Prediction and Classification System
RQO	Resource Quality Objective
SASS5	South African Scoring System version 5
SDC	Source Directed Controls
SIC	Stones in current
SIGNAL	Stream Invertebrate Grade Number Average level
SIMPER	Similarity Percentage
SISS	Sensitivity of Sensitive Species
SOD	Superoxide Dismutase
SOMI	Serra dos Órgãos Multimetric Index
SOOC	Stones out of current
TIN	Total inorganic nitrogen
TSI	Toxic Score Index
UN	United Nations
WRC	Water Research Commission
WWTW	Waste Water Treatment Works

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CHAPTER 1: GENERAL INTRODUCTION AND LITERATURE REVIEW

1.1 Introduction

World population growth continues to escalate rapidly, accompanied by rapid urbanization, industrialization and increased demand for food (Chamie, 2004). These factors have consequently led to over exploitation and pollution of already stressed freshwater resources leading to deterioration in water quality, a reduction in water quantity and degradation of freshwater biodiversity habitats (Jewitt, 2002; Hassan *et al.*, 2005). For example, industrialization over the past half century has led to huge increases in the discharge of toxic chemicals into fresh water bodies, some of whose toxicity are partly or totally unknown (Hassan *et al.*, 2005). In addition, many of these chemicals are persistent and could transform into by-products that may have adverse effects on water resources (Hassan *et al.*, 2005). As a result, the ability of freshwater ecosystems to provide clean and reliable sources of water, maintain the natural water cycle and the biological food web as well as provision of food and recycling of nutrients have been severely impaired (Hassan *et al.*, 2005). These have limited the amount of useable water available for biodiversity, for further economic and social developments as well as natural ecosystems functioning (Jewitt, 2002; Hassan *et al.*, 2005).

Several African countries have already reached, or passed, water stress thresholds where further economic development is hampered by shortage of water supply (Falkenmark, 1989; Ashton, 2002; Donkor, 2003). The present population growth trends and patterns of water use may cause more countries to experience chronic or acute water scarcity by the year 2025 (Ashton, 2002). The problem of water scarcity on the continent is further compounded by increased deterioration in water quality due to pollution, inadequate management strategies and lack of political will to implement and enforce existing policies (Falkenmark, 1989; Donkor, 2003; Swatuk, 2005; Adedeji and Ako, 2009). The reduction in water quantity and quality pose serious threats to sustainable use of water resources in Africa (Ashton, 2002). The challenges associated with water quantity and quality may have adverse negative economic impacts in most parts of southern and northern regions of Africa, where most countries witness incessant variable and erratic rainfall patterns, extreme temperatures and high evaporation rates coupled with problems of water pollution, inadequate management strategies and policies (Day, 1998; Donkor, 2003; Swatuk, 2005; Varis and Abu-Zeid, 2009).

Water resources in most parts of South Africa are limited and this is exacerbated in that the country is located in predominantly semi-arid part of the world (NWRS, 2004).The climate varies from desert and semi-desert in the west to sub-humid along the eastern coastal area with an average annual rainfall of about 450 mm (NWRS, 2004). The potential evaporation ranges from 1100 mm to 3000 mm, which is considerably higher than the average annual rainfall of the country (Davies and Day, 1998; Dallas, 2000; NWRS, 2004). It has been predicted that South Africa will experience severe water scarcity by 2030 (Mukheibir and Sparks, 2003) probably due to its climate, pollution and the increase demand for water to meet the needs of the growing human population. People living in South Africa rely mostly on surface water for their domestic, industrial and agricultural water supplies and although much water is abstracted directly from dams, the country's rivers are still over exploited due to scarcity of perennial lentic water bodies (Davies and Day, 1998). The over exploitation of river ecosystems in South Africa has impacted severely on their ecological integrity (Roux *et al.,* 1999; Thiere and Schulz, 2004; Oberholster *et al.,* 2008; Mantel *et al.,* 2010a; Simaika and Samways, 2010).

The Swartkops River in the Eastern Cape is an important freshwater ecosystem in South Africa and it is considered a vital ecological asset supporting an estuary rich in biological diversity (Taljaard *et al.,* 1998). The river also supplies water for agricultural requirements, particularly for irrigational purpose in its catchment (DWAF, 2003). It drains several industrial areas, including Uitenhage, Despatch and KwaNobuhle, and suffers varying degrees of perturbations resulting from human induced changes. Human activities in its catchment have resulted in a number of both direct and indirect impacts, which include impoundments, industrial and domestic effluent discharges, deforestation and agricultural land use (Taljaard *et al.,* 1998; DWAF, 1996a). Consequently, there is persistent deterioration in environmental water quality and a loss of biodiversity habitats in the Swartkops River (DWAF, 1996a; Baird *et al.,* 2004).

The anticipated further industrialization of the Eastern Cape economy, and increased urbanization of the population within the Swartkops River catchment in particular (DWAF, 2003; NWRS, 2004), may result in further deterioration in the environmental water quality of the river. Therefore, there is urgent need for proactive measures in order to incorporate the increasing water demand and pollution problems into management plans so as to conserve the river and its biota.

In this study, the South African Scoring System version 5 (SASS5), a multimetric approach, Chironomidae community assessment and screening of deformities in Chironomidae larvae were applied to the bioassessment of the Swartkops River health (particularly water quality). The approaches and results are introduced and discussed on the context of current water resource policy, law and implementation strategy in South Africa.

1.2 Water Resources Management in South Africa

The South African national Department of Water Affairs (DWA) is the official government department responsible for the implementation of policies and programmes related to South African water resources management. The DWA is responsible for ensuring that everyone in South Africa has access to good quality water and water for protection of ecosystems. The department is therefore the custodian of South African water resources.

1.2.1 The South African National Water Policy (NWP) and the National Water Act (NWA, No. 36 of 1998)

The South African National Water Policy (NWP) was formulated through a review process initiated in 1994 and finally adopted by Cabinet in 1997 (DWAF, 1997). It recognises equal access to good quality water by all South Africans as its central principle and identifies sustainability and equity as pivotal in the protection, use, development, conservation, management and control of water resources. That is, basic human and environment needs should be met and exploitation of water in all aspects including quality, quantity and reliability of supply must be sustainable in the long term (Palmer, 1999; Dallas, 2000). The NWP is given a legal backing through the National Water Act (Act No. 36 of 1998) being the legal instrument that relates to the management of South Africa's water resources (NWA, 1998). It also provides a legal mandate for the ecological assessment of South African water resources (NWA, 1998). The NWA is founded on principles which take into account efficient management of the country's water resources and equal access to good quality water by all South Africans. The Minister of Water and Environment Affairs is mandated by the NWA to draw the road map for the achievement of the principles enshrined in the NWP and by the year 2004, the first edition of the National Water Resource Strategy that contains clearly defined steps to accomplish the principles of the NWP, was released (NWRS, 2004).

1.2.2 The National Water Resource Strategy (NWRS)

The South African National Water Resource Strategy (NWRS) sets out the strategies, objectives, plans, guidelines and procedures for the protection, conservation, development, management and control of water resources within the framework of existing relevant government policies (NWRS, 2004). The Strategy provides actions to be taken to manage future water needs under four objectives, which include the establishment of a national framework for the management of water resources; establishment of frame work for catchment management strategies; provision of water resources management information to members of the public; and identification of water resource development opportunities and constraints. The NWA, NWP and the NWRS form the basis of the integrated water resources management in South Africa.

1.2.3 Integrated Water Resources Management (IWRM)

The Integrated Water Resources Management (IWRM) approach recognises the multi-faceted issues of social, environment, economic, policies and gender associated with water and therefore advocates a more comprehensive, integrated and holistic approach to deal with water resources management and has become an internationally accepted approach for managing water resources (UN, 2008). This has led to a shift from the traditional fragmented approach in which water resources were managed separately for different users and by different institutions without integration and coordination among relevant stakeholders (UN, 2008). IWRM has been defined as "a process which promotes the coordinated development and management of water, land and related resources, in order to maximize the resultant economic and social welfare in an equitable manner without compromising the sustainability of vital ecosystems" (NWRS, 2004 p 10). The IWRM definition provided above recognises the principles of equity, sustainability and efficiency in the management of water resources as well as collaboration among government agencies, institutions and all relevant stakeholders at international, national and catchment levels (NWRS, 2004).

Management of environmental water quality forms an important component of IWRM and thus becomes necessary to implement programmes that provide data needed for appropriate management interventions in managing environmental water quality (Palmer, 1999). The Resource Directed Measures (RDM) and the Source Directed Controls (SDC) are two approaches to ensure efficient management of water resources in South Africa, and

environmental water quality management forms an integral part of these two complementary approaches (NWRS, 2004). The RDMs are designed to protect water resources by managing water quality and quantity, condition of in-stream and riparian habitats, condition and distribution of the aquatic biota (NWRS, 2004). The RDM has four components: a system for classifying water resources; determination of Ecological Categories; determination of the Reserve and setting of Resource Quality Objectives (RQO). The classification of water resources is set to strike a balance between long-term ecological integrity and continuing water use for socio-economic developments. Water resources are classified into three management classes which include natural, moderately used or impacted and severely used or impacted while the Ecological Categories are classified as natural, good, fair and poor (Thirion, 2008). The Reserve identifies water requirements in terms of quantity, quality and reliability of supply for basic human needs and the functioning of the aquatic ecosystems (Hughes, 2005). The Basic Human Need Reserve provides for the essential needs of individuals served by the water resource in question and includes water for drinking, food preparation and personal hygiene. The Ecological Reserve relates to the water required in terms of quality and quantity to protect and maintain the aquatic ecosystems of the water resource.

The Resource Quality Objectives (RQO) set water management objectives for the resource according to its management class (NWA, 1998; NWRS, 2004). On the other hand, the Source Directed Controls (SDC) impose limits on the use of water resources through tools such as licenses, registrations and authorisations issued to water users either as individuals or corporates (NWRS, 2004). SDC is a strategy designed to avoid over exploitation of water resources in order to achieve the desired level of protection. It is the essential link between the protection of water resources and the regulation of their use (NWRS, 2004). The RDM and the SDC therefore serve as the means by which parts of the principles of the IWRM can be achieved.

As a practical means for routine monitoring and assessment of environmental water quality within the RDM and the SDC, the South African government has implemented a number of national water quality monitoring programmes with three core functions namely: data acquisition, data management and storage, and information generation and dissemination (DWAF, 2004). These monitoring programmes include Eutrophication Monitoring Programme (DWAF, 2002a; Rossouw *et al*., 2008), Radioactivity Monitoring Programme

(Sekoko *et al*., 2006), Toxicity Monitoring Programme (Murray *et al*., 2003), Physico-chemical Monitoring Programme, Microbial Monitoring Programme (DWAF, 2002b) and River Health Programme (RHP, 2006). These programmes contribute toward sustainable management and utilisation of fresh water resources. The River Health Programme (RHP), initiated by DWAF in collaboration with the Department of Environmental Affairs and Tourism (DEAT) and the South African Water Research Commission (WRC) uses biological organisms as its primary indicators to ascertain the ecological conditions of rivers while relying on physical and chemical factors for their interpretation (RHP, 2006).

1.3 The South African National River Health Programme (RHP)

The design of the River Health Programme (RHP) was initiated in 1994 by the then Department of Water Affairs and Forestry (RHP, 2006). The Programme was designed to generate the information needed regarding the ecological conditions of riverine ecosystems in South Africa (Roux *et al.,* 1999; RHP, 2006). Its overall goal was to expand the ecological basis of information on aquatic resources in order to support the rational management of the systems (Roux *et al.,* 1999; Dallas, 2000). The RHP utilises responses of in-stream biota to characterize the impacts of disturbances in aquatic ecosystems and incorporates several components of the biota, including fish, macroinvertebrate and riparian vegetation as its primary indicators in the monitoring of health of river systems while abiotic indicators such as habitat, geomorphology, hydrology and water chemistry form the framework within which the biotic results are interpreted (Roux *et al.,* 1999). The rationale is that the integrity of the in-stream biota provides a direct, holistic and integrated measure of the integrity of the river in which they live (Karr and Chu, 1997; RHP, 2006). Assessment tools with different indicator organisms such as fish, the Fish Assemblage Integrity Index (Kleynhans, 1999); riparian vegetation, the Riparian Vegetation Index and macroinvertebrates, the South African Scoring System (Dickens and Graham, 2002) have been developed for assessment of environmental water quality within the RHP. These tools utilize the responses of aquatic biota to characterise impacts of anthropogenic activities on in-stream biota. Responses of aquatic biota to changes in riverine ecosystems are useful in estimating past and present ecological conditions of rivers and streams (Barbour, 1999; Oberholster *et al.,* 2008; Arimoro, 2009; Hermoso *et al.,* 2010) and the field of biological monitoring (or biomonitoring) is devoted to the understanding of the response of in-stream biota to pollution

and other perturbations that might result from anthropogenic activities (Rosenberg and Resh, 1993; Karr and Chu, 2000; Novotny *et al.*, 2005; Bonada *et al.*, 2006).

1.3.1 Biological monitoring

Biomonitoring of aquatic ecosystems began to gain popularity after the saprobic system which established the conceptual basis for biomonitoring methods, and was based on the sensitivity of aquatic organisms to organic pollution (Bonada *et al.*, 2006). The traditional means of assessing the impacts of pollution on water bodies were through the measurement of physical and chemical parameters. However, it became clear that such measurements could not provide ecological information because the synergistic effects of pollution on aquatic biotic community may not be fully and easily assessed through chemical and physical measurements (Rosenberg and Resh, 1993; Vermeulen, 1995; Kasangaki *et al.*, 2006). Furthermore, only physical and chemical measurements cannot form the basis for biodiversity conservation. Biological assessment of stream conditions provides information needed for the conservation of biodiversity (Simaika and Samways, 2009). In South Africa for example, the Dragonfly Biotic Index (DBI) has been developed for prioritising and assessing site conditions for conservation purpose (Simaika and Samways, 2009).These short comings of physical and chemical water measurements necessitated the use of biological organisms to assess the impacts of anthropogenic activities on water quality in aquatic ecosystems and have given rise to a branch of ecology called biological monitoring (or biomonitoring). Biomonitoring is a product of the assumption that the response or "health" of biota is a reflection of the "health" of the environment in which they live (Rosenberg and Resh, 1993; Bonada *et al.*, 2006). It uses resident biota such plants, animals and micro-organisms to evaluate effects caused by anthropogenic stress on aquatic ecosystems. Stressed water bodies are often dominated by tolerant organisms with corresponding reduction in the number of sensitive ones. Biomonitoring uses the "health" or responses of biological organisms to evaluate changes in the environment that could provide indications of environmental stress and hence the need for remedial actions in stressed environment (Rosenberg and Resh, 1993). Biomonitoring approaches include a) community indices based on multivariate analyses (e.g. ordination, hierarchical clustering, analysis of similarity [ANOSIM], similarity percentage [SIMPER],); b) diversity indices (e.g. Shannon and Simpson indices) and c) biotic indices (e.g. Stream Invertebrate Grade Number Average

Level [SIGNAL, Australia], River Invertebrate Prediction and Classification System [RIVPACS, UK], the South African Scoring System [SASS]) (Jorgenson *et al.*, 2005).

Organisms that are sensitive to changes in their environment are called indicator organisms (Bonada *et al.,* 2006). Indicator organisms are differentially sensitive to environmental stress and are therefore suitable for biomonitoring programmes (Baptista *et al.,* 2007; Justus *et al.,* 2010). In addition, specific endpoints such as changes in growth rate, behaviour, expression of fluctuating asymmetry, morphological deformities and biochemical markers in indicator organisms are also used to detect changes in the environment (Bonada *et al.,* 2006; Faria *et al.,* 2006; Lee *et al.,* 2006; Ochieng *et al.,* 2008). These endpoints are called biomarkers (Bonada, *et al.,* 2006).

Different organisms have been used as biological indicators including algae, diatoms, fish and macroinvertebrates (Rosenberg and Resh, 1993; Barbour *et al.,* 1999). Macroinvertebrates live on the substrate, in vegetation and other available habitats of freshwater bodies for at least part of their lifecycle and are large enough to be seen with unaided eyes. They are usually employed in biomonitoring programmes in a variety of ways, such as monitoring changes in genetic composition, bioaccumulation of toxicants, toxicology testing in the laboratory and field measurement of changes in population dynamics, community composition or ecosystem functioning (Rosenberg and Resh, 1993; Bonada *et al.,* 2006). They have several advantages over other indicator organisms, which include their ubiquitous occurrence, abundance and ease of collection; a species richness that offers a wide spectrum of environmental responses; relatively sedentary lifestyles and therefore representativeness of local conditions; provide an indication of environmental conditions over varying time periods; differential sensitivity to a variety of pollutants and consequent capability of a graded response to a broad spectrum of stress; and serve as critical path-way for the transport and utilization of energy and matter in aquatic ecosystems (Camargo *et al.,* 2004; Bonada *et al.,* 2006).

Use of macroinvertebrates in biomonitoring of South Africa's water resources became popular with the development of the South African Scoring System (SASS), and was later incorporated into the river health programme as one of its biological indices. SASS is used as a tool for the biological assessment of environmental water quality in South Africa's riverine ecosystems (Chutter, 1998; Dickens and Graham, 2002).

1.3.2 The South African Scoring System (SASS)

The South African Scoring System (SASS) is a biotic index based on the presence of selected families of aquatic macroinvertebrates and their perceived sensitivity to water quality changes (Chutter, 1998). SASS is a modification of the Biological Monitoring Working Party (BMWP) Scoring System, and has undergone several improvements resulting in the current South African Scoring System version 5 (SASS5) (Chutter, 1994; Dickens and Graham, 2002). Macroinvertebrate families are awarded scores based on their perceived sensitivity in the range of 1 to 15 in increasing order of their sensitivity to water quality changes. SASS5 results are expressed both as an index score and as average score per recorded taxon (ASPT) value. SASS has been rigorously tested (Dallas, 1997; 2004; Vos *et al.*, 2002) and is widely used in South Africa as a tool for assessing river health conditions (Dickens and Graham, 1998; Fouche and Vlok, 2010; Mantel *et al.*, 2010a; 2010b). Its wide acceptance and application as a biomonitoring tool may be attributed to the following reasons:

i) It is easy and simple to operate; it does not require sophisticated equipment.

ii) The method is cheap and results are easily interpreted and conveyed to water resources managers.

iii) Sampling is generally non-destructive except where representative collections are required (Davies and Day, 1998)

iv) It provides an insight into the biological status of rivers in terms of environmental water quality (Chutter, 1998; Dickens and Graham, 2002).

However, because SASS is a rapid bioassessment method developed for rapid assessment of environmental water quality, it has some limitations. For example, it is a single biotic index and as such, it masks ecological information from all levels of the ecosystem that is, from individual, population and community levels. Several studies have shown that a multimetric approach (i.e. the use of multiple metrics from different ecosystems levels and functions) rather than a single biotic index would provide more robust ecological information needed for the management of water resources (Lücke and Johnson, 2009; Sanchez-Montoya *et al.*, 2010). In addition, because of its rapid assessment nature, taxonomic resolution in SASS is at the family level. Therefore, changes in species composition, abundance and distribution within a family in relation to environmental water quality changes may not be detected

(Roach *et al.,* 2000). These limitations posed the need for the exploration and possible application of other intensive methods that could be used along with SASS in the assessment of environmental water quality in the Swartkops River.

1.3.3 A Multimetric approach

Metrics are measurable parts or processes of a biological system that have been shown to change in value in response to changes caused by anthropogenic activities in an ecosystem (Ofenböck *et al.,* 2004). Multimetrics (i.e. the combined used of multiple metrics) have been frequently used for assessing water quality deterioration in rivers and streams (Klemm *et al*., 2002; Vlek *et al.,* 2004; Baptista *et al.,* 2007; Trigal *et al.,* 2009). The approach was first used in the USA for the assessment of biotic integrity of fish communities (Karr, 1981), and later for fish and macroinvertebrates (Ohio EPA, 1988a-c; Yoder and Rankin, 1995). A multimetric approach allows for integration of ecological information from individual, population, community and ecosystem levels to give a better picture of anthropogenic impacts on macroinvertebrate community structures and also the combination of metrics reduces the weaknesses of individual single metric (Karr and Chu, 1997; Ofenböck *et al*., 2004). Although it is possible to convert the various aspects of macroinvertebrate communities to a single quantitative score (Verdonschot 2000; Baptista *et al.,* 2007), several researchers have used the method to assess the impacts of various kinds of pollution without such conversion (Ravera, 2001; Böhmer *et al.,* 2004; Camargo *et al.,* 2004; Lücke and Johnson, 2009). This could be as a result of the criticisms such conversion has received that useful ecological information may be lost while reducing to a single score (Sutter, 1993; Polls, 1994; Bonada *et al.,* 2006) and to also allow the combination of metrics to reduce the weaknesses of individual single metric (Klemm *et al.,* 2002).

The different aspects of macroinvertebrate communities that may be included in a multimetric approach include measures of abundance, composition, richness, diversity, biotic indices and functional feeding groups (Klemm *et al.,* 2002; Camargo *et al.,* 2004). Measures of abundance is an enumeration method that deals with the counting of number of individuals within a taxon and composition gives the percentage of individuals within a taxon in relation to the total number of individuals in all recorded taxa in a sample while richness deals with the number of taxa in a sample (Klemm *et al*., 2002). Richness often considers a change in dominance of one or more taxa due to pollution or any other disturbance (Verdonschot, 2000). Diversity measures are metrics considered to decrease with increasing disturbances

(Verdonschot, 2000). Sensitivity measures rely on the assignment of sensitivity scores to taxa based on their perceived sensitivity or tolerance to pollution. Diversity indices can be grouped as either dominance indices or information statistics indices (Ogbeibu, 2005). Dominance indices are weighted towards the abundance of the commonest species (e.g Simpsons index) while information statistics indices such as Shannon index reflects individual taxon abundance (Ogbeibu, 2005; Gray and Delaney, 2008). The functional measures use the alteration in dominant feeding type under different types of disturbances to assess impacts of stress on in-stream macroinvertebrates (Verdonschot, 2000). For metrics to be useful to assess changes in environmental water quality, they should be able to respond and discriminate such changes from natural variations (Klemm *et al.,* 2002; Ofenböck *et al.,* 2004).

The multimetric approach has received considerable attention with the development of multimetric indices for use in the assessment of environmental water quality around the globe (Klemm *et al.,* 2002; Böhmer *et al.,* 2004; Camargo *et al.,* 2004; Ofenböck *et al.,* 2004; Baptista *et al.,* 2007; Trigal *et al.,* 2009). The Index of Biotic Integrity 12 (IBI 12) based on 12 sets of metrics has been developed for water quality assessment in all stream types in Germany (Böhmer *et al.,* 2004). The IBI 12 was found to detect the state of impairment of every stream type with Spearman's correlation value of R = 0.76 and was particularly sensitive to sewage effluent with Spearman's correlation value of R = 0.87 (Böhmer *et al.,* 2004). In Austria, stressor specific multimetric indices were developed for the assessment of Austrian rivers and streams (Ofenböck *et al.,* 2004). These authors found that the resulting indices were able to distinguished reference and slightly impaired sites from perturbed sites with accuracy of between 99.8% and 100%. In Brazil, Serra dos Órgãos Multimetric Index (SOMI) was developed for the assessment of water quality in the Serra dos Órgãos region, South-east Brazil (Baptista *et al.,* 2007). The index was found to be a robust easy-to-apply tool for biomonitoring programmes. Vlek *et al.* (2004) developed similar index for the assessment of water quality in the Netherlands.

Several studies have considered the performance of different metrics either for the assessment of a single stressor type or a combination of multiple stressors including the impacts of coal mining García-Criado *et al.* (1999); acid mine drainage, Gray and Delaney (2008); and sewage effluent, (Ravera, 2001; Lücke and Johnson, 2009). Freshwater habitat conditions (Simaika and Samways, 2011). Comparison of biological metrics and indices is important in

order to assess which metrics and indices are most suitable for the assessment of a particular stressor. Despite the considerable information that abounds on the use of multimetrics, the approach has received little or no attention in the assessment of environmental water quality in South Africa water resources.

1.3.4 Chironomidae community bioindication

Among families of aquatic macroinvertebrates, Chironomidae are probably the most widely distributed and species rich family constituting between 10 % and 50 % of the biomass of aquatic macroinvertebrates (Armitage *et al*., 1995; Cranston, 1996; Harrison, 2002; Porinchu and MacDonald, 2003). They occupy extremely varied biotopes because of their ability to tolerate a wide array of environmental water quality gradients and their extraordinary ecological range and environmental sensitivity make them particularly useful for assessing and interpreting changes in water quality of aquatic ecosystems (Dickman and Rygiel, 1996; Bhattacharyay *et al.*, 2006). Consequently, several studies have used them as indicators of environmental water quality changes (Janssens de Bisthoven and Gerhardt, 2003; Mousavi *et al*., 2003; Adriaenssens *et al*., 2004; Janssens de Bisthoven *et al*., 2005, Luoto, 2010). One of the limitations of SASS being a rapid assessment protocol, as mentioned earlier, is that its taxonomic resolution is at the family level and changes in species composition, abundance and distribution within the family in relation to environmental water quality changes cannot be detected (Roach *et al.,* 2000). This is primarily because SASS is a rapid assessment method and was not intended for detailed and rigorous assessments of streams (Dickens and Graham, 2002). However, despite the huge species richness in the family Chironomidae in South Africa water resources (Harrison, 2002), and several studies elsewhere (Adriaenssens *et al*., 2004; Wright and Burgin, 2009; Luoto 2010) that have revealed that the family Chironomidae has both pollution sensitive and tolerant taxa, the family has been assigned a highly tolerant score of 2 in SASS5 (Dickens and Graham, 2002). Therefore, more refined taxonomic assessment of this family could provide relevant information on their range of sensitivity to environmental water quality changes in South African water resources.

Changes in chironomid community composition may reveal evidence of industrial, agricultural, organic pollution and habitat degradation in aquatic ecosystems (Diggins and Stewart, 1993; Luoto, 2010). Diggins and Stewart (1998) used chironomid larval community composition to monitor the impact of trace elements in the Buffalo River, New York. They reported that mean chironomid density decreased with increasing trace elements

concentrations. Janssens de Bisthoven and Gerhardt (2003) investigated chironomid community structure in two unpolluted streams (Ovedsan and Skaralidbacken) and one chemically and thermally polluted stream (Ybbasrspan). The unpolluted streams had 13 and 16 taxa with *Microrendipes pedellus* dominating while the degraded stream had only 5 taxa dominated by *Procladius choreus*. Changes in diversity and structure of Chironomidae communities have been investigated along a gradient of heavy metal contamination and *Procladius* spp., *Tanytarsus* spp., and *Chironomus anthracinus* appear to be good indicators of heavy metal contaminations (Mousavi *et al.,* 2003). Adriaenssens *et al*. (2004) examined the diversity of chironomid communities and their role as indicators of water quality changes for the assessment of particular river types and water quality in Flanders, Belgium. These authors, grouped chironomid populations into three indicator groups, which include indicators of good water quality, indicators of waters enriched with nutrients and organic matter and taxa that were indifferent to changes in water quality. Janssens de Bisthoven *et al.* (2005) examined chironomid *faunistic* composition in sites impacted with acid mine drainage (AMD) and arsenic pollution. They reported 18 - 22 taxa at AMD impacted sites and 22 taxa at arsenic impacted sites compared to the 25 taxa recorded at the reference site. These authors also observed that AMD sites were characterized by a high proportion of the subfamilies Chironominae and predatory Tanypodinae and asserted that these subfamilies may be useful bioindicators for AMD pollution. Wright and Burgin (2009) reported changes in chironomid community in response to both organic and heavy metal pollution in the upper Grose River catchment. These authors concluded that Australian chironomids display strong pollution response. Chironomids had also been found to respond strongly to nutrient enrichment in Finland (Luoto, 2010). Although chironomids are particularly useful in the assessment of environmental water quality, a major limitation associated with the use of this group of macroinvertebrates is the inherent difficulties in their identification to species level (Harrison, 2002).

Assessment of environmental water quality based on changes in community structure either at species, genus or family levels of taxonomy can only detect altered environmental conditions when species or families of macroinvertebrates are lost from a site (Faria, *et al.*, 2006). Community structure assessments are essentially measures of lethality, and concentration of contaminants must be high enough to result in the disappearance of sensitive groups before community response assessment can detect impacts of stressors. Lethal end points are not suitable for use as early warning indicators of contaminations in streams (Faria,

et al., 2006). At sub-organism levels, biomarkers respond to sub-lethal concentrations of contaminants and are considered early warning signals for environmental water quality deterioration. Chironomids present a range of biomarkers that can be used in the assessment of environmental water quality.

1.3.5 Biomarkers in Chironomidae

Biomarkers are specific endpoints examined in indicator species (Rosenberg and Resh, 1993). Chironomids present an array of biomarkers including changes in life-history parameters, behavioural, biochemical and morphological responses. Different life-history parameters in chironomids such as growth rate, larval development time, fecundity, mortality, survival and behaviour have been used in ecotoxicological studies involving a variety of chemicals including heavy metals, radioactive elements, pesticides and arsenic (Heinis *et al.*, 1990; Postma and Davids, 1995; Janssens de Bisthoven *et al.*, 1998; 2001; Vermeulen *et al.*, 2000; Meregalli and Ollevier, 2001; Martinez *et al.*, 2006; Dias *et al.*, 2008). Heinis *et al.* (1990) exposed midge *Glyptotendipes pallens* to cadmium and quantified behavioural response by means of impedance response mechanisms. Slight deviation from the normal feeding behaviour was observed at concentrations of 0.1 to 1.0 mg/l of Cd and behaviour became erratic at concentrations of 5.0 to 10.0 mg/l. Postma and Davids (1995) exposed larvae of *Chironomus riparius* to different concentrations of cadmium over nine consecutive generations and reported increased larval development time, reduced hatchability of eggs and sharp decrease in fecundity. Growth rate was significantly retarded at concentration of 17 nMCd (1.7×10^{-5}mg/l Cd) of cadmium. Copper and lead have been reported to have negatively impacted on survival and mortality of *Chironomus riparius* (Janssens de Bisthoven *et al.*, 1998). Moulting in chironomids has been used as an end point to assess toxicity of metals. Vermeulen *et al.* (2000) recorded a decrease in moulting success of up to 52 % in *Chironomus riparius* exposed to 100 µgPb/l of lead and 29 % moulting retardation was observed when exposed to 500 µgHg/l of mercury. Meregalli and Ollevier (2002) exposed chironomid larvae to three different concentrations of 17α-ethynylestradiol and reported no effect on the survival of *Chironomus riparius*. Martinez *et al.* (2006) used larval length and larval head width as endpoints in assessing the toxicity of arsenic. By being exposed to arsenic, larval growth rate was affected by having smaller sizes and slower development rate in contaminated containers than those in the control experiment. Effects on survival, development time and growth were examined when chironomid larvae were

exposed to uranium and were all shown to be negatively affected by an increase in concentration of uranium (Dias *et al.,* 2008).

Interest in molecular assessment of sub-organism response to stress has grown rapidly. The advantage of molecular markers is that they provide internal monitors that respond to external stressors prior to the expression of other morphological responses (Butterworth 1995; Lee *et al.,* 2006a). Expression of heat shock protein (HSP) and haemoglobin (Hbs) have been examined as indicators of cadmium, nonylphenol, bisphenol-A, 17 α-ethynyl estradiol, bis (2-ethylhexyl) phthalate, endosulfan and paraquat dichromate induced stress in *Chironomus riparius* and *Chironomus tentans* (Hudson and Ciborowski, 1995; Lee *et al.,* 2006a). Higher incidences of active nucleoli in polytenic chromosomes have been associated with chironomid larvae exposure to pesticides (Meregalli *et al.,* 2002). Michailova *et al.* (2006) studied the genotoxic action of copper on the polytene chromosome of *Chironomus riparius* exposed to three concentrations, 0.005, 0.01, 0.05 mg/l of aqueous copper. They reported a significantly higher frequency of functional alterations, specifically decondensed centromeres and telomeres and reduction in activity of Balbiani rings, in treated materials compared to control. Changes in antioxidant enzyme activities and haemoglobin content in *Chironomus riparius* have been employed as biomarkers of physical (hypoxia, hyperoxia) and chemical (potassium dichromate, fenitrothion) stress (Choi *et al.,* 2000). According to these authors, hypoxia, hyperoxia and sub-lethal concentrations of potassium dichromate and fenitrothion caused an increase in Cu, Zn-superoxide dismutase (SOD) and Mn-SOD activities and a simultaneous decrease in glutathione peroxidise (GSH-Px) activities. They also observed a parallel increase in haemoglobin concentration in the haemolymph. Acetylcholinesterase (AChE) and glutathione *S*-transferase (GST) were used as biomarkers when *Chironomus riparius* larvae were exposed to four concentrations (0, 5, 10 and 50 ng/g) of the organophosphate insecticide pirimiphos methyl (Crane *et al.,* 2002). At 10 ng/g, these authors noted significant reduction in AChE activity while GST remained unaffected. Hahn and Schulz (2002) examined the synthesis of ecdysteroid in *Chironomus riparius* exposed to the endocrine disruptor bis (tri-*n*-butyltin) oxide (TBTO) and reported sex specific effects. There was significant reduction in the synthesis of ecdysteroid in female larvae at all concentrations (50, 500, 5 000 ng/ l) whereas elevated synthesis of ecdysteroid in male larvae at 500 ng/l was observed. Lee *et al.* (2006b) used expression of proteomic biomarkers such as *S*-adenosylmethionine decarboxylase, *O*-methyltranferase, phenol hydroxylase, thioesterase, zinc metalloprotease and aspartase as indicators of cadmium toxicity to *Chironomus riparius.*

Enzyme activities such as the activation of catalase and glutathione-*S*-transferase have been used as biomarkers in *Chironomus riparius* when exposed to bisphenol A and ethynyl estradiol (Lee and Choi, 2007). Apart from biochemical markers, several researchers have employed morphological markers (or responses) in chironomids to evaluate the effects environmental degredations (Lenat, 1993; Vermeulen, 1995; Nazarova *et al.,* 2004; Ochieng *et al.,* 2008) in aquatic ecosystems.

Morphological responses in chironomids to increased environmental stress and degradation have been used as early warning signals of environmental water quality deterioration and sediment toxicity. Warwick (1988) associated the darkening of chironomid head capsule pigmentation and the thickening of body wall cuticle to the presence of high concentrations of pollutants. Several studies have shown high correlations between incidences of chironomid head capsule deformities and levels of pollution (Lenat, 1993; Diggins and Stewart, 1998; Nazarova *et al.,* 2004; Ochieng *et al.,* 2008). Morphological deformities are thought to be responses to chemical stressors only (Lenat, 1993; MacDonald *et al.,* 2006).

1.3.6 Morphological deformity in Chironomidae

The term "deformity" refers to morphological features that depart from the normal Chironomidae larval configuration (Warwick, 1985; Nazarova *et al.,* 2004). Effects produced by mechanical wear, breakage or abrasion are usually not included in deformity screening and are recognised by their "chipped" or "rough" edges (Vermeulen, 1995; Bird, 1997; Nazarova *et al.,* 2004). Because chironomid larvae live in close association with sediments and often feed on organic detritus and associated algae, deformities in chironomids can be used as biomonitoring tool for assessing sediment toxicity and environmental water quality deterioration (Vermeulen, 1995). Morphological deformities in chironomids represent sub-lethal effects of exposure to contaminants and are considered an early warning indicator of environmental water quality deterioration caused by chemical contaminants. Assessments of these deformities offer an effective and cost friendly means of assessing impacts of environmental stressors on aquatic ecosystems (Meregalli *et al.,* 2002). The assumption behind the use of deformities as a biomonitoring tool is that, incidences of deformities in chironomid communities higher than 8 % of the total number of larvae can be taken as an indicator of environmental contamination (Nazarova *et al.,* 2004; Ochieng *et al.,* 2008).

The earliest reports of deformities in the mouthparts and the thickening of the body wall in chironomids exposed to a wide array of chemical contaminants came from the sediments of Lake Erie and Lake Ontario in Canada (Hamilton and Saether, 1971). Paleolimnological studies by Warwick (1980) estimated the background incidence of chironomid deformities to vary between 0 and 0.8 % from the Bay of Quinte in Canada. However, Warwick 1980's estimation was based on fossil materials and incidences of deformities in slightly contaminated areas which are use as reference sites in field studies have been reported to vary between 0 and 8% above which, site is taken to be contaminated (Warwick 1985; Vermeulen, 1995; Nazarova *et al.,* 2004; Ochieng *et al.,* 2008). Chironomid deformities were used to evaluate the impacts of sewage effluent in northern Nova Scotia, Canada (Mac Donald *et al.,* 2006). In America, interest in the use of deformities for the assessment of sediment stress and environmental water quality has grown rapidly. Lenat (1993) utilized mentum deformities of *Chironomus* larvae to evaluate the effects of toxicity and organic loading in North Carolina streams while Diggins and Stewart (1993) reported incidences of chironomid deformities in the Buffalo River in New York. The impacts of various effluents on the morphology of chironomid larvae in the Yamaska River have been examined by Bird (1994). Diggins and Stewart (1998) further assessed the impacts of trace elements in Buffalo River using chironomid larval deformities. Martinez *et al.* (2002) examined the potential of chironomid deformities as biological indicators of heavy metal contamination in the Coeurd' Alene River Idaho in USA, and found them to be important indicators of heavy metal contamination.

In New Zealand, Jeyasingham and Ling (2000) utilized chironomid larval deformities to evaluate the impacts of contaminants and seasonal influence on head capsule deformities in *Chironomus zealandicus*. Deformities have also been used to assess the impacts of heavy metals in River Damodar in India (Bhattacharyay *et al.,* 2005 and 2006). However the subject has attracted little attention in Africa (Janssens de Bisthoven and van Speybroeck, 1994; Ochieng *et al.,* 2008). Janssens de Bisthoven and van Speybroeck (1994) reported elevated levels of chironomid mouth part deformities from Lake Victoria in Kenya. Ochieng *et al.* (2008) utilized mouthpart deformities in Chironomidae as indicators of heavy metal pollution in northern Lake Victoria, Uganda. Despite the huge species richness of chironomids in South African water resources (Harrison, 2002), their morphological deformities have not been used to assess environmental water quality changes resulting from human influences.

Morphological structures examined for deformities

Several structures including the mentum, mandible, ligula, paraligula, pecten epipheryngis and antennae in Chironomidae larvae have been examined for deformities. Saether (1979) noted the occurrence of aberrant mouthparts, thickened cuticle and darkened head capsule pigmentation in several chironomid larvae from heavily polluted Canadian Lakes. Warwick (1985) examined deformities in the antennae of *Procladius* species in contaminated rivers while Servia *et al.* (1998) documented loss of segments, displacement of sensory organs and abnormal structures of unknown homology in the antennae of *Prodiamesa olivacea* in the organically polluted River Sar in Spain. Jeyasingham and Ling (2000) observed antennal deformity in arsenic and organically polluted Hamilton and Ngaroto Lakes in New Zealand. In chemical and thermally polluted rivers, Janssens de Bisthoven and Gerhardt (2003) noted antennal deformities. Bhattacharyay *et al.* (2005) reported similar conditions in the antennae of several chironomid species in the heavy metal polluted River Damodar in India. Chironomid antennae have proved to be extremely sensitive to pollution in many of these studies, but were sensitive only in situations of low level contamination and beyond this they cease to respond morphologically to increased chemical stress (Warwick, 1988).

The structures that are frequently used and have imparted the greatest amount of information on sediment toxicity and environmental water quality deterioration are the mouthparts. In trying to classify North Carolina streams, Lenat (1993) used mentum deformities as a basis for stream classification. The ligula, mentum, mandible, paraligula and pecten epipheryngis have been examined by several researchers (Warwick, 1985; Servia *et al.*, 2000; Janssens de Bisthoven *et al.*, 2003; Veroli *et al.*, 2008). The argument in favour of these structures is that they are easily prepared for examination and are available as fossil materials allowing for assessment of environmental health through time (Warwick, 1988; Burt, 1998; Jeyesingham and Ling, 2000).

Causative agents of chironomid deformities

Several environmental contaminants have been reported as causal agents for deformities observed in chironomid larvae. Diggins and Stewart (1998) associated high mouthpart deformities rate to trace element contamination in the Buffalo River, New York, where occurrence of deformities increased significantly with exposure to high trace elements levels. Jeyasingham and Ling (2000) in their examination of incidences of head capsule deformities

in *Chironomus zealandicus* in some water bodies of New Zealand attributed the high incidences of deformities recorded in the polluted sites to different pollutants such as organic pesticides and arsenic. Martinez *et al.* (2002) in assessing the potential association between menta deformities and trace elements in the Coeurd' Alene River, reported deformity rates between 3.8 and 10.3% at contaminated sites compared to 0.9% recorded at control or uncontaminated stations. There was a significant correlation between metal concentrations and deformity rates for all metals including As, Cd, Cu, Pb and Zn except Ni. They suggested that the larvae were able to regulate Ni by excretion. In India's River Damodar, there was positive correlation between chironomid mouthpart deformities and the concentration of copper (Bhattacharyay *et al*., 2006). Such positive correlations have also been obtained between copper, zinc and mouthparts deformities in Lake Victoria (Ochieng *et al*., 2008). Chironomid deformities have been attributed to organic pollution (Servia *et al.,* 1998; 2000; MacDonald *et al.,* 2006); heavy metals (Lenat, 1993; Jassen de Bisthoven and Gerhardt, 2003; Nazarova *et al.* 2004; Ochieng *et al.,* 2008) and acid mine drainage (Janssens de Bisthoven *et al.*, 2005).

Several laboratory studies have demonstrated the deformity inducing capacity of different compounds. Madden *et al.* (1992) induced deformities in *Chironomus* sp. larvae using DDT and Dacthal® and a positive correlation between increasing DDT concentrations and mentum deformities was noted but there was no effect of DDT on antennal deformities. Dacthal® induced both mentum and antennal deformities, but no clear dose-response curve was detected. Janssens de Bisthoven *et al.* (1998) demonstrated the deformity induction capacity of copper and lead, and reported abnormalities in the mentum and mandibles. Vermeulen *et al.* (2000) carried out experiments exposing *Chironomus riparius* to sediment spiked with lead, mercury and β-sitosterol. Significant deformations in the pecten of chironomid larvae in aquaria containing lead and mercury were observed but deformation of the mentum was not significantly higher than those in the control although a concentration response relationship in the mentum of larvae exposed to mercury was observed. *Chironomus riparius* was exposed to 4-n-nonylphenol and the frequencies of mentum deformity increased significantly with increasing concentrations of the compound (Meregalli *et al.,* 2001). Meregalli and Ollevier (2001) exposed *Chironomus riparius* to 17α-ethynylestradiol but no adverse effect on survival and deformities were found. Martinez *et al.* (2003) obtained an inverse relationship when *Chironomus tentans* was exposed to three different concentrations of cadmium in which higher incidences of deformities were observed in the lower concentration. Arsenic has

been reported as a deformity causal agent in *Chironomus tentans* (Martinez *et al.*, 2006). Dias *et al.* (2008) also observed an inverse relationship between *Chironomus riparius* mouthpart deformities and four concentrations of uranium in which deformities were highest in the lowest concentration.

Chironomidae deformities: are responses contaminant-specific?

Different types of morphological deformities have been reported in both field and laboratory studies using chironomids for the assessment of environmental water quality deterioration and sediment toxicity and they may be attributed to different contaminant types (Warwick, 1988). In Vermeulen's 1995 review, it was observed that proportional occurrence of different deformity types shifted somewhat consistently according to the type of prevailing pollution. For example, in sites contaminated with heavy metal pollution, the Köhn gap deformity has a high proportion of occurrence compared to sites polluted with organic sewage (Lenat, 1993; Servia *et al.*, 2000; Martinez *et al.*, 2002; MacDonald *et al.*, 2006). It seems that mentum gaps, or the so-called Köhn gaps, are mostly induced by heavy metal contamination and may have high discriminatory power to indicate the presence of heavy metals (Vermeulen, 1995). However, there appears to be some degree of overlap among different types of deformity induced by different heavy metals. For example, Janssens de Bisthoven *et al.* (1998) in a multigenerational experiment, tied split medial mentum teeth to a combination of copper and lead exposure. Janssens de Bisthoven *et al.* (2001) associated split medial mentum teeth and premandible deformities to cadmium contamination. Missing and split teeth were the most frequent deformity type when *Chironomus tentans* was exposed to copper (Martinez *et al.*, 2003).

Some studies have shown that increase in contaminant concentration could induce more severe deformities. Martinez *et al.* (2003) reported fused teeth, Köhn gaps and missing teeth as the most frequent deformity types in tanks with 9μgg^{-1}Cd dry weight of cadmium. At a concentration of 39μgg^{-1}Cd dry weight of cadmium deformed mandibles, asymmetrical menta, missing teeth and fused teeth were reported as the predominant deformity types but at concentration of 61μgg^{-1}Cd dry weight, missing teeth and highly deformed mandibles were reported as the prevalent deformity types. When *Chironomus riparius* was exposed to arsenic at three different concentrations, 30, 130 and 260μgg^{-1}As dry weight, different deformity types dominated at the different levels of concentrations (Martinez *et al.*, 2006).

Contaminant or concentration specificity of deformities may prove advantageous for its analytical capacities, but to a large extent, it remains a hypothesis because most deformity types such as split medial mentum teeth, missing teeth, fused teeth and asymmetrical mandibles seemed to be pollutant-nonspecific and have been reported with several kinds of contaminants (Vermeulen, 1995). For example, Janssens de Bisthoven *et al.* (2005) detected missing teeth, fused teeth among chironomid populations in sites impacted with acid mine drainage and MacDonald *et al.* (2006) had also reported incidences of missing teeth in organically polluted sites. Fused teeth, extra median tooth, missing teeth have been reported in chironomid populations exposed to arsenic (Martinez *et al.*, 2006). It therefore seems that, to a large extent, deformity types in chironomid larvae are non-contaminant specific.

Chironomidae larval deformities: teratogenic or mutagenic?

"Mutagens" are defined as DNA damaging agents that induce gene mutations that can be passed on from parents to offspring while "teratogens" are agents that alter normal cellular differentiation or growth processes which result in malformed foetus but are normally not passed on from parents to offspring as opposed to mutagenic changes that are hereditary (Hughes, 1996). Martinez *et al.* (2003) emphasized the need to determine whether deformity causal agents are teratogenic or mutagenic because chironomids breeding among adults carrying altered genes would result in genetically deformed offspring and deformities observed will be as a result of heredity and not a consequent of exposure to contaminants in the environment.

A number of studies have addressed the question of whether the mode of deformity induction in chironomid larvae is teratogenic or mutagenic (Frank and Köhn, 1982; Dickman and Rygiel, 1996; Groenendijk *et al.*, 1998; Martinez *et al.*, 2003; 2004). Frank and Köhn (1982) reported deformity frequency of 40.3% in chironomids larvae taken from a polluted canal. However, when these larvae were reared to adult stage in clean sediments and the adults allowed to breed, deformity frequency in their offspring (larvae) declined to less than 2%. Similarly, Dickman and Rygiel (1996) observed a decline in deformity rate to the control level among the offspring of parent population taken from polluted sites when these offspring were reared in clean sediments. Groenendijk *et al.* (1998) reported that mentum gaps in the offspring population of chironomids taken from a contaminated site dropped below rates

observed in the parent population. Jeyasingham and Ling (1997) in control substrates under laboratory conditions cultured the F_1 and F_2 progeny of a parent population that was taken from arsenic and pesticide polluted sites of Hamilton Lake and Lake Ngarato and recorded a decrease in the frequency of deformities among the larvae of the successive F_1 and F_2 generations. Martinez *et al.* (2004) reported an incidence of deformity of 14% in a chironomid parent population reared in zinc contaminated sediment but the frequency declined to 1.7 % in the F_1 generation larvae that were raised in clean sediment. However, they observed no significant difference in deformity rate between a chironomid parent population raised in lead spiked sediment and progeny reared in clean sediment. The mode of action of copper in chironomid larval deformities was earlier reported as mutagenic (Martinez *et. al.*, 2003). It therefore seems that while some deformity causal compounds are teratogenic others may be mutagenic and the mutagenic ones may have the tendencies of being carried throughout the life stages of the population.

Chironomidae larval deformities: indices used in bioindication

With growing interest in the use of chironomid morphological deformities for the assessment and monitoring of environmental water quality deterioration and sediment toxicity, researchers have developed different indices using chironomid mouthpart deformities. A widely used chironomid deformities quantification index at the community level is the proportion of deformed individual or deformity incidence (DI) expressed in percentage irrespective of the structure although most work that have used the DI have focused on the mouth parts. The DI is given as DI = d/n X100%, where d is the number of deformed larvae and n the total number of larvae examined (Hämäläinen, 1999).

Warwick (1985) developed the first index which was based on the severity of *Chironomus* larvae antennal deformities. This index was called index of severity of antennal deformations (ISAD). The index is expressed as ISAD =∑IMR/n where IMR (index of morphological response) is the total of deformity class values for each larvae, ∑IMR is the sum of the class value of all deformed larvae examined and n is the total number of larvae examined. The IMR is determine in six basic steps; the loss of genuine segments, reduction in the length of antenna, displacement or loss of ring organ, fusion of the apex of the basal segment, displacement of the accessory organs and presence of structures of unknown homology. Each of these deformity types are assigned to class value according to their apparent severity. These class values range from 1 to 21. Based on ligula deformities of *Procladius,* Warwick

(1991) developed another index called index of severity of ligula deformations (ISLD). The ISLD is expressed as ∑IMR/n (all notations have same meaning as above). Class values range from 1 for the mildest deformity type to 64 for the most severe deformities. Dermott (1991) developed an index of severity of all deformities (ISAD) by modifying Warwick's 1985 index. This index accounts for deformities in nine different structures and the value of 1 is assigned to all specimens both deformed and non-deformed, and additional weight ranging from 1 to 4 are given for each deformity type according to perceived levels of severity.

Lenat (1993) in an attempt to classify streams by means of chironomid larval deformities, designed a scoring system called the toxic score index (TSI) which takes into consideration the severity of deformities in the mentum of *Chironomus* larvae. *Chironomus* mentum deformities were grouped into three classes: class I, slight deformities which were difficult to distinguished from breakage or abrasion of the teeth; class II, severe and clearly apparent deformities including extra teeth, missing teeth, large gaps and distinct asymmetry; class III consisted of larvae with severe deformation, including at least two class II types of deformity. The toxic score assigned higher weighting to more severe deformation. The index is expressed as: Toxic score index (TSI) = No. of class I +2(No. of class II) + 3(No. of class III)/N where N is the total number of larvae examined. Lenat (1993) suggested that TSI = 25 should be taken as threshold value above which a site should be considered toxically contaminated and that the frequency of severe deformity i.e. class II and class III is generally greater than 6% at toxic sites. This index was successfully used to classify North Carolina's streams into different categories based on levels of toxic contaminations (Lenat, 1993).

Bhattacharyay *et al.* (2005) developed the sensitivity index of antennae of sensitive species $(SISS)_{antennae}$ based on a modification of the Warwick (1988) index. The index uses antennae of sensitive species for the assessment of environmental degradation at the family and subfamily level. The Index is expressed as: $(SISS)_{antennae} = 1/N \{\sum IMRsp1 + \sum IMRsp2 \ldots\ldots \sum IMRspn\}$ where $(SISS)_{antennae}$ = sensitivity of antennae of sensitive species; N = total number of chironomid larvae of sensitive species; sp1- spn are sensitive species. However, all these indices except the $(SISS)_{antennae}$ have received criticism from Hämäläinen (1999) that they are highly redundant with the DI showing strong linear relationship (r^2 = 0.77- 0.99) with the DI and that they might therefore contribute little additional information. Nevertheless, Lenat's 1993 toxic score index (TSI) has gain popularity among researchers (Servia *et al.,* 2000; Al-Shami *et al.,* 2010). Servia *et al.* (2000) applied the toxic score

system to evaluate the impacts of contaminants on *Chironomus riparius* in river Sar, north west Spain. These authors have also calculated a toxic score threshold of 8.7 for *Prodiamesa olivacea* corresponding to the 25 for *Chironomus* spp. Al-Shami *et al.* (2010) employed the toxic score index to evaluate the effects of pollutant on the severity of mentum deformities in *Chironomus* spp. in river Juru, Malaysia. Based on the TSI, these authors evolved a modified toxic score index (MTSI). The MTSI assigned additional weighting to deformities according to the position of the teeth in the mentum. They reported that both the TSI and MTSI showed strong correlations with environmental variables but that the correlation was stronger for the MTSI. Apart from the toxic score system, the other indices are highly technical and has therefore inhibited their wide spread application (Rosenberg and Resh, 1993).

1.4 Rationale and Significance of the Study

Managing environmental water quality is a key component of integrated water resources management in South Africa (NWRS, 2004) and therefore requires the development and application of improved management tools. The application of a multimetric approach which integrates information from different levels of the ecosystem and also reduces weakness of individual metrics could provide useful ecological information for improved water resources management in the Swartkops River. In addition, responses of species or genera within a family, to environmental water quality changes could be useful in biomonitoring programmes. Assigning water quality tolerance limits at the family level could mask individual species sensitivity and lead to erroneous conclusions regarding the water quality status of a site. The family Chironomidae is widespread in South African fresh water bodies with huge species richness (Harrison, 2002). Despite considerable studies in other part of the world (Janssens de Bisthoven and Gerhardt, 2003; Mousavi *et al*., 2003; Adriaenssens *et al*., 2004; Janssens de Bisthoven *et al*., 2005, Luoto, 2010) that have shown varying sensitivity of species in this family, with some being extremely sensitive to environmental water quality changes, their range of sensitivity in relation to water quality changes in South African water resources has not been explored. The assessment of species or genera in this family in relation to environmental water quality changes in South African water resources and the Swartkops River in particular could be useful in using them as indicator species.

Furthermore, improved management of water resources requires early warning signals of environmental water quality deterioration. Morphological deformities in chironomids represent sub-lethal response to environmental water contaminants and are therefore

considered early warning signals of environmental water quality deterioration. Although Chironomidae larval deformities have been successfully used in other parts of the world as a biomonitoring tool, its potential as an indicator of pollution stress in South African water resources has not been explored.

In this study, chironomids were screened for deformities because of their huge species richness and varying environmental sensitivity. Chironomids are particularly well suited for deformity screening because this family of aquatic macroinvertebrates contain highly tolerant species that could occur at "heavily" polluted sites. Such species are needed in order to have large enough sample size at polluted sites for meaningful and robust statistical analyses which could aid in drawing sound inferences. In addition, the configurations of the structures screened for deformities have been well documented in chironomids (Lenat, 1993; Janssens de Bisthoven and Gerhardt, 2003; Nazarova *et al.* 2004; Ochieng *et al.,* 2008).

The Swartkops River is an important freshwater resource in South Africa draining heavily industrialised and urban areas in the Eastern Cape. Therefore, given the highly industrialised nature of activities surrounding the Swartkops River, the increasing human population within the catchment and being an important freshwater ecosystem in South Africa, there is need for a holistic and improved management approach. Assessing environmental water quality in the Swartkops River using information from different levels of taxonomic resolution (i.e. family, genus or species) and organisation (i.e. community, population, organism and sub-organism levels) would contribute substantially to an improved water quality management of the River and better understanding of the impacts of anthropogenic activities on the river's macroinvertebrate assemblages. In this study therefore, a multi-metric biomonitoring approach, that included morphometric responses of chironomids, was applied at four sites in the Swartkops River; one relatively clean "reference site" and three impacted sites, with a specific aim and a clear set of objectives.

1.5 Aim and Objectives of the Study

1.5.1 Aim

The aim of this study was to assess anthropogenic impacts in the Swartkops River using different macroinvertebrate based biomonitoring approaches.

1.5.2 Objectives

i) To provide a river health assessment of the Swartkops River using the South African Scoring system version 5 (SASS5) in order to determine the current environmental water quality status of the Swartkops River.

ii) To use selected macroinvertebrate metrics to assess macroinvertebrate responses to environmental water quality changes in the Swartkops River with a view to assessing whether these metrics provide further useful information that may complement SASS5 in biological water quality assessment of the Swartkops River.

iii) To investigate Chironomidae community responses to environmental water quality changes in order to explore the bioindication potential of the Swartkops River chironomid assemblages.

iv) To document and illustrate deformities in larval of different genera or species of Chironomidae.

v) To compare incidences of deformities among genera or species of Chironomidae as well as different morphological structures to establish structural sensitivity and sensitivity of dominant Chironomidae genus or species to the development of deformity.

vi) To evaluate seasonal variations in the incidences of Chironomidae head capsule deformities.

vii) To contribute baseline information for using head capsule deformity in chironomid larvae as a biomonitoring tool in South African freshwater resources.

1.6 Thesis Structure

Chapter 1: This is a genenral introduction and literature review chapter. Water resources situations are introduced, water resources management strategy in South Africa as well as the different approaches applied in this study are critically reviewed. Rationale, aim and objectives of the study as well as the thesis structure are presented.

Chapter 2: A description of the study area, major anthropogenic influences in the Swartkops River are presented in this chapter. Measurement of physical and chemical water quality

variables, sampling methods, Chironomidae mounting techniques and statistical analyses employed are described.

Chapter 3: Results for the measured physical and chemical water quality variables, South African Scoring System version 5 (SASS5), multimetric approach and Chironomidae community assessments are presented and critically discussed in this chapter.

Chapter 4: Results for morphological deformities in Chironomidae larvae are presented and critically discussed in this chapter.

Chapter 5: Results obtained from all the approaches applied are critically discussed in this chapter; conclusion and recommendations drawn based on the findings of this study.

CHAPTER 2: MATERIALS AND METHODS

2.1 Introduction

The chapter briefly describes the study area and the major anthropogenic influences in the Swartkops River catchment and provides details of the study sites. The different methods employed in the study are explained in detail, starting with a description of the protocol employed for macroinvertebrate sampling and the different biological indices applied to assess the macroinvertebrate data in the study are discussed. A description of the method adopted for mounting head capsules of chironomids and subsequent screening of morphological deformities is provided. The chapter concludes with a description of methods used to measure physical and chemical water quality variables and a discussion of statistical analyses applied for the study.

2.2 Study Area Description

The Swartkops River is located in the Eastern Cape of South Africa and has its origin in the Groot Winterhoek Mountains. The river is an important ecological asset, supporting an estuary that provides important breeding habitats for water birds and fish (Taljaard *et al.*, 1998). But owing to its location, draining several urban and industrial areas, and a number of activities in its catchment, it suffers varying degrees of human induced impacts such as industrial and domestic effluent discharges, impoundments, deforestation and agricultural land use which directly or indirectly contribute to the deteriorating water quality of the river (DWAF, 1996a; 2003). The municipal areas of Uitenhage, Despatch and Kwanobuhle are all within the Swartkops River catchment (Binning and Baird, 2001). The river arises from the confluence of two rivers, the Kwazunga River to the north and the Elands River to the southwest. These two rivers (i.e. the Kwazunga and Elands Rivers) originate in the Groot Winterhoek Mountains and join just above Uitenhage in an area called Kruisrivier to form the Swartkops River, which discharges into the India Ocean at Algoa Bay in Port Elizabeth (Figure 2.1) (DWAF, 1996a). The Brak and Chatty Rivers are two smaller tributaries that originate in the plains to the north of Port Elizabeth and join the Swartkops River after the confluence of the Elands and Kwazunga. The Brak River forms a confluence with the Swartkops River at Uitenhage while the Chatty River joins the Swartkops just before the Swartkops village after the tidal limit at Perseverance and thus has no influence on the

freshwater component of the Swartkops River (Figure 2.1). The River catchment is about 1555 km^2 (Haigh, 2002) with a mean annual runoff of 84.2 $\times 10^6$ m^3 (Bate *et al.*, 2004).

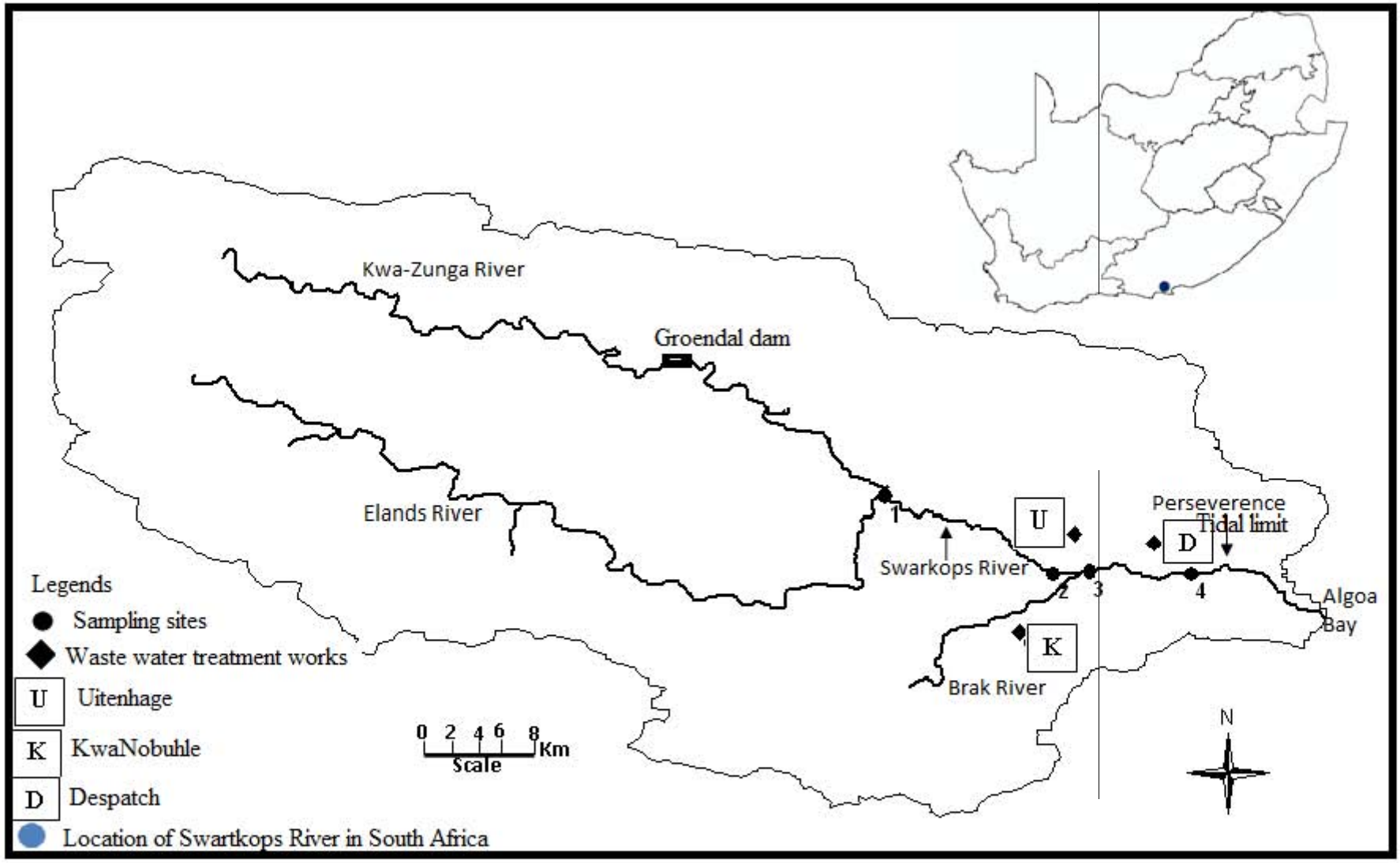


Figure 2.1: Map of Swartkops River showing sampling sites, urban areas and waste water treatment works

Generally, the climate is warm and temperate with a mean daily temperature of about 6^{o}C in July and 27^{o}C in January (Nelson Mandela Bay Municipality, 2006). The catchment receives rain throughout the year but the highest rainfall usually occurs in June and October (Haigh, 2002). Pattern of rain fall in the catchment is highly variable with at least a minimum monthly average of 60 mm (Nelson Mandela Bay Municipality, 2006).

Geologically, the Swartkops Basin consists of an upper base of Cretaceous shale and mudstone, overlain by marine sedimentary deposits in the high lying regions and by various alluvial deposits on the floodplain (Fromme, 1988). Underlying the entire basin are table mountain group quartzite and sandstones and the Cretaceous System forms an easily erodible trough consisted of coarse conglomerates inter bedded with sandstone and mudstone of Enon formation (marine and fluvial origin), greenish-grey slate, siltstones and sandstones of Kirkwood formation (fluvial origin) (DWAF, 1996a; Bornman and Klages, 2005).

Soils of the Swartkops River catchment vary but are of mainly alluvial derived from the Uitenhage group (Haigh, 2002). The soil texture consists mainly of fine sand, loam and clay of consolidated dune sands and lime-rich sandy clay which are easily erodible (DWAF, 1996a). The soils in the Swartkops River floodplain are deep and are well suited for agriculture (DWAF, 1996a).

The natural vegetation of the Swartkops River catchment is dominated by Bushveld or Succulent thicket (Kleynhans *et al.*, 2005). However, the Swartkpops River catchment natural vegetations have been extensively impacted by the encroachment of exotic or invader vegetation such as *Acacia* spp. and *Eucalyptus* spp. (DWAF, 1996a). In the Swartkops River system, particularly at Uitenhage and Despatch, there is extensive and dense growth of *Phragmites australis*, *Eichhornia crassipes*, or water hyacinth, and *Salvinia molesta* (DWAF, 1996a). The extensive growth of water hyacinth and other invader or exotic vegetations in the river system can reduce the aesthetic and recreational value and also impact on the aquatic ecosystem habitats.

2.2.1 Anthropogenic influences on the Swartkops River

Although the upper catchment of the Swartkops River lies within a pristine inaccessible area of the Groot Winterhoek Mountains, the lower catchment is subjected to varying degrees of pollution. Effluent discharges into the Swartkops River include effluents from Uitenhage, Despatch and Kwanobuhle waste water treatment works (WWTW) (Taljaard *et al.*, 1998;

DWAF, 1996a). The Uitenhage and Despatch waste water treatment works release approximately 6 822 500 m^3 and 835 000 m^3 per annum of treated waste water into the river respectively (DWAF, 1996a). The Brak River receives treated effluent of about 2 847 000 m^3 per annum from the Kwanobuhle waste water treatment works and discharges it into the Swartkops River below Uitenhage (DWAF, 1996a). According to Binning (1999), about 50 000 to 100 000 litres of wash water from evaporation ponds of the wool processing factory in Uitenhage seep into the Swartkops River. Although the tannery industry at Uitenhage discharges effluent into evaporation ponds, it is believed that this effluent could have profound effects on the water quality of the river due to seepage (Binning, 1999). In addition, a number of storm-water canals flow into the Swartkops River from nearby roads and informal settlements and often carry raw sewage and other pollutants into the river (DWAF 1996a; Bornman and Klages, 2005). These combined discharges have substantially contributed to the high nutrients levels and overall poor water quality status of the Swartkops River. Different agricultural practices have also impacted on the river's water quality. About 15 percent of the catchment is subjected to agricultural practices including cultivation and livestock farming and these constitute diffuse sources of nutrients due to the application of fertilizers and animal waste (de Villiers and Thiart, 2007). A recent fish kill, and the excessive plant growth such as water hyacinth and *Enteromorpha* in the river are indicative of eutrophication (Taljaard *et al.*, 1998; Ndoni, 2009). Furthermore, high levels of faecal contamination and trace metals have been reported in the Swartkops River (Taljaard *et al.*, 1998; Binning and Baird, 1999). The elevated levels of trace elements may be linked to run-off from industries and roads as well as emission from burning of fossil fuel and automobiles in the catchment (Taljaard *et al.*, 1998).

There are numerous man-made obstructions along the course of the Swartkops River and its tributaries but the greatest of these is the Groendal Dam with a storage capacity of 12×10^6 m^3 (Fromme 1988; Bate *et al.*, 2004). Other obstructions in the form of causeways also exist along the length of the Swartkops River. The causeway at Nivens Bridge serves as a weir impeding the natural flow of the river and has resulted in the formation of an artificial pool.

As a result of its geology, the Swartkops River is suitable site for sand and gravel mining. The mining activities taking place in the Swartkops River catchment particularly at Uitenhage and Despatch could have profound ecological impacts on the physical and biological processes of the river. Physical impacts of sand mining include bed degradation, modification

of channel morphology, bed coarsening and destruction of aquatic and riparian habitat (The Ojos Negros Research Group, 2004; Bornman and Klages, 2005). Physical habitat disruptions alter natural biological processes by causing changes that favour some species over others and could cause overall decline in biological diversity. Mining in the Swartkops River catchment could also have severe impacts on its water quality. Environmental water quality impacts resulting from mining activities may include increased turbidity due to re-suspension of sediment, sedimentation due to stockpiling, oil spills or leakages from excavation machinery and transportation vehicles and dumping of organic waste (Bornman and Klages, 2005). The high vehicle traffic as a result of mining activities at the Uitenhage and Despatch reaches of the river may have other negative impacts on the environment.

2.2.2 Sampling sites

The study was conducted at four sampling sites (sites 1, 2, 3 and 4) (Figure 2.1) over a period of one year. Site 1 (S 33^{o} 45^{1} 08.4" E 25^{o} 20^{1} 32.6") is located upstream of Uitenhage and was chosen to represent least impacted conditions (reference site) in agreement with Reynoldson *et al.* (1997), Dallas (2000) and based on an expert judgement taking into considerations the availability of macroinvertebrate habitats, extent of impacts, ease of accessibility and area that falls within the same ecoregion with the other sampling sites. Within the accessible areas in the Swartkops River, this site represents the best available condition in the river. The biotope diversity at the site could be considered as good, although gravel, sand and mud (GSM) were not widely represented (Figure 2.2). While looking for an appropriate reference site (i.e. selection of site 1), it was important to select a site that falls within the same level II ecoregion with the other sampling sites because of natural variations in macroinvertebrate assemblages among sites in different ecoregions (Kleynhans *et al.*, 2005; Dallas and Day, 2007). Within an ecoregion, there are relative similarities in both biotic and abiotic components of the ecosystem and thus, macroinvertebrate samples collected at sites within the same ecoregion should be similar (Kleynhans *et al.*, 2005).

The reference site is located 16.4 kilometres downstream of the Groendal dam (Figure 2.1). Such an impoundment could impact on the macroinvertebrate composition of the site. For example, Bredenhand and Samways (2009) reported high proportions of Ecnomidae, Hydropsychidae, Hydraenidae and a significant drop in species diversity of Ephemeroptera, Plecoptera and Trichoptera (EPT) taxa at sites downstream of a dam in the Eerste River.

However, considering the factors mentioned above for the selection of the site, its use as a reference condition for this study was unavoidable.

Figure 2.2: Site 1, showing the biotopes sampled.

Site 2 (S 33^{o} 47^{1} 29.0" E 25^{o} 24^{1} 26.4") is located in the industrial city of Uitenhage. The site is severely impacted with domestic, agricultural, sewage and industrial effluents. The waste water treatment work near Nivens Bridge is located close to the site and livestock farming and other agricultural practices are evident around it. There is extensive growth of water hyacinth and other aquatic weeds at the site. Although there is evidence of habitat degradation at the site (Figure 2.3), the adequacy of sampling biotope diversity may be considered as generally good.

Figure 2.3: Site 2 at Uitenhage, showing the biotopes sampled on the left and the extent of pollution and habitat degradation on the right.

Site 3 (S 33^{o} 47^{1} 11.8" E 25^{o} 27^{1} 58.7) is located further downstream, also within the industrial city of Uitenhage. The site is also impacted with industrial, domestic, sewage and agricultural effluents and there is extensive growth of water hyacinth and other aquatic weeds (Figure 2.4). The Uitenhage waste water treatment work and an automotive industry are situated close to this site and there is evidence of discharges to the river. Agricultural practices around this site include livestock farming and cultivation of crops. The adequacy of sampling biotope diversity may be considered as generally good although the GSM biotope were not well represented.

Figure 2.4: Site 3 at Uitenhage, showing the biotopes sampled on the left and extensive growth of aquatic weeds on the right.

Site 4 (S 33^{o} 47^{1} 34.0” E 25^{o} 27^{1} 58.7”) is located at Despatch close to a sand mining industry. The site is impacted with agricultural and municipal runoff as well as by effluents from the Despatch waste water treatment works. These have consequently resulted in a thick growth of aquatic weeds and discoloured water at the site (Figure 2.5). There is a small culvert at the site resulting in flow modification. Habitat degradation is also evident in the vicinity of the site probably due to the sand mining activities. The adequacy of sampled biotope diversity at this site could be considered as good although GSM, stones in- and out-of current were not extensively represented.

Figure 2.5: Site 4 at Despatch, showing the biotopes sampled on the left and extensive growth of water hyacinth on the right.

2.3 Macroinvertebrate Sampling

Macroinvertebrate samples were collected seasonally over a period of one year beginning in late August (spring), late November (summer) 2009, March (autumn) and July (winter) 2010. Collection of macroinvertebrates samples was conducted in accordance with the SASS5 protocol (Dickens and Graham, 2002) for the South African Scoring System version 5 (SASS5). The protocol requires the collection of macroinvertebrates from three distinct biotopes, namely: stones (stones in- and out- of current), vegetation (marginal and aquatic vegetation) and sediment (gravel, sand and mud) hereafter referred to as stones, vegetation and GSM. Macroinvertebrate sampling was done using a SASS5 net (30 X 30 cm frame with mesh size 1000 µm). Although the SASS5 protocol requires the collection of one sample from each biotope, three replicates were taken from each of the three biotopes (stones, vegetation and GSM) making it a total of nine samples from each site per season. This was done in order to ascertain the representativeness of a single sample, to assess the samples with selected metrics, diversity indices and to provide for robust statistical analysis of the selected metrics and diversity indices. However, only the first sample collected from each biotope was used for total site SASS5 computation.

Collected macroinvertebrates were tipped into a white SASS5 tray that was half filled with river water. Families of macroinvertebrates present were identified by the river side, recorded on a SASS5 sheet, preserved in 70% ethanol and transported to the laboratory for sorting, abundance counts and to ascertain the accuracy of field identification. Chironomids from all sampled biotopes were sorted and identified as far as possible using identification keys described by Wiederholm (1983), Cranston (1996) and Harrison (2002).

2.4 Biological Indices Applied for Water Quality Assessment

The South African Scoring System version 5 (SASS5) and selected macroinvertebrate metrics and diversity indices were used as biological indices in the assessment of water quality in the Swartkops River. A third index, Integrated Habitat Assessment System (IHAS) was also used to aid the interpretation of SASS5 results.

2.4.1 South African Scoring System version 5 (SASS5)

South African Scoring System (SASS) is a biotic index based on the presence of families of aquatic macroinvertebrates and their perceived sensitivity to water quality changes (Chutter, 1998). SASS has undergone several modifications resulting in the current South African Scoring System version 5 (SASS5) (Dickens and Graham, 2002). In SASS5, macroinvertebrate families are awarded scores based on their perceived sensitivity from score 1 to 15 in order of their sensitivity to water quality deterioration and the results are expressed both as an index score (SASS5 score) and as average score per recorded taxa (ASPT value). The SASS5 score is calculated by adding the scores of all recorded taxa while ASPT value is calculated by dividing the total SASS5 score by the number of recorded taxa (Dickens and Graham, 2002). Although ASPT value is more consistent and reflects the biological water quality of a river more accurately than SASS5 score (Dickens and Graham, 2002), SASS5 scores are more reflective of water quality status of polluted rivers (Dickens and Graham, 2002). Because of possible spatial variations in macroinvertebrate assemblages among rivers in different ecoregions (Kleynhans *et al.*, 2005), Dallas (2007a) has developed guidelines that take into account geographical and longitudinal variations in the interpretations of SASS5 data. Thus each level I ecoregion divided into upper and lower zones has its own biological bands for the interpretation of SASS5 data (Dallas, 2007a). The Swartkops River is located within the Southern eastern coastal belt (lower zone) ecoregion and the biological bands for

this region according to Dallas (2007a) were used for the interpretations of SASS5 data in this study.

Table 2.1: Ecological bands for Southern eastern coastal belt (lower zone) ecoregion Dallas (2007a)

Biological Band	**Water quality category name**	**Description**	**Range of SASS5 scores**	**Range of ASPT values**
E/F	Seriously/ critically modified	Seriously/ critically modified	< 62.9	< 5
D	Poor	largely modified	63 - 81.9	5.1 - 5.3
C	Fair	moderately modified	82 - 99.9	5.4-5.9
B	Good	largely natural with few modifications	100 - 148.9	6.0-7.0
A	Natural	Unmodified	149 - 180	7.1-8

2.4.2 Integrated Habitat Assessment System

Integrated habitat assessment system (IHAS) is an index used for the assessment of physical habitat structure of streams and rivers and its aim is to provide information on the quality, quantity and diversity of habitats available to macroinvertebrates (Ollis *et al.,* 2006). IHAS was developed to be used along with SASS in biomonitoring programmes (McMillan, 1998). Although the index in itself is not a water quality assessment index, it provides an assessment of the adequacy and diversity of habitats for the interpretation of SASS5 results. The IHAS scoring system is based on 100 points, divided into two sections: sampling habitat (55 points) and stream condition (45 points). The sampling habitat section is further divided into three sections: stones in- current (SIC) (20 points), vegetation (15 points) and other habitats including GSM, bedrock and algal presence (20 points). The stream condition provides information about the physical characteristics of the stream such as stream depth, width, velocity, surrounding impacts and water colour.

IHAS assessment was conducted at each site throughout the study period by completing the IHAS form. Because IHAS is completely subjective, depending on the assessor's observation and expertise, upon completion of the IHAS form by one member of the field trip team, it was handed over to another member of the team for verification to ensure that the assessment is a true reflection of the site's habitat condition.

2.4.3 Selected macroinvertebrate metrics and diversity indices

Metrics are measurable units or processes of a biological system that change in value with the level of pollution in streams and rivers (Ofenböck *et al.,* 2004). Selected metrics and indices were used to explore macroinvertebrate data to ascertain whether additional useful information on the macroinvertebrate assemblage and water quality can be obtained to compensate for the extra time and efforts. Although there are various metrics, 19 metrics in four categories, including measures of abundance, composition (or relative abundance), richness and diversity, were chosen for this study (Table 2.2). The selected metrics and indices are based on taxonomic groupings and are perceived to be more reliable than metrics based on other groupings such as functional feeding groups (Klemm *et al.,* 2002). Functional feeding group metrics were not included in the study because the taxonomic resolution was considered insufficient to reliably and accurately assign each taxon to a functional feeding group. Besides, most functional feeding group metrics are highly variable and do not respond to impacts in a predictable fashion (Karr, 1999). Furthermore, several authors have cautioned against their use as indicators of water quality (Karr, 1999; Klemm *et al.,* 2002; Gabriels *et al.,* 2010). Although Palmer *et al.* (1996) were able to show that individual species had a strong relationship with water quality variables, these authors could not demonstrate a pattern in functional feeding groups in relation to water quality in the Buffalo River, South Africa.

Table 2.2: Selected metrics of macroinvertebrate abundance, community composition, richness and diversity applied to the macroinvertebrate data collected. Metrics are defined according to Klemm *et al.* (2002), Camargo *et al.* (2004) and Baptista *et al.* (2007).

Metrics	**Definition**
Abundance measures	
Number of EPT individuals	Absolute number of individuals in Ephemeroptera, Plecoptera and Trichoptera taxa
Number of Trichoptera individuals	Absolute number of individuals in Trichoptera taxa
Number of ETOC individuals	Absolute number of individuals in Ephemeroptera, Trichoptera, Odonata and Coleoptera taxa
Number of Chironomidae + Oligochaete individuals	Sum of the absolute number of individuals in Chironomidae and Oligochaete taxa
Number of Gastropoda individuals	Number of individuals in Gastropoda
EPT/Chironomidae ratio	Ratio of EPT individuals to Chironomidae individuals
Measures of composition (relative abundance)	
%EPT	Percentage of individuals in EPT taxa relative to entire sample
% Chironomidae + Oligochaete	Percentage of individuals in Chironomidae + Oligochaete taxa relative to entire sample

Metrics	Definition
% Oligochaete + Hirudinea	Percentage of total individuals in Oligochaete and Hirudinea relative to entire sample
% Corixidae	Percentage of individuals in Corixidae relative to entire sample
% Trichoptera	Percentage of individuals in Trichoptera taxa relative to entire sample
% ETOC	Percentage of individuals in Ephemeroptera, Trichoptera and Odonata relative to entire sample
Richness measures	
ETOC richness	Absolute number of taxa in Ephemeroptera, Trichoptera, Odonata and Coleoptera
Hemiptera + Diptera richness	Absolute number of taxa in Hemiptera and Diptera
EPT richness	Absolute number of taxa in EPT
Diversity measures	
Simpson diversity index	Weighted towards the abundance of commonest families (Ogbeibu, 2005)
Shannon diversity index	Information statistic index which takes account of the contribution of individual taxa to the diversity while assigning greater weight to most dominant taxa (Ogbeibu, 2005)

Metrics	Definition
Margalef's family richnes index	Accounts for both number of taxa and individuals and is independent of sample size (Ogbeibu, 2005)
Equitability (or evenness)	Measures the relative even distribution of abundance of taxa within a sample (Clarke and Warwick, 1994)

The selection of metrics was based on their performance to discriminate between impaired and unimpaired sites from several studies (Klemm *et al.,* 2002; Vlek *et al.,* 2004; Ofenböck *et al.,* 2004; Baptista *et al.,* 2007; Gray and Delaney, 2007) and their varying sensitivity to environmental water quality impairment. The strength of metrics is their ability to integrate information from individual, population, community and ecosystem levels and to reduce weaknesses of individual single metric (Klemm *et al,* 2002; Vlek *et al.,* 2004). According to Ofenböck *et al.* (2004) useful metrics should be ecologically relevant to the biological assemblage or community under study, and be sufficiently sensitive to stressors to provide a response that may be discriminated from natural variation.

Two categories of diversity indices i.e. dominance and information-statistics indices were employed in this study. Dominance indices are weighted towards the abundance of commonest families e.g. Simpson index while information-statistics indices reflect individual taxon abundance while they still assign greater weight to the commonest families e.g. Shannon index (Clarke and Warwich, 1994; Gray and Delaney, 2007).

2.5 Screening Head Capsule Deformities in Chironomidae Larvae

In the laboratory, preserved macroinvertebrate samples were gently spread on a white tray and all chironomid larvae sorted, under a hand-held magnifying glass, using fine forceps. Sorted chironomids were kept in specimen vials containing 70% ethanol to prevent the head capsule from becoming dry as dried and shrunken head capsules are difficult to mount (Dickman and Rygiel, 1996). The head capsules were mounted for taxonomic identification using mouth parts and other structures according to the keys described by Wiederholm

(1983), Cranston (1996) and Harrison (2002).The mounting procedures of Ochieng *et al.* (2008) were followed but cold potassium hydroxide (KOH) was used instead of hot KOH (Warwick, 1988). The larvae were removed from the 70% ethanol solution and cleared in 10% cold (w: v) KOH solution for about 15- 20 minutes (Warwick, 1988). Cold KOH as a clearing agent was found to produce better results than warm KOH. The clearing was followed by three time's consecutive dehydration using 96% ethanol and finally in absolute ethanol until the head capsules become clear. The larvae were transferred into a solution of xylene for 10 minutes and the entire larva was then placed on labelled mounting slides containing a drop of Canada balsam. Under a dissecting microscope, the head capsule, ventral side up, was removed from the body segment and was placed separately on a different mounting slide with the same label as the corresponding body segment. A cover slide was placed on the slide containing the capsule, pressed gently while carefully applying a rotary motion to flatten the head capsule in order to expose the mouth parts (Warwick, 1988). Slides were left for about five to eight days to air dry. Specimens were examined for further identification and screened for deformities in the antenna, mentum, ligula, mandible, prmandible and paraligula under an Olympus compound microscope (B X 51) equipped with an Altra 20 soft imaging system digital camera. All broken or ambiguous deformities were considered as normal specimens and only clearly deformity types derived from a combination of Lenat, (1993); Janssens de Bisthoven *et al.* (1998); Martinez *et al.* (2002; 2003; 2004); Bhattacharyay *et al.* (2005) and Ochieng *et al.* (2008) were identified as deformed specimens. Separated body segments were mounted separately and were used during identification of specimens. Depending on the specimen and the structure being observed, a magnification of X 10 or X 40 was used and all image analysis and photos were taken using the software analySIS Five soft imaging system.

2.5.1 Morphological features examined

The purpose of this section is to give a brief description of the different chironomid morphological structures that were screened for deformities. The mentum is a double walled sclerotized and usually toothed medioventral plate of the head capsule posterior to other mouth parts (Armitage *et al.,* 1995) (Figure 2.6). The mandible is a paired appendage which operates in an oblique plane between the labrum and the maxilla in most chironomid genera and is toothed, with an outer dorsal (apicodorsal) tooth, apical tooth and variable number of inner teeth, usually between two to three, although the dorsal tooth is absent in some genera

(Cranston, 1996). In Tanypodinae, the ligula was examined for deformities in place of the mentum because the mentum is poorly developed in this sub-family. The ligula is a conspicuous toothed sclerotised structure located on the floor of the mouth of larvae of Tanypodinae and is flanked laterally by a pair of toothed paraligula (Harrison, 2002). Table 2.3 shows the arrangement of teeth in the mentum, ligula, mandible, paraligula and the number of antennal segments as well as the position of the ring organ among the commonest genera encountered in this study.

Chironomid antennae are often well developed structure consisting of three to seven segments with most genera having five segments (Cranston, 1996). In most genera, the antenna bears lauterborn organs on the second segment. They are paired compound organs, consisting of peg sensillum and two series of thin digitiform fan-like sensilla (Harrison, 2002).They are usually located apically and opposite to each other (Armitage *et al.*, 1995). The antenna is usually divided into a basal pedestal and a thinner flagellum consisting of the antenna segments. In most genera, the lauterborn organ and the style are both situated on second segment while the blade and the accessory blades on the basal segment (Wiederholm, 1983).

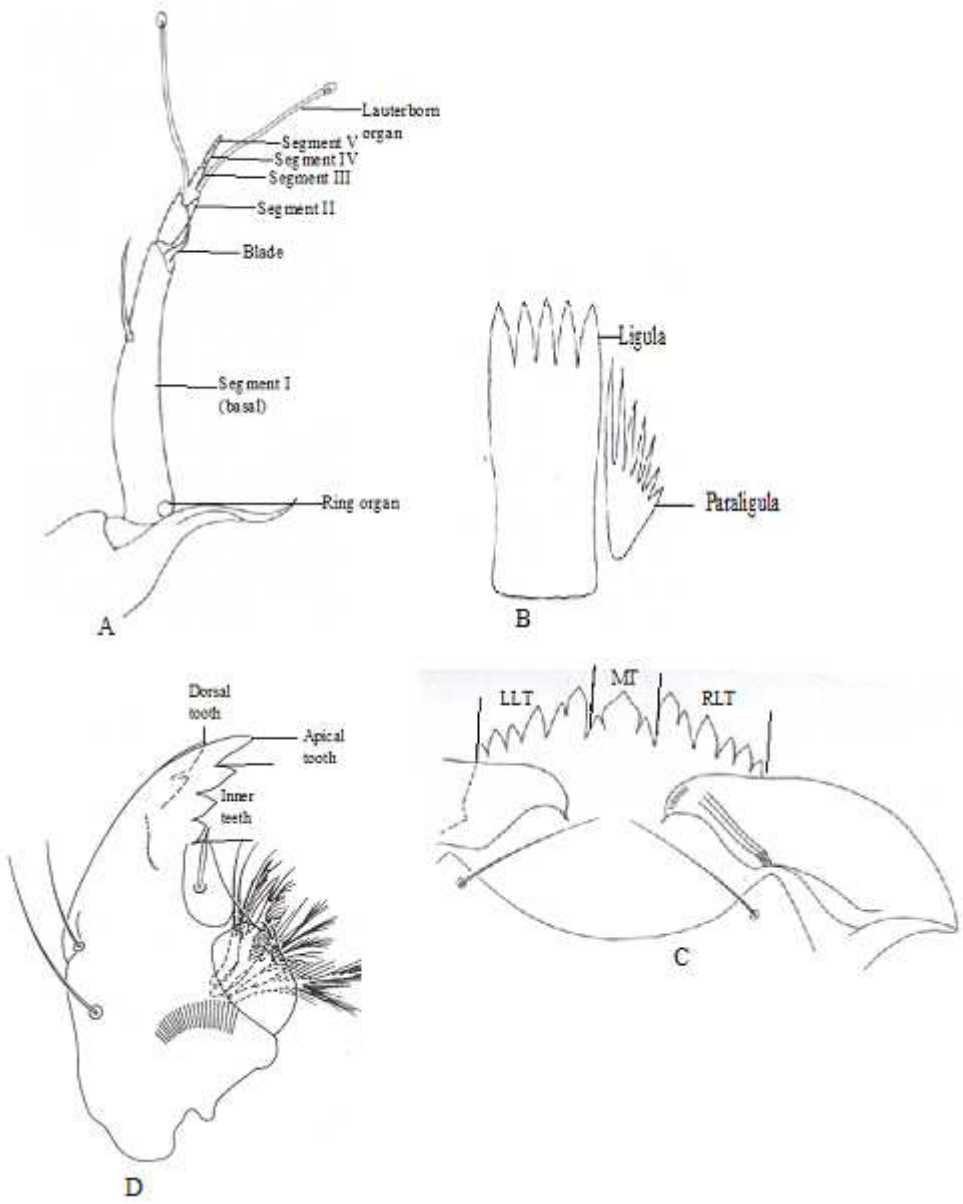


Figure 2.6: Antenna, mentum, ligula, mandible and paraligula of Chironomidae. A: Antenna of Tanytarsus B: Ligula and paraligula of Tanypus. C: Mentum of Chironomus showing position of teeth (MT is median teeth, RLT and LLT are right and left lateral teeth respectively) D: Mandible of Chironomus. Structures scanned from Wiederholm (1983)

Table 2.3 Arrangement of teeth in the mentum, ligula, mandible, paraligula and the number of antenna segments as well as the locations of ring organs among the commonest chironomid genera. Summarised from Wielderholm (1983).

Genus	**No. of median teeth (mentum)**	**No. of teeth (ligula)**	**No. of paired lateral teeth (mentum)**	**No. of inner teeth (mandible)**	**Apical tooth (mandible)**	**Dorsal tooth**	**No. of teeth (Paraligula)**	**No. of antenna segment**	**Position of ring organ in antenna**
Chironomus	1 trifid	N/A	6	3	Yes	Yes	N/A	5	In proximal 1/2 of basal segment
Dicrotendipes	1 laterally crenate	N/A	6	4	Yes	Yes	N/A	5	in proximal 1/3 of basal segment
Polypedilum	2	N/A	7	2	Yes	Yes	N/A	5	In proximal 1/4 of basal segment
Kiefferulus	1 trifid	N/A	6	3	Yes	Yes	N/A	5	In proximal 1/3 of first segment
Tanytarsus	1 simple, bifid or trifid	N/A	5	2 or 3	Yes	Yes	N/A	5	In proximal portion of first segment
Tanypus	N/A	5	N/A	3	Yes	No	5 – 14	4	In middle of apical 1/3 of basal segment
Ablabesmyia	N/A	5	N/A	1	Yes	No	1 bifid	4	Middle of basal segment
Clinotanypus	N/A	5 – 7	N/A	2	Yes	No	3 – 4	4	Base of apical 1/10 of basal segment

Genus	**No. of median teeth (mentum)**	**No. of teeth (ligula)**	**No. of paired lateral teeth (mentum)**	**No. of inner teeth (mandible)**	**Apical tooth (mandible)**	**Dorsal tooth**	**No. of teeth (Paraligula)**	**No. of antenna segment**	**Position of ring organ in antenna**
Cricotopus	1	N/A	6, rarely 5or 7	3	Yes	No	N/A	4 or 5	In basal 1/3 of first segment
Orthocladius	1	N/A	Usually 6, sometime 7-9	3	Yes	No	N/A	6	In basal 1/4 of first segment
Cardiocladius	1	N/A	5	4	Yes	No	N/A	5	In basal 1/4 of first segment
Paratrichocladius	1	N/A	6	3	Yes	No	N/A	5	In basal 1/3 of first segment

2.6 Measurement of Water Quality Physical and Chemical Variables

Water quality analyses were carried for each macroinvertebrate sampling event. On site, dissolved oxygen (DO), electrical conductivity (EC), turbidity, temperature and pH were measured using Cyberscan DO300, Cyberscan Con300, Orbeco-Hellige 966, mercury-in-glass thermometer and Cyberscan pH 300 meters respectively.

2.6.1 Collection and preservation of water samples for chemical analysis

Water samples used for the analysis of chemical variables, except for five days biochemical oxygen demand (BOD_5), were collected in 250 ml plastic bottles; water samples for BOD_5 were collected in sterilised 500 ml glass bottles. Prior to each field trip, the sampling bottles were acid washed according to the Institute for Water Research glassware acid wash protocol. In addition, bottles used for the collection of water samples for BOD_5 were sterilized in an autoclave set to 120^{o}C heated for 15 minutes. Water samples were collected facing upstream of the river as recommended in APHA *et al.* (1971) and the bottles were filled to the neck allowing no head space and transported to the laboratory in an ice-filled cooler box. Samples were preserved at 4 ^{o}C in the laboratory for chemical analysis. All chemical analyses were performed within 24 hours of sample collection.

2.6.2 Analysis of chemical water quality variables

Preserved water samples were taken from the refrigerator and allowed to equilibrate to room temperature before they were processed. Samples were analysed for nitrate-nitrogen (NO_3-N), nitrite-nitrogen (NO_2-N), ammonium-nitrogen (NH_4-N), orthophosphate-phosphorus (PO4-P), five days biochemical oxygen demand (BOD_5) and total inorganic nitrogen. NO_3-N and NO_2-N were analysed according to Velghe and Claeys (1983) on a Shimadzu mini 1240 spectrophotometer at 388nm and APHA *et al.* (1971) method number 354.1 on a Biotek micro plate reader at 540 nm respectively. Spectroquant® phosphate and ammonium concentration test kits catalogue number 1.14848.0001 and 1.14752.0001 were used to analyse for orthophosphate-phosphorus and ammonium-nitrogen according to manufacturer's instructions on a Biotek micro-plate reader at 660 nm. Total inorganic nitrogen (TIN) concentration was obtained by the summation of the individual concentrations of nitrate, nitrite and ammonium (Palmer *et al.*, 2005). BOD_5 was analysed according to APHA (1992). Analyses were conducted for three replicates for each sample and averaged. This was done

because average readings were considered more representative and also to reduced variability in the measured results.

For the analysis of phytoplankton chlorophyll *a*, 200 ml of river water was filtered onto a Whatman GF/F filter paper and analysed fluorometrically using a Turner Design 10 – AU digital fluorometer according to Arar and Collins (1997). Analysis of phytoplankton chlorophyll *a* was done for three replicates and readings were averaged. For the determination of periphyton chlorophyll *a*, three stones were picked randomly from the river bed and a flattened surface area scraped using a scraper and a pre-measured surface measurer of 6.16 cm^2. The scraped stone area was rinsed with distilled water onto a beaker and filtered with a Whatman GF/F filter paper and analysed fluorometrically with a Turner Design 10 – AU digital fluorometre according to Arar and Collins (1997).

2.7 Statistical Analysis

The purpose of this section is to present the different statistical analyses that were used to explore water quality variables and the macroinvertebrate data in order to make relevant statistical comparisons among sites and seasons. Prior to analysing the data in the different statistics software employed in this study, data were captured in Excel (Microsoft 2007 office) and exported or copied to relevant packages.

2.7.1 Box-and-whisker plots

Box-and-whisker plots are graphs that display summary statistics such as median, quartiles and extreme values and permit the visualization of metric ranges variation between sites (Baptista *et al.,* 2007). The South African Scoring System version 5 (SASS5), average score per taxon (ASPT) and the selected metrics (Table 2.2) ability to discriminate between site 1 (reference site) and downstream sites (i.e. sites 2, 3 and 4) was assessed using box-and-whisker plots. Box-and-whisker plot computations were done using Statistica software package version 9. The degree of overlap of medians and the inter-quartile ranges (IQRs) between site 1 (reference site) and downstream sites was considered as an indicator of the discriminatory ability of SASS5, ASPT and metrics (Klemm *et al.,* 2002). Two levels of discrimination among metrics between site 1 (reference site) and downstream sites (i.e. sites 2, 3 and 4) were considered satisfactory. The first sets of metrics were those in which there was no overlap in inter-quartile ranges between site 1 and sites 2, 3 and 4 while the second sets where those in which there was an overlap in the inter-quartile ranges but the medians

were outside of the inter-quartile ranges (Baptista *et al.*, 2007). Among the selected metrics, only those that discriminate site 1 (i.e. reference site) from all three downstream sites were retained for further analysis. To test for metrics ability to detected subtle difference between sites, the same criteria were used to assess whether metrics that distinguished site 1 from sites 2, 3 and 4, were able to distinguish between the three downstream sites (i.e. sites 2, 3 and 4). Prior to box-and-whisker plot analysis, the nine macroinvertebrate replicates representing different SASS5 biotopes, stones, GSM and vegetation, from each site per season were pooled to form three SASS5 replicates making it a total of 12 replicates for each site in all seasons. The discriminatory ability test was done irrespective of seasons. The pooling of samples was done so that metrics could bear resemblance to SASS.

2.7.2 Kruskal -Wallis test

The Kruskal-Wallis test is a non-parametric univariate statistics used to compare the means and/or medians between two or more samples (Ogbeibu, 2005). The test was used to further test whether there were statistical significant differences ($p < 0.05$) among metrics with satisfactory discriminatory ability in order to confirm the visual results of Box-and whisker plots. Differences in median SASS5 scores, number of taxa and average score per taxon (ASPT) among sampled SASS5 biotopes were also tested using Kruskal-Wallis multiple comparison. This test was also applied to test for differences in chironomid diversity indices between sampling sites. The Kruskal-Wallis test was conducted using the Statistica software package version 9.

2.7.3 Analysis of variance

Analysis of variance (ANOVA) is a parametric test used to compare the means between two or more samples. ANOVA was used to test the null hypothesis that there is no difference in the means of the water quality variables among the four sampling sites. One-way ANOVA was used to test whether there were significant differences ($p < 0.05$) between water quality variables from the four sampling sites. When water quality variables were significantly different, Tukey Honestly Significant Different (HSD) test was used to indicate sites that differed.

The incidence of chironomid deformity expressed as percentage was calculated separately for each genus and structure as the proportion of deformed larvae to the total number of larvae while community incidence of deformity was calculated for each structure as the percentage

of the number of all deformed larvae irrespective of genera to the total larvae sampled for each site (Janssens de Bisthoven and Gerhardt, 2003; Ochieng *et al.*, 2008). Because calculation of incidence of deformities was sample size dependent, prior to its calculation replicate samples collected were pooled for each site so as to have a large enough sample size for meaningful statistical analysis. Incidences of deformities were arcsine transformed and ANOVA was used to compare deformities among sites, seasons and structures for dominant chironomid genera. Because chironomid genera vary in sensitivity in expression of morphological deformities, site to site comparison was restricted to genera occurring across two or more sites. This same principle was applied for seasonal comparison. ANOVA was conducted using the Statistica software package version 9.

2.7.4 Simple correlation

Pearson correlation was used to correlate SASS scores and ASPT values with the concentrations of water quality variables: nitrate – nitrogen (NO_3-N), nitrite – nitrogen (NO_2-N), ammonium – nitrogen (NH_4-N), orthophosphate – phosphorus (PO_4-P), total inorganic nitrogen (TIN), dissolved oxygen (DO), five days biochemical oxygen demand (BOD_5), chlorophyll *a*, electrical conductivity (EC), turbidity and temperature. Pearson correlation was also used to establish the relationships between metrics with satisfactory discriminatory ability and the concentrations of measured water quality variables. It was also used to elucidate the relationship between water quality and Chironomidae deformities. Prior to correlation analysis, biological data were log (x+1) transformed while environmental data were normalised after log (x+1) transformation by subtracting the mean value and dividing by the standard deviation over all sample for that environmental variable (Clarke and Warwick, 1994). Normality of environmental variables was performed in order to assign all variables equal weight irrespective of their scale of measurement. Log (x+1) transformation was chosen for the biological data because it prevent the domination of few abundant species while still retaining the contributions of both dominant and rare species in the analysis (Clarke and Warwick, 1994).

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

ANOSIM test is analogous to the classical parametric analysis of variance (ANOVA) and it is a non-parametric permutation procedure used to test for significant difference of non-normally distributed data set (Clarke and Warwick, 1994). Two-way ANOSIM nested

procedure was used to test whether there were significant differences ($p < 0.05$) in macroinvertebrate abundance and composition among sampled biotopes and seasons within a site. One-way ANOSIM was used to test whether significant difference exist between replicates sampled from the same biotope within a site. ANOSIM to test whether there was significant difference between replicates sampled from the same biotopes was conducted at the site level ($n = 12$) with replicate numbers as factor. The analysis could not be conducted at each site at per biotope per season level ($n = 3$) because of few groups. ANOSIM was also applied to the chironomid community data set. It was used to test whether significant difference exist between sites in chironomid composition and abundance during each of the four sampling seasons.

SIMPER analysis is used to identify taxa responsible for observed similarity and dissimilarity between sample groups (Clarke and Warwick, 1994). This analysis was conducted to identify taxa responsible for the observed similarity or dissimilarity between replicates, biotopes, sites and seasons. ANOSIM and SIMPER analyses were conducted using the computer programme PRIMER 5 version 5.2.9.

2.7.6 Cluster analysis

Cluster analysis is a multivariate method that aims to classify or group samples or sites according to their similarity such that samples, sites or replicates of a sample with similar biological community composition form distinct clusters from those of other sites or samples. Hierarchical agglomerative clustering was used to group sites for each sampling season based on macroinvertebrate community structure. Hierarchical agglomerative clustering usually uses a similarity matrix to fuse samples into groups and further fuses the groups into larger clusters, starting with the highest mutual similarities and gradually lowering the similarity level at which groups are formed and resulting in a single cluster containing all samples (Clarke and Warwick, 1994). The results of hierarchical clustering are given pictorially in dendrograms, with the x-axis representing the full set of samples and the y-axis the level of similarities of samples. Bray-Curtis similarity matrix was used because it allows all species, common or rare, to contribute to the final level of similarity whilst still assigning greater weight to the commonest species (Clarke and Warwick, 1994).

2.7.7 Ordination

An ordination is a pictorial representation of samples in the form of a map usually in two or three dimensions, in which the distances among samples represent the degree of similarities or dissimilarities of their community structure (Clarke and Warwick, 1994). Nearer samples are often more similar in community structures than distant samples. The relationships among sampling sites and seasons based on physical and chemical water quality variables were elucidated using correlation principal component analysis (PCA). PCA was used for the ordination of sites and seasons based on water quality variables because it is a conceptually simple method well suited for ordinations of environmental variables (Clarke and Warwick, 1994). In addition, correlation PCA was chosen because it assigns all environmental variables equal weight irrespective of their scale of measurement so that different scales of measurement do not impact on the final results of the PCA ordinations (Clarke and Warwick, 1994).

2.7.8 Canonical correspondence analysis (CCA)

CCA is a multivariate statistical analysis to elucidate the relationships between biological community and their environment (ter Braak and Verdonschot, 1995). CCA is frequently used to determine which environmental variable(s) is/are important in structuring the biological community. In this study, CCA was applied to elucidate the relationship between the chironomid community assemblage and the measured physical and chemical water quality variables with a view to determining the important variables responsible for the observed spatial and temporal distribution of the chironomid community. A Monte Carlo permutation test with 199 random permutations was used to determine the environmental axis that significantly correlated with the biological variables. CCA and cluster analysis were performed using the computer programme Environment Community Analysis 1.33 package (ECOM) (Pisces conservation Ltd, 2000).

CHAPTER 3 RESULTS: RESPONSE OF MACROINVERTEBRATE COMMUNITIES AS INDICATORS OF ANTHROPOGENIC IMPACTS IN THE SWARTKOPS RIVER

3.1 Introduction

The chapter begins with the presentation of results for the measured physical and chemical water quality variables, analysis of macroinvertebrate communities and river health assessment of the Swartkops River using the South African scoring system version 5 (SASS5), and later explores the results for the macroinvertebrate metrics. The diversity and structure of Chironomidae community in relation to environmental water quality changes are also critically analysed. The chapter concludes with discussions of each of the results presented. The SASS5 results were used to determine the current water quality status of the Swartkops River while the macroinvertebrate data were subjected to multimetric analysis in order to explore whether sensitive metrics could provide further ecological information that may enhance understanding of macroinvertebrate responses to impaired water quality. The diversity and structure of chironomid communities were critically analysed to elucidate the bioindication potentials of this family of aquatic macroinvertebrates in assessing water quality deterioration in the Swartkops River.

3.2 Results

3.2.1 Physical and chemical water quality variables

Means, standard deviations and ranges of measured physical and chemical water quality variables taken at all four sites during the four sampling seasons are presented in Table 3.1. The mean values for dissolved oxygen (DO) were highest at site 2 and lowest at site 4. The DO level at site 3 and 4 were below 4 mg/l throughout the sampling seasons (Table 3.1) indicating conditions of low oxygen. One-way analysis of variance (ANOVA) and Tukey-Honestly post hoc Significant Different (HSD) test conducted on log (x +1) transformed data revealed that with the exception of sites 1 and 2, DO differ significantly between all other sampling sites (Table 3.1). Temperature and pH did not show much seasonal variation, although temperature was consistently higher during summer and autumn seasons and lowest during winter. Electrical conductivity (EC) was significantly higher at sites 2, 3 and 4 while turbidity at site 3 was significantly different ($p < 0.05$) from the rest of the sampling sites (Table 3.1). Five days biochemical oxygen demand (BOD_5) and ammonium - nitrogen (NH_4-

N) were significantly higher at sites 3 and 4 and although ANOVA reveal that there was statistically significant difference ($p < 0.05$) in orthophosphate - phosphorus (PO_4-P) among all four sampling sites, Tukey (HSD) post hoc test could not be conducted to ascertain sites responsible for the observed differences. This is because PO_4-P was detected only during spring at site 1. Chlorophyll *a* phytoplankton and periphyton concentrations were significantly higher at sites 2, 3 and 4. However, there was no statistically significant difference in the mean values of chlorophyll *a* phytoplankton concentration among sites 2, 3 and 4 (Table 3.1). Tukey Honestly Post hoc test revealed that chlorophyll *a* periphyton concentrations at sites 1 and 2 were different from each other and were also different from concentrations at sites 3 and 4. However, no statistically significant difference ($p > 0.05$) was observed in periphyton concentrations between sites 3 and 4 (Table 3.1). Although nitrate-nitrogen (NO_3-N) and nitrite-nitrogen (NO_2-N) were higher at sites 3 and 4, no statistically significant difference was observed among all four sampling sites. Total inorganic nitrogen (TIN) was higher at site 3 and 4. The relatively high BOD_5 and nutrient concentrations coupled with low DO levels at sites 3 and 4 are probably indicative of high organic loads.

Generally, the downstream sites, particularly sites 3 and 4, revealed evidence of environmental water quality impairment with EC, DO, BOD_5, NH_4-N, TIN and chlorophyll *a* (phytoplankton concentrations) showing statistically significant differences ($p < 0.05$) between site 1 and downstream sites 3 and 4 (Table 3.1). However, Tukey HSD analysis revealed no significant statistical difference between sites 1 and 2 for the measured water quality variables except, EC and chlorophyll *a* phytoplankton and periphyton (Table 3.1).

Table 3.1 Means, standard deviations and ranges (in bracket) of measured physical and chemical water quality variables (n = 4). P values are shown for only variables that showed significant differences between sites (different superscript letters per variable indicate significant differences established using the Tukey HSD test). [1] = variable detected only once, [2] = variable detected twice, [3] = variable detected only three times.

Variable	**Site 1**	**Site 2**	**Site 3**	**Site 4**	**p-value**
Dissolved oxygen (mg/l)	6.07 ± 1.13[a] (4.73 – 7.48)	6.99 ± 1.84[a] (5.53 – 9.48)	3.26 ± 0.54[c] (2.63 – 3.89)	2.17 ± 1.3[d] (0.9 – 3.79)	0.001
pH	6.61 ± 1.17 (5.13 – 7.75)	7.12 ± 1.08 (5.69 – 8.25)	7.36 ± 0.42 (6.97 – 7.9)	7.27 ± 0.56 (6.65 – 8.01)	
Temperature (^{o}C)	17.88 ± 4.44 (12.5 – 22.0)	17.71 ±7.72 (9.8 – 27.3)	20.13 ± 4.49 (14.3 – 25.2)	18.55 ± 5.43 (12.2 – 24.0)	
Electrical conductivity (mS/m)	32.58 ± 4.69[a] (28.1 – 39.0)	407.5 ±84.5[bcd] (300 – 488)	212.78 ± 79.9[bce] (154.8 – 331)	268.5 ± 37.6[bc] (234 – 322)	0.000
Turbidity (NTU)	8.75 ± 7.89[a] (3.68 – 10.1)	6.15 ±1.47[a] (4.7 – 8.0)	115.63 ± 139.2[b] (10.5 – 320)	9.35 ± 11.22[a] (2.2 – 26)	0.012
BOD_5 (mg/l)	4.21 ± 1.96[a] (2.16 – 6.86)	8.66 ±5.47[a] (4.78 – 16.68)	14.66 ± 5.15[b] (8.32 – 20.62)	13.58 ± 6.85[b] (7.06 – 22.94)	0.012
Nitrate - nitrogen (NO_3-N) (mg/l)	0.078[2]± 0.06 (0.032 – 0.12)	0.922 ± 1.36 (0.034 – 2.93)	1.45 ± 0.71 (0.408 – 1.98)	2.2 ±2.29 (0.467 – 5.42)	
Nitrite - nitrogen (NO_2-N) (mg/l)	0.014 ± 0.015[3] (0.005– 0.03)	0.07 ± 0.09 (0.008– 0.205)	0.209 ± 0.12 (0.045 – 0.296)	0.088 ± 0.18 (0.028 – 0.42)	
Ammonium - nitrogen (NH_4-N) (mg/l)	0.162 ± 0.17[3a] (0.003– 0.338)	0.575 ± 0.81[a] (0.066 – 1.79)	5.4 ± 2.24[b] (3.86 – 8.83)	3.97 ± 1.99[b] (1.05 – 5.24)	0.000
Total inorganic nitrogen (TIN) (mg/l)	0.171 ± 0.22[a] (0.039 – 0.49)	1.567 ± 2.26[a] (0.169 - 4.92)	7.2 ± 2.51[b] (5.19 – 10.84)	6.34 ± 3.62[b] (1.59 -10.19)	0.001
Orthophosphate - phosphorus (PO_4-P) (mg/l)	0.013[1]	1.11 ± 0.56 (0.463 – 1.5)	7.46 ± 4.05 (2.17 – 11.98)	7.59 ± 1.35 (6.86 – 9.61)	0.001
Chlorophyll *a* phytoplankton (µg/l)	0.856 ± 0.72[a] (0.427– 1.933)	3.82 ± 2.88[b] (1.6 – 7.96)	2.51 ± 0.97[b] (1.53 – 3.62)	1.95 ± 0.53[b] (1.56 – 2.74)	0.000

Variable	Site 1	Site 2	Site 3	Site 4	p-value
Chlorophyll *a* periphyton ($\mu g/cm^2$)	37.02 ± 2.37[a] (20.29– 48.96)	308.08 ±118.2[b] (145.87–428)	421.87 ± 92.55[c] (283.64 –476.27)	410.03 ± 114.7[c] (244.81 – 489.53)	0.028

Principal Component Analysis (PCA) classified the sites into three distinct groups based on the measured physical and chemical water quality variables (Figure 3.1). PCA axis 1 with Eigen-value 6.007 and axis 2 with Eigen-value 1.884 accounted for 50.061% and 15.701% of the total variations respectively. PCA therefore revealed that axes 1 and 2 could explain 65.76% of the variation among measured water quality variables which is an indication of good ordination. PCA grouped site 1 separately from sites 2, 3 and 4 in all the sampling seasons. Samples from site 2 during spring, summer and autumn formed the second distinct cluster while all samples collected at sites 3 and 4 together with winter sample from site 2 formed the third cluster. The separation of the downstream sites (i.e sites 2, 3 and 4) from site 1 is an indication of deteriorating water quality at these sites (Table 3.1), which may be attributed to organic loads, industrial effluents and other anthropogenic activities. Although site 2 comes out separately from site 1 and sites 3 and 4 except for winter, the poorest water quality situation at the site was recorded during winter, which is when it clusters with sites 3 and 4.

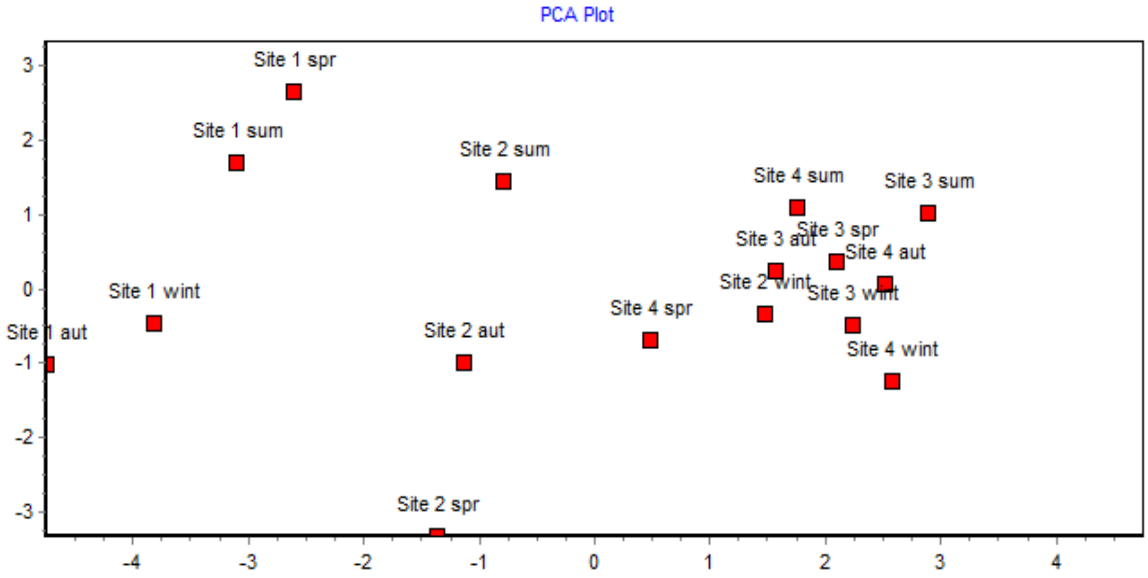


Figure 3.1: PCA ordination bi-plots for water quality variables at the four sampling sites in the Swartkops River for each of the four sampling seasons. Abrreviations: sum = summer, spr = spring, aut = autumn and wint = winter.

3.2.2 Analysis of macroinvertebrate communities in the Swartkops River

Relationships among macroinvertebrate communities sampled at the four sampling sites during the four sampling seasons were elucidated by cluster analysis (Figures 3.2, 3.3, 3.4 and 3.5). In spring, macroinvertebrate assemblages clustered mostly by sites (Figure 3.2). Within sites, replicates taken from the same biotopes were often more closely related. At a Bray-Curtis similarity level of at least 40 %, five distinct clusters were identified. Replicates in cluster 1 were all sampled at site 1 while replicates sampled at sites 4 and 2 formed clusters 3 and 4 respectively and cluster 5 was largely made of replicates sampled at site 3 (Figure 3.2).

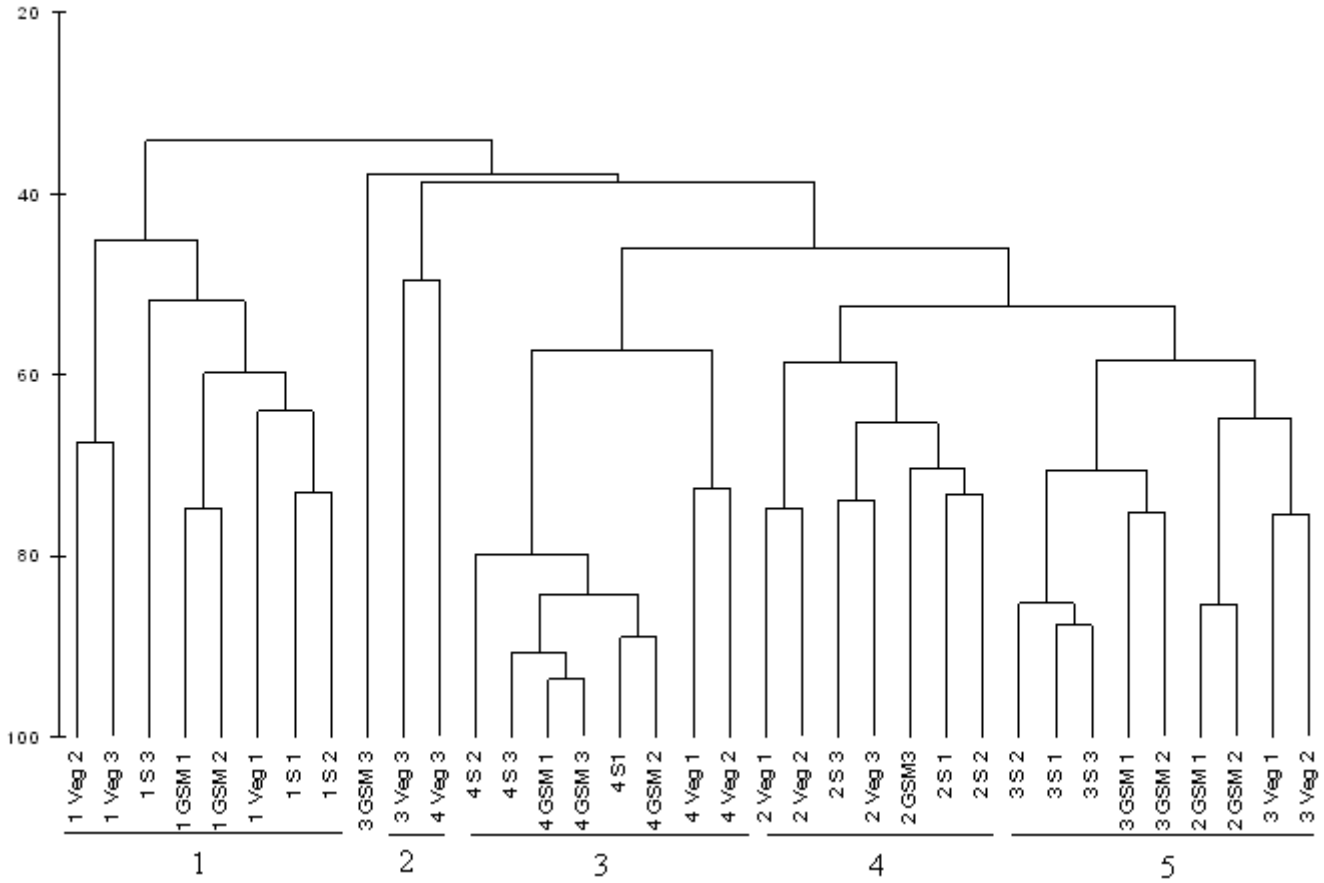


Figure 3.2: Bray – Curtis similarity hierarchical cluster analysis of macroinvertebrate replicates sampled during spring at the four sampling sites in the Swartkops River. Abbreviation format: sites are represented by numbers before biotopes: (1= site 1, 2 = site 2, 3 = site 3 and 4 = site 4), biotopes (S = stones in- and out- of current, Veg = marginal and aquatic vegetation and GSM = gravel, sand and mud) and replicates represented by numbers placed after biotopes: (1 = replicate 1, 2 = replicate 2 and 3 = replicate 3).

During the summer survey, macroinvertebrate communities also clustered mostly by sites and within sites, replicates sampled from the same biotopes were more closely associated (Figure 3.3). At a Bray-Curtis similarity of at least 40 %, four distinct clusters were observed. Replicates sampled at site 2 formed cluster 1 while replicates sampled at sites 1 and 3 formed clusters 2 and 3 respectively. Cluster 4 was largely made of replicates sampled at site 4. Replicates such as replicates 1, 2 and 3 of the vegetation biotope sampled at site 4 did not group with other samples (Figure 3.3). Similarly, replicates 2 and 3 of the stones biotope sampled at site 1 did not form any distinct cluster with other samples (Figure 3.3).

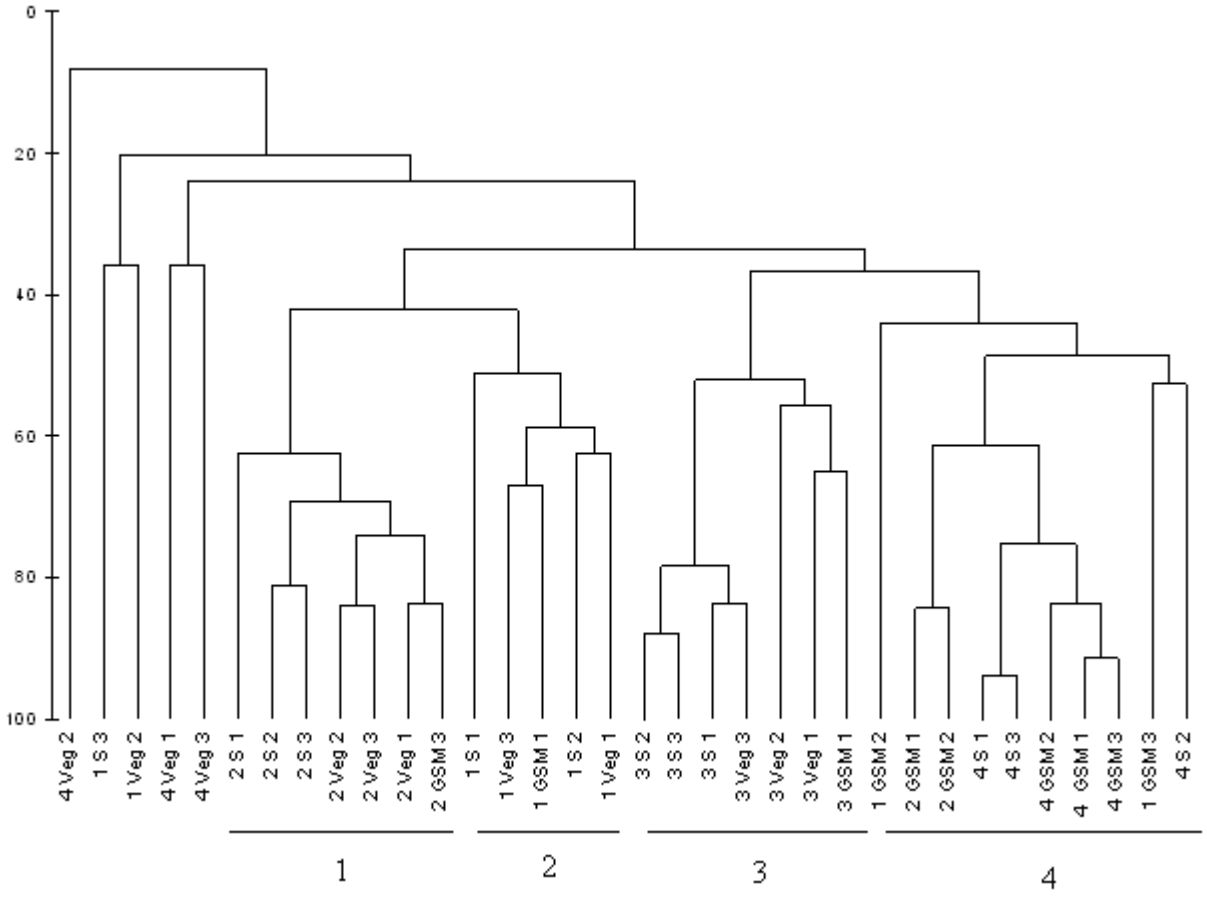


Figure 3.3: Bray – Curtis similarity hierarchical cluster analysis of macroinvertebrate replicates sampled during summer at the four sampling sites in the Swartkops River. Abbreviation format: sites are represented by numbers before biotopes: (1= site 1, 2 = site 2, 3 = site 3 and 4 = site 4), biotopes (S = stones in- and out- of current, Veg = marginal and aquatic vegetation and GSM = gravel, sand and mud) and replicates are represented by numbers placed after biotopes: (1 = replicate 1, 2 = replicate 2 and 3 = replicate 3).

During autumn, macroinvertebrate communities also clustered mostly by sites and within sites, replicates sampled from the same biotopes were more closely linked (Figure 3.4). At a Bray-Curtis similarity of at least 40 %, five different clusters were identified. Clusters 2, 4 and 5 were made of replicates sampled at sites 4, 1, 3 and 2 respectively. Samples in cluster 1 were a mixture of replicates sampled at sites 3 and 4 probably indicating similarity between macroinvertebrate communities at these sites during autumn.

During winter, the pattern of grouping of macroinvertebrate assemblages were less distinct with replicates sampled at sites 3 and 4 clustering to form cluster 2 (Figure 3.5). However, clusters 1 and 3 were made of replicates sampled at sites 1 and 2 respectively. Within these sites (i.e. sites 1 and 2), grouping of replicates sampled from the same biotope were also less distinct.

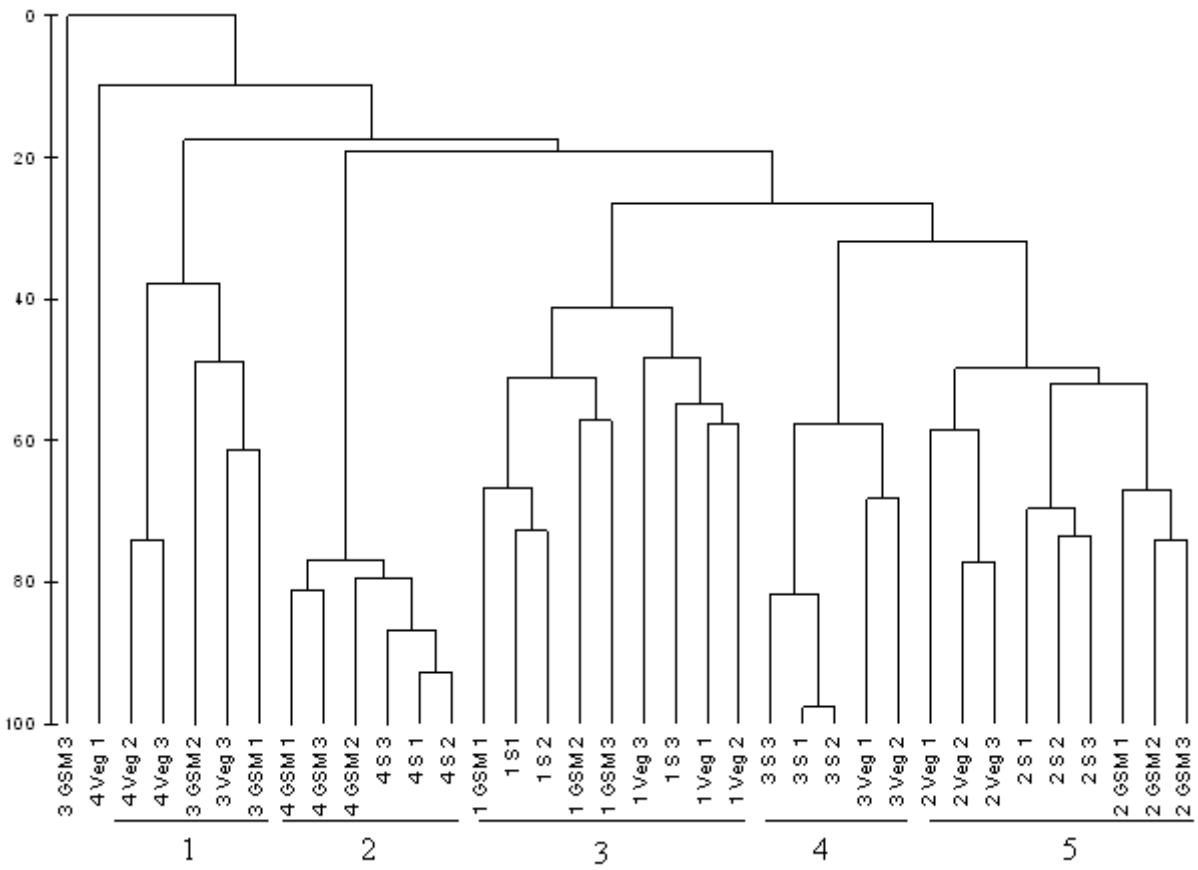


Figure 3.4: Bray – Curtis similarity hierarchical cluster analysis of macroinvertebrate replicates sampled during autumn at the four sampling sites in the Swartkops River. Abbreviation format: sites are represented by numbers before biotopes: (1= site 1, 2 = site 2, 3 = site 3 and 4 = site 4), biotopes (S = stones in- and out- of current, Veg = marginal and aquatic vegetation and GSM = gravel, sand and mud) and replicates are represented by numbers after biotopes: (1 = replicate 1, 2 = replicate 2 and 3 = replicate 3).

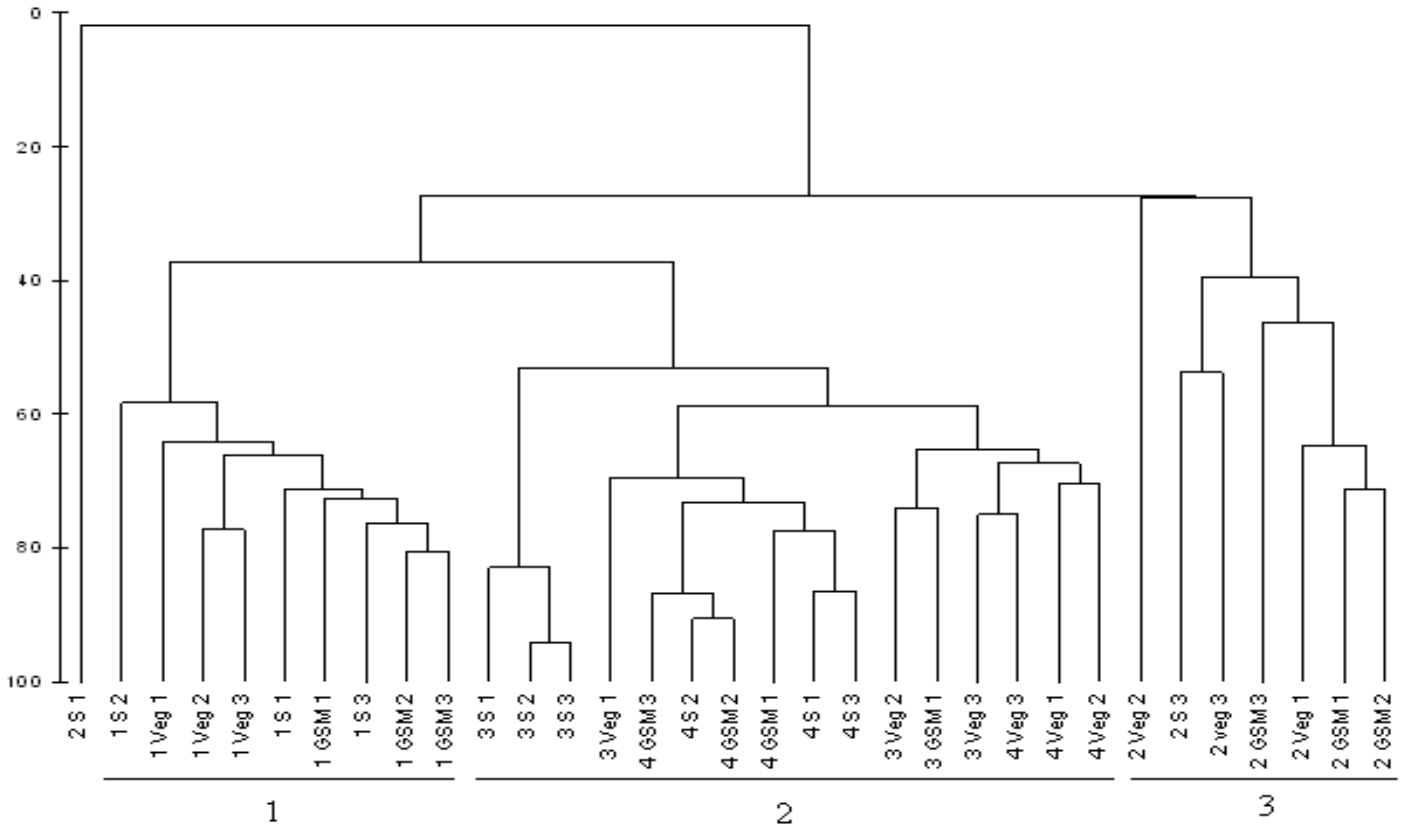


Figure 3.5: Bray – Curtis similarity hierarchical cluster analysis of macroinvertebrate replicates sampled during winter at the four sampling sites in the Swartkops River. Abbreviation format: sites are represented by numbers before biotopes: (1= site 1, 2 = site 2, 3 = site 3 and 4 = site 4), biotopes (S = stones in- and out- of current, Veg = marginal and aquatic vegetation and GSM = gravel, sand and mud) and replicates are represented by numbers placed after biotopes: (1 = replicate 1, 2 = replicate 2 and 3 = replicate 3).

3.2.2.1 Analysis of similarities and differences of macroinvertebrate communities at each of the four sampling sites

Macroinvertebrates were sampled from three SASS5 biotopes: stones, vegetation and GSM. Three replicates were collected from each biotope at each of the sampling sites during each sampling season. However, because of insufficient GSM biotope, only two GSM replicates were collected during spring at site 1, during autumn at site 3 and one each during winter and summer at site 3. Similarity between replicates sampled from the same biotopes and differences between sampled biotopes and seasons at each of the sampling sites were explored using Analysis of similarity (ANOSIM) and similarity percentage (SIMPER) analysis. Analysis of similarity to test whether there were significant difference between replicates sampled from the same biotopes was conducted at the site level ($n = 12$) with replicate numbers as factor. The analysis could not be conducted at each site per biotope per season level ($n = 3$) because of few groups. However, at site 1 where only two GSM replicates were collected during spring, number of GSM group was 11 while at site 3 it was 6 because only two GSM replicates were collected during autumn and one GSM replicate each during winter and summer.

At site 1, ANOSIM revealed that macroinvertebrate replicates sampled from the same biotopes were not significantly different ($p > 0.05$) in terms of abundance and composition. ANOSIM pair-wise test indicated that there were significant differences ($p < 0.05$) between the three sampled biotopes (i.e. stones, vegetation and gravel, sand and mud, GSM) in terms of macroinvertebrate abundance and composition (Table 3.2). SIMPER analysis revealed that similarity between replicates taken from the stone biotope was largely due to the presence of families such as Chironomidae, Simulidae, Baetidae and Libellulidae. Among vegetation replicates, similarity could be attributed to the presence of Chironomidae, Coenagrionidae, Baetidae and Lymnaeidae. The within GSM similarity was largely due to the contributions of the abundance and composition of families such as Chironomidae, Simulidae, Oligochaete, Baetidae and Hydropsychidae to the similarity matrix. SIMPER analysis showed that the faunal differences in terms of abundance and composition, observed between stone and vegetation biotopes could be ascribed to families such as Chironomidae and Simulidae which were more abundant on stone biotope, but less abundant in vegetation biotope. Differences observed between stone and GSM biotopes could be attributed to Chironomidae, Simulidae and Baetidae that dominated the abundance of macroinvertebrate sampled from stone. The abundance of families such as Hydropsychidae and Oligochaete differentiated GSM from stone. Differences

between vegetation and GSM biotopes were largely due to the high abundance of Chironomidae and Libellulidae in vegetation biotope and being less abundant in GSM biotope. Hydropsychidae, Lymnaeidae and Oligochaete discriminated the GSM biotope from vegetation.

Seasonal differences in macroinvertebrate communities were also observed at site 1 (Table 3.2). ANOSIM pair-wise test indicated that in terms of macroinvertebrate abundance and composition, significant difference exist between spring and autumn, summer and autumn, summer and winter and autumn and winter (Table 3.2). SIMPER analysis showed that differences observed between spring and autumn were mostly due to the contributions of Hydropsychidae, Simulidae, Chironomidae, Lymnaeidae, Coenagrionidae and Baetidae to the dissimilarity matrix. These families were more abundant in spring than in autumn. Differences between summer and autumn could be attributed to the abundance of families such as Chironomidae, Lymnaeidae, Oligochaete and Baetidae during summer, and abundance of Simulidae during autumn. Differences in macroinvertebrate composition between summer and winter were mostly due to the abundance of Lymnaeidae and Coenagrionidae in summer and abundance of Simulidae, Chironomidae and Baetidae during winter survey. The contributions of Simulidae, Oligochaete, Chironomidae and Baetidae to the dissimilarity matrix were responsible for the observed differences between autumn and winter. No significant difference between spring and summer, and winter and spring was observed.

Table 3.2: Analysis of similarities and differences between biotopes, and seasons sampled at the four sampling sites in the Swartkops River. Abbreviation: S = stones (stones in- and out- of current), Veg = vegetation (marginal and aquatic), GSM = gravel, sand and mud. Spr = spring, Sum = summer, Aut = autumn, and Wint = winter.

Site 1					
Biotopes	**Average dissimilarity (%)**	**Significantly different?**	**Seasons**	**Average dissimilarity (%)**	**Significantly different?**
S and Veg	60.96	Yes	Spr and Sum	54.43	No
S and GSM	61.75	Yes	Spr and Aut	54.56	Yes
Veg and GSM	68.68	Yes	Spr and Wint	48.3	No
			Sum and Aut	59.76	Yes
			Sum and Wint	55.52	Yes
			Aut and Wint	52.45	Yes
Site 2					
S and Veg	62.47	Yes	Spr and Sum	49.78	No
S and GSM	58.38	Yes	Spr and Aut	62.81	Yes
Veg and GSM	58.66	Yes	Spr and Wint	69.03	Yes
			Sum and Aut	48.62	No
			Sum and Wint	74.4	Yes
			Aut and Wint	75.88	Yes
Site 3					
S and Veg	50.93	Yes	Spr and Sum	53.79	Yes
S and GSM	55.79	Yes	Spr and Aut	59.9	Yes
Veg and GSM	54.12	No	Spr and Wint	47	No

Biotopes	Average dissimilarity (%)	Significantly different?	Seasons	Average dissimilarity (%)	Significantly different?
Site 3					
			Sum and Aut	48.01	No
			Sum and Wint	39.41	No
			Aut and Wint	52.77	Yes
Site 4					
S and Veg	74.54	Yes	Spr and Sum	63.64	Yes
S and GSM	40.06	No	Spr and Aut	60.28	Yes
Veg and GSM	73.35	Yes	Spr and Wint	52.22	Yes
			Sum and Aut	57.9	Yes
			Sum and Wint	67.27	Yes
			Aut and Wint	69.14	Yes

At site 2, ANOSIM analysis to test whether there were significant differences in macroinvertebrate abundance and composition between replicates collected from the same biotopes indicated no significant difference ($p > 0.05$). ANOSIM pair-wise test revealed significant differences between biotopes in terms of macroinvertebrate abundance and compositions (Table 3.2). SIMPER analysis indicated that similarities between replicates taken from the stone biotope were largely due to the abundance of family such as Chironomidae, Oligochaete, Baetidae, Corixidae and Simulidae while the contributions of Chironomidae, Oligochaete, Coenagrionidae, Baetidae, Culicidae and Dytiscidae to the similarity matrix accounted largely for the similarity observed between replicates taken from the vegetation biotope. Faunal similarity between gravel, sand and mud (GSM) replicates could be ascribed to the abundance of families such as Chironomidae, Oligochaete and Corixidae. Differences in terms of macroinvertebrate abundance and composition between stones and vegetation could be

ascribed to families such as Chironomidae and Physidae whose abundances were higher on stones than in vegetation, while abundance of Baetidae, Oligochaete and Coenagrionidae discriminated vegetation from stone biotope. Faunal differences between stones and GSM could be ascribed to the high abundance of families such as Chironomidae, Baetidae, Physidae and Simulidae in stone biotope as opposed to the abundance of Corixidae, Oligochaete and Dytiscidae in the GSM biotope. Significant differences in terms of macroinvertebrate abundance and composition between the vegetation and GSM biotopes could be attributed to families such as Baetidae, Chironomidae, Coenagrionidae, Physidae and Culicidae which were more abundant in the vegetation biotope than in GSM biotope, and families such as Corixidae and Oligochaete which were more abundant in the GSM biotope than in vegetation.

With regard to seasonal differences, ANOSIM pair-wise test indicated significant differences ($p < 0.05$) between spring and autumn, spring and winter, summer and autumn, autumn and winter in terms of macroinvertebrate abundance and composition. However, ANOSIM pair-wise test did not reveal significant differences between spring and summer, summer and autumn (Table 3.2). SIMPER analysis indicated that significant differences between the spring and autumn surveys could be ascribed to families such as Physidae, Simulidae, Oligochaete and Coenagrionidae which were more abundant in spring than in autumn, and families such as Baetidae, Corixidae and Ceratopogonidae that were more abundant in autumn than in spring. Significant differences between spring and winter surveys could be attributed to families such as Chironomidae, Physidae, Simulidae, Oligochaete and Coenagrionidae which were more abundant in spring than in winter, and Culicidae being more abundant in winter than in spring. Differences in terms of macroinvertebrate abundance and composition between summer and winter were as a result of families such as Chironomidae, Corixidae, Physidae, Baetidae, Oligochaete and Dytiscidae that were more abundant in summer than in winter. No family had higher abundance in spring than in summer. The abundance and compositional contributions of Baetidae, Chironomidae, Corixidae, Coenagrionidae, Oligochaete and Culicidae to the dissimilarity matrix between autumn and winter surveys, accounted largely for the observed significant difference between these two sampling seasons.

At site 3, ANOSIM revealed that there were no significant differences ($p > 0.05$) between replicates sampled from the same biotope in terms of macroinvertebrate abundance and composition. ANOSIM pair-wise test indicated significant differences between stone and vegetation biotope, stone and gravel, sand and mud (GSM) biotopes (Table 3.2). ANOSIM

indicated that no significant difference existed between vegetation and GSM biotopes. SIMPER analysis revealed that similarity between stone replicates was largely due to the abundance contribution of family Chironomidae to the similarity matrix while similarities between vegetation replicates and between GSM replicates were as a result of the abundance contributions of families such as Chironomidae, Culicidae and Notonectidae to the respective similarity matrices. SIMPER analysis indicated that macroinvertebrate community differences in terms of abundance and composition that existed between stone and vegetation could be attributed to families such as Oligochaete and Chironomidae which were more abundant on stone biotope than in vegetation, and families such as Culicidae, Notonectidae, Hirudinae and Belostomatidae that were less abundant on stones than in vegetation biotope. Differences between stones and GSM were as a result of families such as Chironomidae and Oligochaete that were more abundant on stones than in vegetation, and families such as Notonectidae and Culicidae which were more abundant in vegetation than on stones.

With respect to seasons, in terms of macroinvertebrate assemblages sampled at this site, ANOSIM pair-wise test revealed that there were significant differences between spring and summer, spring and autumn, autumn and winter in terms of macroinvertebrate abundance and composition. However, ANOSIM pair-wise test did not indicate significant difference between summer and winter, spring and winter, summer and autumn in terms of macroinvertebrate abundance and composition (Table 3.2). SIMPER analysis revealed that significant differences between spring and summer could be ascribed to taxa such as Oligochaete, Culicidae, Hirudinae being more abundant in spring than in summer, and taxa such as Chironomidae and Notonectidae being more abundant in summer than in spring. Significant differences between spring and autumn were largely due to the abundance contributions of Oligochaete, Hirudinae, Chironomidae, Culicidae, Notonectidae and Belostomatidae to the dissimilarity matrix between these two sampling seasons. Autumn and winter differences could be attributed to families such as Culicidae, Belostomatidae and Notonectidae that were more abundant in autumn than in winter, and families such as Chironomidae, Oligochaete and Hirudinae that were less abundant in autumn than in winter.

At site 4, ANOSIM showed that replicates sampled from the same biotopes were not significantly different ($p < 0.05$) in terms of macroinvertebrate faunal composition. ANOSIM pair-wise test revealed that with the exception of stone and GSM biotopes, all sampled biotopes (i.e. stone, vegetation and gravel-sand-mud GSM), and all sampled seasons (i.e. spring, summer,

autumn and winter) were significantly different ($p < 0.05$) (Table 3.2). SIMPER analysis revealed that similarity between the stone replicates was largely due to the abundance of Oligochaete and Chironomidae while similarity between the vegetation replicates was largely a result of the abundance of Chironomidae, Hirudinae and Culicidae. Similarity between GSM replicates was also due to the abundance of Oligochaete and Chironomidae. SIMPER analysis revealed that significant faunal differences between stone and vegetation biotopes in terms of macroinvertebrate abundance and composition were largely due to families such as Oligochaete and Hirudinae on the stone biotope as opposed to the dominance of Chironomidae, Culicidae and Physidae in the vegetation biotope. Significant differences between vegetation and GSM could be ascribed to the dominance of Chironomidae and Culicidae in vegetation and Oligochaete and Hirudinae that dominated the macroinvertebrate abundance in GSM.

In terms of seasonal differences, SIMPER analysis indicated that differences between spring and summer surveys could be attributed to families such as Hirudinae and Physidae which were more abundant in spring than in summer, and Oligochaete and Chironomidae that were less abundant in spring than in summer. Spring and autumn differences were largely due to the abundance contributions of Hirudinae, Oligochaete and Culicidae to the dissimilarity matrix between these two seasons. Differences in terms of macroinvertebrate abundance and composition between summer and autumn could be attributed to families such as Oligochaete and Chironomidae that dominated the abundance of macroinvertebrate in summer and Culicidae and Corixidae that dominated the abundance of macroinvertebrates in autumn. Spring and winter differences could be ascribed to Oligochaete and Physidae which were less abundant in winter than in spring, and Chironomidae and Hirudinae that were more abundant in winter than in spring. The summer survey was largely discriminated from the winter survey by Oligochaete abundance while Chironomidae and Hirudinae differentiated the winter and summer surveys. Differences in macroinvertebrate abundance and composition between autumn and winter surveys were a result of Oligochaete and Culicidae that dominated the abundance of macroinvertebrates in autumn and Chironomidae and Hirudinae that dominated the abundance in autumn.

3.2.3 River health assessment of the Swartkops River using the South African Scoring System version 5 (SASS5) scores, number of taxa and average score per taxon (ASPT)

SASS5 scores, number of taxa and ASPT values were assessed at each of the sampling sites during the four sampling seasons. At site 1, median SASS5 scores and number of taxa for stone biotope were slightly higher than those for the vegetation, and gravel, sand and mud (GSM) biotopes (Figure 3.6). Kruskal-Wallis multiple comparison of significant difference test revealed that median SASS5 scores between stone and GSM differ significantly ($p < 0.05$) (Table 3.3). However, median SASS5 scores between stone and vegetation, vegetation and GSM did not differ significantly ($p > 0.05$) (Table 3.3). Kruskal-Wallis test also indicated that the number of taxa between stone and GSM, vegetation and GSM were statistically different. Number of taxa did not differ significantly between stone and vegetation (Table 3.3). Average score per taxon (ASPT) value did not differ significantly between all three sampled biotopes. Total SASS5 score, number of taxa and ASPT values during the four sampling seasons at site 1 are given in Figures 3.7, 3.8 and 3.9. Total SASS5 score and number of taxa were highest in spring and lowest in winter while ASPT value was highest in winter and lowest in summer. The mean total SASS55 score (Figure 3.10) at this site revealed that the overall water quality category was “good” (Table 3.4) while the mean ASPT value (Figure 3.10) indicated that the overall water quality category was “fair” (Table 3.4).

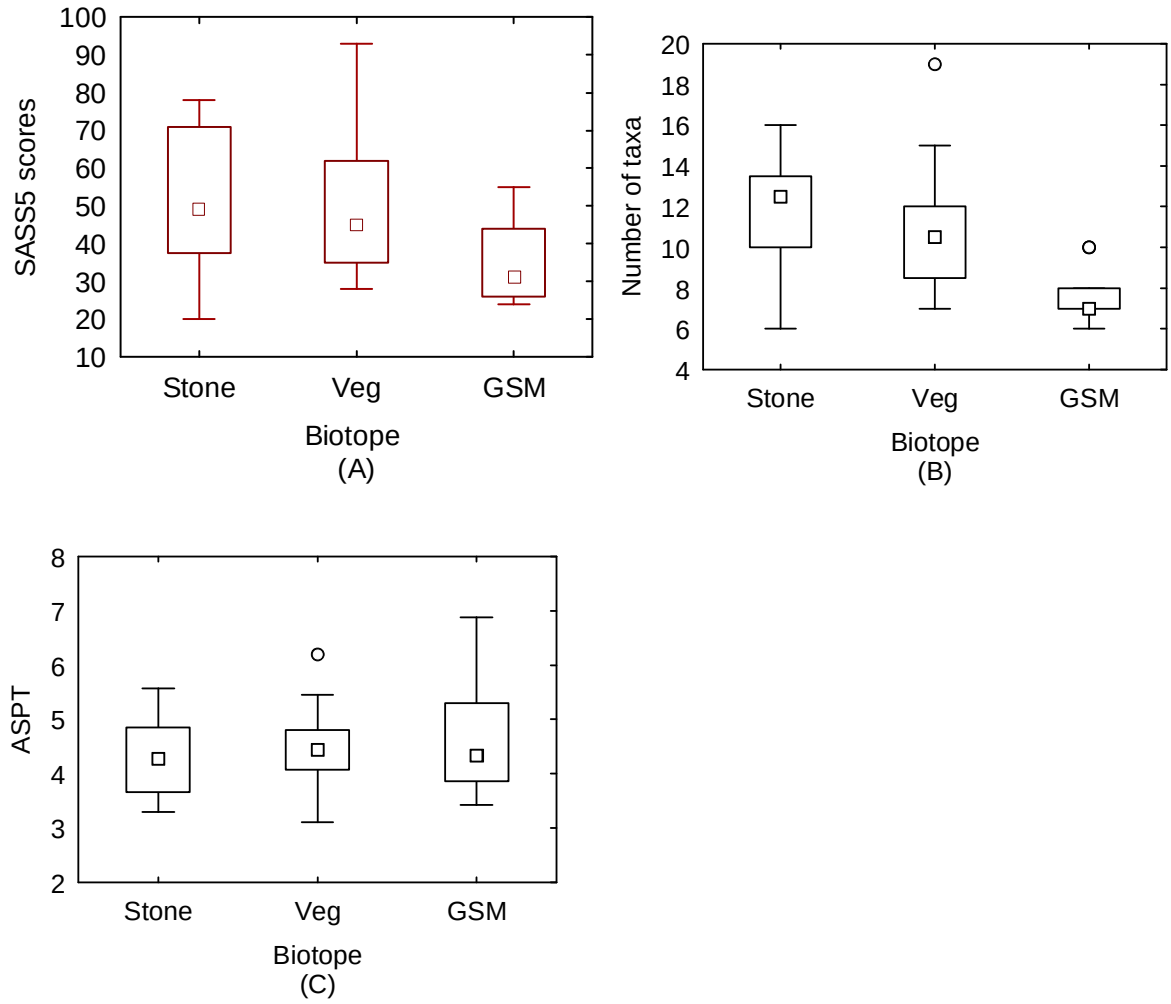


Figure 3.6: Median values for South African Scoring System version 5 (SASS5) score (A), number of taxa (B) and average score per taxon (ASPT) (C) for each of the three sampled biotopes at site 1 in the Swartkops River. Abbreviation: Stone = (stone in- and out- of current), Veg = (aquatic and marginal vegetation) and GSM = (gravel, sand and mud). Small squares represent median numbers, boxes represent interquartile ranges (25-75th percentiles) and circles represent outliers.

Table 3.3: Kruskal-Wallis multiple comparisons of significant difference in SASS5 scores, number of taxa and ASPT values between sampled biotopes at the four sampling sites in the Swartkops River. Biotopes are: stone (stones in- and out- of current), Veg (marginal and aquatic vegetation), GSM (gravel, sand and mud). Yes = significant difference ($p < 0.05$) and No = no significant difference ($p > 0.05$)

Site 1							**Site 2**						
	SASS5 score		**Number of Taxa**		**ASPT**			**SASS5 score**		**Number of Taxa**		**ASPT**	
Biotope	Veg	GSM	Veg	GSM	Veg	GSM	**Biotope**	Veg	GSM	Veg	GSM	Veg	GSM
Stone	No	Yes	No	Yes	No	No	Stone	No	No	No	No	No	No
Veg		No		Yes		No	Veg		No		No		No
Site 3							**Site 4**						
	SASS5 score		**Number of Taxa**		**ASPT**			**SASS5 score**		**Number of Taxa**		**ASPT**	
Biotope	Veg	GSM	Veg	GSM	Veg	GSM	**Biotope**	Veg	GSM	Veg	GSM	Veg	GSM
Stone	Yes	No	Yes	No	Yes	No	Stone	Yes	No	Yes	No	Yes	No
Veg		Yes		Yes		Yes	Veg		Yes		Yes		No

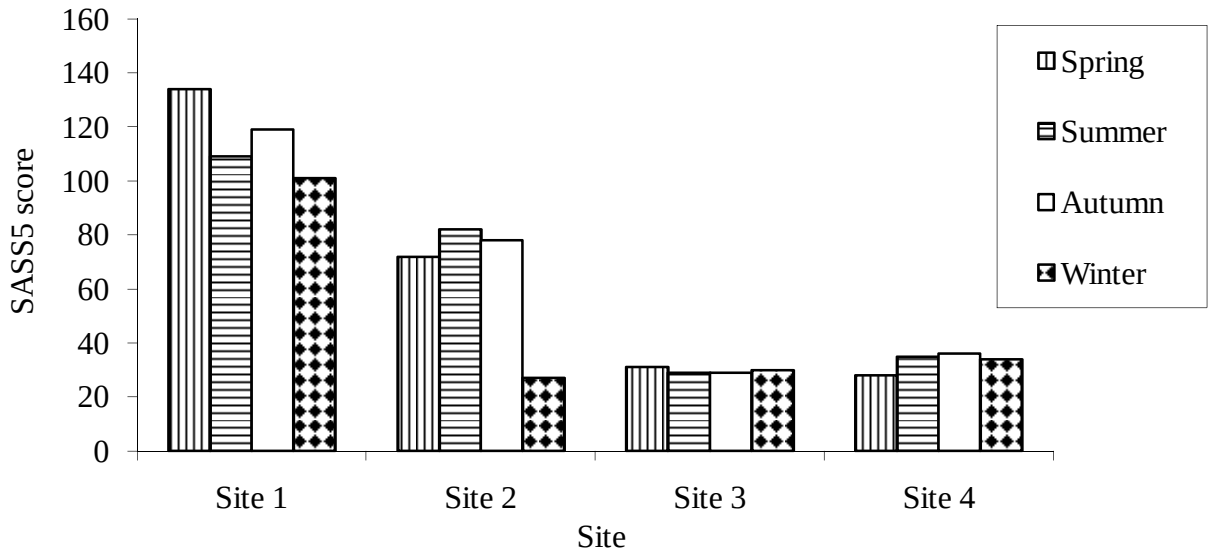


Figure 3.7: Total South African Scoring System version 5 (SASS5) scores at each of the four sampling sites during the four sampling seasons in the Swartkops River.

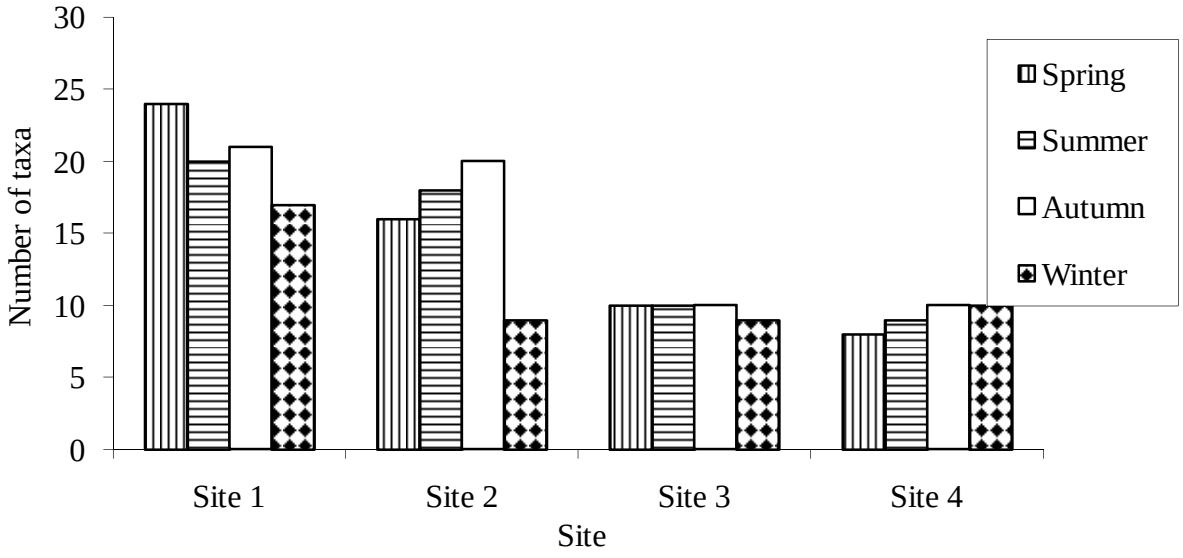


Figure 3.8: Total number of taxa at each of the four sampling sites during the four sampling seasons in the Swartkops River.

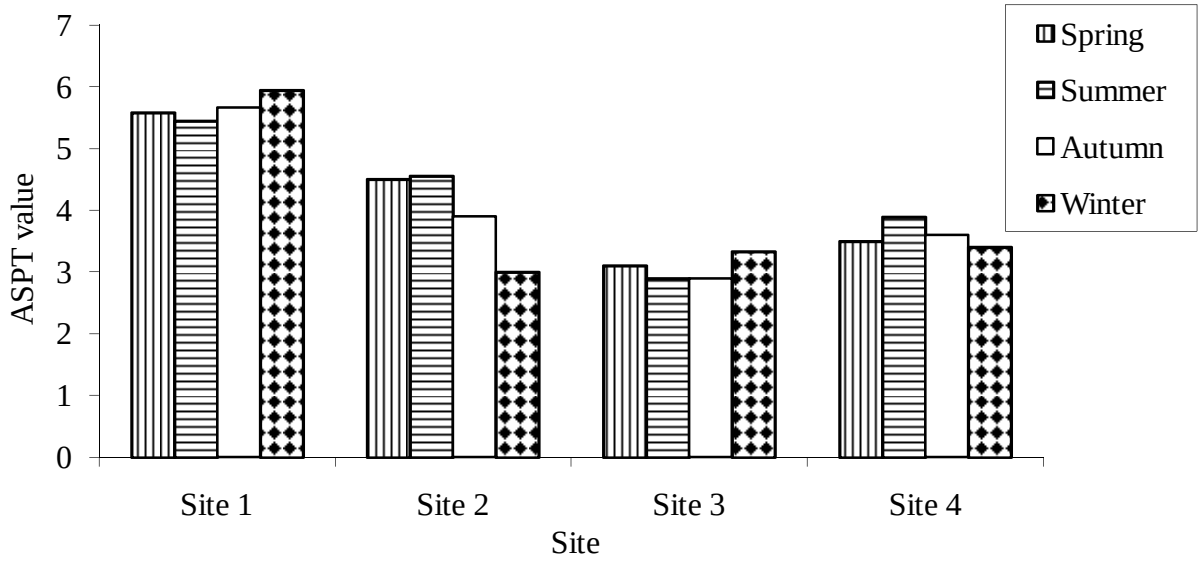


Figure 3.9: Total average score per taxon (ASPT) values at each of the four sampling sites during the four sampling seasons in the Swartkops River.

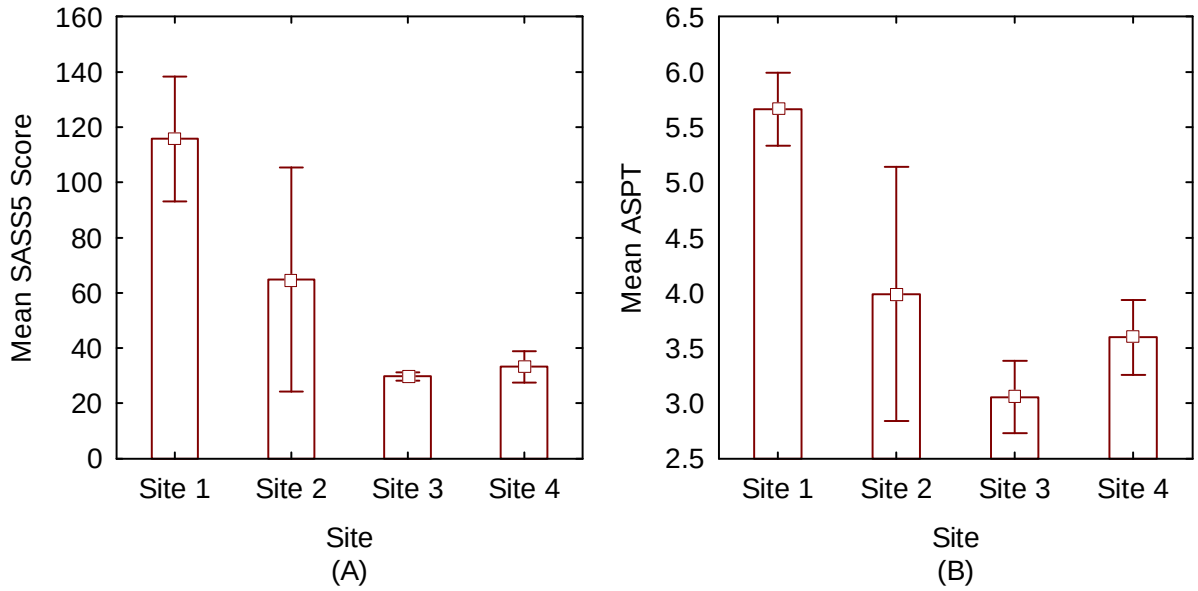


Figure 3.10: Mean values and standard deviations for South African Scoring System version 5 (SASS5) scores (A) and average score per recorded taxon (ASPT) (B), at each of the four sampling sites in the Swartkops River. Small square = mean and range bars = standard deviations.

Table 3.4: Seasonal and overall water quality categories for each of the four sampling sites in the Swartkops River based on SASS5 and ASPT biological bands for the Southern eastern coastal belt (lower zone) ecoregion (Dallas, 2007a)

Site 1					
	Spring	**Summer**	**Autumn**	**Winter**	**Overall quality**
SASS5 score	Good	Good	Good	Good	Good
ASPT value	Fair	Fair	Fair	Fair	Fair
Site 2					
SASS5 score	Poor	Fair	Poor	Critically modified	Poor
ASPT value	Critically modified	Critically modified	Critically modified	Critically modified	Critically modified
Site 3					
SASS5 score	Critically modified	Critically modified	Critically modified	Critically modified	Critically modified
ASPT value	Critically modified	Critically modified	Critically modified	Critically modified	Critically modified
Site 4					
SASS5 score	Critically modified	Critically modified	Critically modified	Critically modified	Critically modified
ASPT value	Critically modified	Critically modified	Critically modified	Critically modified	Critically modified

At site 2, median SASS5 scores and number of taxa for vegetation biotope were higher than those for stone and GSM biotopes (Figure 3.11). However, Kruskal-Wallis multiple comparisons test revealed that SASS5 scores, number of taxa and ASPT were not significant different between the three sampled biotopes (Table 3.3). Total SASS5 scores, number of taxa and ASPT values during the four sampling seasons at this site are given in Figures 3.7, 3.8 and 3.9. Total SASS5 score was highest in summer and lowest during winter while total number of taxa was highest in autumn and lowest in winter. The ASPT was highest in summer and lowest in winter. The mean total SASS5 score (Figure 3.13) at this site revealed that the overall water quality category was “poor” (Table 3.4) while mean ASPT value (Table 3.14) indicated “critically modified” overall water quality category (Table 3.4).

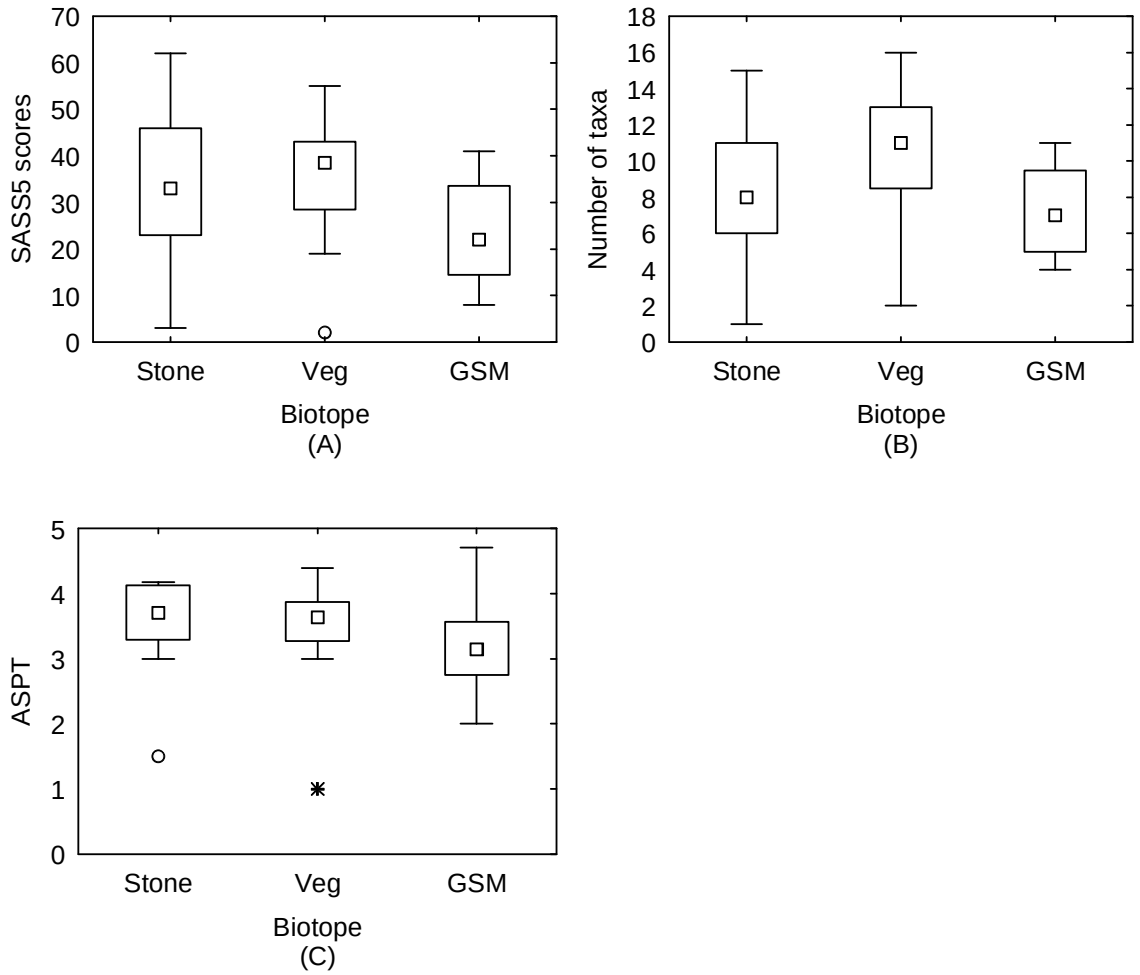


Figure 3.11: Median values for South African Scoring System version 5 (SASS5) score (A), number of taxa (B) and average score per taxon (ASPT) (C) for each of the three sampled biotopes at site 2 in the Swartkops River. Abbreviation: Stone = (stone in- and out- of current), Veg = (aquatic and marginal vegetation) and GSM = (gravel, sand and mud). Small squares represent median numbers, boxes represent interquartile ranges (25-75th percentiles), circles represent outliers and asterisks represent extreme.

At site 3, median SASS5 score, number of taxa and ASPT value were higher in the vegetation biotope than in stones and GSM, but lowest in stone biotope (Figure 3.12). Kruskal-Wallis multiple comparison test revealed that SASS5 scores, number of taxa and ASPT values for vegetation biotope differ significantly ($p < 0.05$) from stone and GSM biotopes (Table 3.3). SASS5 scores, number of taxa and ASPT values between stone and GSM biotopes were not significantly different ($p > 0.05$). Total SASS5 score was highest in spring and lowest in autumn and summer (Figure 3.7). Total number of taxa was highest in spring, autumn and summer but

lowest in winter (Figure 3.8) while total ASPT value was highest in winter and lowest in summer and autumn (Figure 3.9). Mean total SASS5 score and ASPT value (Figures 3.13 and 3.10) indicated “critically modified” overall water quality category at this site (Table 3.4).

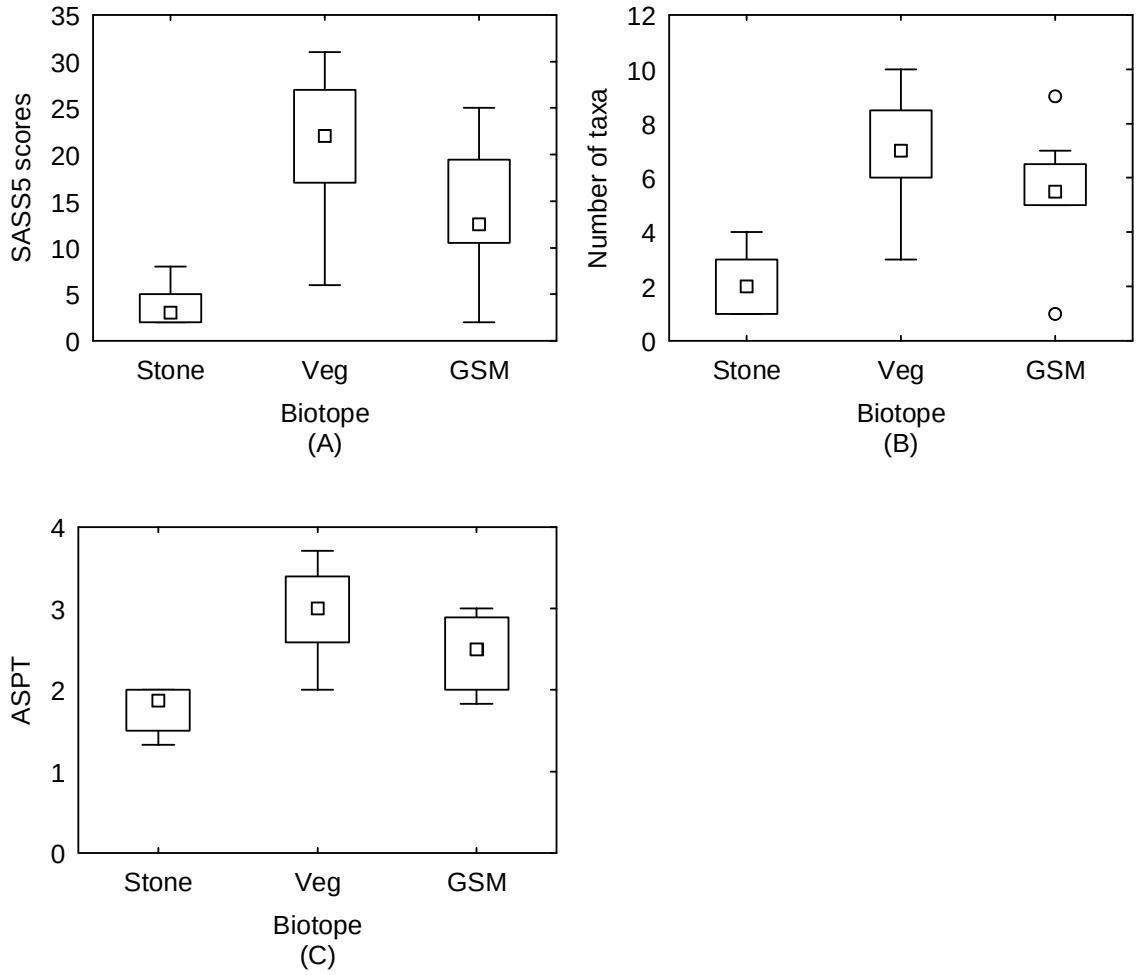


Figure 3.12: Median values for South African Scoring System version 5 (SASS5) score (A), number of taxa (B) and average score per taxon (ASPT) (C) for each of the three sampled biotopes at site 3 in the Swartkops River. Abbreviation: Stone = (stone in- and out- of current), Veg = (aquatic and marginal vegetation) and GSM = (gravel, sand and mud). Small squares represent median numbers, boxes represent interquartile ranges (25-75th percentiles) and circles represent outliers.

At site 4, median SASS5 score, number of taxa and ASPT value were all highest in vegetation biotope (Figure 3.13). Kruskal-Wallis multiple comparison test revealed that SASS5 scores and number of taxa and ASPT values for vegetation biotopes were significantly different from stone and GSM biotopes (Table 3.3). SASS5 scores and number of taxa and ASPT values between stone and GSM biotopes were not significantly different (Table 3.3). Total SASS5 score was highest during autumn and lowest in spring (Figure 3.7) while total number of taxa was highest in autumn and winter but lowest in spring (Figure 3.8). ASPT value was highest in summer but lowest in winter (Figure 3.9). Mean total SASS5 score and ASPT value (Figures 3.13 and 3.14) indicated “critically modified” overall water quality category at this site (Table 3.4).

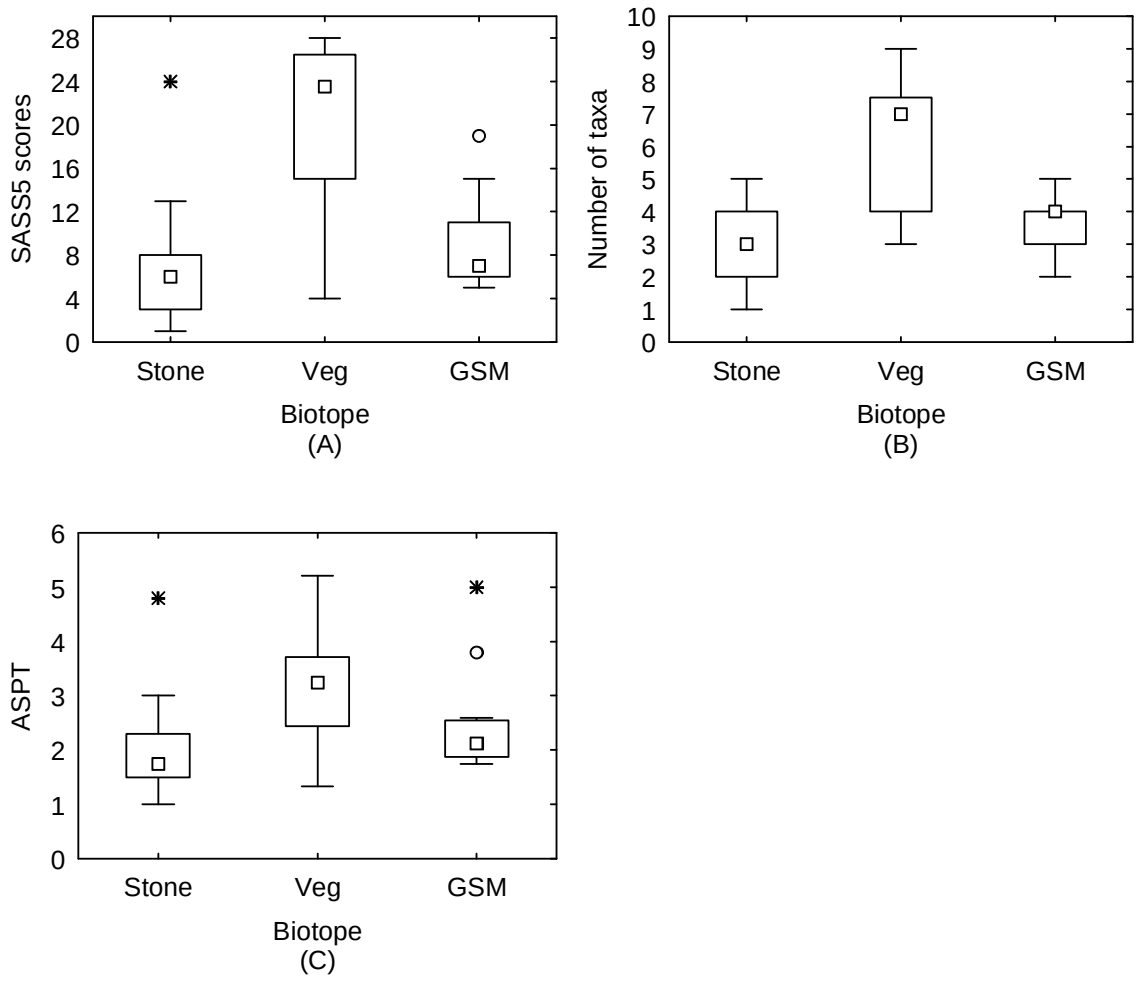


Figure 3.13: Median values for South African Scoring System version 5 (SASS5) score (A), number of taxa (B) and average score per taxon (ASPT) (C), for each of the three sampled biotopes at site 4 in the Swartkops River. Abbreviation: Stone = (stone in- and out- of current), Veg = (aquatic and marginal vegetation) and GSM = (gravel, sand and mud). Small squares represent median numbers, boxes represent interquartile ranges (25-75th percentiles), circles represent outliers and asterisks represent extreme.

3.2.3.1 Discriminatory ability of the South African Scoring System version 5 (SASS5) and average score per taxon (ASPT)

The ability of SASS5 and ASPT to discriminate site 1 from downstream sites (i.e. sites 2, 3 and 4) and to distinguish between sites 2, 3 and 4 was explored using box-and-whisker plots (Figure 3.14). Details of this statistical method are discussed in chapter 2 section 2.7.1 Total SASS5 scores and ASPT values discriminated site 1 from the three downstream sites (i.e. sites 2, 3 and 4). Among the downstream sites, SASS5 scores separated site 2 from both sites 3 and 4 but could not separate sites 3 and 4 from each other. On the other hand, ASPT values separated sites site 3 from sites 2 and 4 but could not discriminate between sites 2 and 4. Thus, both SASS5 scores and ASPT values did not separate all four sampling sites from one another (Figures 3.14). The SASS5 scores and ASPT values were therefore considered very effective in the discrimination of site 1 from all other sites but not effective in the discrimination between all the three downstream sites.

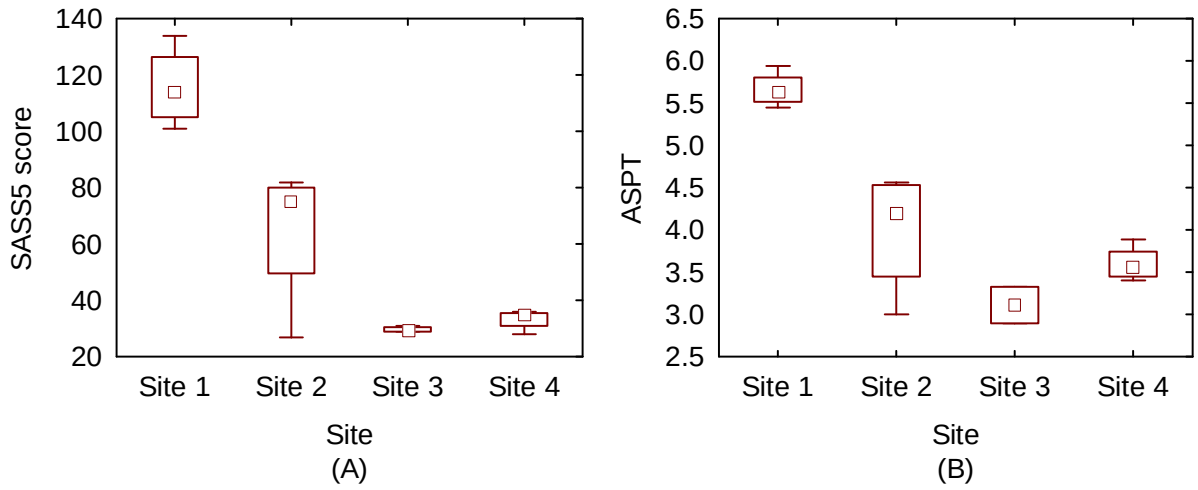


Figure 3.14: Median and quartile values for South African Scoring System version 5 (SASS5) scores (A) and average score per taxon (ASPT) (B), for each of the sampling sites in the Swartkops River demonstrating discriminatory ability of SASS5 score and ASPT values. Small squares represent median numbers and boxes represent interquartile ranges (25-75th percentiles).

3.2.3.2 Response of South African Scoring System version 5 (SASS5) scores and average score per taxon (ASPT) values to environmental water quality variables

Pearson correlation of log (x+1) transformed SASS5 and ASPT scores were used to correlate SASS5 scores and ASPT values to log (x + 1) transformed environmental water quality variables. Both SASS5 scores and ASPT values exhibited significantly strong ($p < 0.05$) positive correlations with the concentrations of dissolved oxygen (Table 3.5). However, there were significant negative correlations between both biotic scores and the concentrations of nitrate-nitrogen (NO_3-N), nitrite-nitrogen (NO_2-N), orthophosphate – phosphorus (PO_4-P), five days biochemical oxygen demand (BOD_5), chlorophyll *a* (periphyton concentrations), electrical conductivity (EC) and total inorganic nitrogen (TIN). The significant correlations of both SASS5 scores and ASPT values with the measured water quality variables are indications of the sensitivity of both indices to changes in the selected water quality variables.

Table 3.5: Pearson's correlation coefficient between water quality variables, the South African Scoring system version 5 (SASS5) scores and average score per taxon (ASPT) value. * = significant at $p < 0.05$, ** significant at $p < 0.01$

Water quality variable	**SASS5**	**ASPT**
BOD_5	-.812**	-.817**
NO_3-N	-.755**	-.742**
NO_2-N	-.671**	-.671**
NH_4-N	-.849**	-.785**
Total inorganic nitrogen	-.886**	-.836**
PO_4-P	-.872**	-.800**
Periphyton chlorophyll *a*	-.693**	-.742**
Phytoplankton chlorophylla *a*	-0.367	-0.451
Electrical conductivity	-.668**	-.732**
Dissolved oxygen	.695**	.507*
Turbidity	-0.331	-0.467

3.2.4 Macroinvertebrate metrics

Overall mean values and standard deviations of metrics for macroinvertebrates sampled at the four sampling sites are presented in Figures 3.15, 3.16, 3.17 and 3.18. Among metrics in the abundance category, the abundances of Ephemeroptera-Plecoptera-Trichoptera (EPT), Ephemeroptera-Trichoptera-Odonata-Coleoptera (ETOC), EPT/ Chironomidae ratio, Trichoptera and Gastropoda were higher at sites 1 and 2 while Chironomidae + Oligochaete abundance were highest at site 3 (Figure 3.16). The dominance of Chironomidae + Oligochaete abundance at site 3 could be as a result of the disappearance of sensitive taxa due to pollution.

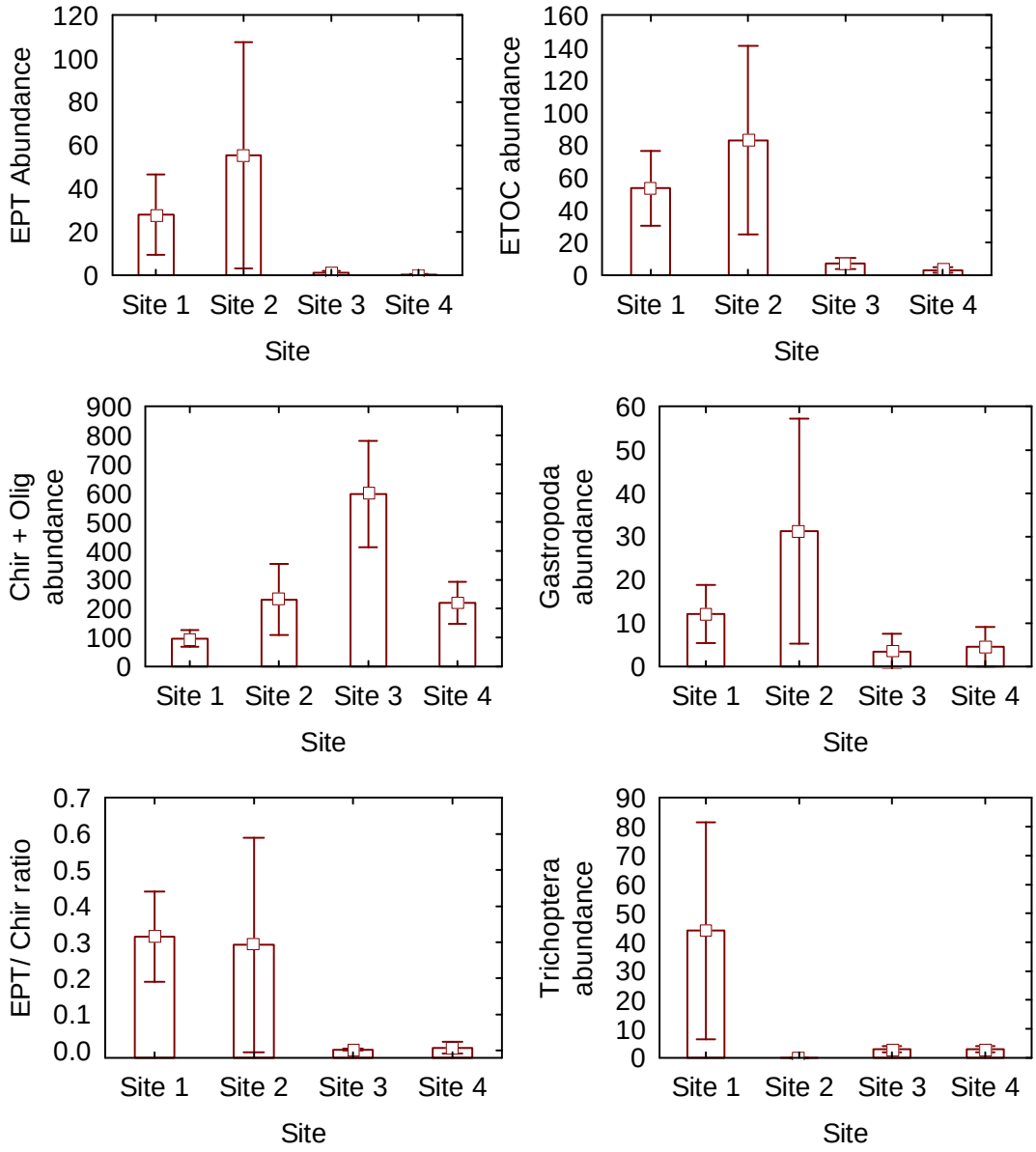


Figure 3.15: Overall mean values and standard deviations for each of the metrics in the abundance category at the four sampling sites in the Swartkops River. Abbreviations: small squares = means, range bars = standard deviations, EPT = Ephemeroptera-Plecoptera-Trichoptera, ETOC = Ephemeroptera-Trichoptera-Odonata-Coleoptera, Chir + Olig = Chironomidae + Oligochaete, ETT/Chir = Ephemeroptera-Plecoptera- Trichoptera/ Chironomidae.

Metrics in the composition (relative abundance) category also show variations between the four sampling sites and seasons (Figure 3.16). The overall mean values for % EPT, % ETOC, and % Trichoptera were highest at site 1 while overall mean values for % Chironomidae + Oligochaete

were higher at sites 3 and 4 and lowest at site 1. Overall means for % Corixidae and % Chironomidae + Hirudinae were highest at sites 4 and 2 respectively.

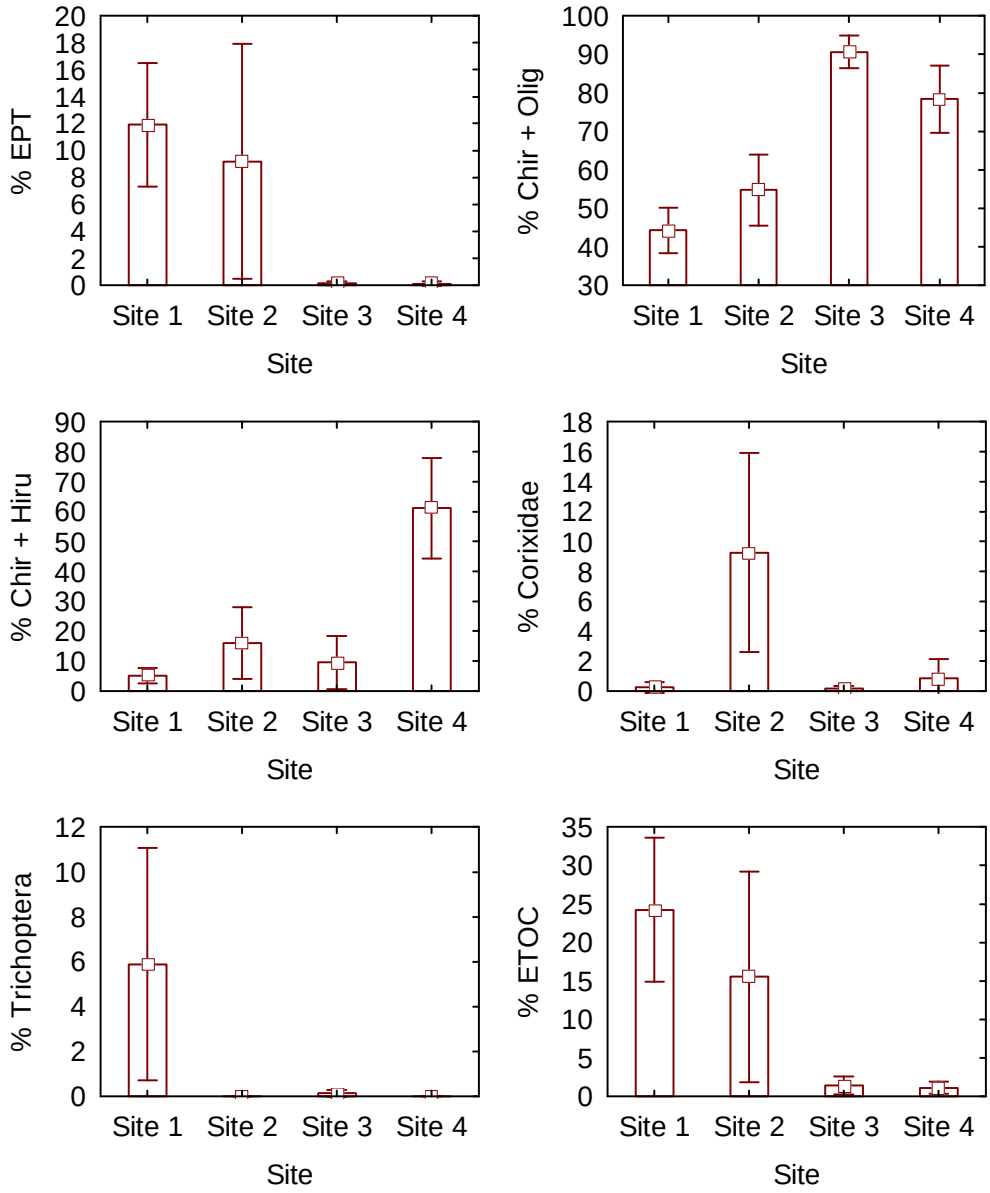


Figure 3.16: Overall mean values and standard deviations for each of the metrics in the composition (relative abundance) category at the four sampling sites in the Swartkops River. Abbreviations: small squares = means, range bars = standard deviation, % EPT = % Ephemeroptera – Plecoptera – Trichoptera, % Chir + Olig = % Chironomidae + Oligochaete, % Chir + Hiru = % Chironomidae + Hirudinae, % ETOC = % Ephemeroptera – Trichoptera – Odonata- Coleoptera

Metrics in the richness category also display variations between the four sampling sites (Figure 17). Among the four metrics in the richness category, overall mean value for Hemiptera + Diptera richness were higher at sites 1 and 2 and lowest at site 3, while those for Margalef‘s family richness index, EPT richness, and ETOC richness were all highest at site 1. The lowest overall mean values for EPT richness and ETOC richness were recorded at site 4 while that for Margalef’s family richness index was observed at site 3.

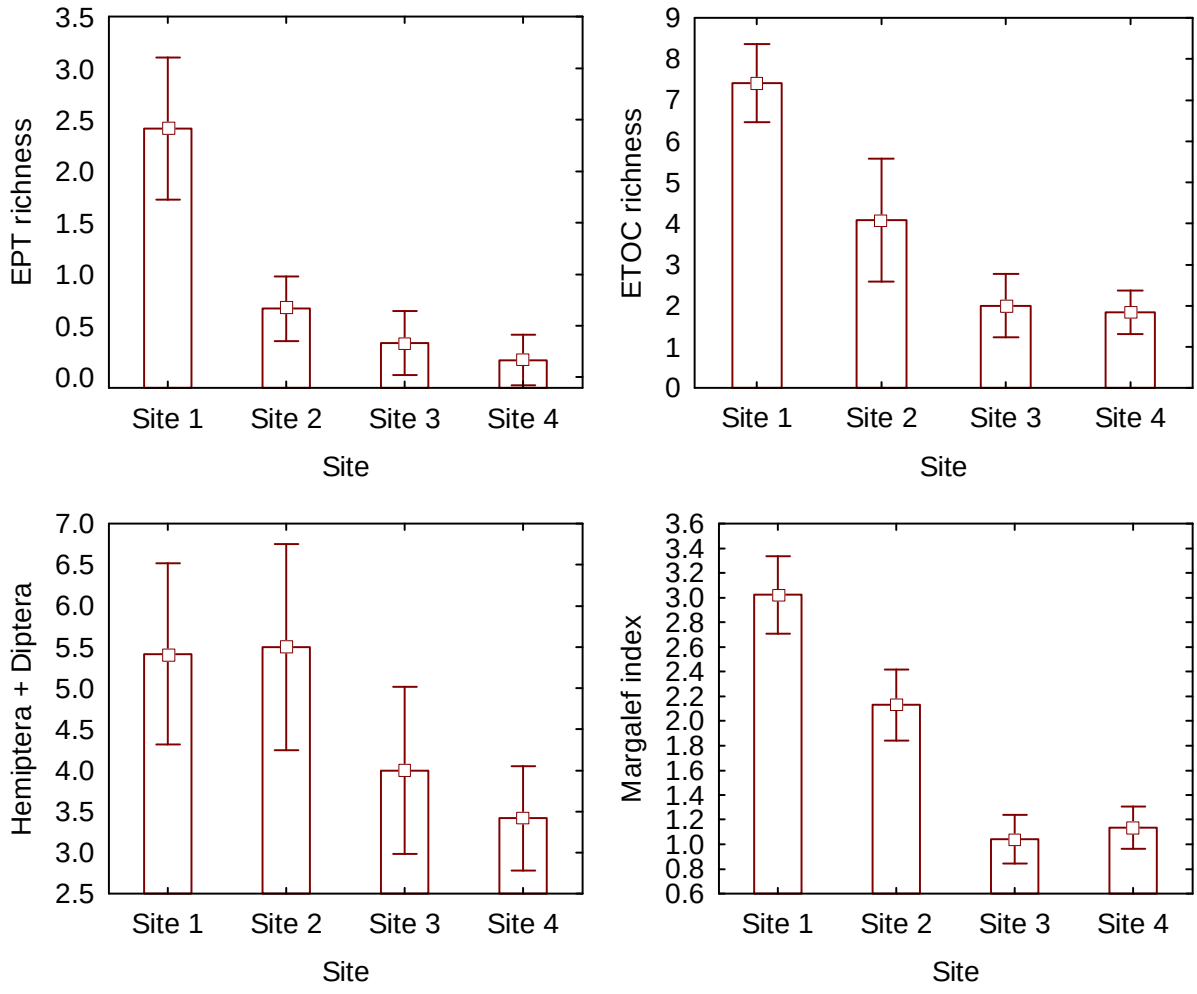


Figure 3.17 Overall mean values and standard deviations for each of the metrics in the richness category at the four sampling sites in the Swartkops River. Abbreviations: small squares = means, range bars = standard deviations, EPT = Ephemeroptera – Plecoptera – Trichoptera, ETOC = Ephemeroptera – Trichoptera – Odonata -Coleoptera

Diversity and evenness (equitability) indices all show the same trends and were all highest at site 1 (Figure 3.18). The overall mean values for Shannon family diversity index, Simpson family diversity index and equitability were all highest at site 1 and lowest at site 3 (Figure 3.19). These indices were also all higher at site 2 than at site 4. Generally, the relatively high diversity indices at site 1 are probably an indication of good water quality at this site. The lowest Shannon, Simpson and equitability (or evenness) indices at site 3 could be as a result of high abundance of Chironomidae +Oligochaete which often dominate the abundance of macroinvertebrate at this site.

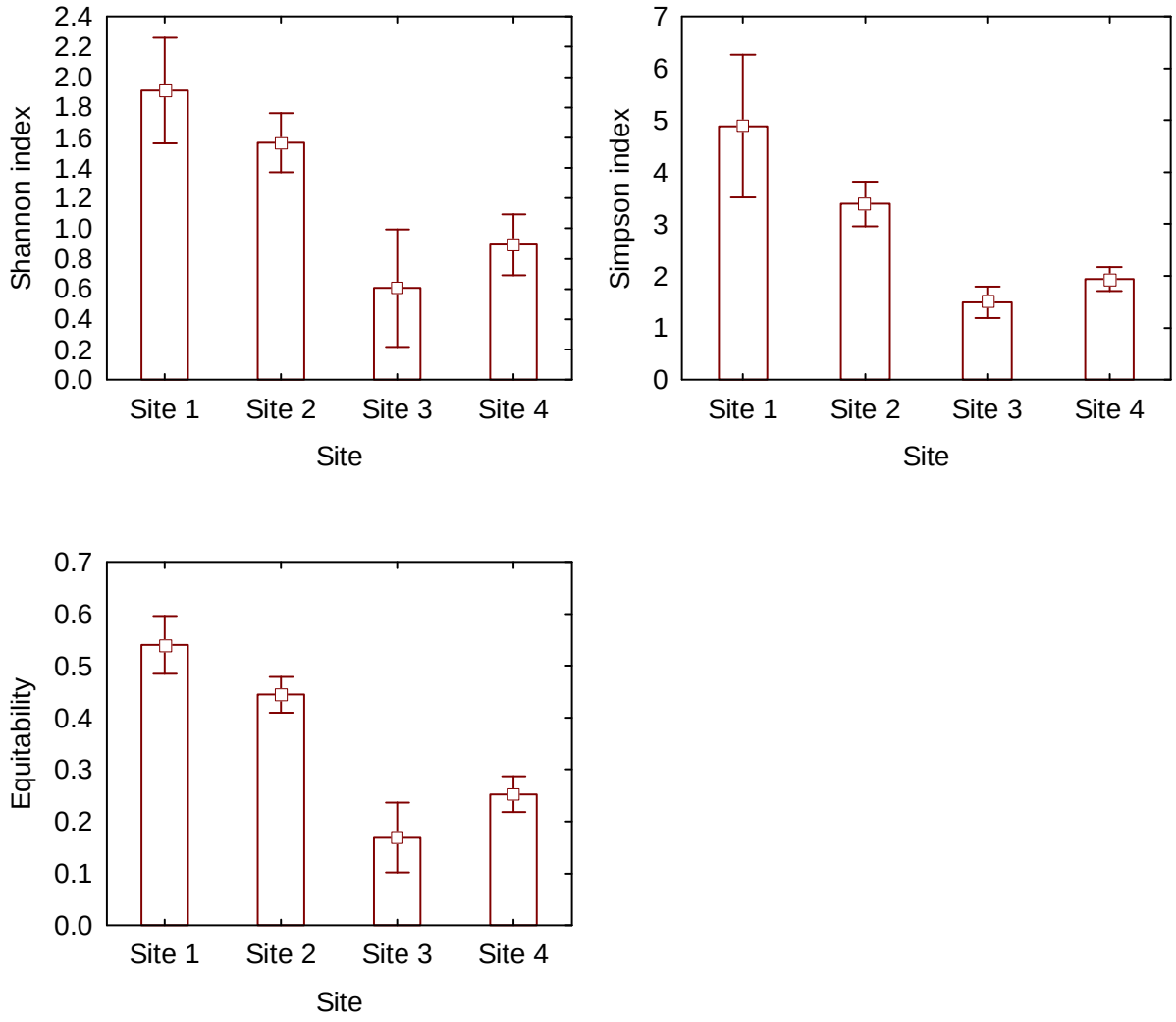


Figure 3.18: Overall mean values and standard deviations for each of the metrics in the diversity and evenness category at the four sampling sites in the Swartkops River. Small squares = means range bars = standard deviations.

3.2.4.1 Test of discriminatory ability of metrics

The ability of metrics to discriminate site 1 from downstream sites (i.e. sites 2, 3 and 4) and to discriminate between sites 2, 3 and 4 was explored using box-and-whisker plots (Figures 3.19 and 3.20). Details of this statistical method are discussed in chapter 2 section 2.7.1. Out of the 19 metrics evaluated, 10 were considered to have satisfactory discriminatory ability (Figure 3.19). In other words, based on the criteria previously discussed in chapter 2, these metrics separated site 1 from the downstream sites and they include Trichoptera abundance, % Chironomidae + Oligochaete, % ETOC, % Trichoptera, EPT richness, ETOC richness, Margalef family richness index, Equitability, Shannon and Simpson diversity indices. Conversely, Gastropoda abundance, EPT abundance, ETOC abundance, EPT/ Chironomidae ratio, % EPT, % Corixidae, % Oligochaete + Hirudinae, Chironomidae + Oligochaete abundance and Hemiptera + Diptera richness could not discriminate site 1 from the downstream sites (i.e. sites 2, 3 and 4) (Figure 3.20). EPT abundance and ETOC abundance for instance, occurred in high numbers at both sites 1 and 2 while Hemiptera + Diptera richness were ubiquitous. Out of the 10 metrics that separated site 1 from sites 2, 3 and 4, six of these were able to discriminate between sites 2, 3 and 4. That is, six metric separated all the four sampling sites from each other and they include EPT richness, % Chironomidae + Oligochaete, Margalef's family richness index, Shannon diversity index, Simpson diversity index and Equitability. The response of the 10 metrics to water quality variables were further investigated to explore their sensitivity to changes in water quality.

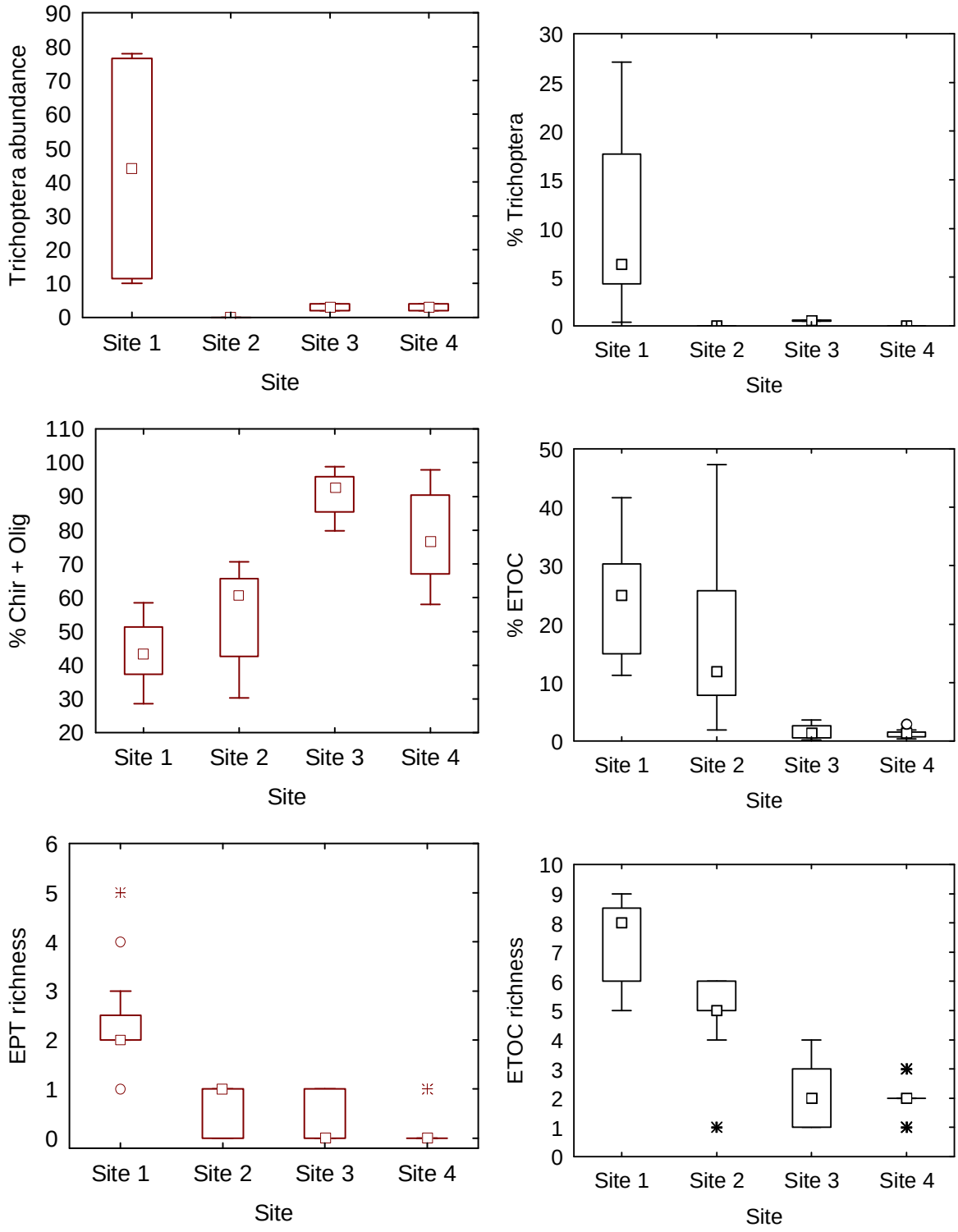


Figure 3.19: Medians (small squares) and quartiles (boxes) for each of the 10 sensitive metrics measured at the four sites in the Swartkops River. Range bars show maximum and minimum non-outliers numbers. Circles and asterisks represent outliers and extremes respectively (Figure continued on next page).

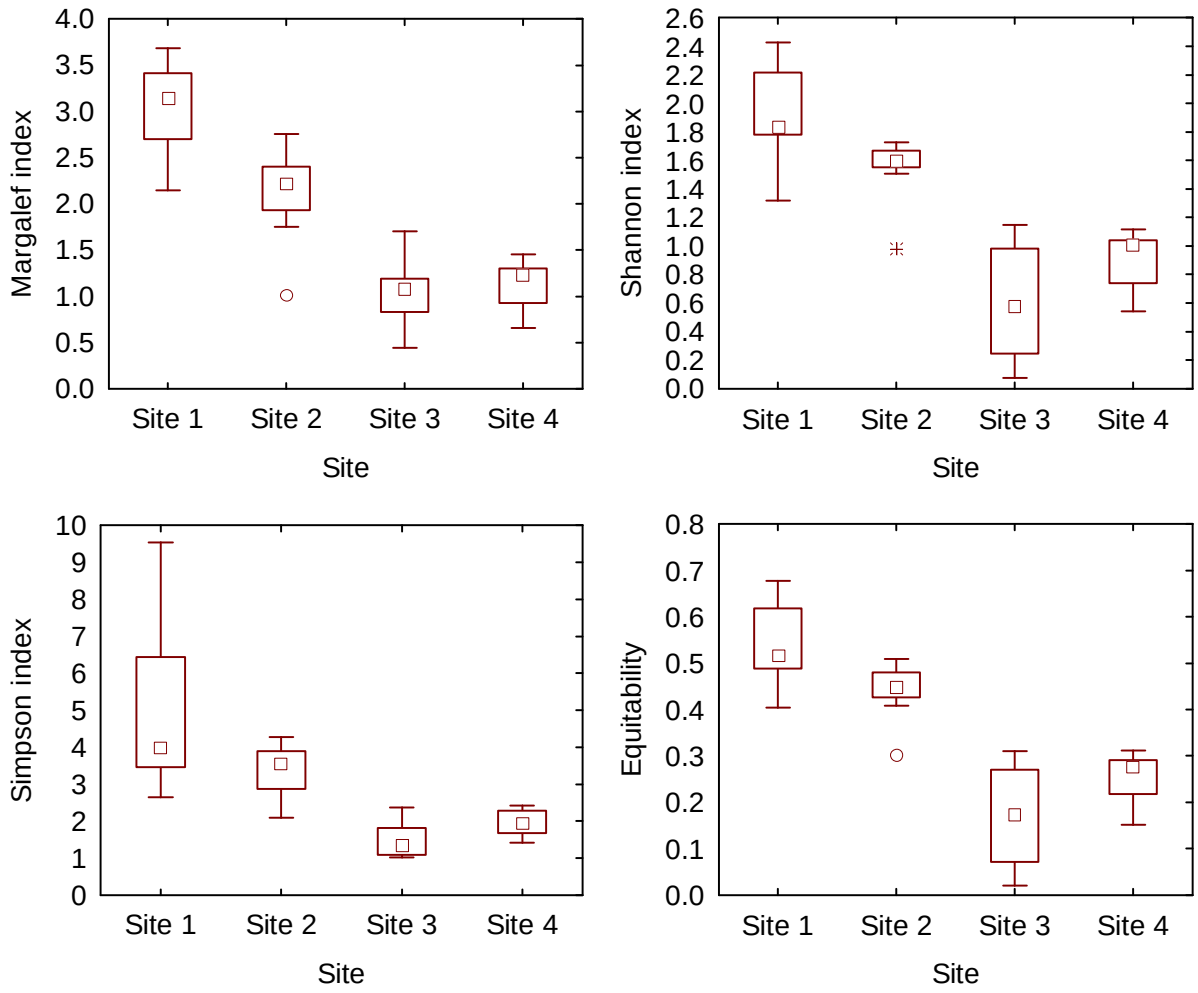


Figure 3.19: Medians (small squares) and quartiles (boxes) for each of the 10 sensitive metrics measured at the four sites in the Swartkops River. Range bars show maximum and minimum non-outliers numbers. Circles and asterisks represent outliers and extremes respectively (Starts from previous page).

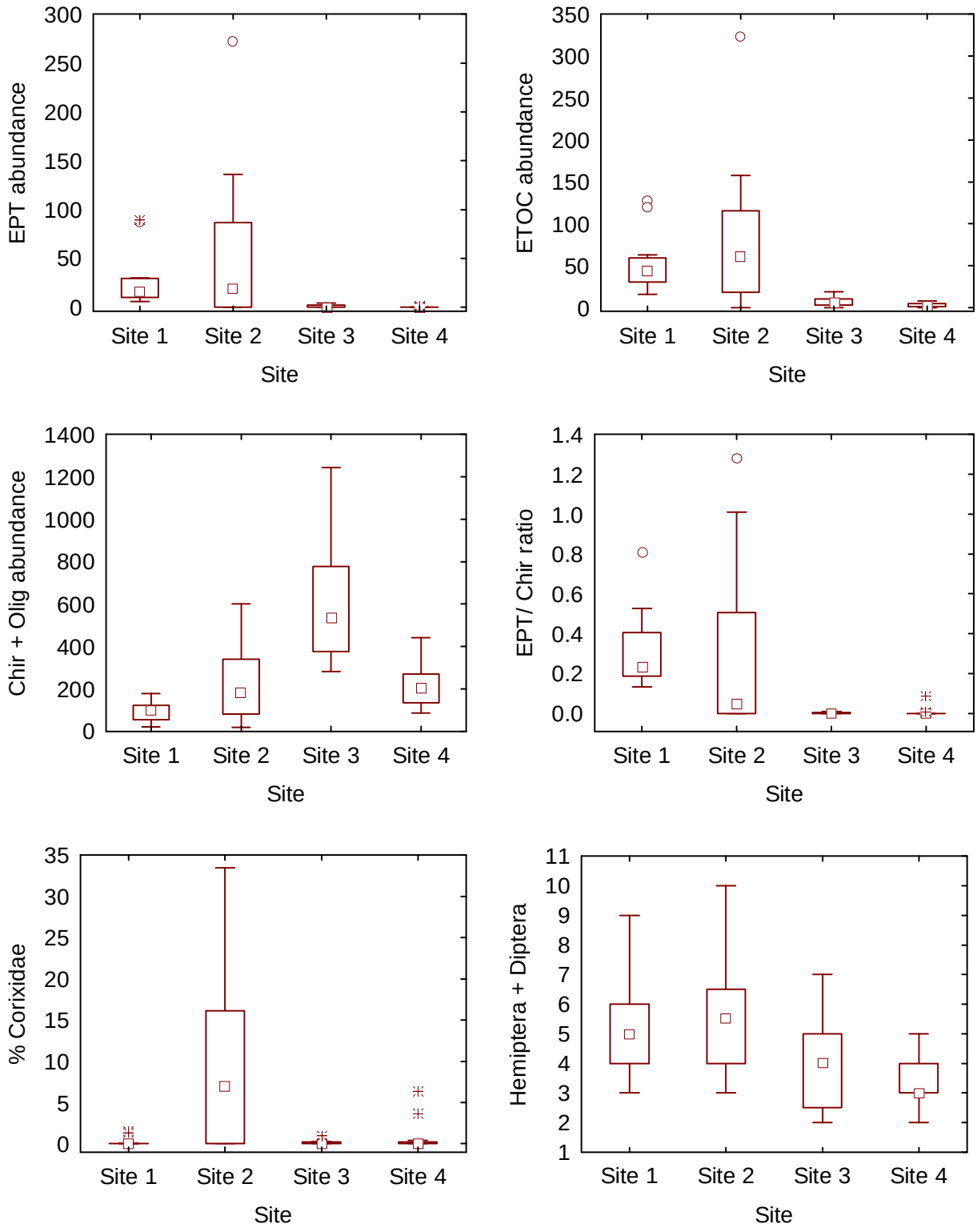


Figure 3.20: Medians (small squares) and quartiles (boxes) for each of the 9 non-sensitive metrics measured at the four sites in the Swartkops River. Range bars show maximum and minimum non-outliers numbers. Circles and asterisks represent outliers and extremes respectively (Figure continued on next page)

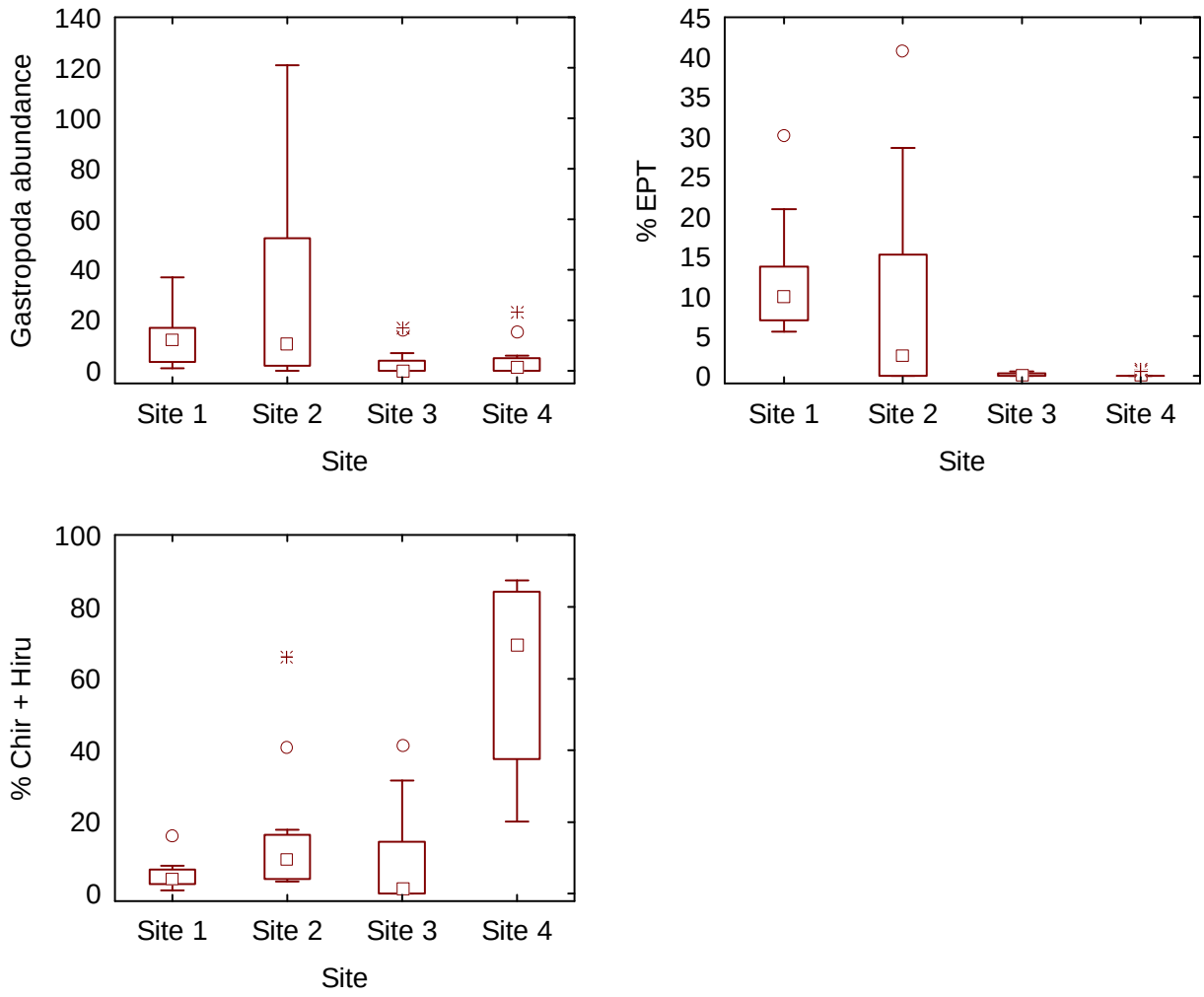


Figure 3.20: Medians (small squares) and quartiles (boxes) for each of the 9 non-sensitive metrics measured at the four sites in the Swartkops River. Range bars show maximum and minimum non-outliers numbers. Circles and asterisks represent outliers and extremes respectively. (Starts from previous page)

3.2.4.2 Response of metrics to environmental water quality variables

Response of the selected metrics to differences in environmental water quality at the four sites was explored using Pearson's correlations. Details of this analysis are given in chapter 2 section 2.7.4. Pearson's correlation coefficient between metrics and water quality variables are presented in Table 3.6. Results revealed that the 10 metrics were sensitive to changes in the measured water quality variables. With the exception of % Chironomidae + Oligochaete, all metrics were negatively correlated with increasing concentrations of nitrate-nitrogen (NO_3-N), nitrite-nitrogen (NO_2-N), ammonium-nitrogen (NH_4-N), total inorganic nitrogen (TIN), orthophosphate-phosphorus (PO_4-P), chlorophyll *a* (phytoplankton and periphyton), five days biochemical oxygen demand (BOD_5) and electrical conductivity (EC). However, increases in the concentrations of these same variables favour the dominance of % Chironomidae + Oligochaete. On the contrary, all 10 metrics except % Chironomidae + Oligochaete increase with increasing dissolved oxygen (DO). Pearson's correlation revealed that increase in DO correlate negatively with % Chironomidae + Oligochaete (Table 3.6).

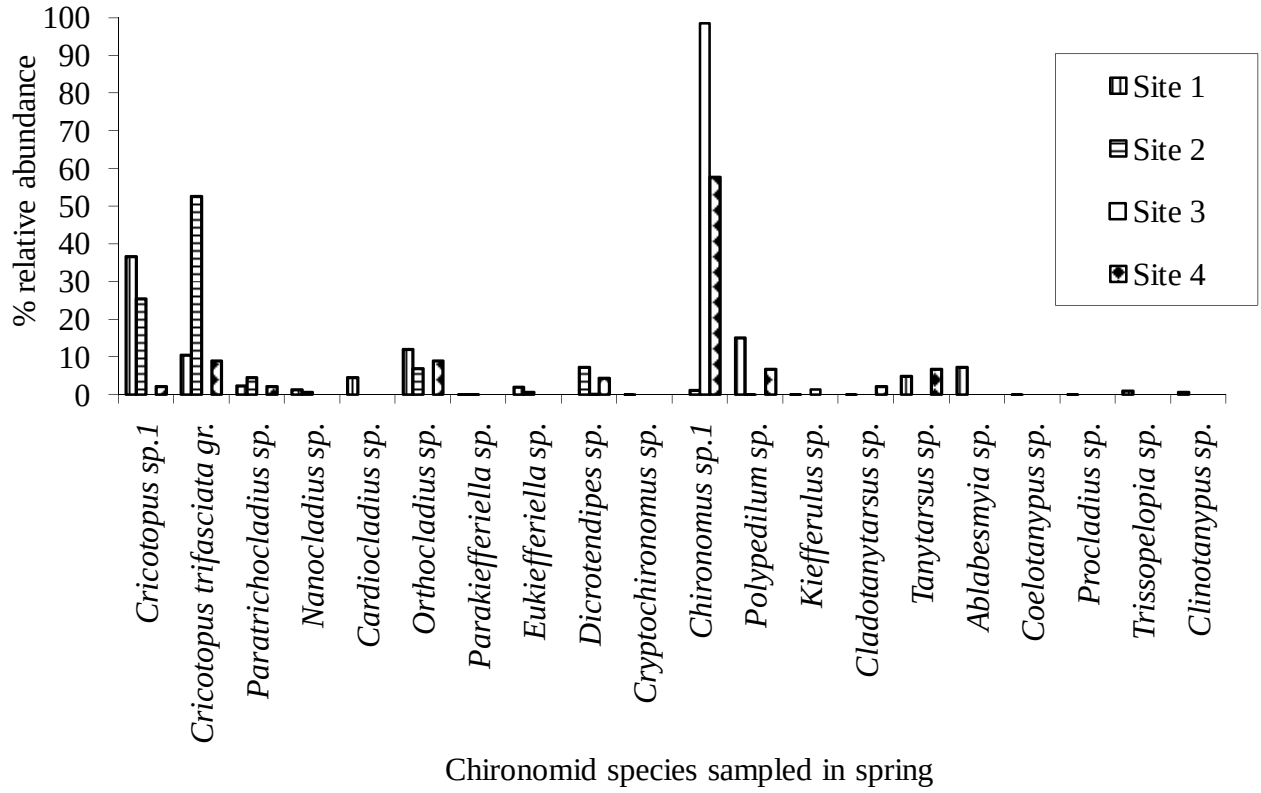
Table 3.6 Pearson's correlation coefficients between water quality variables and mean metric values. * Significant at $p < 0.05$, ** significant at $p < 0.01$. Abbreviations: Peri = periphyton, Phyto = phytoplankton and Turb = turbidity

Metrics	**BOD_5**	**NO_3-N**	**NO_2-N**	**NH_4-N**	**TIN**	**PO_4-P**	**Peri**	**Phyto**	**EC**	**DO**	**Turb**
Trichoptera abundance	-.630**	-.544*	-0.354	-0.483	-.538*	-.615*	-.520*	-.624**	-.885**	0.252	-0.023
%Chiro+Oligocaete	.697**	.647**	.600*	.870**	.855**	.807**	.620*	0.349	0.466	-.779**	0.405
%Trichoptera	-.635**	-.546*	-0.392	-.513*	-.565*	-.657**	-.565*	-.640**	-.901**	0.278	-0.068
%ETOC	-.839**	-.744**	-.659**	-.905**	-.912**	-.909**	-.595*	-0.218	-.549*	.809**	-0.367
ETOC richness	-.812**	-.718**	-.527*	-.766**	-.805**	-.764**	-.610*	-0.343	-.608*	.616*	-0.444
EPT richness	-.843**	-.750**	-.542*	-.797**	-.840**	-.784**	-.687**	-.507*	-.793**	.568*	-0.264
Shannon index	-.698**	-.674**	-.625**	-.877**	-.853**	-.818**	-.579*	-0.329	-0.475	.689**	-.531*
Simpson index	-.649**	-.653**	-.604*	-.853**	-.833**	-.844**	-.698**	-0.425	-.557*	.712**	-0.471
Margalef	-.792**	-.715**	-.612*	-.895**	-.893**	-.904**	-.734**	-0.369	-.633**	.756**	-0.448
Equitability	-.702**	-.693**	-.637**	-.887**	-.869**	-.841**	-.628**	-0.36	-.513*	.713**	-.503*

Out of the 10 metrics, only Ephemeropteran-Plecopteran-Trichopteran (EPT) richness shows significant correlation with all water quality variables but no significant correlations were observed between DO, NO_2-N, NH_4-N and Trichoptera abundance. Similarly, % Trichoptera was not significantly correlated with DO and NO_2-N. The remaining metrics displayed significant correlations with all water quality variables except chlorophyll *a* phytoplankton. EPT richness, Trichoptera abundance, and % Trichoptera were the only metrics sensitive to changes in phytoplankton concentrations. In most cases, correlations of metrics with NH_4-N and PO_4-P were stronger than with NO_3-N and NO_2-N (Table 3.6). The diversity indices, especially Shannon index, were very sensitive to changes in water quality variables. Most of the water quality variables are nutrients and nutrient related indicators and therefore revealed that the 10 metrics could be suitable for the assessment of nutrient enrichment in the Swartkops River.

3.2.5 Temporal and spatial variations in chironomid abundance and distribution

The relative abundance and distribution of chironomid species recorded at each of the sampling sites during each of the sampling seasons are presented in Figure 3.21. A total of 26 taxa representing 3 subfamilies of Chironomidae were recorded during the study period (Appendix A, Table A9). At site 1, the highest numbers of taxa (18 taxa) were recorded during spring and were followed by 10 taxa in autumn. During summer and winter sampling seasons, 9 taxa each were recorded. The observed variations in the number of recorded taxa during the sampling seasons could be attributed to species that did not occur throughout the sampling seasons. For instance, species such as *Paratrichocladius* sp. and *Cladotanytarsus* sp. occurred only in spring and winter while *Nonacladius* sp., *Parakiefferilla* sp., *Eukiefferiella* sp., *Cryptochironomus* sp., *Coelotanypus* sp., *Procladius* sp., *Trissopelopia* sp. and *Clinotanypus* sp. occurred only in spring at site 1 (Figure 3.21). On the other hand, species such as *Cricotopus* sp.1, *Cricotopus trifasciata* gr., *Orthocladius* sp. and *Tanytarsus* sp., were observed throughout the sampling seasons at this site. *Cricotopus* sp. 1 and *Cricotopus trifasciata* gr. were the most dominant species at site 1 during spring, summer and autumn sampling seasons, while *Tanytarsus* sp, was the most abundant taxon at this site in winter (Figure 3.21).



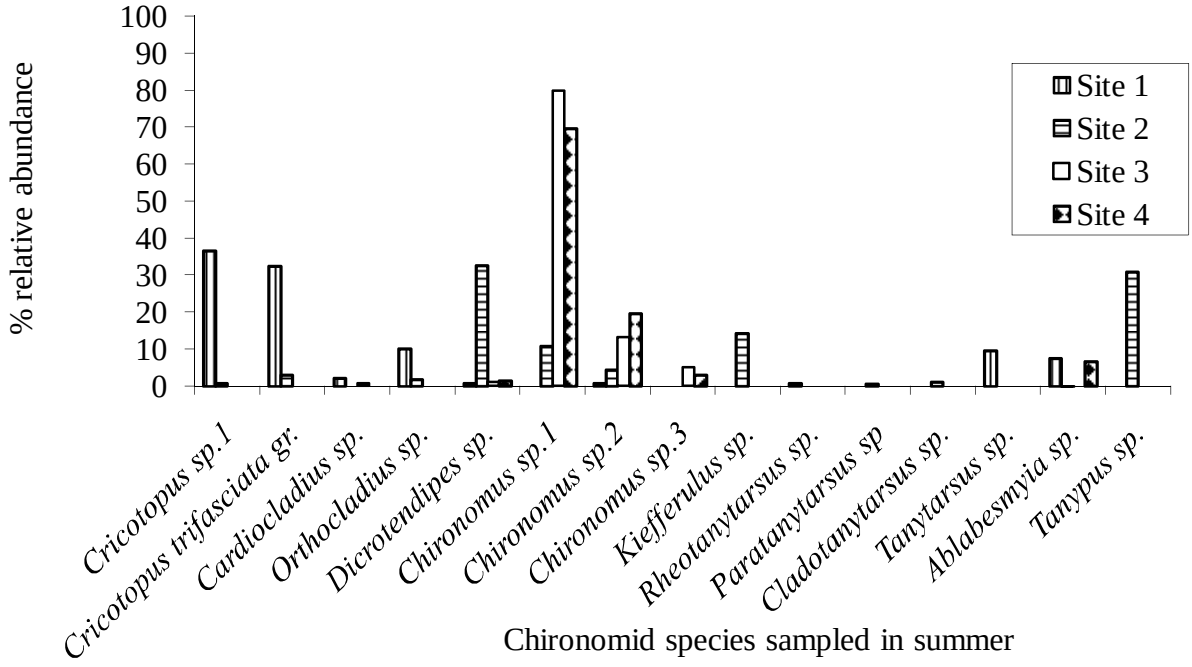


Figure 3.21: Percentage relative abundance of chironomid species recorded during the four sampling seasons at each of the four sampling sites in the Swartkops River. (Figure continued on next page).

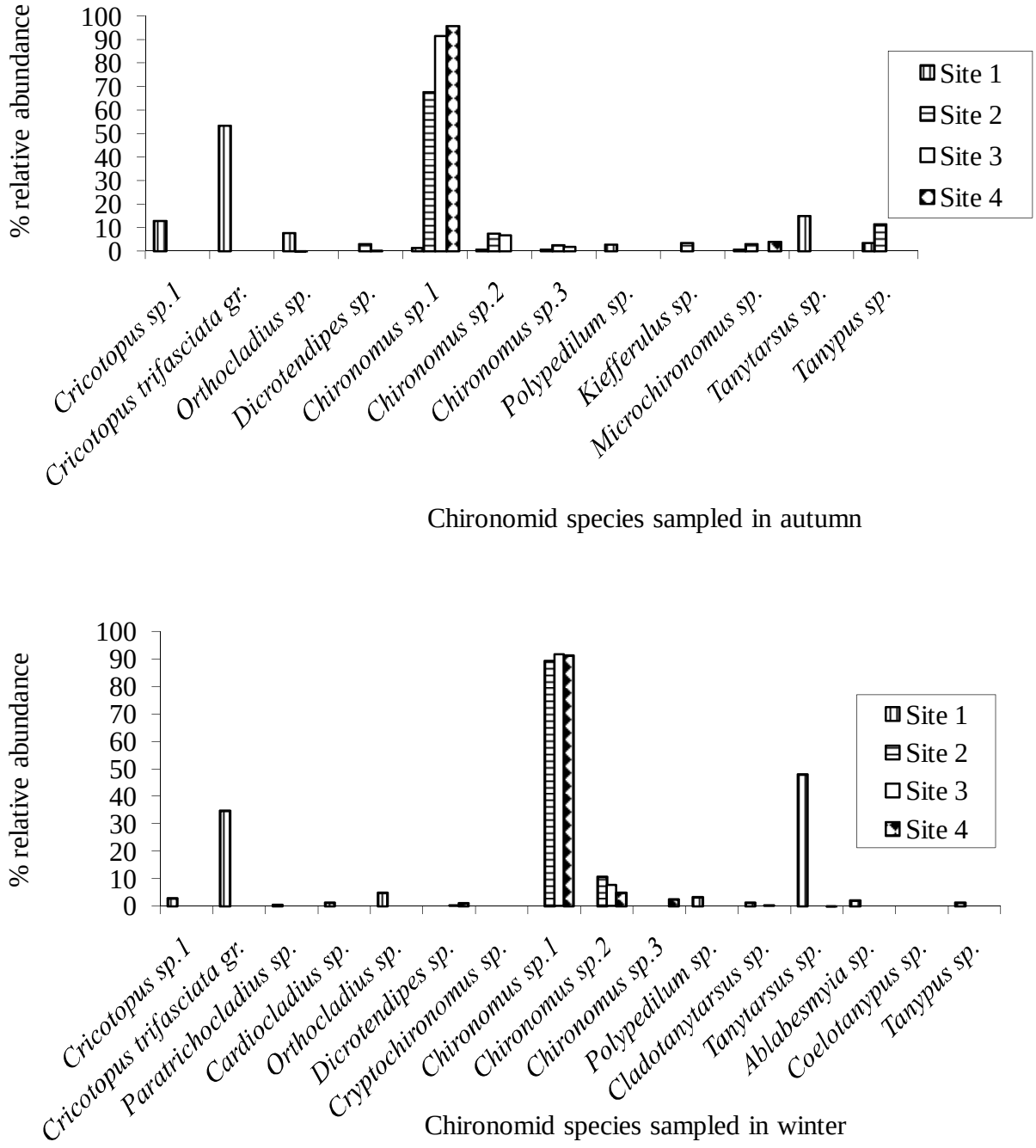


Figure 3.21: Percentage relative abundance of chironomid species recorded during the four sampling seasons at each of the four sampling sites in the Swartkops River. (Figure starts from previous page)

At site 2, contrary to site 1 for which the highest number of taxa was recorded in spring, the highest number of taxa (11 taxa) was observed in summer and was closely followed by spring with 10 taxa (Figure 3.21). Only 2 taxa, *Chironomus* sp. 1 and *Chironomus* sp. 2 were recorded at this site during winter. The site was dominated by *Cricotopus* sp.1 and *Cricotopus trifasciata* gr. in spring while *Dicrotendipes* sp. and *Tanypus* sp. dominated the chironomid composition in summer. *Chironomus* sp.1 and *Chironomus* sp.2 were the dominant species in both autumn and winter. However, some species occurring at the site such as *Paratanytarsus* sp., *Cladotanytarsus* sp., could be considered as occasional occurring either in one or two seasons only with relatively low abundances (Figure 3.21).

Among the sampling sites, site 3 had the highest abundance of chironomids but it was consistently dominated by *Chironomus* sp. 1, *Chironomus* sp. 2 and *Chironomus* sp. 3. In all sampling seasons, *Chironomus* sp. 1 accounted for more than 75 % of the total abundance of chironomid species at this site (Figure 3.21). Nevertheless, species such as *Dicrotendipes* sp. occurred consistently at the site while species such as *Cladotanytarsus* sp., *Cardiocladius* sp. and *Kiefferulus* sp. could be considered rare species at the site occurring in one or two seasons only with relatively low abundances. Overall, the site was dominated by species of the subfamily Chironominae particularly of the tribe Chironomini (Figure 3.22).

Site 4 had the lowest abundance of chironomids of all the sampling sites and the highest numbers of taxa at this site (9 taxa) were recorded during spring. During summer and winter, 5 taxa each were recorded and autumn had the lowest numbers of taxa (Figure 3.21). Similar to site 3, site 4 was dominated by *Chironomus* sp.1, while *Cricotopus* sp.1, *Cricotopus trifasciata* gr. *Orthocladius* sp., *Tanytarsus* sp. and *Cladotanytarsus* sp. occurred either in one or two seasons or in very low relative abundances. Overall, the site was dominated by species of the subfamily Chironominae with relatively few species from the other subfamilies such as Orthocladiinae (Figure 3.22).

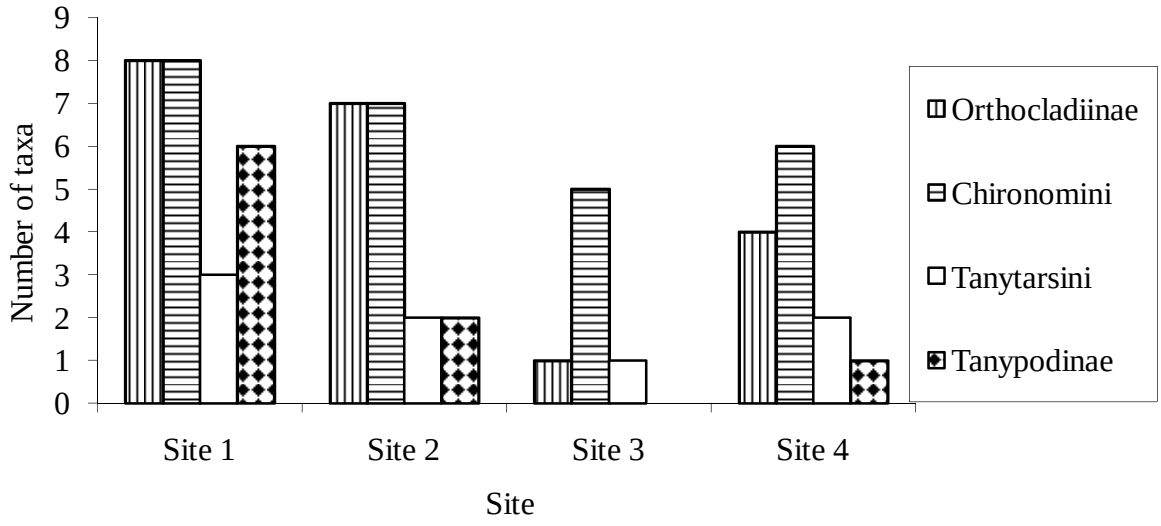


Figure 3.22: Number of taxa recorded for chironomid subfamilies and tribes at the four sampling sites in the Swartkops River. Subfamilies are: Orthocladiinae and Tanypodinae, Tribes: Tanytarsini and Chironomini (subfamily Chironominae).

3.2.5.1 Analysis of differences in chironomid abundance and composition between sampling sites

Overall differences in chironomid abundance and composition between the four sampling sites during the study period were elucidated by analysis of similarity (ANOSIM) and similarity percentage (SIMPER) analysis and are presented in Table 3.7. ANOSIM indicated significant difference ($p < 0.05$) between all sampling sites except between sites 3 and 4. SIMPER analysis revealed that significant difference between sites 1 and 2 could be attributed to species such as *Cricotopus trifasciata* gr., *Cricotopus* sp.1, *Tanytarsus* sp., *Orthocladius* sp., *Polypedilum* sp. and *Ablabesmyia* sp., which were less abundant at site 2 than at site 1 and species such as *Tanypus* sp., *Chironomus* sp.1 and *Dicrotendipes* sp., which were more abundant at site 2 than at site 1. SIMPER analysis also indicated that differences between site 1 and sites 3 and 4, were as a result of *Cricotopus trifasciata* gr., *Cricotopus* sp.1, *Orthocladius* sp. *Tanytarsus* sp. and *Polypedilum* sp., which were absent at site 3 but less abundant at site 4 and *Chironomus* sp. 1 and *Chironomus* sp. 2 being more abundant at sites 3 and 4 than at site 1. The contributions of species such as *Dicrotendipes* sp., *Chironomus* sp.1, *Chironomus* sp.2, *Kiefferullus* sp., *Tanypus* sp. and *Cricotopus trifasciata* gr. to the dissimilarity matrix between site 2, and sites 3 and 4 accounted for the observed differences between these sites.

Table 3.7: Analysis of differences in chironomid abundance and composition between the four sampling sites in the Swartkops River during the study period. Yes = significantly different at $p < 0.05$ and No = not significantly different $p > 0.05$

Sites	**Average dissimilarity (%)**	**Significantly different?**
1 and 2	73.16	Yes
1 and 3	93.11	Yes
1 and 4	82.96	Yes
2 and 3	59.90	Yes
2 and 4	60.89	Yes
3 and 4	45.40	No

3.2.5.2 Chironomid species richness and diversity indices

Means and standard deviations of chironomid species richness, diversity and equitability at the four sampling sites are presented in Figure 3.23. Generally, diversity indices (Shannon and Simpson), richness index (Margalef) and equitability were highest at site 1 and lowest at site 3. These indices were higher at site 2 than at site 4. Kruskall-Wallis multiple comparisons test revealed that equitability, Shannon and Simpson diversity indices between site 1 and 3 were significantly different ($p < 0.05$), while Margalef's species richness index differ significantly between site 1 and sites 3 and 4.

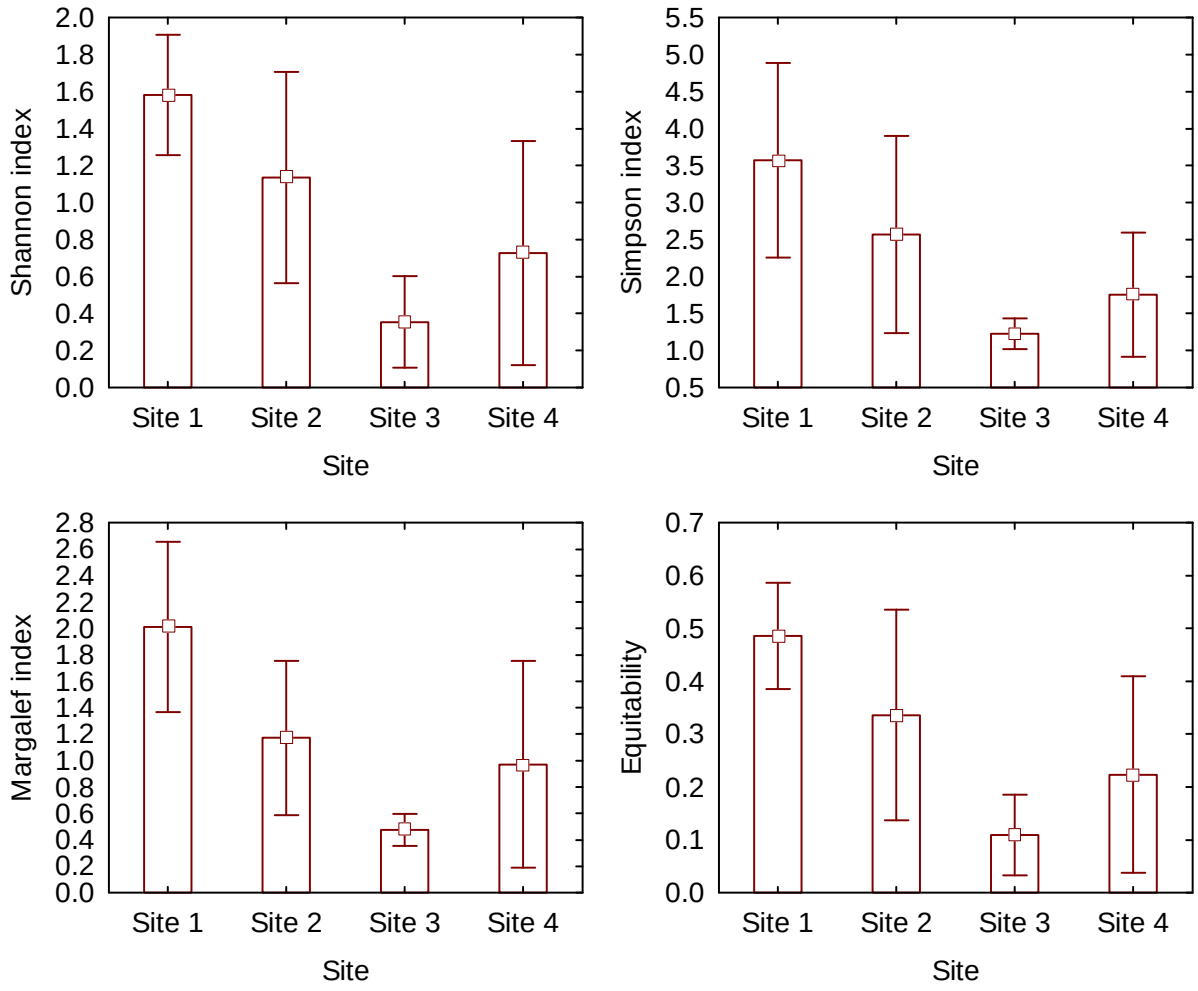


Figure 3.23: Chironomidae means and standard deviations for Shannon, Simpson, Margalef and Equitability indices at the four sampling sites in the Swartkops River. Small squares = means and range bars = standard deviations.

3.2.5.3 Chironomid communities and water quality relationships

Canonical correspondence analysis (CCA) ordination plot revealed strong relationship between chironomid communities and measured physical and chemical water quality variables (Figure 3.24). According to the CCA plot, five days biochemical oxygen demand (BOD_5), turbidity, electrical conductivity (EC), dissolved oxygen (DO), orthophosphate-phosphorus (PO_4-P) and total inorganic nitrogen (TIN) were the most important variables that impacted on the community structures. However, BOD_5, PO_4-P and EC were found to exhibit the highest influence on chironomid community at sites 2, 3 and 4, particularly sites 3 and 4. The CCA ordination plot revealed that DO was positively correlated with most of the chironomid taxa at site 1 (Figure 3.24). Temperature, NO_2-N, NO_3-N, NH_4-N and chlorophyll *a* (phytoplankton and periphyton) were removed from the ordination because they exhibited high multi-colinearity (R^2 = 0.8) with EC, DO, PO_4-P, TIN and BOD_5. The CCA ordination plot for the whole chironomid data set associated the downstream sites (i.e. sites 2, 3 and 4) with chironomid taxa that seemed to be more pollution tolerant and consisted mostly of the tribe Chironomini, particularly *Chironomus* sp.1, *Chironomus* sp.2, *Chironomus* sp.3 and *Dicrotendipes* sp. These species were positively correlated with high BOD_5, PO_4-P and TIN while species such as *Microchironomus* sp. was negatively correlated with EC on both axes 1 and 2. On the other hand, *Tanypus* sp. and *Kiefferulus* sp. were greatly influenced by both EC and DO. Axis 1 of the ordination plot revealed that increased DO was positively correlated with *Cladotanytarsus* sp. while increased EC negatively correlated with *Tanypus* sp. and *Kiefferullus* sp. (Figure 3.24). In addition, DO correlate positively with less tolerant species of chironomids particularly those associated with site 1 and the spring sample of site 2. These species, as shown by axis 1 of the ordination plot, were abundant in oxygen rich sites but their population became reduced as the oxygen level was depleted.

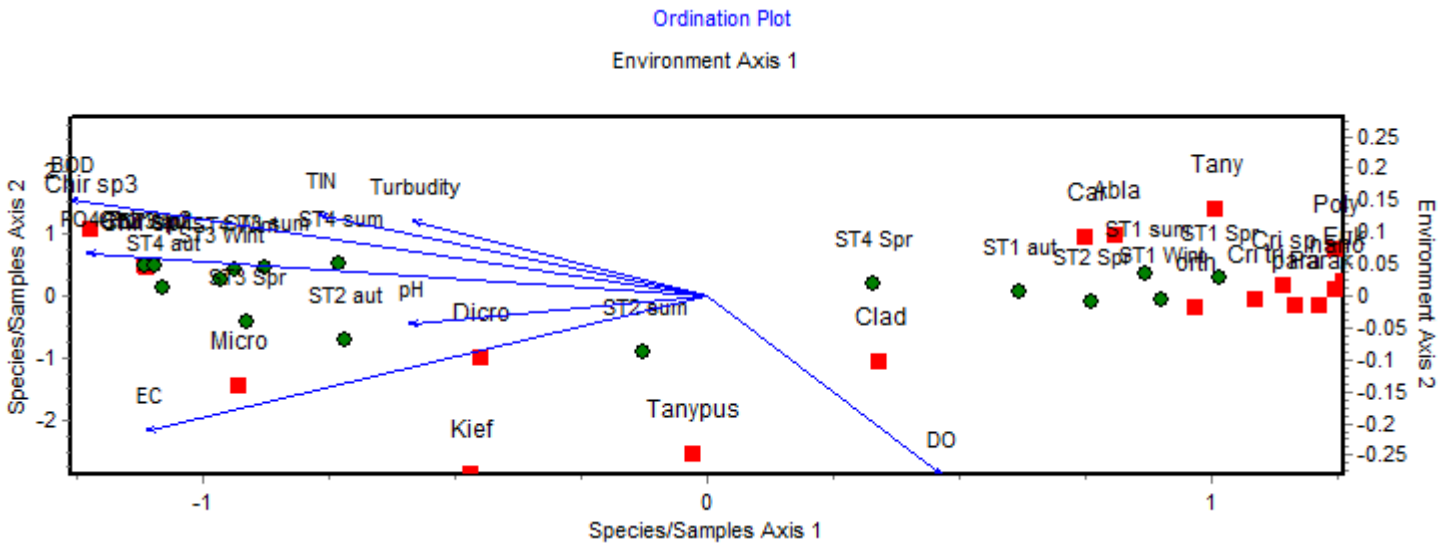


Figure 3.24: CCA ordination tri-plot for chironomid taxa and water quality variables at the four study sites and seasons: Abbreviations: Sites: ST1 = site 1, ST2 = site 2, ST3 = site 3, ST4 = site 4. Seasons: spr = spring, sum = summer, aut = autumn, and wint = winter. Species code: Cri sp = *Cricotopus* sp.1, Cri tr = *Cricotopus trifasciata* gr., Para = *Paratrichocladius* sp., Nano = *Nanocladius* sp., Dicro = *Dicrotendipes* sp., Euk = *Eukiefferella* sp., Abla = *Ablabesmyia* sp., Kief = *Kiefferulus* sp., Car = *Cardiocladius* sp., Tany = *Tanytarsus* sp., Poly = *Polypedilum* sp., Parak = *Parakiefferella* sp., Clad = *Cladotanytarsus* sp., Chir sp1 = *Chironomus* sp.1, Chir sp2 = *Chironomus* sp.2, Chir sp3 = *Chironomus* sp. 3, Orth = *Orthocladius* sp., Micro = *Microchironomus* sp.

The relative magnitude of Eigen values for each of the CCA axis is an indication of the relative importance of the axis. CCA axis 1 accounted for 42.43% of total variance of the data set and in total the first three axes of the ordination accounted for 61.01% (Table 3.8) of the variance suggesting high correlation between community data and water quality variables. The statistical significance of the model was tested using Monte Carlo permutation test and Eigen value for axis 1 was found to be significant ($p < 0.05$) (Table 3.6). Pearson's correlation between species and water quality variables displayed strong positive correlation for all three axes (Table 3.8).

Table 3.8: Properties of the Canonical Correlation Analysis ordination tri-plot

Canonical properties	**Axis**		
	1	2	3
Canonical Eigen value	0.631	0.190	0.087
% variance explained	42.43	12.76	5.825
% Cumulative variance explained	42.43	55.18	61.01
Monte Carlo test p – value	0.01	0.178	0.604
Pearson correlation of species and environmental Scores	0.992	0.946	0.946

3.3 Discussion

3.3.1 Physical and chemical water quality variables

The physical and chemical water quality variables provide a clear distinction between site 1 and sites 2, 3 and 4. The high organic loading and industrial effluent discharges at sites 3 and 4 were evident in the level of five days biochemical oxygen demand (BOD_5), nitrite-nitrogen (NO_2-N), nitrate-nitrogen (NO_3-N), ammonium-nitrogen (NH_4-N), total inorganic nitrogen (TIN), orthophosphate-phosphorus (PO_4-P), electrical conductivity (EC) and dissolved oxygen (DO). Effluents from the Uitenhage and Despatch waste water treatment works (WWTW) are likely the main contributors of the observed high organic loading at sites 3 and 4. The low DO and the relatively high BOD_5 was evident in the obnoxious odour that characterised sites 3 and 4 throughout the sampling periods. This could be as a result of anaerobic decomposition of organic matter resulting in the production of harmful gases such as methane and hydrogen sulphide (Dallas and Day, 2004; Nyenje *et al.,* 2010). These harmful gases could cause the death of sessile macroinvertebrates and eventual depletion of their populations. This probably contributed to the observed poor macroinvertebrate composition that characterised the downstream sites particularly sites 3 and 4. Of particular interest is the increase in BOD_5 from 4.78 mg/l in autumn to 16.68 mgL^{-1} in winter at site 2 (Table 1). Cattle dung, rarely seen at this site, was common during winter and probably contributed to this unexpected increase in BOD_5.

The nutrient levels recorded in this study at the downstream sites, particularly at sites 3 and 4, are in the range capable of causing mesotrophic and eutrophic conditions when compared to the South African water quality guidelines (DWAF, 1996b; Dallas and Day, 2004). The eutrophic condition of the Swartkops River is evident in the large scale blooms of water hyacinth, macrophytes, phytoplankton, periphyton and the obnoxious odour that is apparent at the downstream sites (i.e. sites 2, 3 and 4). It was speculated that the recent fish kill in the Swartkops River was probably a result of its eutrophic conditions (Ndoni, 2009). The relatively high nutrient levels could be attributed to sewage discharges from the Uitenhage and Despatch waste water treatment works (WWTW) as well as run-off from informal settlements. Sources of nutrients in the Swartkops River have been discussed extensively in chapter 2. In addition, previous studies such as DWAF (1996a) and de Villiers and Thiart (2007) have also reported relatively high nutrient levels from the Swartkops River, corroborating the findings in this study.

The relatively high electrical conductivity (EC) at sites 2, 3 and 4 would probably have resulted from discharges of waste water treatment works (WWTW) and automotive industry in the vicinity of these sites. In addition, the soil of the Swartkops River catchment being of marine origin could result in naturally high EC levels. According to DWAF (1996a), the main cause of high EC, apart from anthropogenic sources, in the Swartkops River is the river's natural geology. Macroinvertebrates are particularly sensitive to changes in EC (Dallas and Day, 2004) and this could contribute to disappearance of sensitive taxa from sites 2, 3 and 4.

3.3.2 Macroinvertebrates community structure and replicate samples

Macroinvertebrates are known to exhibit varying degree of sensitivity to environmental water quality deterioration (Bonada *et al.*, 2006; Arimoro and Muller, 2010; Fouche and Vlok, 2010). Thus, their presence is considered a reflection of the water quality status and aquatic health of the environment in which they live. For instance, when families of aquatic macroinvertebrates perceived to be sensitive to water quality deterioration are found at a site, it is assumed that such site is of good quality, at least from environmental water quality point of view. In this study, macroinvertebrate replicate samples taken from sites 3 and 4 were often closely associated, while site 1 and 2 were quiet distinct although site 2 was closely linked to site 4 during spring survey. The distinctiveness of site 1 is due to the presence of macroinvertebrate families such as Baetidae 2sp (i.e. more than two species of Baetiadae) and Hydropsychidae 2sp (more than two species of Hydropsychidae) (Appendix A, Tables A1 and A2). These taxa are indicative of largely unimpaired water quality status. The close association observed between macroinvertebrate communities at sites 3 and 4 during most of the sampling seasons could be attributed to the presence of families of macroinvertebrate such as Simulidae, Oligochaete and Chironomidae that characterised samples from these sites. These families are highly tolerant and could thrive in polluted environment. Of particular interest is the high abundance of chironomids and Oligochaete at sites 3 and 4 respectively (Appendix A, Tables A5 - A8). Chironomids and Oligochaete are regarded as highly tolerant families among aquatic macroinvertebrates because of their ability to survive in oxygen depleted environments (Fouche and Vlok, 2010). This characteristic could have contributed to their dominance at the two sites with high organic loads. In addition, most chironomids feed on fine particulate organic matter, particularly algae, and their increase may be related to increase in periphyton and phytoplankton resulting from high nutrients levels. Furthermore, Dickens and Graham (1998) and Arimoro (2009) have noted that chironomids

are opportunistic and possess the ability to thrive in areas of low competition. Apart from poor oxygen levels at these sites, the relatively high EC recorded throughout the sampling seasons would have also contributed to the poor macroinvertebrate assemblages recorded. As noted by Dallas and Day (2004), EC is one of the most important chemical variables that could profoundly impact on macroinvertebrate community structure.

Generally, results of this study revealed that the dipterans, coleopterans and hemipterans were the most ubiquitous groups of macroinvertebrates throughout the sampling seasons (Appendix A, Tables A1 – A8). They were recorded in all the sampling sites. Most families within these orders such as Dytiscidae, Elmidae, Gyrinnidae, Belostomatidae, Corixidae, Gerridae and Notonectidae are air breathers and thus are capable of replenishing their oxygen supply directly from the atmosphere and are therefore, less affected by depleted oxygen levels in water. This is probably the most plausible explanation for their continuous presence in the downstream sites.

At the four sampling sites, seasonal differences in macroinvertebrate composition and abundance were noted (Table 3.2). Observed seasonal differences could be attributed to changes in abiotic factors such as food availability, hydraulic conditions, biotope availability, temperature and biotic factors such as life history parameters (Dallas, 2004). During winter for example, fewer families of macroinvertebrates were recorded at three of the four sampling sites (Figure 3.8). This could be as a result of increase rains resulting in increase flow thereby impacting on the availability of biotopes. This was more pronounced for the stones in- and out- of current biotope. During winter most of the stones out- of current were submerged thereby reducing the number of macroinvertebrates encountered on the stone biotope during this season. Dallas (2004) had also noted that variation in discharge was more pronounce for the SIC/ SOOC (i.e. stone in- current / stone out- of current) biotope. Also, differences in life history parameters such as insect reproductive patterns and emergence time could also influence seasonal variability in terms of macroinvertebrate abundance and compositions.

Furthermore, SASS5 protocol stipulates the collection of one macroinvertebrates sample per biotope. Replicate macroinvertebrate samples were assessed to ascertain whether a single sample taken from a particular biotope in a site at a particular time as stipulated by SASS5 is a reasonable reflection of macroinvertebrates composition of that biotope at the time of sampling. Analysis of similarity (ANOSIM) did not indicate significant differences between

replicates taken from the same biotope at a site. Therefore, the results in this study support the collection of one sample from each biotope.

3.3.3 South African Scoring System version 5 and sampled biotopes

The results of the present study revealed that with the exception of vegetation and GSM biotopes at site 3, stone and GSM biotopes at site 4, there were significant differences in macroinvertebrate composition between the three sampled biotopes at the four sampled sites (Table 3.2). These differences are expected because of natural variations in hydraulics, substrate, thermal conditions and other ecological characteristics that exist among the different sampled biotopes (Dallas, 2007b). Median SASS5 scores, number of taxa and ASPT values were higher in the stone and vegetation biotopes at sites 1 and 2 (Figures 3.6 and 3.11). This is because these two biotopes supported more sensitive taxa such as Baetidae 2sp at these sites and were more widely available. At these two sites, lowest median SASS5 score, number of taxa and ASPT value were recorded for the GSM biotope. These findings are in agreement with those of Dallas (2007b) who noted that GSM biotope appeared to contribute very little to the overall SASS score in a site and even argued that this biotope could be ignore in bioassessment.

Although Dallas (2007b) reported highest median SASS5 scores, number of taxa and ASPT values for stone biotope in some reference sites in Rivers in Western Cape and Mpumalanga, highest median SASS5 scores, number of taxa and ASPT values were recorded in the vegetation biotope at sites 3 and 4. This is because more taxa were recorded in vegetation biotopes than on stones and GSM at these sites. It could be that macroinvertebrates at these sites use vegetation as “refuge” or ‘shield’ from the high level of pollution that characterised the sites. At all the sampling sites, the lowest SASS5 score, number of taxa and ASPT value were recorded for the GSM biotope. The insufficient GSM biotope particularly at site 3 contributed to the generally low scores recorded for the biotope at the site. The reduced availability of GSM at site 3 could be attributed to vegetation encroachment, thus reducing in-stream habitat availability. This was the main reason why three GSM replicates could not be collected at this site during autumn, winter and summer. Generally, the observed differences in macroinvertebrate compositions, SASS5 scores, number of taxa and ASPT values among the different biotopes, emphasised the importance of sampling all SASS5 biotopes in biomonitoring of rivers using the South African Scoring system version 5 (SASS5).

In terms of total sites scores, SASS5, number of taxa and average score per taxon (ASPT) were all highest at site 1 (Figures 3.7, 3.8 and 3.9). SASS5 scores and number of taxa were highest during spring sampling season at site 1 and this could be a reflection of improved habitat diversity and general stream conditions as reflected by Integrated Habitat Assessment System (IHAS) score during this sampling season at this site (appendix A, Figure A1). The overall SASS5 score revealed that the water quality status at site 1 could be considered as "good" while the ASPT value placed the site in the "fair" water quality category. Although ASPT values are more consistent and should reflect the water quality of a site more accurately than SASS score, Chutter (1998), Dickens and Graham (2002) suggested that SASS scores are more reflective of environmental water quality status of polluted rivers than ASPT values. Consequently, several researchers such as Dickens and Graham (1998) have adopted SASS scores as the basis of interpreting the water quality status of polluted rivers. However, the absence of highly sensitive families at this site is probably a reflection of impacts resulting from low flow and / or habitat alteration. The site is located some distance downstream of the Groendal Dam from which water abstraction takes place and this would have resulted in lowered flows which could impact on the macroinvertebrates at this site. Evidence of habitat alteration is probably not unconnected to heavy vehicles passing across a small culvert situated close to the site.

The low SASS5 and ASPT scores at site 2 is probably a reflection of the relatively impaired water quality at this site. The site is situated a few kilometres downstream from a waste water treatment works discharge. The discharges from this waste water treatment work (WWTW) have negative effects on the environmental water quality at the site. In addition, rubbish from informal settlement upstream of this site could also have contributed to the poor SASS5 scores and ASPT values. However, the presence of families such as Baetidae associated with aquatic vegetation at this site is probably indicative of natural filtering processes taking place among the vegetation biotope as the impacted water flows through them. Nevertheless, the most noticeable change at this site is the significant reduction in SASS5 score, ASPT value and number of taxa during winter, which also correspond to increased nutrients, electrical conductivity (EC), five days biochemical oxygen demand (BOD_5), decrease in dissolved oxygen (DO) and low IHAS score, indicating high organic loads and habitat alterations at this site (Figures 3.7 to 3.9 Appendix A, Tables A3 –A4, Figure A1). The poor water quality status at the site during winter is probably more anthropogenic rather than a natural change due to seasonality. This is evident in the amount of cattle dung found at the site when winter

samples were collected and the presence of an obnoxious odour, which suggested anaerobic decomposition of organic matter. This could probably have contributed to the observed organic enrichment and thus the overall poor water quality status.

At site 3, SASS5 and ASPT scores were generally very low throughout the sampling seasons (Figures 3.7 to 3.9). Consequently, both scores revealed that the water quality at this site is "critically modified". This is not unexpected because of the range of impacts the site receives, which include discharges from the Uitenhage waste water treatment works, automotive industries, livestock farming and run-off from informal settlements as well as reduced habitat availability due to vegetation encroachment. Throughout the sampling seasons, cattle dung was seen all over the site and this could have also contributed to the observed critically modified water quality status.

At site 4, SASS5 and ASPT scores were also very low throughout the sampling seasons. Thus, both SASS5 and ASPT scores revealed that water quality at this site is "critically modified". This is also not unexpected because of discharges from the Despatch waste water treatment works and run-off from informal settlements which have negatively impacted on the water quality at this site. A water resources situational assessment of the Swartkops River by DWAF (1996a) placed all sites within the Uitenhage and Despatch areas as critically or seriously modified. The present findings are in agreement with DWAF (1996a) and revealed that these sites are still critically modified.

3.3.4 Macroinvertebrates metrics

An understanding of how deterioration in environmental water quality affects different aspects of macroinvertebrate communities is crucial in their use as indicators of health of river ecosystems. For example, the present study revealed that deterioration in water quality did not impact greatly on metrics in the abundance category, whereas metrics in the compositions (or relative abundance), richness and diversity categories were severely affected. The results of this study suggest that the multimetric approach in which detailed attention is paid to different aspects of macroinvertebrate communities (i.e. abundance, composition, richness and diversity) could help in exploring how each population or groups of populations, different aspects of macroinvertebrates such as diversity and richness are affected by changes in water quality. For example, Trichoptera abundance was significantly negatively correlated with the concentrations of phytoplankton whereas no such correlation

was observed for NO_2-N concentrations. Similarly, EPT richness was significantly correlated with all measured water quality variables. Evidence of such interactions or correlations between water quality and specific metrics, in which different metrics display varying levels of correlation with measured water quality variables, could easily be masked when these metrics are aggregated to form a simple biotic index. Also, as revealed by the discriminatory ability test, the presence or absence of macroinvertebrate taxa is not enough to determine whether their occurrence is a reflection of the water quality status of a site. By subjecting their occurrences to multimetric analysis, it was possible to determine the extent to which occurrences could reflect the status of a site. Overall, the results suggest that the 10 metrics which proved to be sensitive in this study could be used alongside SASS5 and ASPT in detailed assessment of environmental water quality deterioration in the Swartkops River.

Furthermore, out of the 10 metrics that proved sensitive in this study, only Trichoptera abundance, % Trichoptera and EPT richness exhibited significant correlations with phytoplankton concentrations. While this is suggestive of the relative sensitivity of these metrics to changes in phytoplankton concentrations, it also provides for the weakness of those metrics unable to demonstrate significant correlations with phytoplankton concentrations. The high nutrient levels in the Swartkops River have led to high primary productivity including the growth of phytoplankton which has negatively impacted on the aesthetic value of the River. Low EPT richness, Trichoptera and % Trichoptera could be good indicators of increased phytoplankton concentration. Furthermore, among the 10 metrics, only EPT richness was negatively correlated with all water quality variables except dissolved oxygen (Table 3.6). EPT richness displaying strong negative correlations with organic pollution is not unexpected because the families within the EPT orders are regarded as very sensitive to water quality impairment and loss of taxa within this group indicates perturbation (Vlek *et al.,* 2004). EPT richness is commonly used in water quality assessment worldwide (Vlek *et al.,* 2004; Baptista *et al.,* 2007; Arimoro and Ikomi, 2009). The findings in this study in which EPT richness exhibited sensitivity to water quality impairment are consistent with those of Camargo *et al.* (2004) who reported that EPT richness were among the metrics that exhibited strongest negative correlations with nutrient enrichment in impounded fluvial river ecosystems.

Among metrics in the abundance category, only Trichoptera abundance enabled discrimination of site 1 from sites 2, 3 and 4. The trichopterans are known to be indicators of

relatively clean waters and their presence at site 1 is indicative of good environmental water quality. The inability of EPT, ETOC abundances and EPT/ Chironomidae ratio to allow discrimination of site 1 from sites 2, 3 and 4 is probably due to the high abundances of Baetidae and Coleopterans at site 2. In addition, families in the order Plecoptera are mostly temperate water invertebrates and very few species are known to exist in South African waters (de Moor *et al.,* 2003). Thus, throughout the sampling seasons, not a single individual in this order was caught. Consequently, the metric EPT in this study refers to individuals in Ephemeroptera-Trichoptera. The afore-mentioned reasons probably explain why EPT abundance, ETOC abundance and EPT/ Chironomidae ratio could not distinguish between site 1 and downstream sites 2, 3 and 4.

The general assumption behind the use of richness and diversity indices is that these indices decrease with increase in pollution level. In the present study, both Margalef's family richness index, Shannon and Simpson diversity indices discriminated site 1 from sites 2, 3 and 4 and also exhibited strong correlations with environmental water quality variables. The ability of these indices to discriminate site 1 and the downstream sites could be attributed to the fact that site 1 supported more macroinvertebrate fauna probably favoured by relatively good water quality. These indices were lowest at site 3 which is indicative of the poor water quality which has greatly impacted on macroinvertebrate assemblage at this site. The findings in this study are consistent with those of Camargo *et al.* (2004) who reported that Simpson diversity was among the indices that exhibited strong negative correlations with nutrient values. Ofenböck *et al.* (2004) had also reported that the Margalef's index could be suitable for the assessment of the impacts of organic pollution, channel modification and impoundments. Nevertheless, it is important to note that Simpson diversity index in this study slightly distinguished site 1 from site 2. This perhaps, is probably due to the property of this index which tends to be skewed towards the abundance of the commonest family and thus strongly affected by the most abundant family (Ogbeibu, 2005).

Equitability (or evenness) is an ecological concept which measures the relative abundances of the various families in a sample and it increases as the abundances of families are more evenly distributed in a sample such that maximum equitability is obtained when all families abundance are relatively evenly distributed within a sample (Ogbeibu, 2005). The assumption behind this concept is that, the abundances of species in a sample from an undisturbed environment are often relatively evenly distributed than those from a disturbed or impacted

environment where opportunistic and tolerant taxa would account for greater proportion of species abundance (Ogbeibu, 2005). In the downstream sites (i.e. sites 2, 3 and 4) particularly at sites 3 and 4, the abundance of families was skewed toward the most pollution tolerant taxa such as Chironomidae, Oligochaete and Hirudinae. Thus, equitability was found to be reduced at these sites. Consequently, equitability was found to discriminate all the sampling sites from one another and was also strongly correlated with environmental water quality variables. The relatively high equitability at site 1 suggests that samples at this site were not dominated by the abundance of few families and this could be an indication of good water quality.

Generally, the strong correlations of all 10 sensitive metrics with nutrient levels probably suggest that these metrics could be good indicators of nutrient enrichment. Specifically, increased % Chironomidae + Oligochaete seemed to be a good indicator of depleted oxygen and increased nutrient levels. Conversely, increased EPT richness, % ETOC, % Trichoptera, Trichoptera abundance, diversity and equitability seemed to indicate good water quality. Although electrical conductivity (EC) at the downstream sites of the Swartkops River is naturally high (DWAF, 1996a), waste water and industrial effluents discharges also contributed to the observed high EC. The strong negative correlations of Trichoptera abundance, % Trichoptera, EPT richness and Margalef family richness index with EC probably seemed to suggest that these four metrics could be used as general indicators of industrial effluent discharges in the Swartkops River. Also, equitability displayed strong correlations with most of the measured water quality variables. The concept of equitability is important for inclusion in a multimetric approach for water resources management because it helps to elucidate the consequences of pollution which may lead to the dominance of tolerant and opportunistic taxa in a sample as observed at the downstream sites particularly at sites 3 and 4.

3.3.5 Chironomid communities and diversity

The family Chironomidae include both sensitive and tolerant species (Mousavi *et al.,* 2003; Wright and Burgin, 2009). Consequently, in the present study, there were increasing species richness and diversity of chironomids with decreasing levels of pollution and therefore suggest that chironomid species richness and diversities could be used as indicators of water quality changes. The impact of organic pollution on the reduction of both chironomid species diversity and richness in this study are consistent with the findings of Wright and Burgin

(2009) who reported similar trends. The subfamily Chironominae was richer in species and shows a clear distinction between species of the tribe Tanytarsini and Chironomini in sensitivity to pollution. Species of tribe Tanytarsini were particularly vulnerable to pollution and were completely absent at site 3 (Figure 3.22). The results are in agreement with those of Clement *et al.* (1992) and Mousavi *et al.* (2003) who reported more of tribe Tanytarsini in clean waters. The high abundance of *Chironomus* spp. in the heavily polluted sites 3 and 4 was expected because of their ability to tolerate oxygen depletion and they have been reported in several studies at polluted sites (Adriaenssens *et al.,* 2004; Janssens de Bisthoven *et al.,* 2005; Ochieng *et al.,* 2008; Simião-Ferreira *et al.,* 2009). The high abundance of the genus *Chironomus* at the polluted sites is probably due to its ability to use haemoglobin for oxygen transportation and individuals within this genus are therefore able to survive in oxygen depleted environment (Adriaenssens *et al.,* 2004).

The numbers of species of subfamily Tanypodinae were highest at site 1 and only *Tanypus* sp. and *Ablabesmyia* sp. occurred at site 2. Most species of Tanypodinae were reported to be sensitive to water quality changes (Ochieng *et al.,* 2008) which is also reflected in the present study. Although *Procladius* sp. is known to have varying degree of tolerance to water quality changes, it was found only at site 1 with an insignificant contribution to the total abundance (Figure 3.21). It could be that other ecological conditions that were not measured in the present study were responsible for their rare occurrence. Among the four sampled sites, sites 3 and 4 were the most polluted mostly dominated by *Chironomus* spp. The presence of species such as *Cricotopus* sp. 1, *Cricotopus trifasciata* gr., *Tanypus* sp., *Orthocladius* sp., *Kiefferullus* sp. and *Dicrotendipes* sp at site 2 which were completely absent at site 3 and rare at site 4 seemed to suggest that the water quality at site 2 is less impacted than at sites 3 and 4. Also, the higher chironomid species richness and diversity at site 2 than at sites 3 and 4 equally suggest less impact at this site than at sites 3 and 4.

In general, the downstream sites contained 6 to 20 taxa compared to the 25 taxa at site 1 (Appendix A, Table A9). Site 1 was characterised by the subfamilies Orthocladiinae, Tanypodinae and tribes Tanytarsini and Chironomini (subfamily Chironominae) while sites 2, 3 and 4 were characterised by the subfamily Orthocladiinae and tribe Chironomini (Figure 3.22). *Chironomus* spp., *Dirotendipes* sp., *Kiefferulus* sp. and *Tanypus* sp. seemed to be tolerant to pollution, occurring in high abundance at the downstream sites. In contrast, *Polypedilum* sp., *Tanytarsus* sp., *Orthocladius* sp., *Cricotopus* sp.1, *Cricotopus trifascciata*

gr., and *Ablabesmyia* sp. appeared to be more sensitive taxa, being less common at the downstream sites. Results of this study therefore suggest that the family Chironomidae, identified to species or genus, can be use to assess environmental water quality status in freshwater ecosystems because of their varying degree of sensitivity to changes in environmental water quality.

Ordination techniques, such as CCA, are multivariate statistics used to elucidate the relationships between biological community and their environment (ter Braak and Verdonschot, 1995). Axis 1 of the CCA ordination shows good correlation between species and environment scores (Table 3.8). It also revealed that species sensitive taxa were more positively correlated with high DO level while nutrients and EC were the main factors responsible for the observed structure in downstream sites. Nutrient concentrations and electrical conductivity were also reported as the main defining factors responsible for the distribution of chironomids in Flanders, Belgium (Adriaenssens *et al.,* 2004). However, the present study further revealed that all six water quality variables are important in interpreting the results of the CCA ordination and species such as *Chironomus* spp. and *Dicrotendipes* sp. appeared to be good indicators of water enriched with nutrients and organic matter. Adriaenssens *et al.* (2004) reported similar pattern of occurrence for these species from some nutrient enriched running waters in Flanders, Belgium. In general, chironomid communities in the Swartkops River appeared to be good indicators of water quality changes consisting of species with varying degree of sensitivity to water quality deterioration.

With regard to the sampling seasons, diversity and richness were highest during spring and summer and lowest in winter. It has been reported that most chironomid taxa enter the resting stage during winter and could result in the collection of fewer species (Rossaro, 1991). Thus, some authors (e.g. Adriaenssens *et al.,* 2004) restricted the collection of their chironomids to summer only. This probably explains the observed low diversity and richness during winter.

In this study, chironomids were identified to the lowest possible taxonomic resolution based on available keys. The more refined taxonomic resolution of chironomids provided useful ecological information especially of the bioindication potential of the family Chironomidae which could help in elucidating the need for their use in water resources management. The species level identification also helps to separate all four sampling sites in terms of chironomid abundance and composition that was not possible at the family level of taxonomic resolution. However, there exist some difficulties particularly in identifying them

beyond the family level. Therefore, application of species or genus levels identifications may not be suitable for routine rapid biomonitoring programmes but could form part of detail ecological assessments of water resources. Such detailed assessments will require making South African chironomid species level keys available, training of personnel and cost effective planning.

3.4 Conclusion

The deteriorating environmental water quality status of the Swartkops River has impacted on the macroinvertebrate assemblages particularly at the downstream sites. This is not unexpected because of the ranges of impacts these sites receive which include industrial and sewage effluent discharges, run-off from informal settlement and agricultural activities such as livestock farming. Although anthropogenic impacts and water quality situation of the Swartkops River have been previously documented, and recommendations made for remediation (DWAF 1996a; Taljaard *et al.,* 1998), SASS5 results in this study revealed that the downstream impacted sites are critically modified which suggest lack of implementation of action plans contained in previous studies. In addition, site 1 which was used as reference site in this study was placed in the good water quality category by overall SASS5 score while overall ASPT value revealed that water quality at this site is fair. This is a cause for concern. Site 1 (reference site) is nonetheless not in its natural state and also suffers mild impacts as revealed by SASS5 scores.

Most types of biomonitoring tools could easily discriminate unpolluted from polluted sites. But sites with slightly different levels of pollutions with small differences in macroinvertebrate compositions and abundances would be better highlighted when metrics and indices with powerful discriminatory ability are employed. In the present study, sites 2, 3 and 4 are all polluted but with slight differences in macroinvertebrate compositions. Out of the 10 sensitive metrics, Simpson index, Shannon index, Margalef's family richness index, Equitability, % Chironomidae + Oligochaete and EPT richness discriminated these three sites from one another (Figure 3.19). This information regarding which aspects of macroinvertebrate community could discriminate these sites with slight differences in macroinvertebrate compositions would be masked if these metrics were aggregated into a single score. The 10 metrics proved sensitive in this study and, together with SASS5 and ASPT could be suitable for the assessment of nutrient enrichment in the Swartkops River. Furthermore increased % Chironomidae + Oligochaete seemed to be a good indicator of

environments with depleted oxygen levels while increased Trichoptera abundance, % Trichoptera, EPT richness and Margalef's family richness seemed to be indicators of good water quality in the Swartkops River.

The present study emphasised the importance of species level identification in biological water quality assessment because within a family, species are differentially sensitive to pollution. This is reflected in the differences between the chironomid assemblages at the four sampling sites in which some species were completely absent at some of the sampled sites which suggest differences in water quality at these sites, particularly differences observed between the three downstream sites. Also, if species level data were not available, it is possible to erroneously conclude that chironomids are all tolerant of organic and other forms of pollution when in fact, some species within this species-rich family are sensitive to pollution (Wright and Burgin, 2009; Luoto, 2010,). This study therefore provides evidence of the bioindication potentials of the family Chironomidae and when identified to species or genus, can be used to assess environmental water quality status in the Swartkops River and probably in South African freshwater ecosystems. Although chironomid genus and species levels taxonomic resolutions have potential for bioindication, their identification to such taxonomic resolutions (genus or species) is very time-consuming and may therefore not be suitable for rapid bio-assessment programmes. Nevertheless, it seems that the different subfamilies and tribes within this family respond differently to water quality changes and would therefore have different tolerant limits to pollution. Consequently it might be reasonable to argue that the score of **2** given to the family Chironomidae may not best represent the sensitivity of sub-families and tribes within this family. Since sub-family level chironomid identification is relatively easier to carry out than genus and species identifications, the sensitivity of sub-families to water quality impairment should be assessed extensively in order to split the family Chironomidae into sub-families and these assigned different scores in SASS.

CHAPTER 4: RESULTS: DEFORMITIES IN CHIRONOMIDAE LARVAE AS INDICATORS OF ANTHROPOGENIC IMPACTS IN THE SWARTKOPS RIVER

4.1 Introduction

The chapter begins with a presentation of results for larval deformities in Chironomidae communities, patterns of mentum, ligula, mandible and antennal deformities. Possible differences in susceptibility of selected chironomid species collected from the same sites and structures within a species to deformities are explored. The chapter concludes with an exploration of deformities and water quality relationships as well as a discussion of the results presented.

4.2 Results

4.2.1 Screening Chironomidae communities for larval morphological deformities

A total of 4 838 larvae, representing 26 taxa (Appendix A, Table A9), from the four sampling sites during the four sampling seasons were screened for deformities in the mentum, ligula, mandible, premandible, paraligula and antenna. Among the structures examined, the premandible and paraligula did not displayed deformities throughout the study period and are thus not presented. Community incidences of deformities were calculated for each structure as the percentage of the number of all deformed larvae, irrespective of genus or species, to the total number of larvae sampled for each site per season. Therefore incidences of deformities are expressed in percentages. Results revealed that community incidences of mentum deformity were consistently highest at site 3 throughout the sampling seasons (Figure 4.1). The community incidences of mentum deformity were consistently higher than 8% at sites 2, 3 and 4, indicating possible contamination of the Swartkops River. Analysis of variance (ANOVA) conducted on arcsine transformed data revealed that the mean community incidence of mentum deformity was significantly higher ($p < 0.05$) at site 3. Although there were seasonal fluctuations in the incidences of mentum deformities, ANOVA did not reveal statistically significant differences ($p > 0.05$) between seasons across sites.

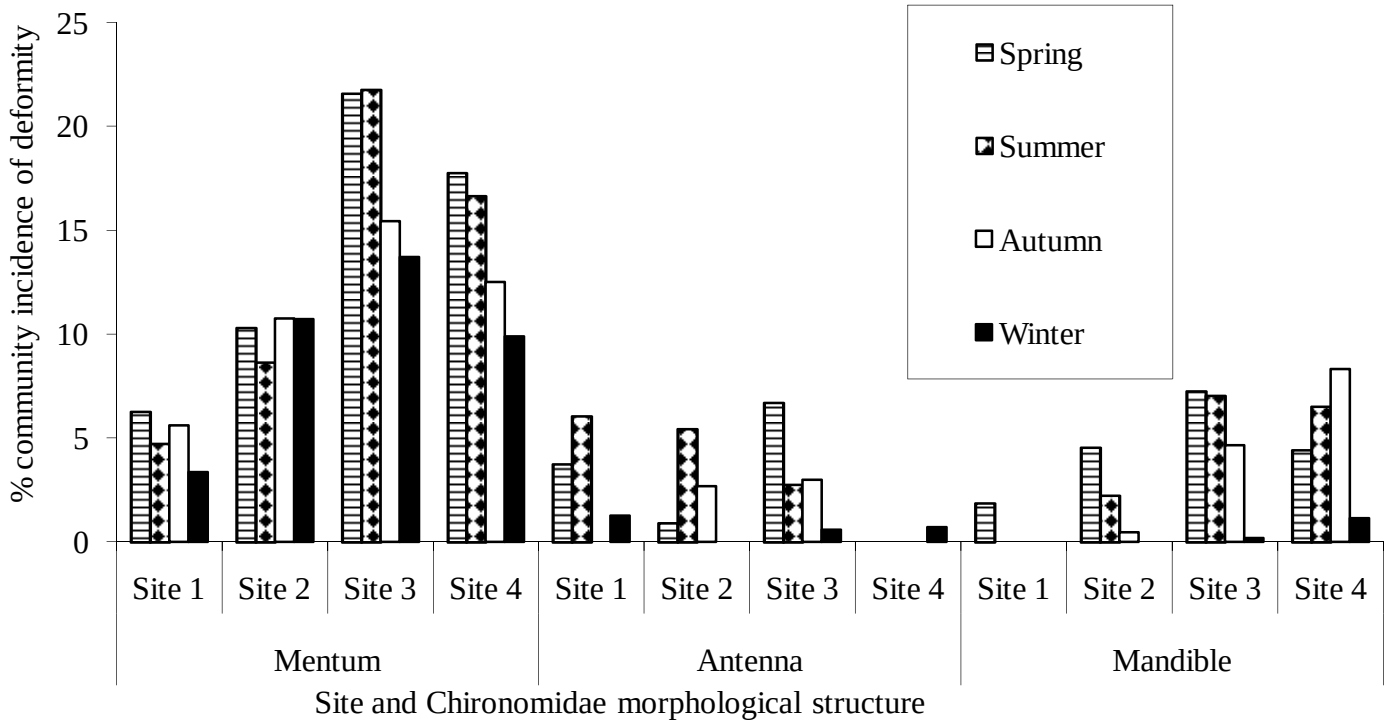


Figure 4.1: Percentage Chironomidae community incidences of mentum, antenna and mandible deformities at each of the four sampling sites in the Swartkops River during the four sampling seasons

The community incidences (%) of mandible deformities are also presented in Figure 4.1. Results revealed that community incidences of mandible deformities were generally higher at sites 3 and 4. Although there were both spatial and temporal variations in the percentage community incidences of mandible deformities, ANOVA conducted on arcsine transformed data did not reveal statistically significant differences ($p > 0.05$) among sites and seasons. In addition, only the community incidence of mandible deformity at site 4 during autumn was higher than 8% (Figure 4.1), which seemed to suggest the need to determine the background levels of deformity for mandible, above which a site could be considered contaminated when screening mandible for deformities.

The community incidences of antennal deformities are also presented in Figure 4.1. The highest community incidence of antennal deformity was recorded at site 3 during spring. However, community incidences of antennal deformities at site 1 were generally higher than sites 2 and 4 (Figure 4.1) except during autumn, where no antennal deformity was observed at site 1. The community incidences of antennal deformities at all sampling sites were consistently below 8 %, suggesting the need for the determination of background incidence of antennal deformity. Besides, although there were spatial and seasonal fluctuations in community incidences of antennal deformities, ANOVA conducted on arcsine transformed data did not revealed significant statistical differences ($p > 0.05$) among sites and seasons. Generally results revealed that with the exception of the antenna, community incidences of mentum and mandible deformities increased from site 1 to the three sites (i.e. sites 2, 3 and 4) downstream of industrial and waste water effluents discharge points and were consistently highest in the mentum (Figure 4.1).

4.2.2 Spatial and temporal patterns of mentum and ligula deformities in Chironomidae larvae

The patterns of mentum and ligula deformities among dominant chironomid taxa at the four sampling sites during the four sampling seasons are presented in Figure 4.2 and illustrated in Appendix B, Figure B1. Detailed descriptions of the various types of deformities are provided in Appendix B, Tables B9 – B12. At site 1, missing teeth and fused teeth were the commonest types of deformities during the four sampling seasons (Figure 4.2). Although split teeth were observed among chironomid taxa at this site, they were less common throughout the sampling seasons than other deformities.

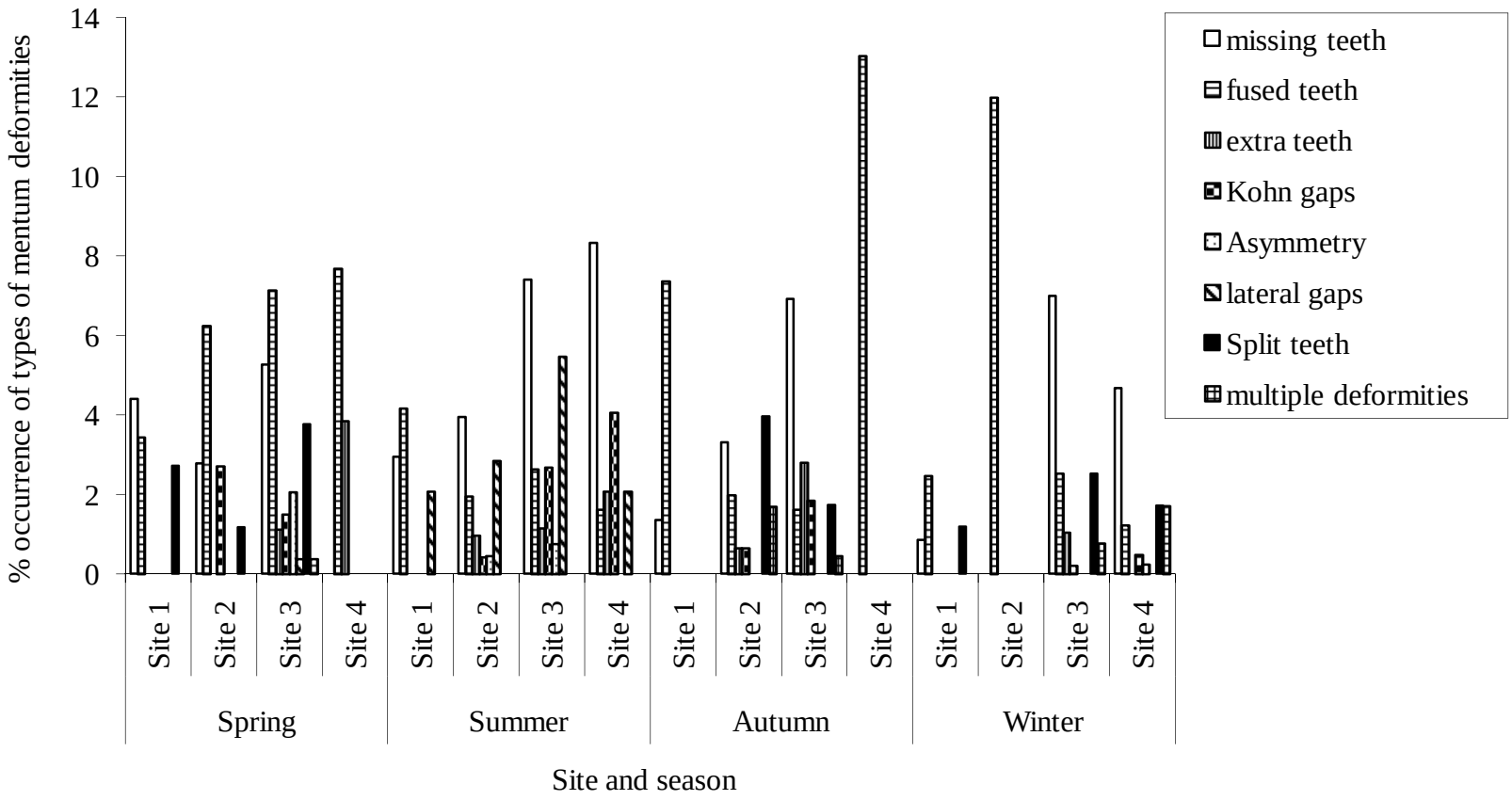


Figure 4.2: Percentage occurrence of deformity types recorded in the mentum of chironomid taxa at each of the four sampling sites in the Swartkops River during the four sampling seasons.

At site 2 during spring, fused teeth, Köhn gaps and missing teeth were the commonest types of deformities while missing teeth was the most dominant type of deformity observed among chironomid taxa during summer and autumn. However, in autumn, multiple deformities (i.e. a single larvae showing more than one type of deformity in the mentum) were observed. During winter, only fused teeth were recorded at this site and although deformity types such as extra teeth, asymmetry and split teeth were observed in other sampling seasons, they were less frequent (Figure 4.2).

At site 3 chironomid taxa were characterised by Köhn gap, asymmetry, fused, missing, split and extra teeth during the four sampling seasons. However, during winter and autumn, multiple deformities were observed among chironomid taxa at this site (Figure 4.2). Generally, more deformities were seen among individuals from this site compared to the other three sites.

At site 4 during spring, chironomid taxa were characterised by fused and extra teeth. In summer, missing teeth and Köhn gaps were the dominant types of deformity seen among chironomid taxa, while fused teeth were the only type of deformity seen in autumn. Chironomid taxa at site 4 during winter were characterised by missing teeth and multiple deformities (Figure 4.2). Generally, results revealed that sites 2, 3 and 4 downstream of waste water treatment works and industrial effluent discharge points were characterised by deformity types such as asymmetry, Köhn gaps and multiple deformities, which were not seen at site 1. In addition, in winter and autumn, the observation of larvae with multiple deformities probably suggests more severe response in these two seasons.

4.2.3 Spatial and temporal patterns of mandible deformities in Chironomidae larvae

The patterns of mandible deformities among dominant chironomid taxa at the four sampling sites during the four sampling seasons are presented in Figure 4.3 and illustrated in Appendix B, Figures B2 while detailed descriptions of the various types of deformities are provided in Appendix B Tables B13 –B16. At site 1 during spring, deformities in the mandible were characterised by extra and split teeth. No mandible deformities were seen during summer, autumn and winter among the dominant chironomid taxa at this site (Figure 4.3).

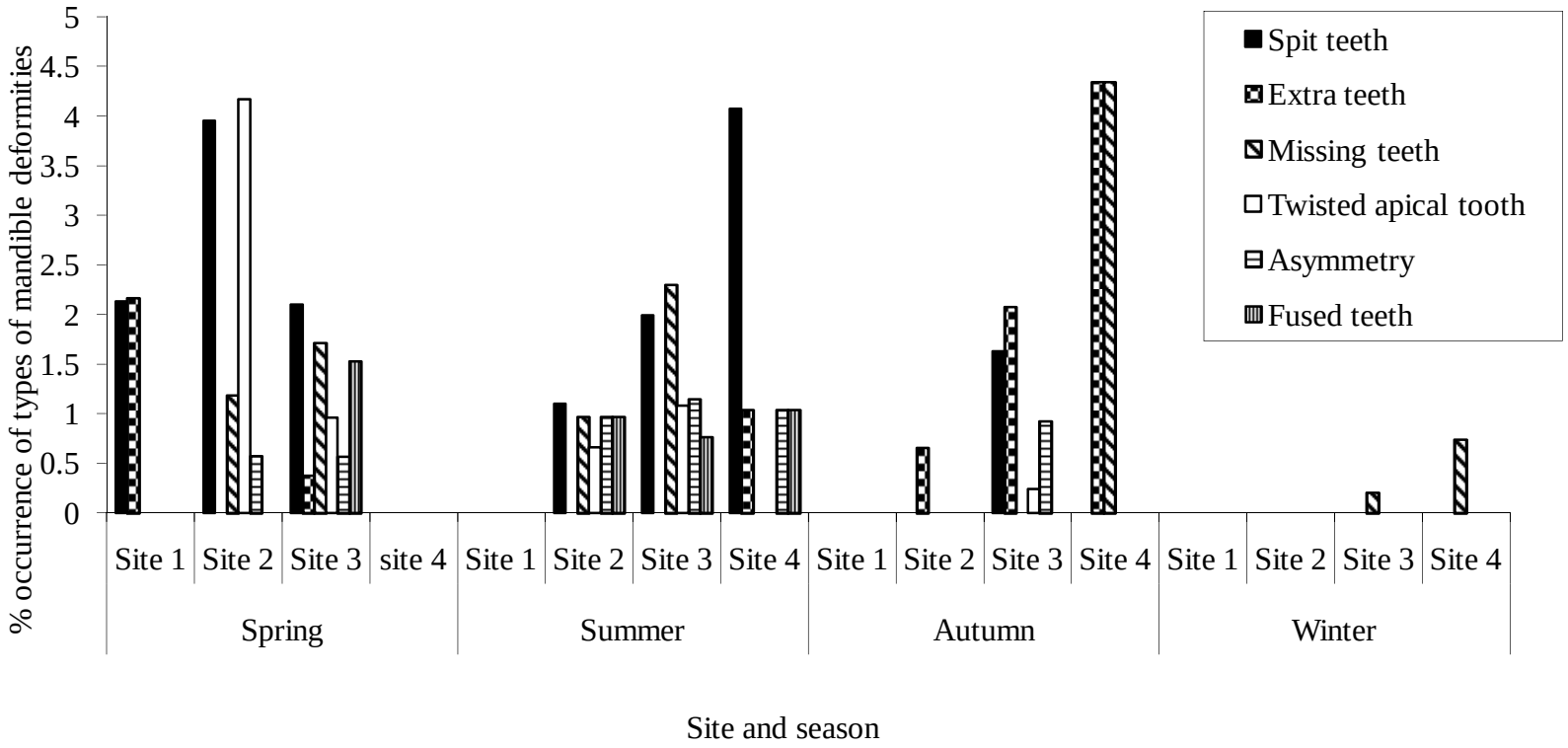


Figure 4.3: Percentage occurrence of deformity types recorded in the mandible of dominant chironomid taxa at each of the four sampling sites in the Swartkops River during the four sampling seasons

At site 2 during spring, split teeth and twisted apical teeth were the dominant deformity types. Although deformities such as missing teeth and asymmetry were seen, they were less frequent (Figure 4.3). In summer, deformity types such as split, missing and fused teeth as well as asymmetry characterised the mandibles of chironomid taxa, whereas only missing teeth was observed in autumn, and no mandible deformities were seen in winter.

At site 3 during spring, the commonest deformities in the mandible of chironomid taxa include split, missing and fused teeth (Figure 4.3). During summer and autumn, deformities in the mandible were mostly characterised by split and missing teeth but only missing teeth were observed in winter. Generally chironomids at site 3 showed deformity types that were not seen at site 1.

At site 4, no deformities were observed in the mandibles of chironomid taxa during spring. However, in summer, autumn and winter, deformities such as split, extra, fused and missing teeth characterised the mandibles of chironomid taxa (Figure 4.3).

4.2.4 Spatial and temporal patterns of antennal deformities in Chironomidae larvae

The patterns of antennal deformities among dominant chironomid taxa at the four sampling sites during the four sampling seasons are presented in Figure 4.4 and illustrated in Appendix B, Figure B3 while detail descriptions of the various types of deformities are provided in Appendix B, Tables B17 - 20 At site 1 during spring, antennae of chironomid taxa were characterised mostly by deformities in the segments and multiple deformities (i.e. an antenna of a single individual larva deformed in more than one structure). During summer, deformities in the lauterborn organ and segments were the most frequent whereas in winter, only the lauterborn organ was deformed. No antennal deformities were observed in autumn (Figure 4.4).

At site 2 during spring, antennae were mostly deformed in the lauterborn organ, segments and ring organ. In summer and autumn, deformities were mostly seen in the segments, lauterborn organ and blade. No antennal deformity was observed in winter (Figure 4.4).

At site 3 during spring, deformities in the segments and multiple deformities were the most frequent among chironomid taxa, while deformities in the lauterborn organ were the commonest in summer. In autumn and winter, antennae of chironomid taxa at the site were characterised mostly by deformities in the segments (Figure 4.4).

At site 4, antennal deformities among chironomid taxa were seen during winter only. They were characterised by deformed segments and style (Figure 4.4). Generally, the various types of antennal deformities were less frequent at sites 3 and 4 compared to sites 1 and 2.

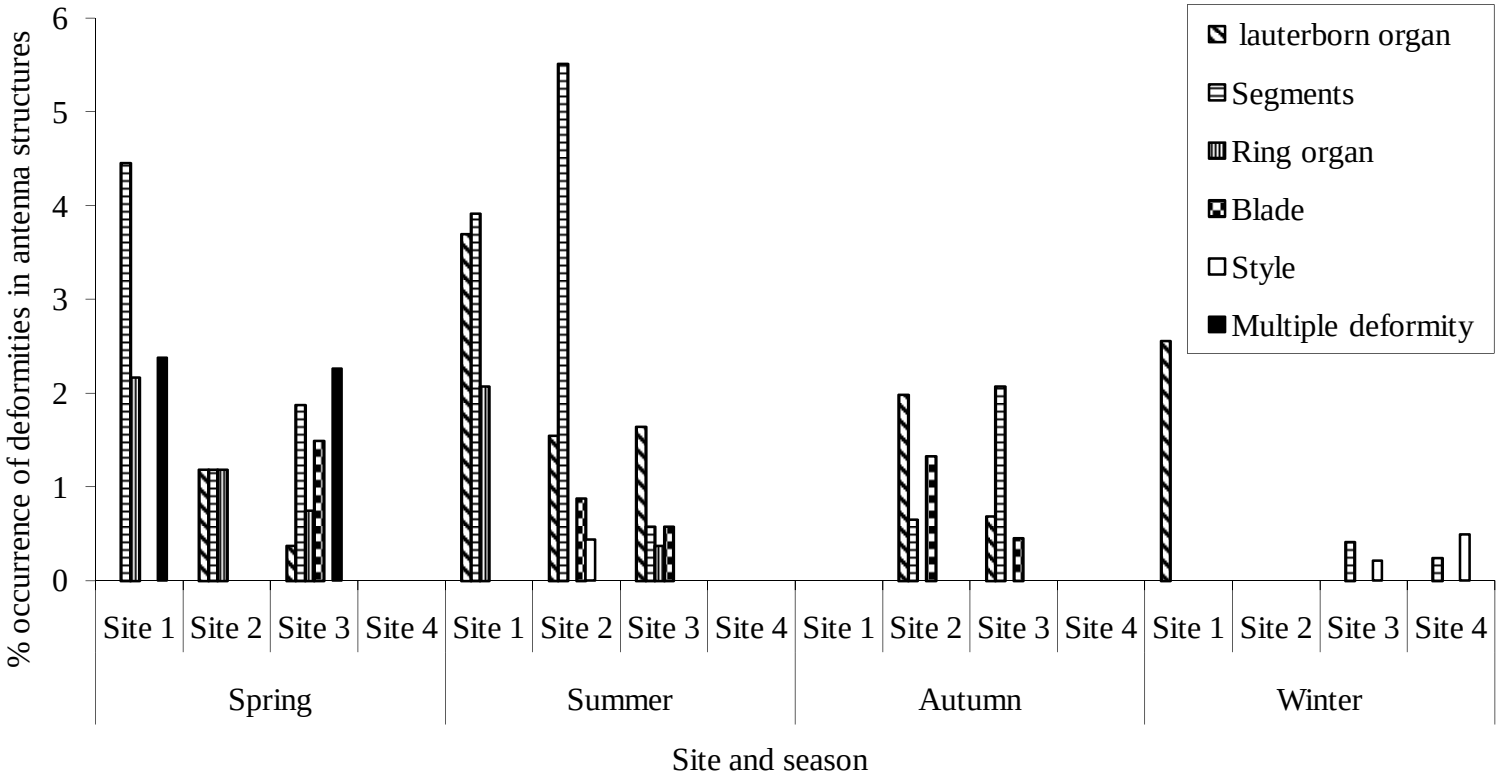


Figure 4.4: Percentage occurrence of deformity types recorded in the antenna of chironomid taxa at each of the four sampling sites in the Swartkops River during the four sampling season.

4.2.5 Incidences of deformities in selected Chironomidae taxa at the four sampling sites

The incidences of deformities calculated as the percentage (i.e proportion) of deformed individuals to the total number of larvae screened within a taxon in selected Chironomidae taxa taken from the same site (i.e. exposed to the same environmental conditions over time) were compared. Taxa were selected for this analysis if there were at least 20 larvae within a site and also occurred in more than one sampling season. Such criteria were necessary because incidences of deformities (i.e. proportion of deformed individuals) is highly dependent on sample size and a very small sample size could result in unrealistically elevated incidence of deformity as observed for some individual taxa with only 2 larvae, with 1 being deformed, resulting in a 50 % incidence of deformity (Appendix B, Table B1). To facilitate the comparison of mean percentage incidence of deformity, it was also important that selected taxa occurred in more than one sampling season to avoid seasonal bias. However, it was also pertinent not to set inappropriately stringent criteria, considering the low abundances of most of the chironomid taxa recorded at the four sites during the four sampling seasons. A sample size of at least 20 larvae per taxa has been used in literature for comparison (MacDonald and Taylor, 2006) and was considered appropriate in this study.

At site 1, incidences of mentum deformities between *Cricotopus* sp. 1, *Cricotopus trifasciata* gr. and *Tanytarsus* sp. were compared (Figure 4.5). Mean incidence of mentum deformity was highest in *Cricotopus trifasciata* gr. but lowest in *Cricotopus* sp.1. The incidences of antennal and mandible deformities were not compared because they did not occur in more than one sampling seasons in all three taxa (i.e. *Cricotopus* sp. 1, *Cricotopus trifasciata* gr. and *Tanytarsus* sp.).

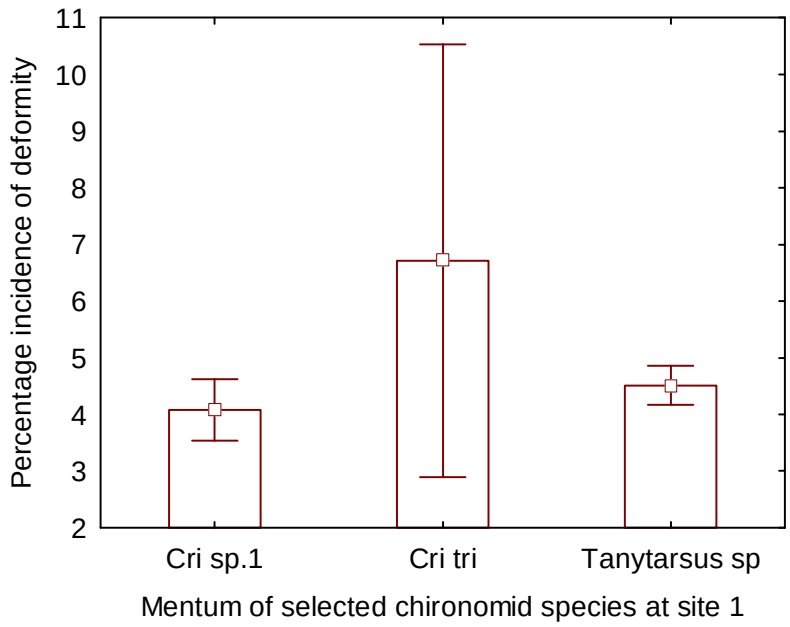


Figure 4.5: Mean and standard deviation for percentage incidences of mentum deformities in *Cricotopus* sp. 1, *Cricotopus trifasciata* gr and *Tanytarsus* sp. sampled at site 1. Abbreviations: Small squares = means, range bars = standard deviations, Cri sp.1 = *Cricotopus* sp. 1 and Cri tri = *Cricotopus trifasciata* gr.

At site 2, mentum and mandible deformities were compared between *Cricotopus* sp.1, *Cricotopus trifasciata* gr., *Dicrotendipes* sp. and *Tanypus* sp. (Figure 4.6). Incidences of deformities in both structures were highest in *Dicrotendipes* sp. but lowest incidences of mentum deformities were observed in *Cricotopus trifasciata* gr., while lowest incidences of mandible deformities were recorded in *Tanypus* sp. The incidences of antennal deformities were not compared because they did not occur in more than one sampling seasons in all four taxa (i.e. *Cricotopus* sp.1, *Cricotopus trifasciata* gr., *Dicrotendipes* sp. and *Tanypus* sp.)

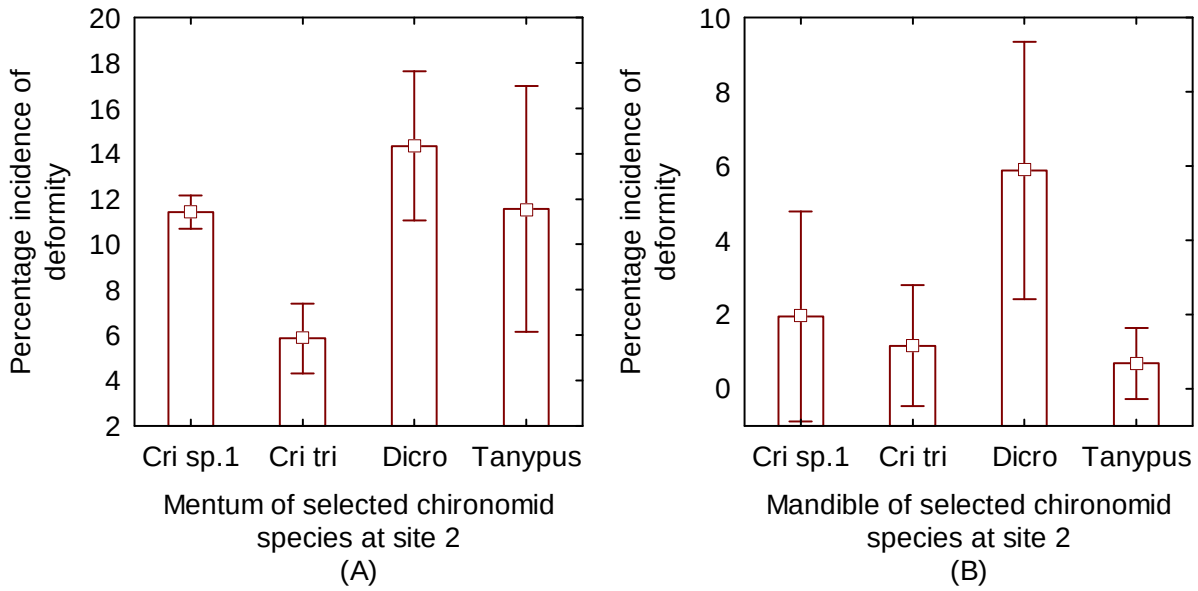


Figure 4.6: Mean and standard deviation for percentage incidences of mentum (A) and mandible (B) deformities in *Cricotopus* sp. 1, *Cricotopus trifasciata* gr., *Dicrotendipes* sp. and *Tanypus* sp. sampled at site 2. Abbreviations: Small squares = means, range bars = standard deviations, Cri sp.1 = *Cricotopus* sp. 1, Cri tri = *Cricotopus trifasciata* gr. and Dicro = *Dicrotendipes* sp.

At site 3, incidences of mentum and mandible deformities were compared between *Chironomus* sp.1 and *Chironomus* sp. 2 (Figure 4.7). Incidences of deformities in both structures were highest in *Chironomus* sp.1. No comparison was made among chironomid taxa at site 4 because *Chironomus* sp.1 was the only dominant taxa that occurred in more than one season at this site. Results therefore suggest that incidences of deformity either in the mentum or mandible varied among chironomid taxa taken from the same sites. However, one-way analysis of variance (ANOVA) conducted on arcsine transformed data (mentum and mandible) did not reveal statistically significant differences ($p < 0.05$) for the mean incidences of deformity between chironomid taxa taken from the same sites.

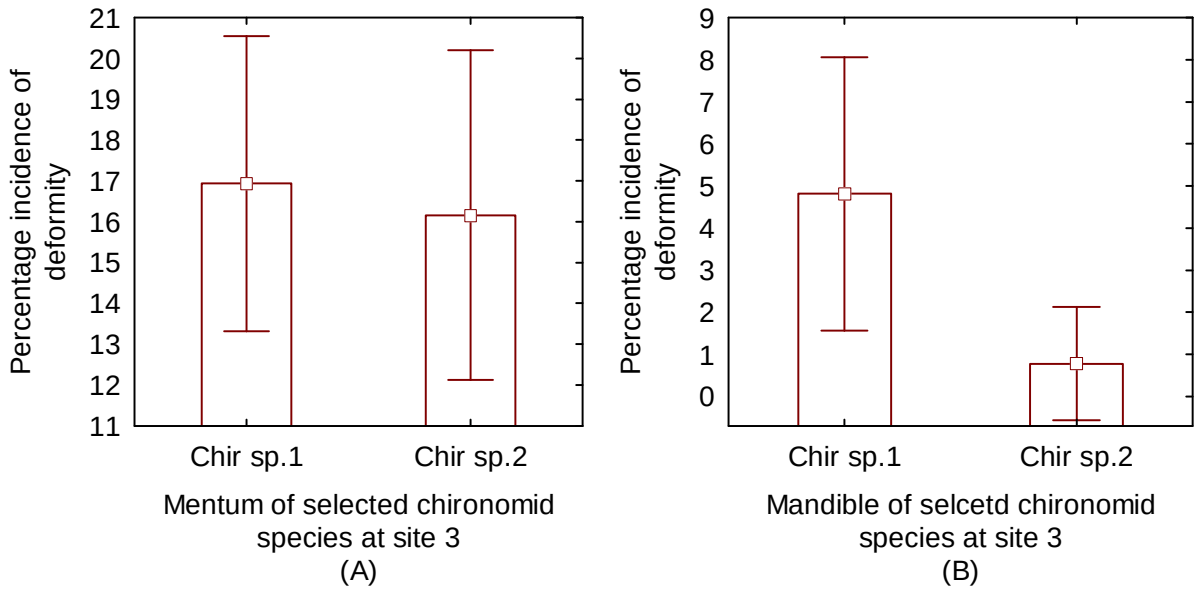


Figure 4.7: Mean and standard deviation for percentage incidences of mentum (A) and mandible (B) deformities in Chironomus sp. 1 and Chironomus sp.2 sampled at site 3. Abbreviations: Small squares = mean, range bars = standard deviations, Chir sp.1 = *Chironomus* sp. 1 and Chir sp.2 = *Chironomus* sp.2

4.2.6 Susceptibility of selected Chironomidae morphological structures to deformity

Susceptibility of mentum, mandible and antenna to deformity among dominant chironomid taxa sampled at each of the four sampling sites was assessed. In order to establish how individual structures within a species or genus taken from the same site (i.e. exposed to the same environmental conditions over time) varies in susceptibility to deformities, incidences of mentum, mandible and antenna deformities were compared. Only structures showing deformities in the same species (from the same site) in more than one sampling seasons were compared. With the exception of *Cricotopus* sp.1 at site 1, results revealed that mean incidences of deformities were generally higher in the mentum than in mandible and antenna (Figure 4.8). However, in *Cricotopus* sp.1 collected at site 1, mean incidence of deformity was higher in the antenna than in mandible and mentum. One-way analysis of variance (ANOVA) conducted on arcsine transformed data showed that, with the exception of *Cricotopus* sp.1 at site 1, mean incidence of deformities in the mentum was significantly higher ($p < 0.05$) than in the mandible and antenna in all other taxa examined at each of the sampling sites. Results

therefore suggest that structures within a taxon taken from the same sites are differentially susceptible to deformities.

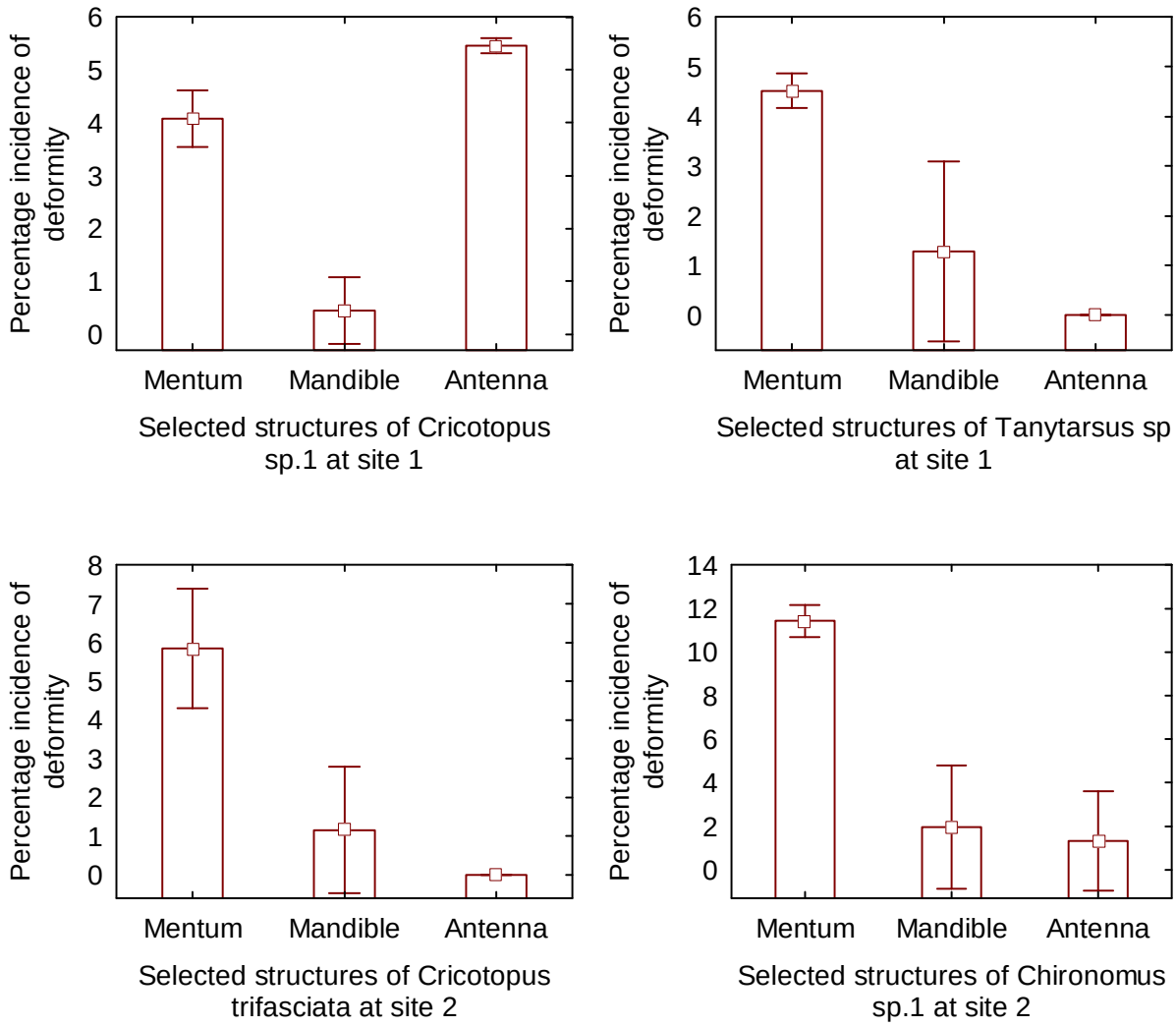


Figure 4.8: Means and standard deviations for percentage incidences of deformities for the mentum, mandible and antenna within a taxon among dominant chironomid taxa taken from the four sampling sites in the Swartkops River. Small squares = mean and range bars = standard deviation (Figure continued on next page)

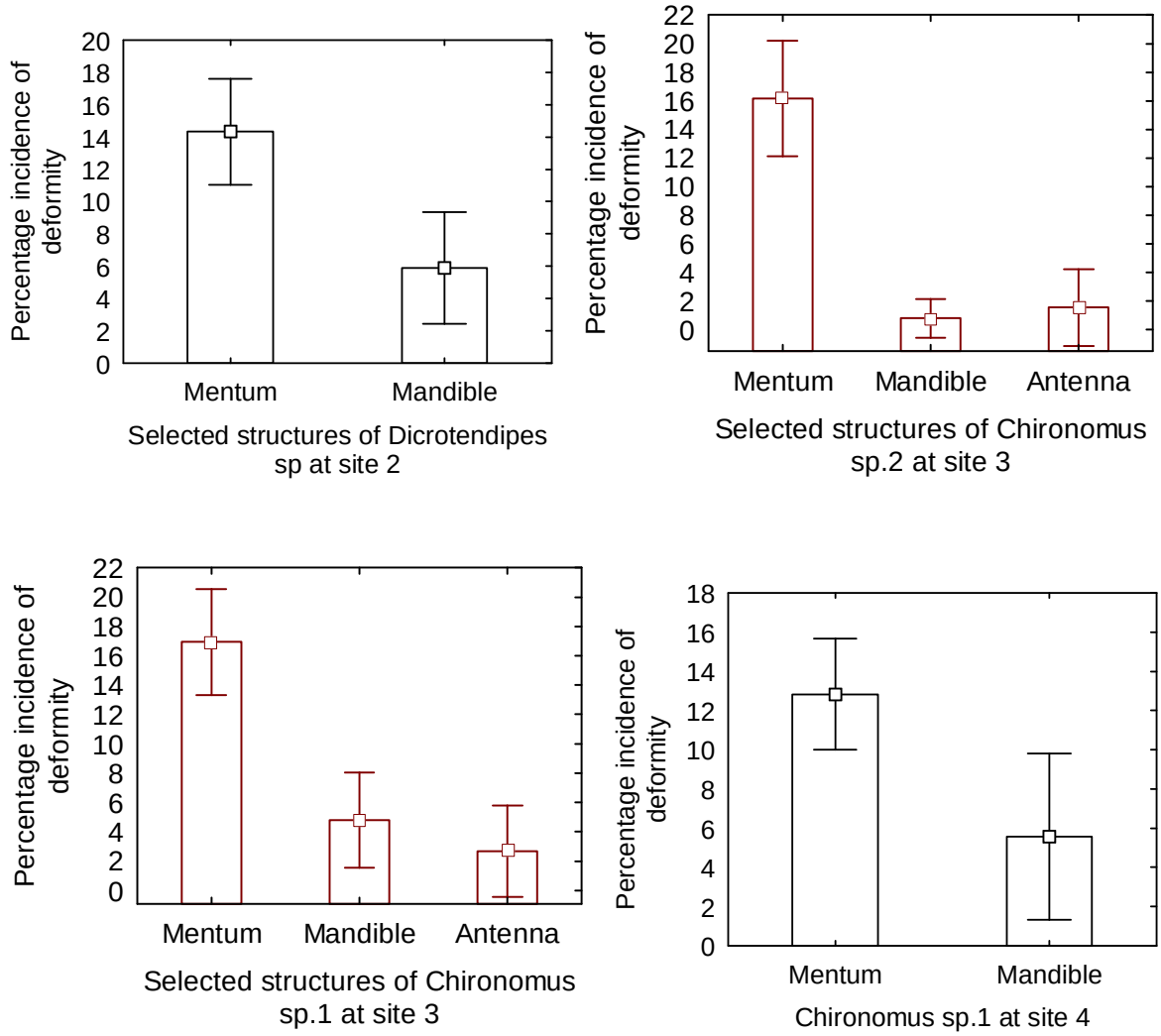


Figure 4.8 Means and standard deviations for percentage incidences of deformities for the mentum, mandible and antenna within a taxon among dominant chironomid taxa taken from the four sampling sites in the Swartkops River. Small squares = mean and range bars = standard deviation (Figure starts from previous page)

4.2.7 Relationships between Chironomidae larval deformities and water quality

Relationships between arcsine transformed community incidences (%) of mentum, mandible and antennal deformities and log (x+1) transformed measured water quality variables were elucidated by a Pearson's correlation analysis. Pearson correlation coefficients between community incidences of mentum, mandible and antennal deformities and measured water quality variables are presented in Table 4.1. A significant negative correlation was found between community incidences of mentum deformities and the concentrations of dissolved

oxygen (DO). Conversely, significant positive correlations were observed between community incidences of mentum deformities and the concentrations of ammonium - nitrogen (NH_4-N), total inorganic nitrogen (TIN), orthophosphate - phosphorus (PO_4-P) and turbidity. Community incidences of mandible deformities displayed significant negative and positive correlations with the concentrations of dissolved oxygen and turbidity respectively. No significant correlation was observed between community incidences of antennal deformities and the concentrations of any of the measured water quality variables.

Table 4.1: Pearson's correlation coefficients between community incidences of mentum, mandible and antennal deformities and measured water quality variables. * = significant at $p < 0.05$ and ** = significant at $p < 0.01$

	DO	**pH**	**Temperature**	**EC**	**Turbidity**	**BOD_5**	**NO_3-N**	**NO_2-N**	**NH_4-N**	**TIN**	**PO_4-P**
% community incidence of mentum deformity	-.562*	.327	.241	.426	.635**	.420	.101	.450	.593*	.579*	.683*
% community incidence of mandible deformity	-.625**	.283	.413	.246	.598*	.461	.119	.124	.499	.484	.468
% community incidence of antennal deformity	.368	.422	.358	-.240	.377	-.203	-.455	-.171	-.299	-.280	-.144

4.3 Discussion

4.3.1 Morphological larval deformities in Chironomidae communities

It has been suggested that incidences of deformities in chironomid community exceeding 8% of the total larvae examined can be taken as indication of in-stream toxic contamination (Warwick, 1988; Nazarova *et al.*, 2004). Thus sites 2, 3 and 4 with the community incidences of mentum deformities exceeding 8% throughout the sampling seasons could be regarded as toxicologically contaminated. However, such an arbitrarily designated reference value as a biological criterion is questionable because ecological conditions vary among aquatic ecosystems (lotic and lentic) and among regions. Therefore, because of differences in ecological conditions among eco-regions, an "ideal" reference site(s) should be determined objectively for each study and deformities obtained at impacted sites should also be objectively compare to those at reference site(s). Such comparison could be done using a box-and-whisker plot of the reference site and impacted sites. If the reference site is well separated from the test or impacted sites (e.g.75 percentile at reference site falls below 25 percentiles at impacted sites) then it could be objectively concluded that observed deformities at impacted sites are probably due to pollution. Furthermore, 8% incidences of deformities in different chironomid communities may not necessarily indicate the same levels of contaminations because as noted earlier, different genera within a community respond differently to pollution and in expression of deformities. For example, a less polluted site dominated by more susceptible genera would likely produce more deformed larvae than heavily polluted sites dominated by pollution tolerant genera. This may lead to erroneous conclusion regarding the level of contamination. A more effective way will be to develop a simple numeric index, similar to the toxic score index TSI (Lenat, 1993), in which genera are assigned weighting according to their sensitivity in expression of morphological deformities. The assignments of sensitivity scores to genera would rely heavily on extensive field and laboratory toxicity test. Lenat 1993's TSI could not be used in this study because it was based on only *Chironomus* sp. and this genus was not recorded in all the four sampling sites.

The importance of seasonal variations in the incidences of deformities has been stressed by Servia *et al.* (2000). In the present study, community incidences of mentum, mandible and antennal deformities were not statistically significantly different ($p > 0.05$) between seasons across sites. However, there were variations (though not significant) in the incidences of deformities among seasons. More deformed antennae and mentum were, in most cases,

observed during spring and summer at the four sampling sites (Figure 4.1). It could be that increased temperature during summer which is likely to increase concentrations and bioavailability of contaminants may increase the number of deformed larvae (Jeyasingham and Ling, 2000). On the other hand, elevated incidences of deformities during spring has been attributed to spring "overwintering" larvae that had been developing at a slower rate under low temperature conditions and were therefore exposed much longer to contaminants (Jeyasingham and Ling, 2000).

The various types of deformities reported in this study have been observed by other authors in different field studies (Martinez *et al.,* 2006; Ochieng *et al.,* 2008; Al-Shami *et al.,* 2010). Deformity types such as Köhn gaps, extra, fused teeth and asymmetry that characterised the mentum of chironomids at polluted sites 2, 3 and 4 were rarely seen at site 1. For instance, no incidence of Köhn gap was observed at site 1 throughout the study period. Groenendijk *et al.* (1998) found strong positive correlation between Köhn gaps and levels of heavy metals in the Dommel River, northern Belgium. The occurrences of this type of deformity in the mentum of chironomids at sites 2, 3 and 4 in this study may be an indication of possible heavy metal contaminations which have been reported from the Swartkops River in an earlier study (Binning and Baird, 2002). Furthermore, the overall elevated incidences of mentum deformities recorded at sites 2, 3 and 4 are consistent with the findings of several authors (e.g. Martinez *et al.,* 2002; Bhattacharyay *et al.,* 2006) who also reported higher incidences of mentum deformities at polluted sites.

Generally chironomids at site 3 showed more deformed mandibles compared to taxa collected at other sites. In addition, the occurrence of deformities such as asymmetry and missing teeth that characterised mandibles of chironomid taxa at site 3 which were not seen at site 1 were probably induced by the relatively high pollution levels at this site.

Although heavy metals have been extensively implicated as causal agents of deformities (Lenat, 1993; Janssens de Bisthoven and Gerhardt, 2003; Nazarova *et al.,* 2004; Ochieng *et al.,* 2008), Servia *et al.* (1998) and MacDonald *et al.* (2006) have also claimed organic pollution to be a possible causal agent of deformities in chironomid larvae. The measured water quality variables in this study are often used as indicators of organic pollution (e.g. Dickens and Graham, 1998) and thus the strong correlation between community incidences of mentum deformities and the concentrations of dissolved oxygen, ammonium-nitrogen, total inorganic nitrogen, orthophosphate-phosphorus and turbidity seemed to support the

claim of Servia *et al*. (1998) and MacDonald *et al.* (2006) that organic pollution is likely to induce deformities in chironomid larvae

4.3.2 Variations in the incidences of deformities among Chironomidae taxa

Incidences of deformity varied among genera of Chironomidae reported in this study. Warwick (1990) first noted that chironomid genera are differentially sensitive and would express varying levels of deformities even if they were collected from the same site. In this study for instance, the mean incidences of mentum deformity in *Cricotopus trifasciata* gr. was higher than those of *Tanytarsus* sp. and *Cricotopus* sp.1 at site 1. This suggests that the application of deformities in chironomids as a biological screening tool requires independent reference or background incidence of deformity for each genus because of differential sensitivity to induction of morphological deformities. However, it could also be that the observed differences between genera is a result of screening deformities among different instar stages of larvae of different genera which was not taken into account in this study. That is, whether the expressions of morphological deformities vary among different chironomid larvae instar stages or as a result of prolonged exposure in the older larvae. This is an area that requires further exploration.

Differences in the expression of deformities among chironomids could be partly attributed to their feeding habits (Warwick, 1991; Nazarova *et al.*, 2004). Most genera within the subfamily Chironominae are detrital feeders (Olafasson, 1992; Armitage *et al.*, 1995) and because of their feeding habits, their mouth parts are often in direct contact with the sediments and the associated contaminant burden and are therefore more likely to express elevated levels of deformities (Bhattacharyay *et al*., 2006). This, perhaps, partly explains the reasons for the observed relatively elevated incidences of deformities among the Chironominae (Figures 4.5 – 4.7). The relatively high incidences of deformity among the Chironominae reported in this study are in agreement with those reported by Bhattacharyay *et al*. (2006) and Ochieng *et al.* (2008) who noted that incidences of deformities among genera within this subfamily were comparatively higher than genera in other subfamilies. Incidences of deformities among the predacious Tanypodinae were relatively less frequent in the present study. Perhaps their feeding habits, being predators (Armitage *et al.*, 1995), do not expose them so much to the sediment bound contaminants. Other studies have also noted low incidences of deformities among the Tanypodinae: Bhattacharyay *et al*. (2006) for example reported fewer numbers of deformed Tanypodinae compared to the Chironomini.

Furthermore, Warwick (1988) suggested that each chironomid genus responds within a certain range of contaminants concentrations, above which it probably starts experiencing mortality. Chironomid species such as *Polypedilum* sp., *Tanytarsus* sp. and *Orthocladus* sp., which were present at site 1 but scarcely seen at sites 2, 3 and 4 probably revealed a community that could not tolerate the pollution levels at these sites. At site 3 for instance, the consistently low abundance of *Dicrotendipes* sp. and the relatively high incidences of mentum deformities probably reflect a species living near its borderline at such a "heavily" polluted site (Appendix B, Tables B2). Although the small sample sizes of *Dicrotendipes* sp. at site 3 reported in this study cannot be used to give a reliable estimate of incidences of deformities in this genus, the low abundances are probably themselves indication of poor water quality and subsequent mortality.

4.3.3 Variations in the incidences of deformities among Chironomidae structures

The incidences of deformities observed among structures examined in this study also varied. Results suggest that there could be differences in sensitivity among structures within a species taken from the same site. Warwick (1985 and 1988) hypothesized that antennae are extremely sensitive organs which only express morphological deformities at very low concentrations of pollutants and suggested that at high levels of pollution antennae develop some sort of resistance to deformities and that deformities in antennae are replaced by deformities in more sclerotized structures such as menta, ligulae and mandibles. The comparatively low incidences of antennal deformity throughout the period of this study may support this claim. By Warwick's (1985 and 1988) hypothesis, it could be that the concentrations of pollutants at all sampling sites (including site 1) were above the ranges for which antenna could express elevated levels of morphological deformities. This may explain the observed relatively low incidences of antennal deformities in this study. If this explanation is correct, then it is important to investigate the mechanisms behind such resistance in antennae. It has, however, been speculated that resistance mechanisms in antennae involve the production of biochemical homeostatic chemicals which detoxify toxins (Warwick, 1988). Furthermore, mandible deformities were also relatively low compared to the mentum throughout the study period. The mandible is a heavily sclerotized structure which has been hypothesized to only express morphological deformities at very high pollution levels (Warwick, 1988). The observed low incidences of mandible deformities in this study may be that the levels of contaminants (organic or inorganic) were not high enough

to induce elevated levels of deformities in the mandibles. However, results revealed that incidences of mandible deformities were consistently higher at sites 2, 3 and 4, downstream of industrial and sewage effluent discharge points, suggesting possible severe impacts at these sites. The community incidences of deformities in the menta and ligulae were consistently higher than those observed in the antennae and mandibles at all the sampling sites (Figure 4.1). The results in this study suggest that the mentum seemed to have a wider range of morphological response expressing deformities (depending on the species or genus) at all the four study sites. Therefore, in studies that involve a pollution gradient, the mentum could be the structure of interest. In summary, application of deformities in Chironomidae larvae as a biomonitoring tool probably requires determination of reference incidence of deformity for each genus and structures within a genus.

4.4 Conclusion

The screening of deformities in Chironomidae larvae revealed evidence of deteriorating environmental water quality in the Swartkops River. The method is particularly useful because it could detect sub-lethal effects of pollution at sub-organism levels and may serve as early warning indicators to water resources managers. The study illustrates the importance of tabulating deformities separately for each genus and structure because of possible differences in expression of deformities.

The elevated incidences of deformities recorded at sites 2, 3 and 4 signals possible chemical contaminants in the Swartkops River. Out of the structures screened for deformities in this study, the mentum proved most useful because it appears to have a wider range of sensitivity, is easy to prepare for examination and deformities can be quantified rapidly. The study of chironomid deformities added value to the lines evidence gathered in the multi-criteria investigation of pollution in the Swaterkops River.

CHAPTER 5: GENERAL DISCUSSIONS, CONCLUSIONS AND RECOMMENDATIONS

5.1 Introduction

Over the past decades, there has been an increase in the pollution of freshwater resources as a result of growing human population, increased urbanisation and industrialisation (Chamie, 2004; Hassan *et al.*, 2005). The Swartkops River is no exception, as it drains a highly urbanised and industrialised catchment and thus its in-stream biota have consequently suffered varying degree of anthropogenic impacts (DWAF 1996a; Binning and Baird 2001). Macroinvertebrates have long been used as indicators of water quality deterioration (Rosenberg and Resh, 1993; Bonada *et al.*, 2006; Justus *et al.*, 2010) and improved management of the Swartkops River water resources would benefit from different levels of macroinvertebrate based assessment approaches that can provide better understanding of the impacts of water quality deterioration on the River's biotic communities. South African national water law and policy requires the protection of water resources by managing water quality, conditions of in-stream and riparian habitats, conditions and distribution of aquatic biota (NWA, 1998; NWRS, 2004). Macroinvertebrates are an important component of the aquatic ecosystems because they serve as a critical intermediate path-way for the transportation and utilization of energy and matter; they respond differently to a variety of pollutants and provide an indication of water quality over varying time periods (Wallace and Webster, 1996; Camargo *et al*., 2004; Bonada *et al.*, 2006). Consequently, their presence, absence, abundance and distribution reflects the health of the aquatic environment in which they live (Bonada *et al.*, 2006; Fouche and Vlok, 2010; Arimoro and Muller, 2010). Therefore, an investigation, particularly with regard to water quality, of the Swartkops River macroinvertebrate communities could provide relevant information needed for the protection of water resources as required by the South African National Water Act (Act No. 36 of 1998) and the National Water Resource Strategy (NWRS, 2004).

In this study, different macroinvertebrate based biomonitoring approaches were applied to better understand the responses of the Swartkops River macroinvertebrate communities to water quality deterioration. The results are critically discussed in this chapter, and conclusions and recommendations drawn based on the findings.

5.2 The Swartkops River Health Conditions

The South African National River Health Programme (RHP) was designed to provide better understanding of the impacts of anthropogenic activities on in-stream biota and to generate relevant ecological information needed for sustainable management of South African freshwater resources (RHP, 2006). The RHP uses the responses of in-stream biota to determine the health of riverine ecosystems (Dallas, 2000; RHP, 2006). In the present study, the South African Scoring System version 5 (SASS5), an index within the river health programme based on the perceived sensitivity of macroinvertebrate taxa to water quality changes, was applied in order to determine the Swartkops River health conditions.

Assessing river health using SASS5 requires the sampling of macroinvertebrates from three different biotopes namely: stones (stones in- and out-of current), vegetation (marginal and aquatic) and gravel, sand and mud (GSM) (Dickens and Graham, 2002). The results of the present study revealed that, with the exception of vegetation and GSM biotopes at site 3 and stone and GSM biotopes at site 4, macroinvertebrate composition differed significantly between the three sampled biotopes at all four sampled sites (Table 3.2). These differences could be attributed to natural variations in hydraulics, substrate, thermal conditions and other ecological characteristics that exist among the different sampled biotopes (Dallas, 2007a). Similarly median SASS5 scores, number of taxa and average score per taxa (ASPT) also varied between the sampled biotopes at the four sites (Figures 3.6 and 3.11) These observed differences in macroinvertebrates composition as well as SASS5 scores, number of taxa and ASPT values underscore the importance of sampling all SASS5 biotopes in biomonitoring of rivers using the South African Scoring system version 5 (SASS5).

Replicate macroinvertebrate samples taken were assessed to ascertain whether a single sample taken from a particular biotope at a site at a particular time is a reasonable reflection of macroinvertebrates composition of that biotope at the time of sampling. Analysis of similarity (ANOSIM) did not indicate significant differences between replicate samples taken from the same biotope at a site. The South African Scoring System version 5 (SASS5) protocol recommends that only one sample be taken from each biotope for SASS5 assessment. The findings in this study therefore support the collection of one sample from each biotope as there were no significant differences among replicates sampled from the same biotopes. These results are consistent with those of Maseti (2005) who also did not find

significant differences in macroinvertebrate composition among replicate samples taken from the same biotope in the Buffalo and Inxu Rivers.

Currently in South Africa, assessment of river health using the South African Scoring System version 5 (SASS5) within the River Health Programme (RHP) assigns a water quality category to each site within a river based on SASS5 scores and ASPT values (Dallas, 2007a). The water quality categories which include natural (unmodified); good (largely natural with few modifications); fair (moderately modified); poor (largely modified); and seriously and critically modified are considered to reflect the river health condition. Based on this approach, total SASS5 score revealed that water quality at site 1 was "good" while ASPT value indicated "fair" water quality category at the site. Similarly, total SASS5 score indicated poor water quality at site 2, while ASPT revealed "critically modified" water quality at this site. Both scores (i.e. SASS5 score and ASPT value) indicated "critically modified" water quality category at sites 3 and 4 (Table 3.4). However, based on the discriminatory ability test approach, total SASS5 scores and ASPT values discriminated site 1 from the downstream sites (i.e. sites 2, 3 and 4). Among the downstream sites, SASS5 scores separated site 2 from sites 3 and 4 but could not separate sites 3 and 4. ASPT values separated site 3 from sites 2 and 4 but could not discriminate between sites 2 and 4. Although SASS5 results revealed evidence of water quality deterioration that may have negatively impacted on the Swartkops River macroinvertebrate assemblages, based on both approaches (i.e. assignment of water quality categories and discriminatory ability test) both SASS5 scores and ASPT values, could not discriminate between all four sampling sites although sites 2, 3 and 4 were demonstrably poorer than site1 (Table 3.4 and Figure 3.14).

In the present study, the significant correlations ($r^2 = \pm 0.5$; $p < 0.05$) between SASS5 scores / ASPT values and the concentrations of dissolved oxygen (DO), nitrate-nitrogen (NO_3-N), nitrite-nitrogen (NO_2-N), total inorganic nitrogen (TIN), orthophosphate-phosphorus (PO_4-P), five days biochemical oxygen demand (BOD_5) and electrical conductivity (EC) indicated the sensitivity of SASS5 to changes in the measured water quality variables.

One priority area of biomonitoring is to develop simple and rapid assessment techniques whose results are easily conveyed to, and understood by, water resources managers (Bonada *et al.*, 2006). SASS5 is a simple and rapid assessment technique whose results are always presented as single biotic index scores and thus, are easily interpreted and information conveyed to water resources managers. However, in situations where resources are limited

and water resources mangers need to prioritise restoration efforts, more rigorous and intensive methods that could distinguish subtle differences between polluted sites can applied for management purposes. Information obtained from such rigorous and intensive methods may not however, be easily understood by water resources managers and, this could limit their application. Since most types of biomonitoring tools could easily discriminate unpolluted from polluted sites, in this study the multimetric approach, based on macroinvertebrates, was investigated to ascertain whether further ecological information could be obtained that could help in discriminating between the downstream polluted sites (i.e. sites 2, 3 and 4). These sites are slightly different in levels of pollution with small differences in macroinvertebrate compositions and abundances. Such information, when obtained, could complement SASS5 results in biomonitoring of the Swartkops River.

5.3 Application of Multimetric Approach to Assess Swartkops River Macroinvertebrate Communities

The multimetric approach was used to integrate information from individual, population and community levels to provide a better understanding of impacts of water quality changes on the Swartkops River macroinvertebrate assemblages. In this study, 19 metrics were selected from 4 categories which include abundance, composition, richness and diversity. Out of the 19 metrics evaluated, 10 of these, with at least one each from the selected categories, proved to have satisfactory discriminatory ability (i.e. discriminated site 1 from sites 2, 3 and 4) and six of these 10 metrics, detected subtle differences between sites 2, 3 and 4 (Figure 3.19) and they also displayed significant correlations to changes in the measured water quality variables across the four sites.

Results further revealed that metrics in the abundance category were less affected by changes in water quality compared to metrics in the other categories. Apart from the abundance of Trichoptera, all metrics in the abundance category, were ubiquitous and could not distinguish site 1 from sites 2, 3 and 4 (Figure 3.19). This suggests that metrics in this category are not sensitive and should therefore be used with caution within the framework of water quality management. In addition, factors such as sampling effort and size of sampled area could easily affect abundances of metrics and can easily contribute to high variability (Barbour *et al.*, 1999; Sánchez-Montoya *et al.*, 2010).

Three metrics in the richness category (i.e. Ephemeroptera-Plecoptera-Trichoptera EPT richness, Ephemeropera-Trichoptera-Odonata-Coleoptera ETOC richness, and Margalef's

family richness index) discriminated site 1 from sites 2, 3 and 4 and were also strongly correlated with changes in physical and chemical water quality variables across the four sites. Metrics in this category are widely used in biomonitoring programmes because they are presumed to respond to human induced changes in a predictable fashion, usually decreasing in values with increase in the level of pollution (Klemm *et al.,* 2002; Suriano *et al.,* 2010). Based on the discriminatory ability test, out of the three metrics in the richness category with satisfactory discriminatory ability, EPT richness and Margalef's family richness index were able to discriminate between sites 2, 3 and 4 (Figure 3.19), which seemed to suggest that these two metrics could detect subtle differences between polluted sites.

Diversity indices take into account two aspects of macroinvertebrate ecology, i.e. richness and evenness (Ogbeibu, 2005). Unlike biotic indices, they were not designed to respond to any specific type of anthropogenic impact but they are often used in macroinvertebrate based biomonitoring of rivers and streams (Camargo *et al.,* 2004; Gray and Delaney, 2008; Suriano *et al.,* 2010). In this study, two diversity indices (i.e. Simpson and Shannon) with different properties were used to explore the diversity of the Swartkops River macroinvertebrate assemblage in relation to water quality changes. Simpson index is always skewed towards the abundance of the commonest families while Shannon index usually reflects the abundance of individual taxa while it still assigns greater weight to the commonest families (Ogbeibu, 2005; Gray and Delaney, 2008). Both indices discriminated between all four sampling sites, which seemed to suggest that these two indices could detect subtle differences between sites with slightly different levels of pollution.

Equitability (evenness) was considered in this study as a possible metric that could be applied for water resources management because it helps to elucidate the consequences of pollution which may lead to the dominance of tolerant and opportunistic taxa in a sample. That is, the assumption behind this concept is that, the abundances of species in a sample from an undisturbed environment are often relatively evenly distributed compared to those from a disturbed or impacted environment where opportunistic and tolerant taxa would account for greater proportion of species abundance (Clarke and Warwick, 1994; Ogbeibu, 2005). In the downstream sites (i.e. sites 2, 3 and 4) particularly at sites 3 and 4, the abundance of families was dominated by the most pollution tolerant taxa such as Chironomidae, Oligochaete and Hirudinae and consequent reduction in equitability at these sites. Equitability was found to

discriminate between all four sampling sites, suggesting its ability to detect small differences between sites.

In general, results suggest that the application of the multimetric approach provides additional information on how the Swartkops River macroinvertebrate abundance, richness, composition and diversity responded to changes in environmental water quality. Specifically, out of the 10 metrics that separated site 1 from sites 2, 3 and 4, six of these were able to distinguish between sites 2, 3 and 4. That is, six metric separated all four sampling sites from each other and these metrics include EPT richness, % Chironomidae + Oligochaete, Margalef's family richness index, Shannon diversity index, Simpson diversity index and equitability. These six metrics, contributed information not provided by SASS5 results. That is, they revealed evidence of significant differences between sites 1 (reference site) and polluted sites (sites 2, 3 and 4) as provided by SASS5 results and evidence of marginal differences between sites 2, 3 and 4 (Figure 3.19). Although SASS5 results revealed the same level of pollution for both sites 3 and 4 (Table 3.4), the multimetric approach indicated site 3 as the most polluted with lower Shannon diversity index, Simpson diversity index, Margalef's family richness index and equitability that were marginally discriminated from sites 2 and 4 (Figure 3.19). Also, higher % Chironomidae + Oligochaete at site 3 was marginally discriminated from those at sites 2 and 4. Therefore, depending on the goals of the assessment programme, available resources and priorities, macroinvertebrate data could be subjected to multimetric analysis to obtain such additional information, particularly of marginal differences between polluted sites, which could enhance decision making regarding the impacts of anthropogenic activities on macroinvertebrate assemblages and overall aquatic ecosystem health.

5.4 Chironomidae as Bioindicators of Water Quality in the Swartkops River

One of the limitations of SASS5 as a rapid bioassessment protocol and the multimetric approach as used in this study is that macroinvertebrates are only identified to the family level. Consequently, both SASS5 and the metrics and indices applied did not detect changes in the abundance, distribution and composition of macroinvertebrates at lower taxonomic levels such as genus and species. Lenat and Resh (2001) have emphasised the importance of genus and species level identification in water quality assessment because, within a family, species are differentially sensitive to changes in water quality and assigning tolerance limit at the family level would masked individual species sensitivity and could lead to erroneous

conclusion regarding water quality condition of a site. These authors highlighted their argument by comparing the classification of sites by a genus/ species level biotic index to a family level biotic index and concluded that about 40 % of sites identified as excellent, and 20 % regarded as poor by a family level biotic index, were wrongly classified. Such erroneous classification could have profound negative water quality management implication because sites which need urgent restoration intervention could easily be overlooked.

In this study, the family Chironomidae were identified to genus and species levels with a view of exploring their bioindication potential (i.e. changes in Chironomidae communities in relation to water quality). Among families of aquatic macroinvertebrates, Chironomidae are probably the most abundant, widely distributed and species rich family with an extraordinary ecological range (Armitage *et al*., 1995; Cranston, 1996; Harrison, 2002; Porinchu and MacDonald, 2003; Luoto, 2010). Refined taxonomic assessment of chironomids in relation to water quality changes could help in elucidating the need to use them in water quality assessment and provide information not supplied by a family level identification.

Results revealed that chironomid communities of the Swartkops River, at genus and species levels of taxonomic identifications, were sensitive to changes in water quality decreasing in species diversity and richness from site 1 to the three downstream sites (Figure 3.23). One shortcoming of the SASS5 results in this study was that SASS5 scores and ASPT values were able to discriminate between site 1 and sites 2, 3 and 4 but could not distinguish between all three downstream sites (i.e. sites 2, 3 and 4). Specifically, both SASS5 scores and ASPT values indicated that water quality at sites 3 and 4 were both critically modified. But on identification of chironomids to the genus and species levels, results revealed that site 4 supported more diverse chironomid communities compared to site 3 that was consistently dominated by *Chironomus* sp.1 *Chironomus* sp. 2 and *Chironomus* sp. 3, indicating possible slight differences in the water quality status at these two sites (Figure 3.23). From a management perspective therefore, site 3 could be prioritised for restoration efforts based on the species level information. Also, the presence of species such as *Cricotopus* sp.1, *Cricotopus trifasciata* gr., *Tanypus* sp., *Orthocladius* sp. and *Kiefferullus* sp. at site 2, which were completely absent at site 3 and rare at site 4, seemed to suggest that the water quality at site 2 is less impacted than at sites 3 and 4. This is also supported by the significant differences in measured water quality variables (DO, EC, BOD_5, NH_4-N, TIN and Periphyton) between sites 2, 3 and 4 (Table 3.1).

Among the three subfamilies of chironomids recorded (Tanypodinae, Orthocladiinae and Chironominae), Chironominae had the highest number of taxa over the study period (Figure 3.22). The two tribes Chironomini and Tanytarsini, within the subfamily Chironominae, seemed to exhibit marked differences in sensitivity to water quality deterioration. Species of tribe Tanytarsini were particularly sensitive to pollution and were completely absent at site 3, while the Chironomini: *Chironomus* sp. 1, *Chironomus* sp. 2 and *Chironomus* sp. 3 dominated the chironomid compositions at sites 3 and 4 (Figures 3.21 and 3.22). Similarly, lesser numbers of species within the subfamilies Orthocladiinae and Tanypodinae were recorded at sites 2, 3 and 4 compared to site 1. The results therefore generally suggest that different chironomid species, genera and even subfamilies are likely to respond differently to water quality changes. The implication therefore is that within the family Chironomidae in the Swartkops River, species differ in sensitivity to water quality changes and could have different tolerant limits with which small differences between sites could be detected. This was reflected in the differences in the chironomid assemblages at the four sampling sites in which some species were completely absent at some of the sampling sites. Furthermore, species level identification showed that the species of the genus *Chironomus* dominated sites 3 and 4 and species within this genus are mostly scrapers that feed on detritus and algae (Armitage *et al.,* 1995). The preponderance of these species could be linked to increase organic matter and presence of algal growth and could therefore serve as good indicators of organic pollution and probably of nuisance growth of phytoplankton and periphyton.

The Swartkops River chironomid communities also showed seasonal variations with more species being collected during spring and summer (Figure 3.21). Rossaro (1991) reported that most chironomid taxa enter the resting stage during winter and could result in the collection of fewer species (Rossaro, 1991). Thus, some authors (e.g. Adriaenssens *et al.,* 2004) restricted the collection of their chironomids to summer only. Therefore, in situations where resources could not permit a year round sampling, this should be done during spring and summer as these two seasons are likely to give the most diverse reflection of chironomid assemblages at each site.

The present study therefore demonstrates the importance of species level identification in biological water quality assessment and also provides evidence of the bioindication potential of the family Chironomidae. Given the observed differences in species, genus, tribes and subfamilies between the four sampling site (Figures 3.21 and 3.22), and the huge species

richness of chironomids in South African freshwater (Harrison, 2002), it might be safe to argue that the score given to this family in SASS5 sheet may not best represent the sensitivity of species, genera, tribes and even sub-families within the family. Since sub-family level chironomid identification is relatively easier to carry out than genus and species, the sensitivity of sub-families to water quality impairment should be assessed extensively through field and laboratory studies in order to split the family Chironomidae into sub-families and these assigned different scores in SASS or design a SASS-type system based on chironomids. Such system could provide information such as habitat preference and water quality tolerant limits of individual chironomid species, and specific indicator taxa to different types of pollution, which may include indicators of organic pollution, heavy metal contaminations and habitat degradation. The water resources management benefits of such system would include improved understanding of species relationships to different types of impacts. Such a system however, would not be suitable for rapid bio-assessment but could be adopted as part of a more detailed ecological assessment of freshwaters.

Although chironomids proved to be useful bioindicators of water quality deterioration in the Swartkops River, there are a number of factors that could limit their routine application in biomonitoring of freshwater resources. Firstly, the identification of chironomid to genus or species is time demanding and laborious because each individual specimen has to be mounted on a slide under a dissecting microscope, cleared with chemicals, allowed to air dry for several days and then examined under high magnification. Each of these steps requires attention to detail making identification of this group of aquatic macroinvertebrates very time consuming and laborious. This has led some authors (Rabeni and Wang, 2001) to argue that identification of chironomids could take up to 50% of the total time spent in identifying the entire macroinvertebrate samples collected in any stream or river. Secondly, even when time permits, identification of chironomid larvae to genus or species can be difficult and may require consulting with an expert in chironomid taxonomy, making this group of macroinvertebrates difficult to apply as bioindicators by non-specialists. Thirdly, detailed and comprehensive South African chironomid larvae species identification keys are not yet available due to lack of experts in Afrotropical chironomids taxonomy (de Moor, Albany Museum, Grahamstown, *per. comm.*, 2010). Considering these factors in the application of chironomids in freshwater biomonitoring, the monitoring individual or agency needs to decide on the trade-off between the benefits of using this group as biological indicators of water quality changes and the time costs or gaining the additional information. Key factors in

deciding whether to use chironomids in routine freshwater biomonitoring will certainly depend on the goal of the assessment programme, available time and financial resources, priorities and possibly availability of specialist chironomid taxonomists. For example, if the goal and priority of the assessment programme is to establish specific indicator taxa for specific types of pollution or to document the species assemblages of a particular river for future references, then species level identification of chironomids would be beneficial.

5.5 Morphological Deformity in Chironomidae as Early Warning Indicators of Water Quality Changes in the Swartkops River

Integrated water resources management (IWRM) requires a holistic approach to assess the impacts of water quality deterioration on in-stream biota (NWRS, 2004). Such a holistic approach would require that effects of contaminants on aquatic ecosystems are detected early enough in order to take appropriate mitigation measures. The approaches mentioned above are community based, and depend on presence or absence of macroinvertebrates to detect effects of pollution. In other words, these approaches rely on potentially lethal endpoints. Lethal end points are not suitable for use as early warning indicators of contaminations of freshwater resources because concentration of contaminants must be high enough to ultimately result in the disappearance of sensitive groups before such endpoints can effectively detect impacts of pollution on aquatic macroinvertebrates (Faria *et al.*, 2006).

Morphological deformities in Chironomidae larvae are regarded as early indicators of water quality deterioration because they are sub-lethal endpoints (Janssens de Bisthoven and Gerhardt, 2003; Nazarova *et al.,* 2004; Ochieng *et al.,* 2008). Deformities in Chironomidae larvae are thought to be effects of exposure particularly to chemical contaminants (Martinez *et al.,* 2006; Dias *et al.,* 2008). However, substrates to which larvae are exposed could also influence the levels of deformities in chironomids (Bird, 1997). It will therefore be useful to investigate whether factors not directly related to water quality such as predation; habitat degradation and competition could also influence deformities. Based on fossils records, the natural incidences of chironomid deformities in unpolluted waters were reported to range between 0 and 0.8% from the Bay of Quinte, Canada (Warwick, 1980). However, because of increased industrialisation, urbanisation and agricultural activities, pristine water bodies are scarce and numerous field studies (e.g. Warwick, 1988; Nazarova *et al.,* 2004; Ochieng *et al.,* 2008) have reported background levels of deformities in least impacted sites to range between 0 and 8%, above which a site could be considered contaminated. The elevated levels of community incidences of deformities, particularly of the mentum at sites 2, 3 and 4 in this

study, are indicators of both point and diffuse pollution sources and possibly heavy metals which have been reported from the Swartkops River in an earlier study (Binning and Baird, 2001), while the observed incidences of deformities at site 1 could be considered as early indicators of water quality deterioration. Several studies (e.g. Diggins and Stewart, 1998; Martinez *et al.*, 2002; Bhattacharyay *et al*., 2006) have implicated heavy metals as causal agents of chironomid deformities. However, under field conditions, it is too simplistic to attribute observed incidences of deformities to a single or group of chemical(s) because input of chemicals into the natural environment not only encompasses their pure state but also their by-products as a result of degradation, products of synergistic and antagonistic interactions (Warwick, 1991; Hassan *et al*., 2005). As a result, in reality, organisms are exposed to a wide array of chemical contaminants in the aquatic environment. Therefore, the elevated mentum deformities observed at sites 2, 3 and 4 could be biological effects of exposure to a wide array of pollutants either acting synergistically, antagonistically, independently or possibly even other environmental stressors, which may or may not be linked to water quality. Nevertheless, the significant correlations between community incidences of mentum deformities and the concentrations of dissolved oxygen (DO), orthophosphate-phosphorus (PO_4-P), ammonium-nitrogen (NH_4-N), total inorganic nitrogen (TIN) and turbidity suggest that organic inputs into the Swartkops River are also likely to induce deformities in chironomids. These deformities reflect a biological community with unfit individuals.

Chironomidae community species richness and diversity were lowest at site 3 suggesting greatest impacts at this site. The Chironomidae community results are supported by the consistently highest community incidences of mentum and mandible deformities recorded at site 3 (Figure 4.1). That is, chironomid taxa at this site were more deformed compared to taxa at the other three sampled sites. Since deformities could be important ecological endpoints of fitness, the ability of chironomid taxa at site 3 to perform normal ecosystem functions such as recycling of nutrient could be impaired. Also, because most of the deformities recorded were associated with the mentum and mandible (Figure 4.1), the ability of deformed chironomids to feed could be affected, and this can contribute to depletion of their population and ultimate reduction in species diversity and richness. Many chironomids feed on algae (Armitage *et al.*, 1995) and their inability to ingest feed materials such as diatoms, blue-green algae due to deformed mouth parts could contribute to increased concentrations of periphyton as was observed at sites 3 and 4 (Table 3.1). Information on individual fitness in an ecosystem can be helpful in water resources management because, a

fit and healthy community depends on the level of fitness of constituent individuals. Therefore, the elevated incidences of deformities at sites 2, 3 and 4 particularly site 3, indicate an ecosystem not functioning at an optimal level due to impaired individual species health.

Incidences of deformity varied among genera of chironomids reported in this study, with taxa within tribe Chironomini subfamily Chironominae expressing comparatively higher incidences of deformities. These taxa are mostly detrital feeders (Armitage *et al.*, 1995) and their mouth parts are often in close contact with sediment bound contaminants and, this could contribute to elevated incidences of deformities. Differences in the expression of morphological deformities among chironomid genera highlight the importance of screening and tabulating deformities separately for each genus. Such differences, as previously mentioned in chapter 4, suggest that the application of Chironomidae larvae deformities as a biological screening tool requires independent reference or background values for each genus. However, the different instar stages of larvae which were not considered in this study could influence the incidences of deformities observed among genera. It will be useful in the future therefore, to investigate relationship between deformities and larval instar stages.

Several authors (e.g. Lenat, 1993; Bird, 1997; Al-Shami *et al.*, 2010) that have used deformities in Chironomidae larvae as indicators of aquatic health have focused on the genus *Chironomus*. Although this genus is widely distributed and could be useful in assessing water quality impairments, it may not always be in sufficient numbers, especially at relatively clean sites (Appendix A, Table A9). Therefore it remains important to screen deformities in other genera. The occurrence of deformities in other genera such as *Tanytarsus* sp. *Cricotopus* sp.1, *Cricotopus trifasciata* gr. and *Tanypus* sp. in the present study highlights the importance of deformities screening in other chironomid genera because each genus may have different reference or background levels of deformities, suggesting different sensitivities to pollutants. In addition, screening deformities in other chironomid genera allows for comparison of sensitivity of different genera in expression of morphological deformities. Knowledge of differences in the expression of morphological deformities among chironomid genera could help in determining sensitivity rating.

Although in Vermeulen's (1995) review it was observed that proportional occurrence of different deformity types shifted somewhat consistently according to the type of prevailing pollution, there has not been any concerted effort to link different types of deformity to a

particular contaminant and there appear to be some degree of overlap among different deformities induced by different types of contaminants (Janssens de Bisthoven *et al.*, 2005; MacDonald *et al.*, 2006). However, it has been noted that occurrence of mentum gaps, or the so called Köhn gaps, are characteristics of sites contaminated with heavy metals (Vermeulen, 1995; Groenendijk *et al.*, 1998). Consequently, the occurrence of Köhn gaps at sites 2, 3 and 4 in this study is probably indication of possible heavy metal contaminations at these sites which have been reported from the Swartkops River in an earlier study (Binning and Baird, 2001). Furthermore, deformity types such as asymmetry, extra teeth and multiple deformities that characterised the mentum of chironomid taxa at sites 4 and 3 which were not seen at site 1 and 2 probably suggest that these types of deformities were induced by high level of pollution. Overall, the discriminatory capacity of Chironomidae deformities as biological screening tool could be enhanced if deformity types and severity are linked to different types of contaminants or levels of concentrations. If this were possible, then the occurrence of a given type of deformity could be trace to the presence of a specific type of contaminant. Such information (i.e. cause and effects relationship between types of deformity and contaminants) could have profound positive impact in water resources management because it could determine the types of dominant contaminants present in water bodies which may help to easily pin-point possible point sources of discharge associated with such specific type of contaminant. To determine cause and effects relationship would require more intensive sampling and concurrent eco-toxicity testing.

The incidences of deformities observed among structures examined in this study varied, with the antenna and mandible experiencing comparatively low incidences of deformities throughout the study periods (Figure 4.1). It has been hypothesised however, that antenna and mandible only express morphological deformities at “very low” and “high” levels of contaminations respectively (Warwick, 1985 and 1988). The occurrence of low incidences of deformities in the antenna at “heavily” polluted sites has been attributed to the development of resistance (Warwick, 1985). The development of antennal resistance to contaminants could affect the utility of antennal deformities as a biological screening tool and increased levels of antennal deformities at reference sites could confound interpretation of results. It is therefore important to screen deformities in more than one morphological structure in order to make a definite conclusion whether elevated levels of deformities observed at a site are due to pollution. However, the mentum, which expressed deformities at all sites, seemed to respond morphologically at varying levels of pollution probably suggesting that it could be a very

sensitive structure which does not easily develop resistance to pollution. Furthermore, mandible deformities, similar to the antenna, were also relatively low throughout the study period. The mandible is a heavily sclerotized structure that only expresses morphological deformities at "high" pollution levels (Warwick, 1988), which probably suggest that mandible could be of great importance in biomonitoring of freshwater resources in cases of "extreme" pollution.

In order to account for possible seasonal variations, some authors (e.g. Servia *et al.*, 2000) have emphasised the importance of screening deformities across seasons. Although there were seasonal variations in the community incidences of mentum, mandible and antennal deformities, these were not statistically significant in the present study. However, there were fluctuations (though not significant) in the incidences of deformities among seasons. The community incidences of mentum and antennal deformities were, in most cases, higher during spring and summer at each of the four sampling sites (Figure 4.1). Increased temperature during summer is likely to increase concentrations and bioavailability of contaminants (Jeyasingham and Ling, 2000), and deformities are likely to be elevated during this season. On the other hand, elevated incidences of deformities during spring has been attributed to spring "overwintering" larvae that had been developing at a slower rate under low temperature conditions and were therefore exposed much longer to contaminants (Jeyasingham and Ling, 2000). Contrary to the observations of Servia *et al.* (2000) who reported high incidences of deformities during winter, in the present study, in most cases, lower incidences of deformities were observed in winter and autumn. However, in winter and autumn, more larvae showed multiple deformities in a single individual larva, which probably suggest more severe responses during these seasons (Figure 4.2). Such severe responses are likely to be caused by prolonged exposure to contaminants as a result of slower rate of larval development under low temperature conditions.

Previous authors have suggested that incidences of deformities in chironomid community exceeding 8% of the total larvae examined should be taken as indication of toxic contamination (Warwick, 1988; Nazarova *et al.*, 2004). However, adoption of such an arbitrarily designated reference value as a biological criterion could lead to an erroneous conclusion because of differences in ecological conditions among aquatic ecosystems and regions. Also, direct comparison of different communities of genera with varying morphological responses could further lead to erroneous conclusions. To resolve these, as

mentioned earlier in chapter 4, an appropriate reference sites should be selected for each study and deformities observed at impacted site should be objectively compared with those at the reference site and, if elevated levels of incidences of deformities are observed at impacted sites after comparison with reference site (e.g. the 75th percentile at reference site falls below 25th percentiles at impacted sites), it could then be concluded that observed deformities at impacted sites are probably due to impacts of pollution.

5.6 Application of the Approaches Used in this Study in Integrated Water Resources Management in South Africa

The South African National Water Act (NWA) (Act No. 36 of 1998) requires sustainable use of water resources and the protection of aquatic ecosystem health in order to maintain the integrity of aquatic ecosystems and the water cycle. Aquatic ecosystems include the water, biota (both in-stream and riparian), sediments, physical and chemical components and their interactions (Palmer, 1999; Jewitt, 2002). The need to manage the whole aquatic ecosystem holistically led to the adoption of the Integrated Water Resources Management approach (NWRS, 2004). Integrated Water Resources Management (IWRM) recognised the aquatic ecosystem as the base from which resources are derived (Jewitt, 2002) and that its protection is necessary in order to achieve the enshrined principles of equitable access to water resources for basic human needs and for other developmental purposes (NWA, 1998). IWRM therefore incorporates the assessments of the biological condition of water resources as well as physical and chemical measurements in the determination of ecosystem health (Roux *et al.,* 1993; Jewitt, 2002; NWRS, 2004). Assessment of biological communities provide an "accurate" and "integrated" measurement of an ecosystem health because biota are subjected to the totality of anthropogenic influences in the environment and integrate their effects over time (Barbour *et al.*, 1999; Bonada *et al.,* 2006; Baptista *et al.,* 2007; Justus *et al.,* 2010). Macroinvertebrates are well known for their ability to reflect the environmental conditions in which they live (Rosenberg and Resh, 1993; Wallace and Webster, 1996; Bonada *et al.*, 2006) and were therefore applied in this study to assess the Swartkops River health conditions.

Different macroinvertebrate based approaches which include the South African Scoring System version 5 (SASS5), a multimetric approach, Chironomidae community assessments and screening of deformities in Chironomidae larvae were applied to assess anthropogenic impacts on the Swartkops River. These approaches allowed the effects of pollutants on the Swartkops River macroinvertebrates to be scaled at different levels of biological organisation,

which included sub-organism, organism, population and community levels as well as at different taxonomic resolutions. Out of the four sites sampled during the study period, results revealed that site 1 was the least impacted with highest SASS5 scores and ASPT values, richness, diversity, equitability and EPT taxa as well as least incidences of deformities. Site 2 could be considered as the next least impacted with higher SASS5 scores, richness, diversity and equitability and lower incidences of deformities than sites 3 and 4. SASS5 results (i.e. SASS5 scores and ASPT values) revealed that both sites 3 and 4 were critically modified but the multimetric approach, Chironomidae community assessment and incidences of deformities in Chironomidae indicated sites 3 as the most impacted of the four sampling sites with least biological diversity, richness, equitability and highest incidences of deformities. Overall, all the approaches applied revealed impaired ecosystem health in the Swartkops River, which has also affected individual species health and fitness.

Based on the results of this study, there is urgent need to improve water quality in the Swartkops River so as to sustain, or possibly even restore, its ecosystem functions. Specific management interventions such as investigation of compliance of point source discharges, management of diffuse sources of pollution are urgently needed. In addition, regular monitoring of water quality in the Swartkops River is required. Such regular monitoring could help in evaluating the success of management strategies.

5.6 Conclusions

The environmental water quality of the Swartkops River is impaired due to anthropogenic impacts such as industrial and sewage effluent discharges, run-off from informal settlements and agricultural activities such as livestock farming, and has consequently impacted on the macroinvertebrate communities. SASS5 results revealed clear evidence of impaired water quality in the Swartkops River, with site 1 being the least impacted and well discriminated from sites 2, 3 and 4. Water quality at sites 2, 3 and 4 were critically modified and SASS5 results could not discriminate between all of these three sites.

The application of multiple metrics in the present study showed how macroinvertebrate abundance, composition, richness and diversity were affected by water quality in the Swartkops River. This provides information, particularly of subtle differences between the downstream polluted sites, which would otherwise be masked if these metrics were aggregated into a single score. Based on the results of this study, measures of abundance of specific groups of macroinvertebrates may be less important in distinguishing the sampling

sites than richness, composition and diversity. Out of the 10 metrics with satisfactory discriminating ability, six were able to discriminate small differences between the downstream sites and were therefore very sensitive to impaired water quality.

Genera and species within the family Chironomidae in the present study displayed varying degrees of sensitivity to water quality deterioration and therefore provided evidence of the bioindication potential of the Swartkops River chironomid assemblage. The species level identification revealed evidence of differences in water quality at the downstream sites and indicated site 3 as the most impacted, with significantly low species diversity, richness and equitability. The results of the study also emphasised the importance of species level identification in biomonitoring of freshwater resources because of the observed species differences at the four sampling sites, and if species level data were not present, subtle differences between sites may not be detected. Also, with species level data, information of functional feeding group and food materials is more accurately determined (Armitage *et at.,* 1995). Species within the genus *Chironomus* are mostly scrapers that feed on organic matter and algae (Armitage *et al.,* 1995) and were highly abundant at site 3. The high abundance of species within this genus could serve as specific indicator of organically enriched site. This is supported by the comparatively high concentrations of five days biochemical oxygen demand (BOD_5), nutrients and low dissolved oxygen recorded at this site. The strong positive correlations observed between BOD_5, orthophosphate – phosphorus (PO_4-P), total inorganic nitrogen (TIN) and *Chironomus* sp. 1, *Chironomus* sp. 2 and *Chironomus* sp. 3 (Figure 3.24) also support this argument. Therefore, species level identification could help identify indicators of specific types of pollution.

The screening of deformities in Chironomidae is particularly useful because the approach provided evidence of sub-lethal effects of pollution on the Swartkops River chironomid communities and may serve as early warning indicators of deteriorating water quality. Morphological deformities also provide indication of species health and fitness of chironomids, which could impact on their ability to feed, and to perform ecological roles such as serving as a path-way for the transportation and utilisation of energy and matter in the Swartkops River. Furthermore, the elevated community incidences of deformities recorded at sites 2, 3 and 4 are probably indication of possible chemical contamination of the Swartkops River, while incidences of deformities recorded at site 1 are considered early warning indicators of water quality deterioration. Incidences of mentum deformities were significantly higher at site 3, which seemed to support Chironomidae community results and multimetric

approach that have indicated site 3 as the most impacted. The results also revealed the importance of screening deformities in other chironomid genera other than *Chironomus.* The mentum, of all the structures examined for deformities, proved useful because it appears to have a wider range of morphological response, displaying deformities at all the sampling sites (i.e. from most to least impacted). In addition, the mentum is easy to prepare for examination and does not require a great deal of technicality or high microscope magnification (though this depends on the size of the specimen) to quantify deformities.

In general, the different approaches applied in this study provided better understanding of anthropogenic impacts on the Swartkops River macroinvertebrates with regard to water quality. These approaches allowed the effects of impaired water quality to be examined at different biological organisation as well as taxonomic resolutions. By applying SASS5, biological water quality at each of the sampled site in Swartkops River was determined while the multimetric approach and Chironomidae species identification provided evidence of marginal water quality differences between sites 3 and 4 that accounted for differences in species compositions at these sites. The multimetric approach also provided an insight to how deterioration in environmental water quality affects different aspects of macroinvertebrate communities such as richness, composition, abundance and diversity. The approach revealed that richness (particularly of EPT richness), diversity and equitability were the most affected by water quality deterioration while abundance and composition were the least affected. Species identification also revealed that genus *Chironomus* was the most tolerant of all the chironomids sampled and could serve as an indicator of organically polluted sites. The deformities in Chironomidae observed in this study provided indication of possible toxic contamination of the Swartkops River and also reflected individual species health or fitness at each of the sampled sites.

Based on the results of this study site 3, being the most impacted by anthropogenic activities, should be prioritised for restoration by water resources managers and concerted efforts be made to investigate compliance of point source discharges and management of diffuse pollution sources at the site. Water resources restoration goals could also be set for the Swartkops River based on results of this study. One of such is to ensure that mitigation measures are in place until macroinvertebrates diversity, richness, equitability and EPT taxa reappear, and incidences of chironomid deformities drop, to a level reasonably comparable to site 1.

5.7 Recommendations

The following recommendations are made for the purpose of incorporating the outcomes of this study in the implementation of Integrated Water Resources Management (IWRM) of the Swartkops River, and for further research.

1) The six metrics namely: EPT richness, Shannon diversity index, Simpson diversity index, Margalef's family richness index, equitability and % Chironomidae + Oligochaete, which were able to distinguished subtle differences between sites 2, 3 and 4 should be included with SASS5 in routine biomonitoring of the Swartkops River.
2) Results from this study show that the different subfamilies within the family Chironomidae respond differently to water quality changes. Because identification of chironomid to the sub-families is relatively easier to carry out than genus and species, the sensitivity of sub-families to water quality impairment should be investigated on a wider geographical scale with a view to possibly splitting the family Chironomidae into sub-families on the SASS score sheet and that these sub-families be assigned appropriate sensitivity scores in SASS. Although this might make SASS less rapid, the benefits of obtaining more relevant scores need to be balanced against the additional identification time.
3) For improved management of freshwater resources, screening of deformities in chironomids should be incorporated into the South African national River Health Programme, and the possibility of extending the method to other macroinvertebrate groups be investigated.
4) In cases where time and other resources do not permit examinations of deformities in other structures, the mentum should be used because of its ability to respond morphologically to different levels of pollution. In addition, it is easy to prepare for examination and does not require great deal of technicality to quantify deformities.
5) In order to improve the discriminatory capacity of Chironomidae deformities as a biological screening tool, concerted efforts should be made to link different types of deformity and severity to possible causal agents, i.e. specificity of a cause and effect relationship. Such investigation could help to elucidate whether a particular type of contaminant could induce specific type of deformity. If cause and effect relationship is well established, mitigation measures will be better directed towards specific cause of observed water quality impairment.

6) Although the present study provides evidence of bioindication potential of the family Chironomidae, their application in biomonitoring of freshwater resources should be considered along with available resources in terms of personnel, time and funds as well as priorities.

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APPENDICES

Appendix A: Number of macroinvertebrates for each replicates of SASS5 biotopes, relative abundance of chironomids, and IHAS scores at the four sampling sites during the four sampling seasons.

Table A1: Number of macroinvertebrates for each replicates of the three SASS5 biotopes during spring and summer at site 1 in the Swartkops River. Abbreviation: Biotopes: S = stone (stone in- and out- of current), V = vegetation (marginal and aquatic) and G = gravel, sand and mud (GSM). Numbers attached to biotopes represent replicates: 1 = replicate 1, 2 = replicate 2 and 3 = replicate 3

	Spring								**Summer**								
Taxon	S1	S2	S3	V1	V2	V3	G1	G2	S1	S2	S3	V1	V2	V3	G1	G2	G3
ANNELIDA																	
Oligochaeta	3	4	8	1			4	15			1				1	7	18
Hirudinea				1				1	1		1						
EPHEMEROPTERA																	
Leptophlebidae			1														
Baetidae	1	5	2			3		4	6	2		2	2	8			4
Baetidae > 2sp				28			26								24		
ODONATA																	
Coenagrionidae	2	1		17	8	12		1		3	2	16	4	11	1		1
Aeshnidae						1								1			
Gomphidae					1										2		
Libellulidae		5	5	11	10	19				5	3	3		1	2		
Chlorolestidae	1	2	2	1	3	5											
Belostomatidae														2	1		
Naucoridae										7							
Nepidae												1					
Pleidae										4							
Veliidae										1		10		4		11	
Gerridae													2				

	Spring								**Summer**								
	S1	S2	S3	V1	V2	V3	G1	G2	S1	S2	S3	V1	V2	V3	G1	G2	G3
TRICHOPTERA																	
Ecnomidae	1		3					2									
Hydropsychidae	5	10				2	3		3	3				4		3	
Hydropsychidae >2sp			9	4			63	65							10		
Leptoceridae				1		1											
COLEOPTERA																	
Dytiscidae				1					1	1	1		1	3			
Gyrinidae												1		1			
Hydraenidae				3			2										
Hydrophilidae									1			3					5
Elmidae								2									
DIPTERA																	
Ceratopogonidae	3	1			1										1	3	1
Chironomidae	83	71	52	68	13	55	18	19	45	30		18	4	32	54	13	3
Culicidae	1		1	9									1	2	2	1	1
Muscidae			1	1									8				
Simulidae	13	6	1	28	2	1	56	17	18	7		6		3	4	1	
Tabanidae															1		
GASTROPODA																	
Ancylidae	1		3							1							
Lymnaeidae	4	2	2	4	6	15		1		6	4	3	22	9	7		
Physidae	1		2		4		1			7		3	1		1		

Table A2: Number of macroinvertebrates for each replicates of the three SASS5 biotopes during autumn and winter at site 1 in the Swartkops River. Abbreviation: Biotopes: S = stone (stone in- and out- of current), V = vegetation (marginal and aquatic) and G = gravel, sand and mud (GSM). Numbers attached to biotopes represent replicates: 1 = replicate 1, 2 = replicate 2 and 3 = replicate 3

	Autumn									**Winter**								
Taxon	S1	S2	S3	V1	V2	V3	G1	G2	G3	S1	S2	S3	V1	V2	V3	G1	G2	G3
ANNELIDA																		
Oligochaeta	5	8					3		5	2	3	6				1	4	2
Hirudinea	2	1	1		1			1			1							
CRUSTACEA																		
Potamonautida	2	2	1				1						1					
EPHEMEROPTERA																		
Baetidae	3	1	3		8	2				11	8	8		3	10	4	14	10
Baetidae > 2sp				16									17					
ODONATA																		
Coenagrionidae	1			6	2		3				2		1	3	3			
Aeshnidae			3									1						1
Cordulidae			4	2							2		1		1			
Gomphidae				1														
Libellulidae	3	1	8	5	2	3	4	2		3		2	1	5	3		5	
Chlorolestidae		1	2	3														
LEPIDOPTERA																		
Pyralidae					1								1					
HEMIPTERA																		
Corixidae			1		1													
Naucoridae																		
Veliidae	1	1	2	2	1		3											1
Gerridae										1	2							

	Autumn									**Winter**								
Taxon	S1	S2	S3	V1	V2	V3	G1	G2	G3	S1	S2	S3	V1	V2	V3	G1	G2	G3
TRICHOPTERA																		
Ecnomidae																		
Hydropsychidae	1	2						1	1		1	1		4				
Hydropsychidae 2sp				6												10		
COLEOPTERA																		
Dytiscidae															1		1	1
Gyrinidae	2				1	1	1	1	1		3		1					
Hydrophilidae							1											
Elmidae								1										
DIPTERA																		
Ceratopogonidae	1		3	9	1		3	1		1		2						
Chironomidae	64	29	11	3	10	2	27	8	4	19	18	65	34	25	48	22	50	35
Culicidae			1															
Muscidae											1							
Simulidae	60	42	4				25	7	2	5	4	16	4	25	26	8	12	24
Anthericidae												1		1				
GASTROPODA																		
Lymnaeidae	3	1	1		13					1				4	1			
Physidae	1		1	2	2	3	1	1	2	1			1					

Table A3: Number of macroinvertebrates for each replicates of the three SASS5 biotopes during spring and summer at site 2 in the Swartkops River. Abbreviation: Biotopes: S = stone (stone in- and out- of current), V = vegetation (marginal and aquatic) and G = gravel, sand and mud (GSM). Numbers attached to biotopes represent replicates: 1 = replicate 1, 2 = replicate 2 and 3 = replicate 3

	Spring									**Summer**								
Taxon	S1	S2	S3	V1	V2	V3	G1	G2	G3	S1	S2	S3	V1	V2	V3	G1	G2	G3
ANNELIDA																		
Oligochaeta	13	16	17	4	6	10	20	22	5	18	3		14	10	15	40	18	12
Hirudinea	5	6			2				3		1		1	3	1			
CRUSTACEA																		
Potamonautida		2	1								1							
EPHEMEROPTERA																		
Baetidae		2		1	1					1	9	13		30	16		4	7
Baetidae													80					
ODONATA																		
Coenagrionidae		1	6	35	16	22	1	3			5	2	16	6	7			6
Lestidae										3								
Aeshnidae						1								1	3			
Cordulidae				2	4													
Gomphidae				2			1											
Libellulidae		1		5	6	4					1	1	1					
HEMIPTERA																		
Belostomatidae				3										20	10		2	
Corixidae										29	19	20	74	33	17	53	90	51
Naucoridae										2								
COLEOPTERA																		

	Spring									**Summer**								
Taxon	S1	S2	S3	V1	V2	V3	G1	G2	G3	S1	S2	S3	V1	V2	V3	G1	G2	G3
Dytiscidae	3		1	1		1	3	4		2	7	9	22	2	5	2	2	16
Gyrinidae																		
Hydraenidae																		
Hydrophilidae													1		1			
Elmidae		1																
DIPTERA																		
Ceratopogonidae	1			1						1	1	1						
Chironomidae	128	148	50	28	46	68	9	7	13	56	93	176	316	124	98	156	187	226
Culicidae				2			1	1			3		18	4	7			15
Muscidae						1								7				
Simulidae	8	15	5	1	6	59	1		10	11	54	15	3		1			
Tipulidae										3								
GASTROPODA																		
Ancylidae	1			1	3					3	1	2	3	1	2			17
Physidae	2	8	4	24	17	10	6	3	4	43	109	15	22	9	8		1	45

Table A4: Number of macroinvertebrates for each replicates of the three SASS5 biotopes during autumn and winter at site 2 in the Swartkops River. Abbreviation: Biotopes: S = stone (stone in- and out- of current), V = vegetation (marginal and aquatic) and G = gravel, sand and mud (GSM). Numbers attached to biotopes represent replicates: 1 = replicate 1, 2 = replicate 2 and 3 = replicate 3

	Autumn									**Winter**								
Taxon	S1	S2	S3	V1	V2	V3	G1	G2	G3	S1	S2	S3	V1	V2	V3	G1	G2	G3
ANNELIDA																		
Oligochaeta	1				5		18	13	18			9	21		23	2	4	1
Hirudinea	1	1	3	3			1	1						1				
CRUSTACEA																		
Potamonautida	1		1															
EPHEMEROPTERA																		
Baetidae		37	21			47		10	24									
Baetidae > 2sp	72			128	136		79											
ODONATA																		
Coenagrionidae	6			17	8	5		1	1								1	
Aeshnidae				3	2	1												
Gomphidae				1														
Libellulidae					1	1		1										
HEMIPTERA																		
Belostomatidae				10	1	3	1							2				
Corixidae	5	6	2	2			60	85	136	1								2
Nepidae				1	1		1											
Notonectidae				6	2	2	5	4	1									
Veliidae				9			6											
Gerridae				2														
COLEOPTERA																		
Dytiscidae			1	15	2	2	3	7	3				1	1				

	Autumn									Winter								
Taxon	S1	S2	S3	V1	V2	V3	G1	G2	G3	S1	S2	S3	V1	V2	V3	G1	G2	G3
Elmidae																		
DIPTERA																		
Ceratopogonidae	1	5		4	1	1		6	24				4			2	3	3
Chironomidae	122	43	74	78	17	8	18	75	25			1	4	2		10	11	
Culicidae				1									3	2	10	2	1	1
Simulidae				1														
Psychodidae																4		
Tabanidae									5									
GASTROPODA																		
Ancylidae		4	1	2			1		1									
Physidae									1							1		

Table A5: Number of macroinvertebrates for each replicates of the three SASS5 biotopes during spring and summer at site 3 in the Swartkops River. Abbreviation: Biotopes: S = stone (stone in- and out- of current), V = vegetation (marginal and aquatic) and G = gravel, sand and mud (GSM). Numbers attached to biotopes represent replicates: 1 = replicate 1, 2 = replicate 2 and 3 = replicate 3

	Spring									**Summer**						
Taxon	S1	S2	S3	V1	V2	V3	G1	G2	G3	S1	S2	S3	V1	V2	V3	G1
ANNELIDA																
Oligochaeta	210	50	48	62	42		12	24	3	2			2			
Hirudinea			4	10	3	21		4		1			7			
EPHEMEROPTERA																
Baetidae													1			1
ODONATA																
Coenagrionidae		1		2	7	12							3			2
Aeshnidae						1										
Libellulidae						3										
HEMIPTERA																
Belostomatidae													26	3	1	
Corixidae												1	1	1		12
Notonectidae					5	23			19				38	11		27
TRICHOPTERA																
Ecnomidae							2									
Hydropsychidae					3				4							
COLEOPTERA																
Dytiscidae																4
Hydrophilidae														6		
Elmidae				4												
DIPTERA																
Chironomidae	279	137	405	21	25	29	75	62	19	415	329	410	462	94	485	362

	Spring									**Summer**						
Taxon	S1	S2	S3	V1	V2	V3	G1	G2	G3	S1	S2	S3	V1	V2	V3	G1
Culicidae	17	8	18	1	1		3	2	1	5		1			8	5
Muscidae														2		
Psychodidae						4										
GASTROPODA																
Physidae				6	5	16	1	11								
Planirbidae				1												

Table A6: Number of macroinvertebrates for each replicates of the three SASS5 biotopes during autumn and winter at site 3 in the Swartkops River. Abbreviation: Biotopes: S = stone (stone in- and out- of current), V = vegetation (marginal and aquatic) and G = gravel, sand and mud (GSM). Numbers attached to biotopes represent replicates: 1 = replicate 1, 2 = replicate 2 and 3 = replicate 3

	Autumn								**Winter**						
Taxon	S1	S2	S3	V1	V2	V3	G1	G2	S1	S2	S3	V1	V2	V3	G1
ANNELIDA															
Oligochaeta			30									100	6	3	5
Hirudinea												56	1	3	9
ODONATA															
Coenagrionidae						1						2		6	
Aeshnidae												1		1	1
Belostomatidae				10	5	18	7	3							
Corixidae												1		1	1
Notonectidae				10	1	14	43	2							1
Veliidae				1	1										
COLEOPTERA															
Dytiscidae				1	3	10		2					1		1
Gyrinidae						1									
DIPTERA															
Chironomidae	282	310	245	261	89	6	17		589	402	208	161	182	141	70
Syrphidae							9	1							
Culicidae				47	6	11	12	6	4				11		4
Psychodidae				1			1								
GASTROPODA															
Physidae													1		

Table A7: Number of macroinvertebrates for each replicates of the three SASS5 biotopes during spring and summer at site 4 in the Swartkops River. Abbreviation: Biotopes: S = stone (stone in- and out- of current), V = vegetation (marginal and aquatic) and G = gravel, sand and mud (GSM). Numbers attached to biotopes represent replicates: 1 = replicate 1, 2 = replicate 2 and 3 = replicate 3

	Spring									**Summer**								
Taxon	S1	S2	S3	V1	V2	V3	G1	G2	G3	S1	S2	S3	V1	V2	V3	G1	G2	G3
ANNELIDA																		
Oligochaeta	56	12	72	4	2		121	61	152	51	10	40				112	166	220
Hirudinea	17	13	18	13	9	8	37	15	34				1					
EPHEMEROPTERA																		
Baetidae					1													
ODONATA																		
Coenagrionidae						2												
Aeshnidae														1				
HEMIPTERA																		
Belostomatidae					1													
Corixidae													3		2	6	1	8
Pleidae						2	1											
Veliidae				1														
COLEOPTERA																		
Dytiscidae				1	1	4								1				
Hydrophilidae				1									3	1				
Elmidae													4					
DIPTERA																		
Chironomidae	4	3	3	7	7	8	3	1	2	18	2	10	6		1	24	60	31
Culicidae													11			1		
Ephydridae																	1	
Muscidae															2			

	Spring									**Summer**								
Taxon	S1	S2	S3	V1	V2	V3	G1	G2	G3	S1	S2	S3	V1	V2	V3	G1	G2	G3
Simulidae						5							1					
GASTROPODA																		
Physidae	4			19	2	15		1										
Planorbidae													6	1	2			

Table A8: Number of macroinvertebrates for each replicates of the three SASS5 biotopes during autumn and winter at site 4 in the Swartkops River. Abbreviation: Biotopes: S = stone (stone in- and out- of current), V = vegetation (marginal and aquatic) and G = gravel, sand and mud (GSM). Numbers attached to biotopes represent replicates: 1 = replicate 1, 2 = replicate 2 and 3 = replicate 3

	Autumn									**Winter**								
Taxon	S1	S2	S3	V1	V2	V3	G1	G2	G3	S1	S2	S3	V1	V2	V3	G1	G2	G3
ANNELIDA																		
Oligochaeta	43	52	45				37	82	50	18	3	17			2	4	3	
Hirudinea										11	58	39	3	10	3	3	65	59
EPHEMEROPTERA																		
Baetidae										1								
ODONATA																		
Coenagrionidae													2	2	3			
Aeshnidae														2	1			
HEMIPTERA																		
Belostomatidae				1				1					6	1	2			
Notonectidae															1			
COLEOPTERA																		
Dytiscidae														4				
Hydrophilidae				1														
Helodidae								1										
DIPTERA																		
Chironomidae	4	2		1	15	6	10	4	12	34	77	30	70	236	286	35	89	107
Syrphidae					2				1									
Culicidae					108	63	1						6	3	1		1	
Muscidae						1					2	1						

	Autumn									**Winter**								
Taxon	S1	S2	S3	V1	V2	V3	G1	G2	G3	S1	S2	S3	V1	V2	V3	G1	G2	G3
GASTROPODA																		
Physidae													1		4			

Table A9: Percentage relative abundance and distribution of chironomids sampled during the sampling seasons at each of the sampling sites in the Swartkops River. Abbreviations: Spr = spring, Sum = summer, Aut = autumn, Wint = winter

	Site 1				**Site 2**				**Site 3**				**Site 4**			
Taxon	Spr	Sum	Aut	Wint	Spr	Sum	Aut	Wint	Spr	Sum	Aut	Wint	Spr	Sum	Aut	Wint
Orthocladiinae																
Cricotopus sp.1	36.6	36.48	13.04	2.87	25.5	0.7							2.22			
Cricotopus trifasciata gr.	10.46	32.43	53.62	34.8	52.6	2.94							8.89			
Paratrichocladius sp.	2.29			0.41	4.56								2.22			
Nanocladius sp.	1.31				0.61											
Cardiocladius sp.	4.58	2.03		1.23						0.77						
Orthocladius sp.	12.09	10.14	7.97	4.92	7	1.82	0.45						8.89			
Parakiefferiella sp.	0.33				0.3											
Eukiefferiella sp.	1.96				0.61											
Chironominae																
Tribe Chironomini																
Dicrotendipes sp.		0.68			7.3	32.59	3.14		0.19	1.07	0.21	0.39	4.44	1.45		1.12
Cryptochironomus sp.	0.32															
Chironomus sp.1			1.45		1.22	10.77	67.71	89.29	98.5	79.91	91.53	91.7	57.78	69.6	95.83	91.24
Chironomus sp.2		0.68	0.72			4.34	7.62	10.71		13.19	6.57	7.72		19.6		4.94
Chironomus sp.3			0.72				2.69			5.06	1.69			2.9		2.47
Polypedilum sp.	15.03		2.9	3.28	0.3								6.67			
Kiefferulus sp.	0.33					14.27	3.59		1.3							
Microchironomus sp.			0.73				3.14								4.16	

	Site 1				**Site 2**				**Site 3**				**Site 4**			
	Spr	Sum	Aut	Wint	Spr	Sum	Aut	Wint	Spr	Sum	Aut	Wint	Spr	Sum	Aut	Wint
Tribe Tanytarsini																
Rheotanytarsus sp.		0.68														
Paratanytarsus sp.						0.56										
Cladotanytarsus sp.	0.33			1.23		1.12						0.19	2.22			
Tanytarsus sp.	4.9	9.46	15.22	48									6.67			0.23
Tanypodinae																
Ablabesmyia sp.	7.19	7.43		2.03		0.14								6.52		
Coelotanypus sp.	0.33															
Procladius sp.	0.33															
Trissopelopia sp.	0.98															
Clinotanypus sp.	0.65															
Tanypus sp.			3.62	1.23		30.77	11.66									
Number of taxa	**18**	**9**	**10**	**9**	**10**	**11**	**8**	**2**	**3**	**5**	**4**	**4**	**9**	**5**	**2**	**5**
Total number of chironomids	**306**	**148**	**143**	**122**	**329**	**715**	**223**	**28**	**540**	**652**	**472**	**518**	**45**	**138**	**24**	**435**

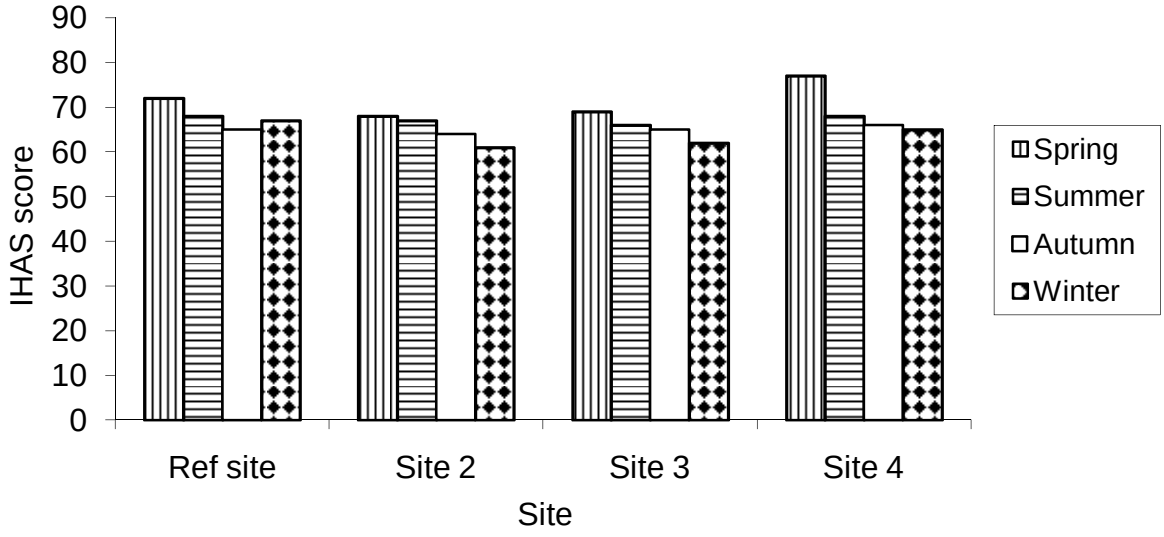

Figure A1: Integrate Habitat Assessment System (IHAS) scores during the four sampling seasons at the four sampling sites in the Swartkops River

Appendix B: Summary and full descriptions of deformities types recorded among chironomids taxa during the four sampling seasons at the four sampling sites in the Swartkops River

Table B1: Summary of incidences (%) of mentum, ligula and antennal deformities among chironomid taxa sampled at the sites 1 and 2 during spring. Numbers in parentheses are percentage deformed while numbers outside parenthesis are actual numbers of larvae deformed

	Site 1					**Site 2**				
Taxa	**No.**	**Mentum**	**Ligula**	**Antenna**	**Mandible**	**No.**	**Mentum**	**Ligula**	**Antenna.**	**Mandible**
Orthocladiinae										
Cricotopus sp.1	112	5 (4.46)		6 (5.36)	1 (0.89)	84	13 (15.48)		3 (3.57)	6 (7.14)
Cricotopus trifasciata gr.	32				1 (3.13)	173	12 (6.94)			4 (2.31)
Paratrichocladius sp.	7					15				
Nanocladius sp.	4					2				
Eukiefferiella sp.	6					2	1 (50)			
Cardiocladius sp.	15	2 (13.33)								
Parakiefferiella sp.	1					1				
Orthocladius sp.	45	3 (6.67)			2 (4.44)	23	3 (13.04)			3 (13.04)
Chironominae										
Tribe Chironomini										
Chironomus sp.						4	1 (25)			
Dicrotendipes sp.						24	4 (16.67)			2 (8.33)
Kiefferulus sp.	1									
Polypedilum sp.	46	7 (15.22)		2 (4.35)	2 (4.35)	1				
Cryptochironomus sp.	1									

	Site 1					Site 2				
Taxa	**No.**	**Mentum**	**Ligula**	**Antenna**	**Mandible**	**No.**	**Mentum**	**Ligula**	**Antenna.**	**Mandible**
Tribe Tanytarsini										
Tanytarsus sp.	15		1 (6.67)	1 (6.67)						
Cladotanytarsus sp.	1									
Tanypodinae										
Ablabesmyia sp.	26		2 (7.69)	3 (6.67)						
Coelotanypus sp.	1									
Procladius sp.	1									
Trissopelopia sp.	3									
Clinotanypus sp.	2									

Table B2: Summary of incidences (%) of mentum, ligula and antennal deformities among chironomid taxa sampled at the sites 3 and 4 during spring. Numbers in parentheses are percentage deformed while numbers outside parenthesis are actual numbers of larvae deformed

	Site 3					**Site 4**				
Taxon	**No.**	**Mentum**	**Ligula**	**Antenna**	**Mandible**	**No.**	**Mentum**	**Ligula**	**Antenna.**	**Mandible**
Orthocladiinae										
Cricotopus sp.1						1				
Cricotopus trifasciata gr.						4				1 (25)
Paratrichocladius sp.						1				
Orthocladius sp.						4	1 (25)			
Chironominae										
Tribe Chironomini										
Chironomus sp.	532	115 (21.62)		36 (6.88)	38 (7.27)	26	3 (11.54)			
Dicrotendipes sp.	1	1 (100)			1 (100)	2				1 (50)
Kiefferulus sp.	4									
Polypedilum sp.						3	3 (100)			
Tribe Tanytarsini										
Tanytarsus sp.						3	1 (33.33)			
Cladotanytarsus sp.						1				

Table B3: Summary of incidences (%) of mentum, ligula and antennal deformities among chironomid taxa sampled at the sites 1 and 2 during summer. Numbers in parentheses are percentage deformed while numbers outside parenthesis are actual numbers of larvae deformed

	Site 1					**Site 2**				
Taxon	**No.**	**mentum**	**ligula**	**antenna**	**mandible**	**No**	**mentum**	**Ligula**	**antenna**	**mandible**
Orthocladiinae										
Cricotopus sp.1	54	2 (3.7)		3 (5.56)		5				
Cricotopus trifasciata gr.	48	4 (8.33)		4 (8.33)		21	1 (4.76)			
Cardiocladius sp.	3									
Orthocladius sp.	15			1 (6.67)		13				
Chironominae										
Tribe Chironomini										
Dicrotendipes sp.	1					233	28 (12.02)		21 (9.01)	8 (3.43)
Chironomus sp.1	1					77	9 (11.69)			4 (5.2)
Chironomus sp.2						31	3 (9.68)			
Kiefferulus sp.						102	3 (2.94)		1 (0.98)	1 (0.98)
Tribe Tanytarsini										
Rheotanytarsus sp.	1									
Paratanytarsus sp.						4				
Cladotanytarsus sp.						8				
Tanytarsus sp.	14			1 (7.14)						
Tanypodinae										
Ablabesmyia sp.	11	1 (9.09)				1				
Tanypus sp.						220	17 (7.73)		17 (7.73)	3 (1.36)

Table B4: Summary of incidences (%) of mentum, ligula and antennal deformities among chironomid taxa sampled at the sites 3 and 4 during summer. Numbers in parentheses are percentage deformed while numbers outside parenthesis are actual numbers of larvae deformed

	Site 3					**Site 4**				
Taxon	**No**	**mentum**	**ligula**	**antenna**	**mandible**	**No.**	**mentum**	**Ligula**	**antenna**	**mandible**
Orthocladiinae										
Cardiocladius sp.	5									
Chironominae										
Tribe Chironomini										
Dicrotendipes sp.	7	1 (14.29)			6 (85.71)	2				
Chironomus sp.1	521	93 (17.85)		14 (2.69)	36 (6.91)	96	16 (16.67)			7 (7.29)
Chironomus sp.2	86	10 (11.63)		4 (4.65)	2 (2.33)	27	3 (11.11)			1 (3.7)
Chironomus sp.3	33	13 (39.39)			2 (6.06)	4	4 (100)			
Tanypodinae										
Ablabesmyia sp.						9				1 (11.11)

Table B5: Summary of incidences (%) of mentum, ligula and antennal deformities among chironomid taxa sampled at the sites 1 and 2 during autumn. Numbers in parentheses are percentage deformed while numbers outside parenthesis are actual numbers of larvae deformed

	Site 1					**Site 2**				
Taxon	**No.**	**mentum**	**Ligula**	**antenna**	**mandible**	**No.**	**mentum**	**Ligula**	**antenna**	**mandible**
Orthocladiinae										
Cricotopus sp.1	18									
Cricotopus trifasciata gr.	74	7 (9.46)								
Orthocladius sp.	11					1				
Chironominae										
Tribe Chironomini										
Dicrotendipes sp.						7	1 (14.29)			
Chironomus sp.1	2					151	16 (10.6)		6 (3.97)	1 (0.66)
Chironomus sp.2	1					17	1 (5.88)			
Chironomus sp.3	1					6				
Polypedilum sp.	4									
Kiefferulus sp.						8	1 (12.5)			
Microchironomus sp.	1					7	1 (14.29)			
Tribe Tanytarsini										
Tanytarsus sp.	21	1 (4.76)								
Tanypodinae										
Ablabesmyia sp.	5									
Tanypus sp.	5					26		4 (15.39)		

Table B6: Summary of incidences (%) of mentum, ligula and antennal deformities among chironomid taxa sampled at the sites 3 and 4 during autumn. Numbers in parentheses are percentage deformed while numbers outside parenthesis are actual numbers of larvae deformed

	Site 3					**Site 4**				
Taxon	**No.**	**mentum**	**Ligula**	**antenna**	**mandible**	**No.**	**mentum**	**Ligula**	**antenna**	**mandible**
Chironominae										
Tribe Chironomini										
Dicrotendipes sp.	1									
Chironomus sp.1	432	64 (14.81)		14 (3.24)	21 (4.86)	23	3 (13.04)			2 (8.7)
Chironomus sp.2	31	6 (19.36)								
Chironomus sp.3	8	3 (37.5)			1 (12.5)					
Microchironomus sp.						1				

Table B7: Summary of incidences (%) of mentum, ligula and antennal deformities among chironomid taxa sampled at the sites 1 and 2 during winter. Numbers in parentheses are percentage deformed while numbers outside parenthesis are actual numbers of larvae deformed

	Site 1					**Site 2**				
Taxon	**No.**	**mentum**	**ligula**	**antenna**	**mandible**	**No.**	**mentum**	**ligula**	**antenna**	**mandible**
Orthocladiinae										
Cricotopus sp.1	7									
Cricotopus trifasciata gr.	85	2 (2.35)								
Paratrichocladius sp.	1									
Cardiocladius sp.	3									
Orthocladius sp.	12									
Chironominae										
Tribe Chironomini										
Chironomus sp.1						25	3 (12)			1 (4)
Chironomus sp.2						3				1 (33.33)
Polypedilum sp.	8	1 (12.5)								
Tribe Tanytarsini										
Tanytarsus sp**.**	117	5 (4.27)		3 (2.56)						
Cladotanytarsus sp.	3									
Tanypodinae										
Tanypus sp.	3									

Table B8: Summary of incidences (%) of mentum, ligula and antennal deformities among chironomid taxa sampled at the sites 3 and 4 during winter. Numbers in parentheses are percentage deformed while numbers outside parenthesis are actual numbers of larvae deformed

	Site 3					**Site 4**				
	No.	**mentum**	**ligula**	**antenna**	**mandible**	**No**	**mentum**	**Ligula**	**antenna**	**mandible**
Taxon										
Orthocladiinae										
Nanocladius sp.						5				
Cardiocladius sp.						1				
Chironominae										
Tribe Chironomini										
Dicrotendipes sp.	2									
Chironomus sp.1	475	64 (13.47)		3 (0.63)	1 (0.21)	406	41 (10.1)		3 (0.74)	3 (0.74)
Chironomus sp.2	40	7 (17.5)				12	1 (8.33)			
Chironomus sp.3						11	1 (9.09)			
Tribe Tanytarsini										
Cladotanytarsus sp.	1									

Table B9: Number and description of mentum and ligula deformities recorded among chironomid taxa at the four sampling sites during spring. Biotopes replicates are also shown

Site	Biotope replicate	Genus	Types of deformity	Description	No. of individual showing deformity
	Stone 1	*Cricotopus* sp.1	fused teeth	fused fifth and sixth lateral teeth	1
Site 1		*Tanytarsus* sp.	missing teeth	missing first right lateral teeth	1
		Orthocladius sp.	fused teeth	fused fourth, fifth and sixth right lateral teeth	1
		Cricotopus sp.1	split tooth	split median tooth	1
	Stone 2	*Cardiocladius* sp.	worn tooth	worn median tooth	2
	Stone 3	*Cricotopus* sp.1	fused teeth	fused fourth, fifth and sixth lateral teeth	1
		Ablabesmyia sp.	split tooth	split left inner tooth	1
			split tooth	split median tooth	1
		Cricotopus sp.1	fused teeth	fused left lateral teeth	1
		Polypedilum sp.	split tooth	split left outer median tooth	1
	Veg. 1	*Ablabesmyia* sp.	split tooth	split right outer tooth	1
				fused second to sixth left lateral teeth	1
				all left lateral teeth fused with first lateral teeth	1
			fused teeth	fused fifth and sixth right lateral teeth	1
				missing outer median tooth	2
		Polypedilum sp.	missing teeth	missing sixth right lateral teeth and third to fifth left lateral teeth	1
			fused teeth	fused fifth and sixth left lateral teeth	1
			missing teeth	missing sixth left lateral teeth	1
	Veg. 3	*Orthocladius* sp.			
Site 2			missing teeth	missing second left lateral teeth	1
	Stone 2	*Cricotopus* sp.1		missing third lateral teeth	1

			Kohn gap	gaps on the median teeth	3
			fused teeth	fused fifth and sixth left lateral teeth	3
				fused first and second left lateral teeth	1
			split tooth	split first lateral teeth	1
			lateral gap	lateral gap on left lateral teeth	1
		Cricotopus trifasciata gr.		first and second right lateral teeth fused	1
			fused teeth	fused fifth and sixth right lateral teeth	1
		Cricotopus sp.1	fused teeth	fused third, fourth, fifth and sixth right lateral teeth	1
		Cricotopus trifasciata gr.	Kohn gap	gaps on the median teeth	2
			fused teeth	fused fourth, fifth and sixth left lateral teeth	4
		Orthocladius sp.	fused teeth	fused fifth and sixth left lateral teeth	1
	Stone 1	*Chironomus* sp. 1	split tooth	split median tooth	1
	Veg. 2	*Dicrotendipes* sp.	fused teeth	fused first and second left lateral teeth	3
		Cricotopus trifasciata gr.	split tooth	split first lateral teeth	1
			lateral gap	gaps in left lateral teeth	1
				fused fifth and sixth right lateral teeth	1
		Cricotopus sp.1	fused teeth	fused first and second right lateral teeth	1
		Orthocladius sp.	fused teeth	fused fifth and sixth right lateral teeth	1
	Veg. 3	*Eukiefferiella* sp.	fused teeth	fused fourth and fifth lateral teeth	1
		Cricotopus triafasciata gr	split tooth	split left median tooth	1
	Stone 2	*Dicrotendipes* sp.	missing teeth	fifth and sixth left lateral teeth missing	1
			fused teeth	fused fifth and sixth left lateral teeth	1
		Cricotopus			.
	GSM 3	*triafasciata* gr			
Site 3			Asymmetry	asymmetry between first lateral teeth	3
	Stone1	*Chironomus* sp. 1	fused teeth	fused fifth and sixth left lateral teeth	4

			fused lateral teeth	1
			fused third and fourth right lateral teeth	6
			fused fourth, fifth and sixth lateral teeth	1
			fused third and fourth left lateral teeth	1
			missing sixth right lateral teeth and third to fifth left lateral teeth	1
		missing teeth	missing first right lateral teeth	1
			split first left lateral teeth	1
			split median tooth	1
		split tooth	split first right lateral teeth	1
		Others	distortion in the basic configuration of mentum	1
		Extra teeth	extra teeth on left lateral teeth	1
		fused teeth	fused outer median and first lateral teeth	3
Veg. 1	*Chironomus* sp. 1	split tooth	split median tooth	1
		fused teeth	fused fourth, fifth and sixth left and right lateral teeth	1
		missing teeth	missing sixth left lateral teeth	3
Veg. 2	*Chironomus* sp. 1	split tooth	split median tooth	1
			missing outer median tooth	1
			missing fifth left lateral tooth	1
			missing sixth left lateral teeth	1
		missing teeth	missing first right lateral teeth	1
		Extra teeth	extra tooth on median tooth	1
		lateral gap	lateral gap on left lateral teeth	1
		fused teeth	fused first and second right lateral teeth	1
GSM1	*Chironomus* sp. 1	Kohn gap	gaps on the median teeth	1
		Asymmetry	asymmetry between first lateral teeth	7
			fused fifth and sixth left lateral teeth	1
Stone 3	*Chironomus* sp. 1	fused teeth	fused lateral teeth	4

			fused third and fourth right lateral teeth	2
			fused fourth, fifth and sixth lateral teeth	1
			fused third and fourth left lateral teeth	
		missing teeth	missing sixth right lateral teeth and third to fifth left lateral teeth	12
			missing first right lateral teeth	4
		split tooth	split first left lateral teeth	4
			split median tooth	3
			split first right lateral teeth	2
		Kohn gap	gaps in the median teeth of the mentum	5
		Others	distortion in the basic configuration of mentum	1
		extra tooth	extra tooth on the central median tooth	2
	Dicrotendipes sp.	Kohn gap	gaps in the median teeth of the mentum	1
Stone 2	*Chironomus* sp. 1	Asymmetry	asymmetry between first lateral teeth	2
		fused teeth	fused fifth and sixth left lateral teeth	2
			fused lateral teeth	1
			fused third and fourth right lateral teeth	2
			fused fourth, fifth and sixth lateral teeth	3
			fused third and fourth left lateral teeth	1
		missing teeth	missing sixth right lateral teeth and third left lateral teeth	1
			missing first left lateral teeth	1
		split tooth	split first left lateral teeth	1
			split median tooth	2
			split first right lateral teeth	1
		Kohn gap	gaps in the median teeth of the mentum	1
		extra tooth	extra tooth on the central median tooth	2

	GSM 2	*Chironomus* sp. 1	missing teeth	missing central median tooth and second left lateral teeth	1
				missing sixth left lateral teeth	1
			fused teeth	fused fifth and sixth left lateral teeth	1
				fused fourth and fifth right lateral teeth	1
			lateral gaps	gaps in the left lateral teeth	1
			split tooth	split median tooth	1
	GSM 3	*Chironomus* sp. 1	split tooth	split median and first right lateral teeth	1
Site 4	Veg. 2	*Polypedilum* sp.	split tooth	split median tooth	1
	Veg. 3	*Polypedilum* sp.	split tooth	split median tooth	2
	Veg. 1	*Chironomus* sp. 1	fused teeth	fused fifth and sixth left lateral teeth	1
				all left lateral teeth fused with first lateral tooth	1
			extra teeth	two extra teeth on central median tooth	1
		Tanytarsus sp.	fused teeth	all left lateral teeth fused with first lateral tooth	1
	Stone 3	*Orthocladius* sp.	fused teeth	fused fifth and sixth left lateral teeth	1

Table B10: Number and description of mentum and ligula deformities recorded among chironomid taxa at the four sampling sites during summer. Biotopes replicates are also shown

Sites	biotope replicate	Genus	Deformity type	Description	No. of individual showing deformity
Site 1	Stone1	*Cricotopus trifasciata* gr.	fused teeth	first and second right lateral teeth fused	1
			split tooth	split first lateral tooth	1
		Ablabesmyia sp.	split tooth	split inner tooth	1
	stone 2	*Cricotopus trifasciata* gr.	missing teeth	missing sixth lateral tooth	1
			fused teeth	fused fifth and sixth lateral teeth	1
	GSM2	*Cricotopus* sp. 1	missing teeth	missing fourth, fifth and sixth left lateral teeth	1
				missing fifth right lateral tooth	1
Site 2	Veg. 2	*Kiefferulus* sp.	split	split median tooth	1
			missing teeth	missing fifth left lateral tooth	1
		Dicrotendipes sp.	missing teeth	second left lateral tooth missing	1
				missing first and second right lateral teeth	1
				missing second right lateral tooth	1
			split tooth	split fourth left lateral tooth	1
	Veg. 1	*Kiefferulus* sp.	missing teeth	missing fifth left lateral tooth	1
		Dicrotendipes sp.	missing teeth	second right lateral tooth missing	2
				missing first and second right lateral teeth	1
				missing second right lateral tooth	1
			split tooth	split fifth left lateral tooth	2

Veg. 3	*Dicrotendipes* sp.	missing teeth	sixth left lateral tooth missing	1
			missing first and second right lateral teeth	1
			missing second right lateral tooth	1
GSM 1	*Tanypus* sp.	split tooth	split inner tooth	2
		fused teeth	fused outer and middle teeth	1
			fused outer and inner left tooth	1
		missing teeth	missing two outer teeth	1
		asymmetry	asymmetry between the two inner teeth	1
	Cricotopus trifasciata gr.	missing teeth	missing first and second right lateral teeth	1
	Chironomus sp.1	missing teeth	missing first left lateral tooth (fused fourth fifth and sixth lateral teeth)	1
GSM 2	*Tanypus* sp.	missing teeth	missing left inner tooth	2
			missing left outer tooth	1
		split tooth	split outer right tooth	4
GSM 3	*Chironomus* sp.1	fused teeth	fused fourth, fifth and sixth right lateral teeth	2
		missing teeth	missing outer median tooth	1
	Tanypus sp.	split tooth	split outer right tooth	3
			split outer left tooth	1
Stone 2	*Dicrotendipes* sp.	worn tooth	loss scleretization of the median tooth	1
		fused teeth	fourth and fifth left lateral teeth	1
		missing teeth	second right lateral tooth missing	1
	Chironomus sp.1	fused teeth	fourth, fifth and sixth right lateral teeth fused	1
	Chironomus sp.2	fused teeth	fused fourth and fifth left lateral teeth	1

	Stone 1	*Chironomus* sp1	fused teeth	all three median teeth fused	1
			extra tooth	two extra median teeth	1
			split tooth	split right median and first and second left lateral teeth	1
		Cricotopus sp1	Kohn gap	Kohn gap	1
	Stone 3	Dicrotendipes sp.	worn tooth	loss scleretization of the median tooth	1
			fused teeth	fourth and fifth left lateral teeth	1
			missing teeth	second right lateral tooth missing	1
				missing first left lateral tooth	2
			split tooth	split sixth left lateral tooth	4
			extra tooth	median tooth	2
			Kohn gap	Kohn gap	1
		Chironomus sp.1	fused teeth	fourth, fifth and sixth right lateral teeth fused	1
		Chironomus sp.2	missing teeth	missing sixth lateral tooth	2
Site 3	Stone 1	*Chironomus* sp.1	extra tooth	extra median tooth, missing tooth and fused tooth	2
			split tooth	split fourth left lateral tooth	2
				split second left lateral teeth	6
			asymmetry	asymmetry between right and left lateral teeth	2
		Chironomus sp.3	split tooth	split right third lateral teeth	2
		Chironomus sp. 2	split tooth	split central median tooth	2
			fused teeth	fused first and second lateral teeth	2
	Stone 2	*Chironomus* sp.1	Kohn gap	Kohn gap (twisted median tooth)	2

		split tooth	split first right lateral tooth	3
		fused teeth	fused fifth and sixth right lateral teeth	2
		missing teeth	missing sixth lateral tooth	4
	Chironomus sp.3	missing teeth	missing sixth and fifth left lateral teeth	9
Stone 3	*Chironomus* sp.1	extra tooth	extra median tooth, missing tooth and fused tooth	2
		split tooth	split fourth left lateral tooth	4
			split second left lateral teeth	3
		asymmetry	asymmetry between right and left lateral teeth	2
		Kohn gap	Kohn gap	2
		fused teeth	fused third and fourth left lateral teeth	3
	Chironomus sp.3	split tooth	split right third lateral teeth	2
	Chironomus sp.2	split tooth	split central median tooth	2
		fused teeth	fused first and second lateral teeth	2
	Chironomus sp1	missing teeth	missing fourth, fifth and sixth left lateral teeth	2
			missing sixth lateral teeth	2
		split tooth	split first right lateral teeth	2
Veg. 1	*Chironomus* sp.1	missing teeth	missing sixth left lateral teeth	7
			missing third lateral teeth	2
		Kohn gap	Kohn gap (twisted median tooth)	5
		split tooth	split second left lateral teeth	2
		fused teeth	fused fourth and fifth right lateral teeth	2
	Dicrotendipes sp.	missing teeth	missing sixth left lateral teeth	1
Veg. 3	*Chironomus* sp.1	missing teeth	missing sixth left lateral teeth	4

				missing third lateral teeth	3
			Kohn gap	Kohn gap	3
			split tooth	split second left lateral teeth	4
			fused teeth	fused fourth and fifth right lateral teeth	2
			extra tooth	extra median tooth	1
	GSM1	*Chironomus* sp.1	missing teeth	missing fourth left lateral teeth	1
				missing fourth and sixth left lateral teeth	1
				missing sixth left lateral tooth	4
			fused teeth	fused first and second lateral teeth	2
				fused fifth and sixth right lateral teeth	1
			Kohn gap	Kohn gap	2
			split tooth	split first right lateral teeth	1
			extra tooth	extra median tooth	1
		Chironomus sp.2	split tooth	split sixth lateral teeth	2
Site 4	GSM 2	*Chironomus* sp.1	missing teeth	missing sixth left lateral teeth	3
				sixth teeth on both sides of mentum missing	1
				missing left median tooth	1
			extra tooth	extra central median tooth	1
		Chironomus sp.2	fused teeth	fused fourth, fifth and sixth right lateral teeth	1
			Kohn gap	Kohn gap	2
		Chironomus sp.3	missing teeth	missing sixth lateral tooth	2
			split tooth	split right second lateral tooth	1
	GSM 1	*Chironomus* sp.1	Kohn gap	Kohn gap	1
			fused teeth	fused fifth and sixth left lateral teeth	1

		Chironomus sp.1	extra tooth	extra median tooth	1
	GSM3	*Chironomus* sp.1	split tooth	split outer left median tooth	1
			missing teeth	missing sixth left lateral tooth	2
		Chironomus sp.3	fused teeth	fused fourth and fifth lateral teeth	1
	Stone 1	*Chironomus* sp.1	missing teeth	missing fifth right lateral tooth	1
			split tooth	split first lateral tooth	1
	Stone 3	*Chironomus* sp.1	Kohn gap	Kohn gap	2

Table B11: Number and description of mentum and ligula deformities recorded among chironomid taxa at the four sampling sites during autumn. Biotopes replicates are also shown

Site	biotope replicate	Genus	deformity type	Description	No. of individual showing deformity
Site 1	Stone 1	*Cricotopus trifasciata* gr.	missing teeth	missing second left lateral teeth	1
			fused teeth	fused fifth and sixth right lateral teeth	2
				fused third and fourth right lateral teeth	1
				fused third and fourth left lateral teeth	2
	Stone 3	*Cricotopus trifasciata* gr.	fused teeth	fused fifth and sixth left lateral teeth	1
	GSM 1	*Tanytarsus* sp.	fused teeth	fused fourth and fifth right lateral teeth	1
Site 2	Veg. 1				
		Chironomus sp.1	Kohn gap	Kohn gap	1
			fused teeth	fused fourth and fifth left lateral teeth	1
			missing teeth	missing left median tooth	1
			split tooth	split central median tooth	1
			extra teeth	extra median tooth	1
		Chironomus sp.2	split tooth	split first-third lateral teeth	1
	Veg. 2	*Chironomus* sp.1	missing teeth	missing third left lateral teeth	2
				missing left median tooth	1
	Veg. 3	*Chironomus* sp.1	fused teeth	fused third and fourth right lateral teeth	1
	Stone 1	*Chironomus* sp.1	split tooth	split first left lateral tooth	1
				split fourth left lateral tooth	1
				split left median and first lateral teeth	1

			fused teeth	fused fifth and sixth right lateral teeth	1
		Kiefferulus sp.	fused teeth	fused first and second right lateral teeth	1
		Microchironomus sp.	missing teeth	missing sixth left lateral tooth	1
	Stone 3	*Dicrotendipes* sp.	fused teeth	fused sixth, fifth and fourth right lateral teeth	1
		Chironomus sp.1	missing teeth	missing second right lateral tooth	1
			fused teeth/ missing teeth	fused first and second right lateral teeth, missing fourth left lateral tooth	1
			split tooth	split first right lateral tooth	1
		Tanypus sp.	twisted tooth	twisted outer lateral tooth	1
	GSM 2	*Tanypus* sp.	split tooth	split outer tooth	2
			twisted tooth	twisted outer lateral tooth	1
Site 3	Veg. 1	*Chironomus* sp.1	missing teeth	missing left median tooth	2
				missing first right lateral tooth	2
				missing first and second right lateral teeth	1
				missing right median tooth and twisted first right lateral teeth	1
				missing third right lateral tooth	1
				missing second lateral tooth	1
				missing second left lateral tooth	1
			Kohn gap	Kohn gap	1
			fused teeth	fused sixth, fifth and fourth lateral teeth	1
			split tooth	split first left lateral tooth	1
				split second first lateral tooth	1
	Veg. 2	*Chironomus* sp.2	split tooth	split left median tooth	1
		Chironomus sp.1	extra teeth	extra central median tooth	1

		missing teeth	missing first left lateral tooth	1
			missing right median tooth	1
		Kohn gap	Kohn gap	1
	Chironomus sp.3	missing teeth	missing fourth right lateral tooth	1
		others	distortion in overall configuration	1
Stone 2	*Chironomus* sp.1	missing teeth	missing central median tooth	1
			missing fifth left lateral teeth	4
			missing first left lateral and fourth right lateral tooth	2
			missing first right lateral tooth	1
		extra teeth	extra median tooth	4
		fused teeth	fused first and second left lateral teeth	1
Stone 1	*Chironomus* sp.1	missing teeth	missing first and second right lateral tooth	1
			missing third right lateral tooth	1
			missing fifth right lateral tooth	1
		Kohn gap	Kohn gap	1
		split tooth	split left median tooth	1
			split first left median tooth	1
			split central median tooth	1
			split second left lateral tooth	1
		extra teeth	extra median tooth	1
	Chironomus sp.2	missing teeth	missing sixth left lateral tooth	1
	Chironomus sp.3	missing teeth	missing first right lateral teeth	1
Stone 3	*Chironomus* sp.1	extra teeth	extra third left lateral teeth	2
			extra median tooth	2

			missing teeth	missing sixth lateral teeth	1
				missing second left lateral teeth	2
				missing second right lateral teeth	1
				missing third and fourth right lateral teeth	2
			fused teeth	fused second and third left lateral teeth	3
				fused fifth and sixth left lateral teeth	1
			Extra teeth/fused teeth	extra median tooth, fused third and fourth right lateral teeth	2
			Kohn gap	Kohn gaps	4
		Chironomus sp.2	missing teeth	missing first lateral teeth	1
			extra tooth	extra median tooth	3
	Veg. 3	*Chironomus* sp.1	Kohn gap	Kohn gap	1
			fused teeth	fused fourth, fifth and sixth right lateral teeth	1
			missing teeth	missing fifth left lateral tooth	1
				missing sixth left lateral tooth	1
			split tooth	split second left lateral tooth	1
Site 4	GSM 2	*Chironomus* sp.1	fused teeth	fused fifth and sixth right lateral teeth	1
				fused fourth, fifth and sixth left lateral teeth	1
	Veg. 2	*Chironomus* sp.1	fused teeth	fused third and fourth left lateral teeth	1

Table B12: Number and description of mentum and ligula deformities recorded among chironomid taxa at the four sampling sites during winter. Biotopes replicates are also shown

Site	Biotope replicate	Genus	Deformity type	Description	No. of individual showing deformity
Site 1	Stone 1	*Tanytarsus* sp.	fused teeth	fused fourth and fifth right lateral teeth	1
	Veg. 1	*Polypedilum* sp.	Split tooth	right central median tooth	1
	GSM 3	*Tanytarsus* sp.	missing teeth	Fifth left lateral tooth	1
			fused teeth	fourth and fifth left lateral teeth	1
		Cricotopus trifasciata gr.	fused teeth	Fourth to sixth right lateral teeth	1
	GSM 2	*Cricotopus trifasciata* gr.	split tooth	median tooth	1
	GSM 1	*Tanytarsus* sp.	fused teeth	Fourth and fifth right lateral teeth	1
	Veg. 3	*Tanytarsus* sp.	fused teeth	Fourth and fifth right lateral teeth	1
Site 2	Stone 3	*Chironomus* sp.1	fused teeth	fused median teeth	1
	GSM 2	*Chironomus* sp. 1	fused teeth	Fused fifth and sixth right lateral teeth	2
Site 3	GSM 1	*Chironomus* sp.1	missing teeth	Sixth left lateral tooth	2
				Sixth right lateral tooth	1
				Fifth and sixth left lateral teeth	2
				third left lateral tooth	2
			fused teeth	fifth and sixth left lateral teeth	1
		Chironomus sp.2	missing teeth	first right and sixth left lateral teeth	1
			split tooth	third right lateral tooth	1
	Stone 2	*Chironomus* sp.1	missing teeth	third right lateral tooth	1
				first left and fifth right lateral teeth	1

			sixth right lateral tooth	4
		fused teeth	central and left median teeth, split central median tooth	2
		Extra teeth	left median tooth	4
		split tooth	split median tooth	2
			second left lateral tooth	4
	Chironomus sp. 2	fused/ missing teeth	fused first and second left lateral teeth / missing fifth left lateral tooth	1
		split tooth	split first left lateral tooth	1
		missing teeth	fifth and sixth left lateral teeth	1
Stone 1	*Chironomus* sp.1	missing teeth	right median teeth	2
			third and fourth left lateral tooth	3
		extra tooth	left median tooth	1
		fused teeth	fifth and sixth left lateral teeth	2
			fused third and fourth right lateral teeth	2
		extra / missing teeth	extra right median tooth / missing sixth left lateral tooth	1
Stone 3	*Chironomus* sp. 1	split tooth	first left lateral teeth	**2**
			first and fourth left lateral teeth	**1**
		missing teeth	sixth right lateral teeth	**5**
	Chironomus sp.2	missing teeth	first right lateral and fourth left lateral teeth	**1**
Veg. 3	*Chironomus* sp.1	missing teeth	sixth left lateral tooth	1
			fifth left lateral tooth	1
			second left lateral tooth	1
		split tooth	central median tooth	1
		fused teeth/	median teeth/ extra third left lateral teeth	1

			extra teeth		
		Chironomus sp.2	split tooth	first left lateral tooth	1
	Veg. 2	*Chironomus* sp.1	missing teeth	missing left median teeth	2
			fused teeth	left median and central teeth	2
				fifth and sixth right lateral teeth	3
			Kohn gap	mentum gaps	1
	Veg. 1	*Chironomus* sp. 1	missing /split teeth	missing third left lateral tooth and split first left lateral tooth	1
			missing teeth	missing right median teeth	2
				missing sixth left lateral teeth	3
Site 4	GSM 3	*Chironomus* sp. 1	missing teeth	sixth left lateral tooth	2
			split teeth	first right lateral tooth	1
			fused teeth	first and second right lateral teeth	1
				fourth to sixth left lateral teeth	1
		Chironomus sp. 3	fused teeth	fused median teeth	1
	GSM 2	*Chironomus* sp.1	Split teeth	first left lateral tooth	1
			split/ missing teeth	second left lateral tooth / missing sixth left lateral tooth	1
			Kohn gap	mentum gap	1
			Kohn gap/ missing teeth	mentum gap/ missing right median tooth	2
	GSM 1	*Chironomus* sp.1	missing teeth	central median tooth	3
				fourth left lateral tooth	2
				second left lateral tooth	1
		Chironomus sp. 2	fused teeth	left and central median teeth	1

	Veg. 2	*Chironomus* sp.1	missing teeth	third left lateral tooth	1
				sixth lateral teeth on both sides of mentum	1
			Kohn gap	mentum gap	1
			extra teeth	median tooth	1
			split tooth	left median tooth	1
	Veg. 1	*Chironomus* sp.1	missing teeth	sixth left lateral tooth	1
			Asymmetry	Between left and right first lateral teeth	1
			split teeth	third right lateral tooth	1
	Veg. 3	*Chironomus* sp.1	split tooth	central median tooth	2
				left median tooth	1
			missing teeth	second right lateral tooth	2
				sixth right lateral teeth	3
	Stone 2	*Chironomus* sp.1	fused teeth	first and second right lateral teeth/ fourth and fifth right lateral teeth	1
				fourth and fifth right lateral teeth	2
	Stone 1	*Chironomus* sp. 1	extra teeth	right median tooth	2
			missing teeth	right median tooth	1
	Stone 3	*Chironomus* sp.1	missing teeth	missing fifth left lateral teeth	2
			missing / split teeth	missing sixth lateral tooth/ split left median tooth	1

Table B13: Number and description of mandible deformities recorded among chironomid taxa at the four sampling sites during spring. Biotopes replicates are also shown

Sites	Biotope replicate	Genus	Type of deformities	Descriptions	No. of individual showing deformity
Site 1	Stone 1	*Cricotopus trifasciata* gr.	split tooth	split apical tooth	1
	Stone2	*Orthocladius* sp.	split tooth	split apical tooth	2
		Cricotopus sp.1	split tooth	split apical tooth	1
	Veg. 3	*Polypedilum* sp.	extra tooth	extra tooth on left mandible	1
			split tooth	split apical tooth	1
Site 2	Stone 1	*Cricotopus* sp.1	split tooth	split apical tooth	1
		Orthocladius sp.	split tooth	split apical tooth	1
	Stone2	*Cricotopus* sp.1	missing teeth	missing apical tooth	1
			split tooth	split apical tooth	1
		Cricotopus trifasciata gr.	split tooth	split second left inner tooth	1
				split apical tooth	2
	Veg. 2	*Dicrotendipes* sp.	split tooth	split apical tooth	1
		Orthocladius sp.	split tooth	split apical tooth	1
	Veg. 3	*Cricotopus* sp.1	split tooth	split apical tooth	3
		Orthocladius sp.	split tooth	split apical tooth	1
	Veg. 1	*Dicrotendipes* sp.	twisted apical tooth	twisted left apical tooth	1
	stone 2	*Cricotopus trifiasciata* gr.	asymmetry	between left and right apical tooth	1

Site 3	stone 1	*Chironomus* sp .1	fused teeth	fused apical and first inner mandibular teeth	1
			split tooth	split apical tooth	4
			missing teeth	missing one inner mandibular teeth	3
			Extra teeth	extra one teeth	1
			others	twisted apical; tooth	1
	Veg. 1	*Chironomus* sp. 1	missing teeth	missing one inner mandibular teeth	2
	Veg. 2	*Chironomus* sp. 1	fused teeth	fused apical and dorsal teeth	1
	stone 3	*Chironomus* sp.1	fused teeth	fused apical and first inner mandibular teeth	1
			split tooth	split apical tooth	5
			missing teeth	missing one inner mandibular teeth	3
			Extra teeth	extra one teeth	1
			others	twisted apical; tooth	4
			fused teeth	fused apical and dorsal teeth	4
		Dicrotendipes sp.	split tooth	split apical tooth	1
	stone 2	*Chironomus* sp. 1	missing teeth	missing one inner mandibular teeth	1
			split tooth	split apical tooth	2
	GSM 2	*Chironomus* sp. 1	Asymmetry	between left and right apical tooth	2
	GSM 3	*Chironomus* sp. 1	Asymmetry	Between left and right apical tooth	1
			fused teeth	fused apical and dorsal teeth	1

Site 4	GSM 1	*Cricotopus trifasciata* gr.	twisted apical tooth	twisted apical tooth	1
	Stone 1	*Dicrotendipes* sp.	Asymmetry	between left and right apical tooth	1

Table B14: Number and description of mandible deformities recorded among chironomid taxa at sites 2, 3 and 4 during summer. Biotopes replicates are also shown. No individual at site 1 had deformity in the mandible during summer

Site	Biotope replicate	Genus	Type of deformity	Description	No. of individual showing deformity
Site 2	stone 2	*Chironomus* sp.1	asymmetry	between right and left apical tooth	1
		Kiefferulus sp.	asymmetry	between right and left apical tooth	1
		Dicrotendipes sp.	twisted tooth	twisted apical tooth	1
	Stone 1	*Chironomus* sp.1	missing tooth	missing third sub apical tooth	1
	Stone 3	*Dicrotendipes* sp.	split tooth	split apical tooth	2
			twisted tooth	twisted apical tooth	1
		Chironomus sp.1	asymmetry	between left and right apical tooth	1
	Veg. 2	*Dicrotendipes* sp.	asymmetry	Between left and right apical tooth	1
			missing tooth	missing apical tooth	1
	Veg. 1	*Dicrotendipes* sp.	fused tooth	fused third and second inner teeth	1
			split tooth	split apical tooth	1
		Chironomus sp.1	missing tooth	missing third sub apical tooth	1
	GSM1	*Tanypus* sp.	fused tooth	fused first and second sub apical teeth	1
	GSM2	*Tanypus* sp.	split tooth	split apical tooth	2
Site 3	Stone 1	*Chironomus* sp.1	asymmetry	between right and left apical tooth	2
			split tooth	split apical tooth	1
		Chironomus sp.3	fused tooth	fused apical and first inner teeth	1
	Stone 2	*Chironomus* sp.1	missing tooth	missing third inner left mandibular tooth	2
			split tooth	split apical tooth	1
			fused tooth	fused apical and dorsal tooth	1

	Chironomus sp.3	split tooth	split apical tooth	1
Stone 3	*Chironomus* sp.1	split tooth	split first inner left mandibular tooth	2
		missing tooth	missing dorsal tooth	1
			missing second sub apical tooth on the left mandible and third on the right mandible	1
			missing third sub apical tooth on left mandible	2
		twisted tooth	twisted apical tooth	3
		fused tooth	fused third and second inner right mandibular teeth	2
	Chironomus sp.2	asymmetry	between left and right apical teeth	2
Veg. 2	*Chironomus* sp.1	missing teeth	missing third inner tooth	2
		asymmetry	Between left and right apical teeth	1
Veg. 1	*Chironomus* sp.1	split tooth	split apical tooth	2
	Dicrotendipes sp.	split tooth	split apical tooth	1
Veg. 3	*Chironomus* sp.1	twisted tooth	twisted apical tooth	2
		split tooth	split apical tooth	1
		missing tooth	missing third sub apical tooth	2
	Dicrotendipes sp.	split tooth	split apical tooth	2
GSM1	*Chironomus* sp.1	asymmetry	between left and right apical	2
		split tooth	split first sub-apical tooth	3
		missing tooth	missing third sub apical tooth	1
			missing third sub apical tooth on both mandible	1
		fused tooth	fused dorsal and apical teeth	1
	Dicrotendipes sp.	split tooth	split apical tooth	2

			missing tooth	missing third sub apical tooth	1
Site 4	GSM2	*Chironomus* sp.1	extra teeth	extra tooth on left mandible	1
			asymmetry	Between left and right apical tooth	1
	GSM3	*Chironomus* sp.1	split tooth	split apical tooth	2
		Chironomus sp.2	split tooth	split apical tooth	1
	GSM1	*Chironomus* sp.1	fused tooth	fused second and third left mandibular teeth	1
		Ablabesmyia sp.	split tooth	split apical tooth	1
	Stone 1	*Chironomus* sp.1	split tooth	split apical tooth	2

Table B15: Number and description of mandible deformities recorded among chironomid taxa at sites 2, 3 and 4 during autumn. Biotopes replicates are also shown. No individual at site 1 had deformity in the mandible during autumn

Site	biotope replicate	Genus	Types of deformity	Description	No. of individual showing deformity
Site 2	Stone 3	*Chironomus* sp.1	missing teeth	missing third inner tooth	1
Site 3	Stone 2	*Chironomus* sp.1	split tooth	split apical tooth	3
	Stone 1	*Chironomus* sp.1	missing teeth	missing third inner tooth	4
			asymmetry	between left and right apical tooth	1
	Stone 3	*Chironomus* sp.1	split tooth	split left apical tooth	2
			fused teeth	fused second and third inner teeth	2
			missing teeth	missing third inner left tooth	2
	Veg. 1	*Chironomus* sp.1	split tooth	split apical tooth	1
				split second inner tooth	1
	Veg. 2	*Chironomus* sp.1	missing teeth	missing third inner tooth	1
		Chironomus sp.3	split tooth	split first and third inner teeth	1
	Veg. 3	*Chironomus* sp.1	missing teeth	missing third inner tooth	2
	GSM 2	*Chironomus* sp.1		fused apical and first inner tooth	1
			fused teeth	fused second and third inner teeth	1
Site 4	Veg. 2	*Chironomus* sp.1	twisted tooth	twisted apical tooth	1
			missing teeth	missing apical and third inner teeth	1

Table B16: Number and description of mandible deformities recorded among chironomid taxa at sites 2, 3 and 4 during winter. Biotopes replicates are also shown. No individual at sites 1 and 2 had deformity in the mandible during winter

Site	Biotope replicate	Genus	Types of deformity	Description	No. of individual showing deformity
Site 3	Veg. 3	*Chironomus* sp. 1	missing teeth	third inner tooth	1
Site 4	GSM 3	*Chironomus* sp. 2	missing teeth	third inner tooth	1
		Chironomus sp. 3	split tooth	apical tooth	1
	Veg. 2	*Chironomus* sp.1	missing teeth	third inner tooth	2
			split tooth	dorsal tooth	1

Table B17: Number and description of antennal deformities recorded among chironomid taxa at sites 1, 2 and 3 during spring. Biotopes replicates are also shown. No individual at sites 4 had deformity in the antennal during spring

Site	Biotope replicate	Genus	Type of deformity	Description	No. of individual showing deformity
Site 1	Stone 2	*Tanytarsus* sp.	Lost lauterborn organ	Lost lauterborn organ on right antenna	1
	Stone 3	*Cricotopus* sp.1	displaced lauterborn organ	lauterborn organ displaced from segment 2 to segment 3	1
		Ablabesmyia sp.	lost segment	lost fourth and fifth segments	1
				style and lauterborn organ displaced	1
	Veg. 3	*Polypedilum* sp.	Lost lauterborn organ	lauterborn organ and distal segment on right antenna lost	1
	Veg. 1	*Ablabesmyia* sp.	lost segment	lost third segment	1
	Stone 1	*Cricotopus* sp.1	lost segments	lost fourth and fifth segments	5
		Polypedilum sp.	displaced ring organ	displacement of ring organ from basal to second segment	1
Site 2	Stone 1	*Cricotopus* sp.1	displaced lauterborn organ	lauterborn organ displaced from second to segment	1
	Veg. 3	*Cricotopus* sp.1	lost segments	Lost fourth and fifth segment on left antenna	1
			displaced ring organ	displaced ring organ to upper half of basal segment	1
Site 3	Stone 1	*Chironomus* sp.	displaced ring organ	displacement of ring organ and lauterborn organ	3
			lost segment	lost fourth and fifth segments on right antenna	1
				lost segment fifth segment	1
	Veg. 1	*Chironomus* sp1	additional segment	additional segment between first and second segments on left antenna	1
			asymmetry	asymmetry between right and left antenna	1

			lost segment	lost fourth and fifth segments on left antenna	1
			displaced ring and lauterborn organ	displacement of ring and lauterborn organ	2
	Veg. 2	*Chironomus* sp.1	displacement of ring organ	displacement of ring organ	2
			lost segment	lost fourth and fifth segments	1
	GSM1	*Chironomus* sp1	lost blade	lost blade on right antenna	3
				blade, style and accessory blade on left antenna lost	2
	Stone 3	*Chironomus* sp. 1	lost segment	lost fourth and fifth segments on left antenna	1
			displaced ring/lauterborn organ	displacement of ring and lauterborn organ	4
			displacement of ring organ	displacement of ring organ	2
			lost segments	lost fourth and fifth segments	2
			lost blade	lost antenna blade	1
				blade, style and accessory blade on left antenna lost	1
	Stone 2	*Chironomus* sp. 1	lost segments	lost fifth segment on right antenna	1
			displace lauterborn organ	lauterborn organ displaced from tip of second segment to basal segment	1
			reduced blade	blade on right antenna shorter than those of left antenna	2
	GSM2	*Chironomus* sp. 1	lost segment	Lost third to fifth segments on left antenna	1
	GSM 3	*Chironomus* sp. 1	Lost segment	Lost fifth segment on left antenna	1
			lost blade	lost blade on left antenna	1

Table B18: Number and description of antennal deformities recorded among chironomid taxa at sites 1, 2 and 3 during summer. Biotopes replicates are also shown. No individual at sites 4 had deformity in the antennal during summer

Site	Biotope replicate	Genus	Types of deformity	Description	No. of individual showing deformity
Site 1	Veg. 3	*Tanytarsus* sp.	lost segment	lost fourth and fifth segments	1
		Cricotopus trifasciata gr.	Lost segment.	lost fifth segment	2
	stone 2	*Cricotopus* sp.1		displaced lauterborn organ	2
	Stone 1	*Cricotopus trifasciata* gr.		displaced ring organ	1
			Lost segment.	lost fifth segment	1
	GSM 1	*Cricotopus* sp.1	lost segment	lost fifth segments both antennae	1
		Orthocladius sp.	lost segment	lost fifth segment	1
Site 2	Veg. 2	*Kiefferulus* sp.	lost segment	lost third to fifth segments on right antenna	1
		Dicrotendipes sp.	lost blade		1
			lost segment	Lost fourth and fifth segment on right antenna	3
	Veg. 1	*Dicrotendipes* sp.	lost segment	Fourth and fifth segments	3
				Fifth segment on both antenna	1
				Fifth segment on left antenna	2
			Displaced lauterborn organ	displacement of lauterborn organ	2
			lost blade	lost antenna blade on right antenna	2
			displaced style	Style displaced to third segment	1
	Veg. 3	*Dicrotendipes* sp.	lost segment	lost fifth segment on left antenna	2
			Displaced lauterborn organ	displaced lauterborn organ	2
	GSM 1	*Tanypus* sp.	lost style	lost style on both antenna	1

			antenna blade lost	antenna blade lost on left antenna	1
	GSM 2	*Tanypus* sp.	lost segment	Lost fourth and fifth segments	5
				Lost fifth segment	1
			lost lauterborn organ		3
	GSM 3	*Tanypus* sp.	Lost segment	Lost fourth and fifth segments on left antenna	4
	Stone 2	*Dicrotendipes* sp.	lost segment	Lost antenna fifth segment	1
				segment 4 and 5 lost	1
	Stone 1	*Tanypus* sp.	Lost segment	Lost third to fifth antenna segments	2
Site 3	Stone 1	*Chironomus* sp.1	lost blade	Right antenna blade	1
				Right lauterborn organ	1
				Left blade and accessory blade	1
	Stone2	*Chironomus* sp.1	lost segment	fifth segment	1
			lost lauterborn organ and style	lauterborn organ and style	2
	Stone 3	*Chironomus* sp.1	lost segment	Fourth and fifth segments	1
			Displaced lauterborn organ	lauterborn organ on third instead of second segment	3
	Veg. 2	*Chironomus* sp.1	lost segment	Fifth segment	1
		Chironomus sp.2	lost lauterborn organ	Right antenna lauterborn organ	3
	Veg.1	*Chironomus* sp.1		Right antenna blade	1
	Veg. 3	Chironomus sp2	displaced lauterborn organ	displaced lauterborn organ	1
		Chironomus sp1	Lost ring organ	Right basal segment ring organ	2

Table B19: Number and description of antennal deformities recorded among chironomid taxa at sites 2 and 3 during autumn. Biotopes replicates are also shown. No individual at sites 1 and 4 had deformity in the antennal during autumn

Site	Biotope replicate	Genus	Types of deformity	Description	No. of individual showing deformity
Site 2	Veg. 1	*Chironomus* sp.1	lost style and lauterborn organ	Right antenna style and lauterborn organ	2
	Stone 1	*Chironomus* sp.1	displace lauterborn organ	lauterborn organ on third instead of second segment	1
	Stone 3	*Chironomus* sp.1	lost antenna blade	Right antenna	2
			lost lauterborn organ	Right antenna	1
Site 3	Veg. 2	*Chironomus* sp.1	lost blade	Left antenna	1
			lost segment	Fifth segment on left antenna	1
	Veg. 1	*Chironomus* sp.1	lost segment	fourth and fifth left antenna segment	2
			lost blade	Left antenna blade	1
			displaced lauterborn organ	lauterborn organ on third instead of second segment	1
			lost style and lauterborn organ	Left antenna	2
			lost segment	Fifth left antenna segment	2
	Stone 1	*Chironomus* sp.1	Lost segment	lost antenna fifth segment[8]	2
				Fourth and fifth segment [10]	1
	Stone 2	*Chironomus* sp.1	lost segment	Fourth and fifth segment [3]	1

Table B20: Number and description of antennal deformities recorded among chironomid taxa at sites 1, 3 and 4 during winter. Biotopes replicates are also shown. No individual at site 2 had deformity in the antennal during winter

Site	**Biotope replicate**	**Genus**	**Types of deformity**	**Description**	
Site 1	Veg. 1	*Tanytarsus* sp.	lost lauterborn organ	on right antenna	3
					3
Site 3	GSM 1	*Chironomus* sp. 1	lost segment	fifth segment	1
	Stone 2	*Chironomus* sp. 1	lost style	style on left antennae	1
			lost segment	third to fifth segments	1
Site 4	GSM 3	*Chironomus* sp. 1	lost style	on left antenna	1
	Veg. 2		missing segment	Fifth segment on left antenna	1
	GSM 2	*Chironomus* sp. 1	lost style	on left antenna	1

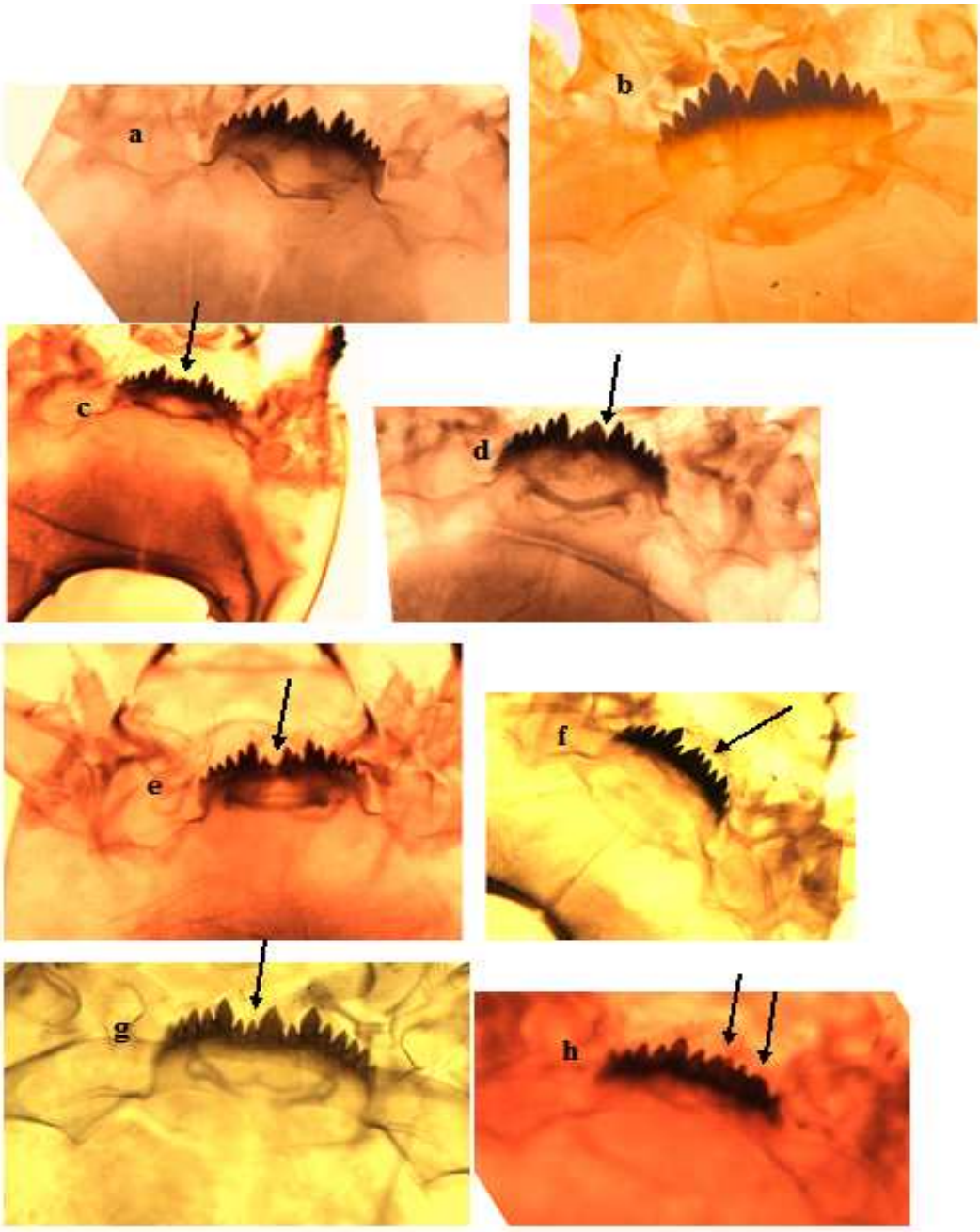

Figure B1: Normal and deformed menta of Chironomidae**. (a)** normal mentum of *Chironomus* sp. 1 (b) normal mentum of *Chironomus* sp. 2 (c) split median teeth (d) missing left median tooth (e) kohn gap (f) fused median teeth (g) Extra right median teeth (h) fused median and third and fourth lateral teeth .

Figure B1 continues: Normal and deformed menta of Chironomidae. (h) missing median tooth (i) missing second left lateral tooth (j) normal mentum of *Cricotopus* sp 1. (k) missing 3rd right lateral tooth (l) kohn gap (m) split 3rd right lateral tooth (n) Normal mentum of *Tanytarsus* sp. (o) missing 5th right lateral tooth .

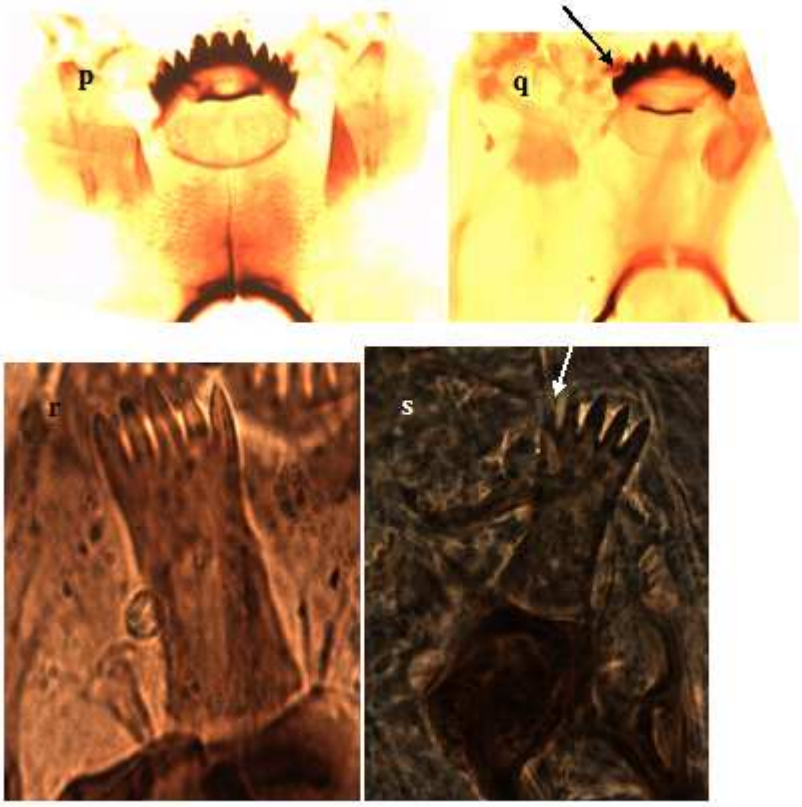

Figure B1 continues: Normal and deformed menta and ligulae of Chironomidae. (p) normal mentum of *Dicrotendipes* sp. (q) missing tooth (r) normal ligula of *Tanypus* sp. (s) split tooth

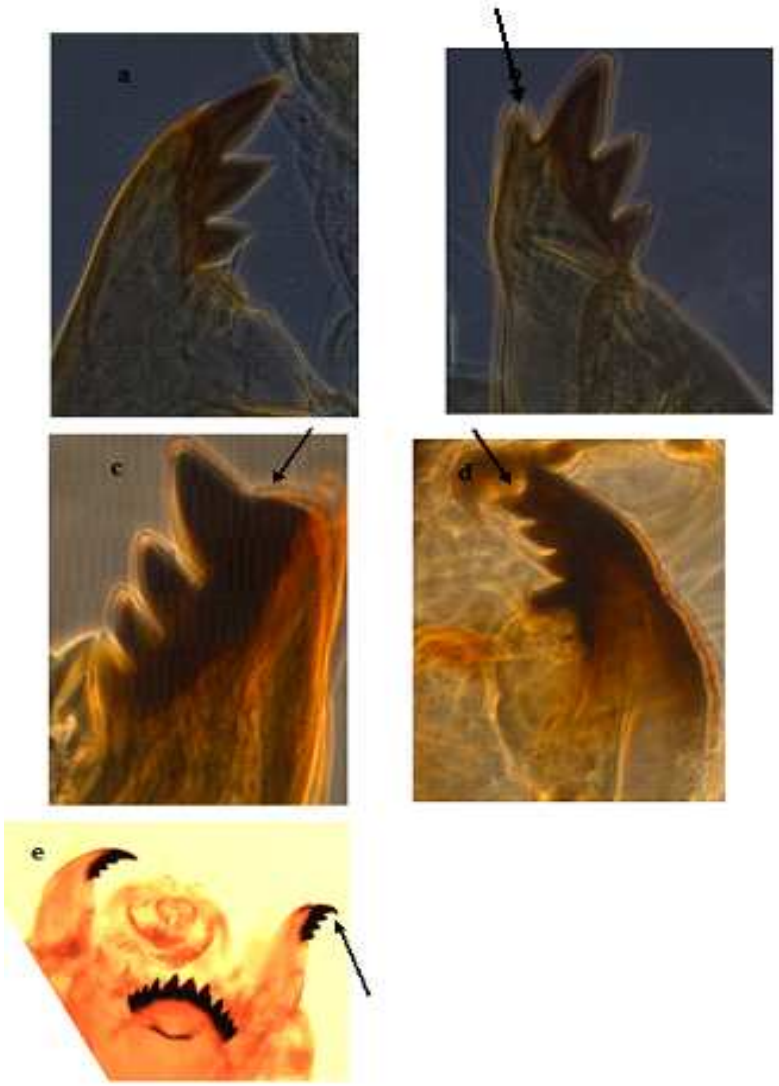

Figure B2: Normal and deformed mandibles of Chironomidae (a) normal Mandibles of *Chironomus* sp1. (b) normal mandibles of Chironomus sp.1 illustrating the dorsal tooth (c) missing dorsal tooth of *Chironomus* sp.1 (d) split apical tooth of *Cricotopus* sp1. (e) twisted apical tooth of *Dicrotendipes* sp.

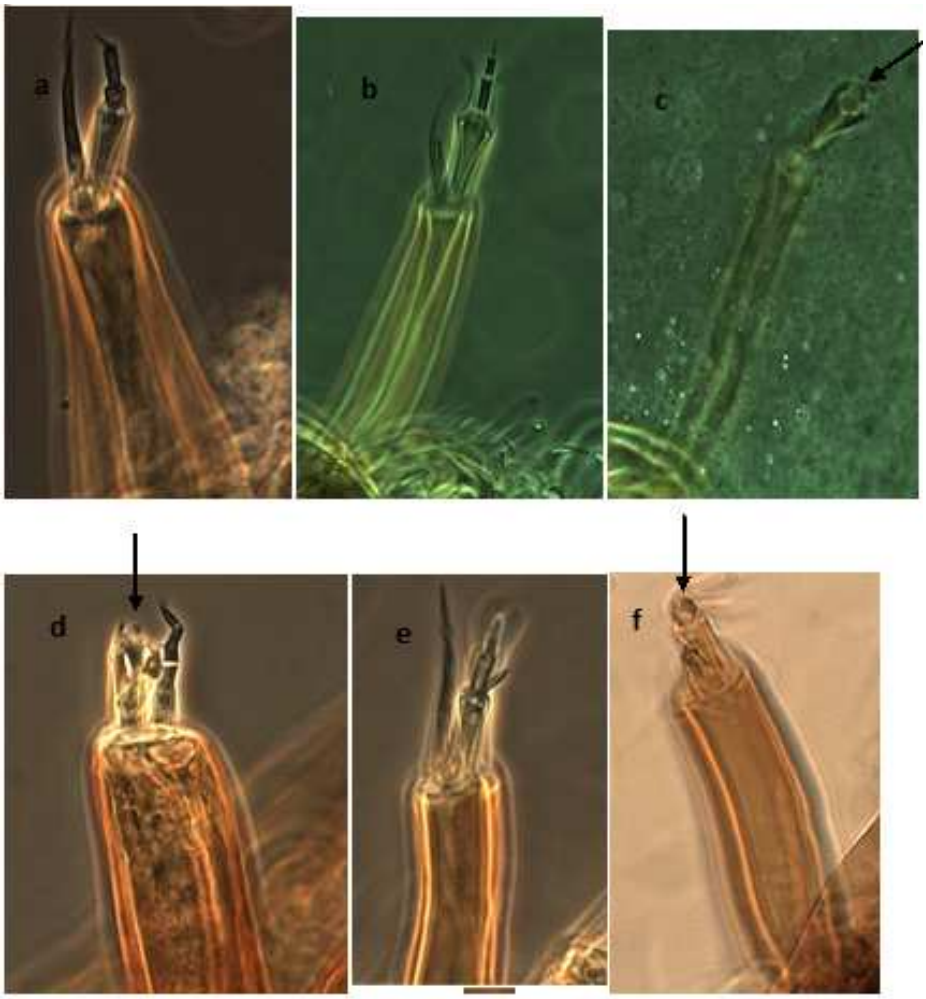

Figure B3: Normal and deformed antennae of Chironomidae (a) normal antenna of *Chironomus* sp1. (b) normal antenna of *Tanytarsus* sp.(c) lost 5th segment in *Tanytarsus* sp. (d) deformed blade of *Chironomus* sp 1. (e) normal antenna of *Dicrotendipes* sp. (f) lost segments and lauterborn organ.