TAXONOMIC AND TRAIT-BASED RESPONSES OF THE ORDERS EPHEMEROPTERA, PLECOPTERA, ODONATA, AND TRICHOPTERA (EPOT) TO SEDIMENT STRESS IN THE TSITSA RIVER AND ITS TRIBUTARIES, EASTERN CAPE, SOUTH AFRICA

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ABSTRACT

Increased urbanization and industrialisation due to human population growth and associated high demand for food have led to widespread disturbances of freshwater ecosystems and associated resources. A widely recognised consequence of these disturbances is the excessive delivery of sediments into the freshwater ecosystems, which severely affects the functioning and integrity of these systems.. The major water quality impairment in the Tsitsa River and its tributaries, situated in the Mzimvubu catchment in the Eastern Cape Province of South Africa, is known to be excessive sediment input. In this study, the application of macroinvertebrates taxonomic-based and trait-based approaches was used to assess the responses and vulnerability of Ephemeroptera, Plecoptera, Odonata and Trichoptera (EPOT) species to settled and suspended sediments stress in eight selected sampling sites in the Tsitsa River and its tributaries. The eight selected sites were Site 1 (Tsitsa upstream), Site 2 (Tsitsa downstream), Site 3 (Qurana tributary), Site 4 (Pot River upstream), Site 5 (Pot River downstream), Site 6 (Little Pot River), Site 7 (Millstream upstream) and Site 8 (Millstream downstream). The methods used in this study involved the analysis of water physico-chemical variables as well as sediment characteristics, derivation of five EPOT metrics, EPOT species-level taxonomic analysis, individual EPOT trait analysis and the development of a novel trait-based approach using a combination of traits. The sampling of EPOT taxa was done using the SASS5 protocols. Identification of EPOT was done to genus/species level and all data were subjected to relevant statistical analysis. The results of ecological categories derived for the physico-chemical variables generally indicated the ecological categories A and B, which was indicative of good water quality conditions. The result of sediment particle analysis revealed four distinct site groups: site group 1 (Tsitsa River upstream and Qurana tributary), site group 2 (Tsitsa River downstream and Millstream upstream), site group 3 (Pot River, both upstream and downstream, and Millstream downstream) and site group 4 (Little Pot River). The species-level taxonomic analysis of EPOT revealed that site group 1 was the most sediment-influenced sites whereas site group 4 was the least sediment-influenced. Species such as *Paragopmhus* sp., *Aeshna* sp. and *Baetis* sp. were considered sediment-tolerant with strong positive association with site group 1. The novel trait-based approach developed in this study proved useful in predicting the responses of EPOT species to sediment stress, and further discriminated between the study sites.

The approach was used to group EPOT species into four vulnerability classes. The result showed that filter feeding EPOT species that have filamentous gills, preferring stone biotopes and feeding on detritus (FPOM) were mostly classified as highly vulnerable to sediment stress and indicated no significant association with the highly sediment-influenced site group 1. The TBA largely corresponded well to the predictions made with the relative abundance of the vulnerable class decreasing in the sediment-influenced sites compared to the tolerant and highly tolerant classes. Overall, the study revealed the importance of the complementary use of taxonomic and trait-based approaches to biomonitoring.

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LIST OF ABBREVIATIONS

ANOSIM	Analysis of similarity
ANOVA	Analysis of variance
AEV	Acute Effect Value
APHA	American Public Health Association
ASPT	Average score per taxon
AUSRIVAS	Australian River Assessment System
BMWP	Biological monitoring working for party
CCA	Canonical correspondence analysis
CEV	Chronic effect Value
CPOM	Coarse particulate organic matter
DCA	Detrended Canonical Analysis
DWAF	Department of Water Affairs
DWS	Department of Water and Sanitation
EC	Electrical Conductivity
EPOT	Ephemeroptera, Plecoptera, Odonata and Trichoptera
EPT	Ephemeroptera, Plecoptera and Trichoptera
EWQ	Environmental Water Quality
FPOM	Fine Particulate Organic Matter
GSM	Gravel Sand and Mud

HBI	Hilsenhoff’s Biotic Index
HFC	Habitat template concept Index
HTC	Habitat Template Concept
IHAS	Integrated Habitat Assessment System Inter-quartile
IWRM	Integrated water resources management
MM1	Multimetric index
NMDS	Non-metric multidimensional scaling National
NWA	National Water Act
NWRS	National Water Resource Strategy
PPPMI	Piabanha-Paquequer-Preto Multimetric Index
RDM	Resource Directed Measures
RIVPACS	River Invertebrate Prediction and Classification System
RQOs	Resource Quality Objectives
RWQOs	Resource Water Quality Objectives
SASS5	South African System version 5
SAWQGs	South African Water Quality Guidelines
SDC	Source directed controls
SIC	Stone-in-current

SIMPER	Similarity percentage
SOOC	Stone-out-of-current
TBA	Traits-based approach
TIN	Total inorganic nitrogen
TWQR	Target Water Quality Range

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DEDICATION

This thesis is dedicated to my mom Mrs Mary Akamagwuna, dad Mrs Jonathan Akamagwuna and to my younger brother Onyeka Akamagwuna.

CHAPTER 1: GENERAL INTRODUCTION AND LITERATURE REVIEW

1.1 Introduction

Freshwater ecosystems continue to deteriorate globally due to a range of human-induced factors, including urbanization, industrialisation and a growing human population (Strayer & Dudgeon, 2010; Vörösmarty *et al.*, 2010; Kopf *et al.,* 2017)**.** Human population growth is projected to increase from seven billion in 2011 to over nine billion by 2050, with implications for food demand and agricultural activities, which could influence land use and the consequent impact on freshwater ecosystem health and integrity (Davis e*t al.,* 2015; FAO 2010; UN, 2016; UNDESA, 2009). Urbanization has caused a severe demand for freshwater resources such as water supply and freshwater ecosystem services, including the use of riverine ecosystems as wastestreams of municipal effluents (Vörösmarty *et al*., 2010; Wagenhoff *et al.,* 2012; Davis *et al.,* 2015). These activities have led to widespread disturbances of freshwater ecosystems, causing habitat degradation and pollution, flow regulation, an overexploitation of resources and the introduction of alien species (Strayer & Dudgeon, 2010; Steffen *et al.,* 2015; Zhang *et al.,* 2017). Landscape degradation can accelerate the input and delivery of nutrients, organics, and other forms of pollutants, including sediments, into the stsream and riverine ecosystems (Zhang *et al.,* 2017). For example, landscape degradation resulting from agricultural activities accounts for 48% of stream pollution from excessive sediment loads in the United States of America (USA) (Sutherland *et al.,* 2012). Similarly in South Africa, landscape degradation is considerred to be the major source of sediment delivery (Le Roux & Sumner, 2013). It has been projected that without improved efficient measures, agricultural pressure on freshwater ecosystems is expected to increase by about 20% by 2050 globally (Bossa *et al.,* 2014).

Domestic and industrial water demands are expected to rise, particularly in cities and countries undergoing rapid economic growth. Water demand for electricity generation will also grow significantly (Bossa *et al.,* 2014). An excessive load of pollutants such as sediments, arising from anthropogenic activities in surrounding catchments, are among the main sources of degradation of the quality of freshwater ecosystems (Larsen *et al.,* 2011; Tiecher *et al.,* 2017).

Sediments (inorganic and organic particles of < 2000 µm in diameter) (Waters, 1995) are a natural component of the aquatic ecosystems, but levels beyond the natural background level of the ecosystems have been implicated as a major stressor of water quality in riverine ecosystems (Walling and Fang, 2003; Conroy *et al.,* 2016; Doretto *et al*., 2017; Vercruysse *et al.,* 2017). For example, elevated sediments in freshwater ecosystems affect channel morphology, water flow and turbidity (Mathers *et al.,* 2017). Many of these changes occasioned by sediment inputs are detrimental to aquatic biota through effects on respiration, micro-habitat, physico-chemical characteristics, food web and food quality, and availability (Bryce *et al*., 2010; Kemp *et al.*, 2011). Fine sediments usually transport contaminants such as heavy metals, polychlorinated biphenyls (PCB), phosphorus, pesticides, pathogens and fallout radionuclides, all of which modify the water physico-chemical conditions of the ecosystems, and act as stressors of varying degrees to aquatic fauna (Yi *et al.,* 2016; Ignatavičius *et al.,* 2017). Overall, these sediment-bound contaminants affects the quality of freshwater ecosystems and reduce their productivity and function (De Lange *et al*., 2005).

Human activities associated with land use practices appear to be the major drivers that cause the delivery of excessive sediments into freshwater ecosystems. These activities may include agricultural practices, mining, forestry, road construction and urban and municipal runoffs (Leslie and Lamp, 2017). For example, the United Nations Education, Scientific and Cultural Organization (UNESCO), have reported that elevated sediments delivery into freshwater ecosystems around the world is mainly due to increasing agricultural activities such as removal of riparian vegetation to increase crop production and livestock grazing (UNESCO, 2011) Furthermore, cultivation of row crops in rich and productive floodplains increases the likelihood of fine sediment surface runoffs and this is particularly true for countries such as the United States of America (USA), Canada, Australia and South Africa (Waters, 1995; Le Roux & Sumner, 2013; Neal & Anders, 2015).

In South Africa, sedimentation of rivers is one of the leading causes of water quality degradation (Le Roux & Sumner, 2013), exacerbated by other interacting factors, which include soil erosivity, slope steepness, flow and rainfall variabilities (Msadala *et al*., 2010). The major causes of elevated sediment loads in South African rivers may be classified into in-channel and non-channel sources, where channel sources are those derived from the beds and banks of rivers and streams as a result of widespread erosion (Basson *et al.,* 2010). Non-channel sources come mainly from the broader landscape and may include activities such as logging, agricultural activities and urban development, which in turn influence soil erosivity and thus delivery of fine sediments into the lotic systems (Le Roux *et al.,* 2008; Lorentz *et al.,* 2012; Gordon *et al*., 2013). Over 70% of South Africa's surface area has been affected by various degrees of soil erosion (Le Roux *et al.,* 2007; Le Roux *et al.,* 2008; Collins *et al.,* 2016), making most riverine systems in South Africa, and in particular in the Eastern Cape province, prone to sedimentation.

The Eastern Cape province of South Africa, where this study was undertaken, is currently ranked among the most severely impacted by soil erosion in the country (Le Roux *et al.,* 2007; Foster *et al.,* 2017). The region is characterised by highly unstable soils that are prone to erosion, evidenced in large-scale gullies that characterise riverine catchments, particularly in the northern part of the province (Msadala *et al*., 2010). Duplex soils, highly sensitive to erosion, with high clay content from the topsoils to the subsoils horizon, are the predominant soil types in the Eastern Cape (Le Roux *et al.*, 2015). The soils are usually vertic, melanic or plinthic in nature and as a result, they inhibit root growth and limit infiltration, which leads to increased runoff and erosion (Le Roux *et al*., 2008; Le Roux *et al*., 2015). Fey & Gilkes (2010) have indicated that more than 90% of the soils in the Eastern Cape are characterised by duplex. Mzimvubu catchment, which includes the Tsitsa River and its tributaries in the Eastern Cape where this study was undertaken, consists of highly erodible duplex soils, placing it among the highest sediment yielding regions in South Africa (Msadala *et al*., 2010).

The Mzimvubu River catchment, comprising Mzimvubu, Tsitsa, Tina, Mzintlava and Knira Rivers in the Eastern Cape of South Africa, covers an area of 19 826 km^2 and provides ecosystem service for the economic development of the mostly rural and peri-urban communities in the catchment. The river catchment also supports a wide array of biodiversity, serving as an important conservation hotspot (Bäse *et al*., 2001) and is being recognised as one of the few remaining rivers in their near-natural condition. For instance, it supports more than 70 vulnerable and endangered plant and animal species (ERS, 2011). In particular, the Tsitsa River and its tributaries, which include the Pot River, Little Pot, Millstream and Mooi Rivers, support several aquatic biota, as well as providing ecosystem services to the resident population (Nel *et al.,* 2011), yet the landscape of the catchment is highly erodible, leading to instream sedimentations, which in turn cause biodiversity and water quality impairments (Le Roux *et al*., 2015). The main water quality impact in the catchment has been attributed to excessive sediment run-off due to the highly erodible nature of the soils in the catchment, poor land management practice and overgrazing (Parwada & Van Tol, 2017).

Despite the fact that elevated sediments input into the Tsitsa River and its tributaries is the major cause of water quality and biodiversity impairments, very few studies, such as Base *et al.* (2001), Madikizela & Dye (2010) & Gordon *et al*. (2013), have explicitly investigated the impacts of elevated sediments on water quality and macroinvertebrates, particularly at the species level (Madikizela *et al*., 2010; Gordon *et al*., 2013). The family-level taxonomic resolution has been the focus of most of the available studies ((Madikizela and Dye, 2010). There have also been no integrations of taxonomic and trait-based evaluations of the potential effects of sediments on macroinvertebrates, particularly at the species level. This study, therefore, focuses on both taxonomic and trait-based responses of the species of the orders Ephemeroptera, Plecoptera, Odonata and Trichoptera (EPOT).

In this study, the EPOT were selected as the object of investigation because they are among the most sensitive taxa to water quality impairments, and are highly diverse in terms of both taxonomic and functional diversity (Waters, 1995; Hogg & Norris, 1991). Further, taxa of the EPOT play an important role in freshwater ecosystems by serving as a link in nutrient cycling in the aquatic food web and can be found in a wide array of habitats (Altermatt *et al.,* 2013). In the context of sediments, the EPOT are known to respond to environmental stressors and an anlysis of the distribution of their trait can proved to be good indicators of sediment stress (Rabení *et al*., 2005; Buendia *et al*., 2013).

The trait-based approach (TBA) is rooted in theoretical ecological concepts such as the habitat template concept (HTC), habitat filtering concept (HFC) and functional diversity, redundancy and uniqueness (Schemera *et al*., 2017). Its application has not gained popularity in South Africa (Odume, 2014; Odume *et al.,* 2018), and therefore this study is among the first few to explicitly apply the approach to a specific aquatic stressor. The TBA is premised on the fact that the underlying interactions between an organism and its external environment are mediated by traits, and thus organisms are able to adapt to and survive in their environment because they possess the right combination of traits. Therefore, explicitly paying attention to traits by focusing on the underlying processes and interactions between the organism and its external environment, can turn descriptive community ecology into a predictive science (Mondy & Schuwirth, 2017). In this regard, traits that mediate between the organism and its environment are seen as key to achieving the goal of predicting an organism's response to environmental change, as well as diagnosing environmental stressors through mechanistically linked traits (Schmera *et al*., 2017).

Therefore, by using species of the orders Ephemeroptera, Plecoptera, Odonata and Trichoptera (EPOT), this study aims to evaluate the taxonomic and trait-based responses of macroinvertebrates to potential effects of sediments in the Tsitsa River and its tributaries, with a view to developing an approach for predicting the potential vulnerability of the EPOT taxa to elevated sediments.

The rest of this chapter is devoted to a detailed literature review, beginning with water resource management in South Africa, followed by environmental water quality (EWQ) as an approach to managing water quality in South Africa. The chapter further reviews sediment as a water quality stressor, paying attention to effects, types and sources of sediments. Biomonitoring, including taxonomic and trait-based approaches, is reviewed. The chapter ends with the rationale of the study, research questions, and aim and objectives, as well as the overall thesis structure.

1.2 Water Resource Management in South Africa

The Department of Water and Sanitation is the custodian of water resource management and policy implementation in South Africa (DWAF, 2010). Three key values namely equity, efficiency and sustainability guide the management of water resources in South Africa. In South Africa, the National Water Act (NWA), Act No 36 of 1998 is the legislative and legal framework guiding the management of water and related resources (RSA, 1998). It sets out the ground rules, procedures, principles and overreaching framework for managing, conserving, controlling, protecting and using water resources in South Africa (RSA, 1998).

The NWA emphasises the equitable, sustainable and efficient management of water resources, in a manner that balances use and protection to ensure that all water users are adequately served by the nation's water resources (DWAF, 2013). To give effect to and realise its objectives, the Act mandates the Minister of Water and Sanitation to develop a National Water Resource Strategy (NWRS), which must be reviewed every five years. The NWRS is a document that provides the roadmap for the realisation of the intent of the Act (RSA, 1998; DWAF, 2013).

1.2.1 The National Water Resource Strategy (NWRS)

The National Water Resource Strategy contains strategies, plans, and procedures on how water and related resources are to be protected, used, developed, conserved, managed and controlled in accordance with the requirements of the NWA (DWAF, 2013). The second edition of the NWRS (NWRS2) builds on the first edition that was published in 2004 (DWAF, 2004). The key objectives outlined in the NWRS2 include i) Use water to support development, elimination of poverty and social and economic inequality, ii) use water to contribute to economic development of the country and for job creation and iii) ensure that water is protected, conserved, managed and controlled sustainably and equitably. To ensure that the objectives set out in the NWRS2 are met, and the principles of the Act are realised, two complementary strategies are adopted in the NWRS2: the Resource Directed Measures (RDM) and the Source Directed Controls (SDC) (DWAF, 2013).

Resource Directed Measures (RDM)

Resource directed measures (RDMs are measures directed at the protection of water resources while ensuring socio-economic growth and development. RDM recognises the increasing stress and anthropogenic impacts (e.g. pollution, population growth, over-utilisation and poor agricultural practices) on the health of aquatic ecosystems, and provides tools and approaches for their management. The RDM is comprised of the following components i) the classification system ii) the classification of significant water resources iii) Reserve determination and iv) setting of Resource Quality Objectives (RQOs).

The classification system provides a set of nationally consistent rules to guide decision-making about water resources classification. Water resources are classified into three major management classes, which reflect the desired levels of use and protection: Class I) water resources that are minimally used and the ecological condition minimally altered from its pre-development condition; Class II) water resources that are moderately used and the ecological condition is moderately altered from its pre-development condition; and Class III) water resources that are heavily used and the ecological condition significantly altered from its pre-development condition.

The Reserve provides for the quantity and quality of water required both to satisfy basic human needs (The Human Need Reserve) and to protect aquatic ecosystems (ecological Reserve). The aim of the Reserve is to secure ecologically sustainable and responsible development and use of the water resources in South Africa. The Reserve is the only water right specified as inviolable in the Act, making it a legally guaranteed provision for both basic human rights in term of access to water and the protection and functioning of the aquatic ecosystem. The provision of the Reserve makes the NWA a groundbreaking Act, departing from a command and control approach, and setting out a sound ecological basis for managing water resources in South Africa. It accords rights to both humans and the aquatic ecosystem.

The last component of the RDM is the setting of the RQOs. The RQOs are defined as measurable quantitative and/or qualitative descriptors of the water resources aligned with management classes. The RQOs relate to all components of the water resources including quality, quantity, geomorphological, and hydraulics biota (in-stream and riparian), as well as socio-economic variables. The RQOs are expressed as numeric or descriptive goals for resource quality within which a water resource must be managed. The RQOs establish comprehensive water quality management targets that relate to the relevant water quality. The SDCs (source directed controls) complement the RQOs by ensuring the goals of the RQOs are met, by setting up regulatory measures such as registration, permits, directives and prosecution, as well as giving economic incentives.

Source Directed Controls (SDC)

The second complementary strategy in the NWRS2 is the SDCs. SDC provides for the regulation of the use of water in South Africa. The SDC focuses on regulating the use of water resources, with the primary purpose of ensuring the objectives that have been set for the water resource (typically defined by the management class and RQOs) are achieved. The SDC tools include regulatory mechanisms such as water quality standards for wastewater discharges, pollution prevention measures, waste use permits, licenses and general authorisation. Progressive implementations of self-regulation using both punitive and economic incentives are encouraged by the DWS.

The RDM and SDC are therefore the two strategies used in the management of water resources in South Africa. They both provide for different tools and approaches for monitoring water quality to realise the overreaching objectives of concurrent use and protection of water resources. One such approach is environmental water quality (EWQ), which combines physico-chemical monitoring and modelling, and biomonitoring and ecological modelling, as well as ecotoxicology (Palmer, 2004).

1.3 Environmental Water Quality (EWQ)

Environmental water quality (EWQ) describes the chemical, physical, and biological characteristics of water, relative to its suitability for ecosystem functionality. EWQ is a three-pillared approach comprising physico-chemical monitoring, biomonitoring and ecotoxicology, which can be collectively or individually used to manage the water quality of aquatic ecosystems. Physico-chemical monitoring may involve the measurement of the physical and chemical attributes of a water resource such as temperature and turbidity, dissolved oxygen and pH as well as nutrient variables such as nitrate-nitrogen, nitrite-nitrogen and phosphate-phosphorus content of water (Palmer, 2004). Biomonitoring utilises the response of resident aquatic biota to provide integrated information on the condition of the aquatic ecosystem. Resident aquatic biota has proven successful in assessing environmental water degradation (Bremner *et al.*, 2006; Arimoro & Muller, 2010). Ecotoxicology provides the information on the cause-effect relationship of specific concentrations of toxicants on the individual organisms, thereby providing a bridge between the physico-chemical and biomonitoring approaches (William *et al*., 2003; Palmer *et al.,* 2004). Of the three approaches to EWQ, this study focuses on physico-chemical and biomonitoring, which are therefore further reviewed.

1.3.1 Water Physico-chemistry

The physico-chemistry of water describes the physical and chemical characteristics, fate and transport of chemical substances dissolved and suspended in water. Water quality monitoring is traditionally done by measuring the concentrations of physico-chemical variables of water such as ammonium (NH_4-N), nitrate (NO_3-N), nitrite (NO_2-N), orthophosphate (PO_4-P), dissolved oxygen (DO), electrical conductivity (EC), temperature, hydrogen ion (pH), turbidity, five-day biological oxygen demand (BOD_5), chemical oxygen demand (COD) and settled and suspended sediment loads.

Measuring physico-chemical variables helps managers to keep track of concentrations of pollutants in water for management purposes and for setting goals and guidelines and is the most widely used method/approach in managing environmental water quality. The presence of high concentrations of specific chemicals has a variety of implications for aquatic ecosystem health and functionality. For example, nutrient loads in the aquatic ecosystem can lead to problems such as eutrophication and algal blooms, provided other conditions such as water temperature, conductivity, sunlight, hydrology of river and hydraulics, are favourable. High loads of both settled and suspended sediment can absorb contaminants, change the geomorphology of the stream channel, and affect aquatic biota (Jones *et al.*, 2012). Given that the rivers where this study was undertaken receive high loads of sediment from the catchments, elevated sediments and aquatic stressors are therefore further reviewed.

1.4 Elevated sediments as water quality stressor in aquatic ecosystems

Sediments are important components of the freshwater ecosystem for nutrient cycling, substrate composition and heterogeneity, all of which, play a part in the integrity of the micro-environment in which, macroinvertebrate are resident (Wood & Armitage, 1997; Murphy *et al.* 2015; Conroy *et al.*, 2016). However, excessive loads of sediment in aquatic ecosystems have deleterious effects on aquatic ecosystems by increasing water turbidity, limiting light penetration and potentially reducing primary productivity, with consequent impacts on the entire food chain (Wood & Armitage, 1997). These effects are associated with the loss of habitat complexity and heterogeneity and the creation of an unstable streambed, which are consequently accompanied by significant changes in faunal composition and a loss in diversity (Downes *et al.,* 2006; Wantzen & Mol, 2013). Elevated in-stream sediments affect aquatic organisms across all trophic levels, through mechanisms that include: i) modification of habitat availability and stability, ii) increases in turbidity and reduction of primary production, iii) impairment of feeding due to a reduction in the energetic value of periphyton and prey density; and iv) impairment of respiration due to a decrease in the oxygen concentrations and impairment of respiratory organs (Graham, 1990; Wood & Armitage, 1997; Mathers *et al.*, 2017).

A recent review by Jones *et al.* (2012) classified fine sediment modes of stress on macroinvertebrates into direct and indirect mechanisms. Direct effects on macroinvertebrates include abrasion of soft body parts, clogging of morphological structures such as filter feeding nets and gills, burial from a constant deposition that affects sedentary and slow-moving organisms and alteration of substrate composition by filling of interstitial spaces with finer sediment particles

(Mathers & Wood, 2016). Indirect effects of sediment disturbance include reduced habitat availability because of increased deposition and change in habitat patches, consequently affecting macroinvertebrate assemblages, and reduced food availability and quality for macroinvertebrates. However, sediment stress on aquatic ecosystems, particularly on macroinvertebrates, is influenced by complex interacting factors. The primary modes by which both suspended and settled sediment impact on macroinvertebrates are summarised in Figure 1.1. Several complex interacting factors affect the degree to which sediments affect macroinvertebrates and these include i) the duration of exposure, ii) the concentration of fine sediments, iii) particle size characteristics and distribution, iv) geochemical composition and particle shapes, and v) the sensitivity of the ecosystem. Gammon (1970) showed that increase in suspended sediments of 40-80 mg/L beyond the natural background level can increase macroinvertebrate drift from 25% up to 90%. In a study by Newcombe and MacDonald (1991), the importance of using exposure duration in addition to exposure concentration in predicting the effects of fine sediment on aquatic biota was emphasised. The authors further revealed that ranked response of aquatic biota was poorly correlated to sediment concentration, although it was strongly correlated with sediment intensity (defined as the concentration multiplied by the duration of exposure). Therefore, in interpreting sediment effects on macroinvertebrates, it is important to take into account the complexities of interacting factors.

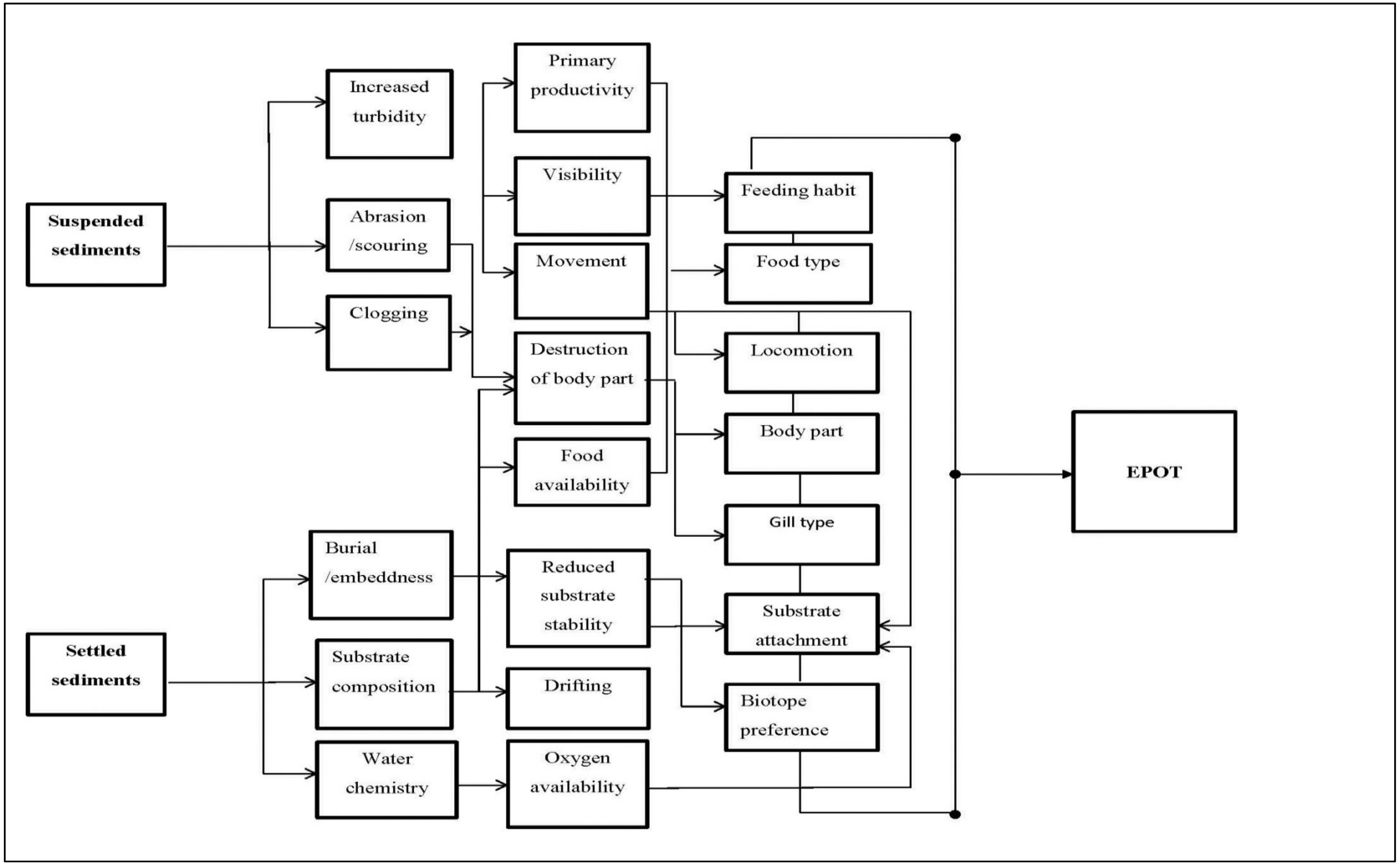


Figure 1.1: Summary of the mechanisms by which settled and suspended sediments affect macroinvertebrates.

E = Ephemeroptera, P = Plecoptera, O = Odonata and T = Trichoptera.

1.4.1 Measurement of suspended and settled sediments

Settled sediments deposited on stream-beds can be measured or quantified by considering the degree of embeddedness of the stream-bed (Wood *et al.,* 2005). The measurement of embeddedness takes into account the depth of coverage of substrate by fine sediments, the size classification of sediments covering the substrate and the percentage of interstitial spaces filled (Waters, 1995). Other means of measuring settled sediments include trap and disturbance techniques, which involve the placement of traps constructed in a metal box in riffle to capture sediment (Duerdoth *et al.,* 2015), the Turner-Hillis point suction method, which involves the use of a point-suction system to remove fine sediments from the stream-bed (Turner *et al.,* 2012) and visual estimation of percent surface fine sediments (Rabení & Zweig, 2005). Suspended sediments concentration is the main contributor of turbidity in rivers and is usually assessed using a measure of turbidity and total suspended solids (TDS) (Bilotta & Brazier, 2008) as a surrogate. Turbidity is defined as the optical property of water resulting in a loss of light transmission due to absorption or scattering (Henley *et al*., 2000). The scattering is caused by particulate matter suspended in the water column that contributes to the colour of the water. These particulate matters in suspension may include algal material, colloids, and plankton and detritus. Turbidity is measured using the nephelometric turbidity unit (NTU). Other devices, such as optical turbidity meters and Mastersizers, are used to measure particle size characteristics as a means of indirect measurement of turbidity. Such procedures involve the sampling of sediment particles and laboratory analysis using a device such as the Mastersizer. Turbidity is a function not only of suspended sediment but also of particle size characteristics, shape and composition, in addition to water colour (Foster *et al*., 1992).

The analysis and measurement of sediments in aquatic ecosystems is an example of the physico-chemical approach to managing water quality. However, there are several limitations to this approach. For example, the physico-chemical approach could be very costly because of the required analytical equipment, and it provides little indication of the ecological response of resident aquatic biota and the overall biophysical health of the ecosystem, being a snapshot of the water quality condition at the time and location in which the measurements were taken. Therefore, in this study, macroinvertebrate species-based biomonitoring was used in combination with physico-chemical monitoring to assess the effects of sediments in the studied river systems.

1.5 Biomonitoring

Freshwater biomonitoring uses resident aquatic biota such as plants, algae, and fish, including macroinvertebrates, to assess the health of aquatic ecosystems (Li *et al*., 2010; Friberg *et al.,* 2011; Odume, 2017). The use of biomonitoring as an approach to water resource management is underpinned by successful identification of appropriate bioindicators whose presence or absence, abundance, diversity and behaviours reflect water quality conditions of the environment in which they live (Odume, 2017). Macroinvertebrates are widely used as bioindicators of water quality (Bonada *et al*., 2006; Murphy *et al*., 2013). The frequent application of macroinvertebrates in freshwater biomonitoring is largely attributed to the following factors: i) their sedentary nature, which causes them to reflect the conditions of the area in which they are collected, ii) their ubiquitous occurrence, abundance and diversity, iii) their short life cycle, which reflects changes in the environment more quickly through changes in population and community structure, iv) their graded response to a variety of pollutants, and v) their vital role in organic matter processing and energy transfer in aquatic ecosystems. Furthermore, macroinvertebrates can be collected and identified easily at least to family level (Odume, 2017). The collecting equipment is relatively affordable, making the use of macroinvertebrates as bioindicators in biomonitoring relatively cheap and inexpensive. In this study, macroinvertebrate-based and trait-based responses were used to assess the effects of sediments on resident aquatic biota in the studied river systems.

1.5.1 Macroinvertebrate-based biomonitoring

Macroinvertebrates can be monitored at different organisational levels from species to community levels. Macroinvertebrates-based biomonitoring approaches include a single biotic indices (Hilsenhoff, 1988), multivariate models (Turak *et al*., 2004; Jorgenson *et al*., 2005) and multimetric indices (Barbour & Yoder 2000). In recent years, trait-based approaches are being developed with the sole aim of enhancing the predictive and diagnostic potentials of biomonitoring approaches (Menezes *et al*., 2016). Biotic indices are based on a subjective and statistical scoring system where each taxon has a presumed sensitivity or tolerance to environmental impairment. Such indices have gained application in many biomonitoring programmes including the Biological Monitoring Working Party System in the UK (BMWP, 1978), Hilsenhoff's Biotic Index (HBI) (Hilsenhoff, 1988) in the USA, and the South African Scoring System (SASS) in South Africa (Chutter, 1972; Dickens & Graham, 2002). The SASS is currently in its fifth version, known as SASS5. In SASS5, macroinvertebrate families are awarded scores based on their perceived sensitivity to water quality (Odume *et al*., 2014). SASS results are represented in the form of index scores and as an average score per taxon (ASPT). These two measures provide a useful indication for interpretation of the quality of river systems (Odume, 2017). The multimetric approach combines metrics such as taxa richness, functional feeding groups, assemblage composition and life history strategies to indicate the health of a water body (Li *et al*., 2010; Odume, 2017). Examples of multimetric indices that have gained wide application include the Piabanha-Paquequer-Preto Multimetric Index (PPPMI) (Baptista *et al*., 2011) that is widely used in Brazil to assess aquatic health, the Multimetric Macroinvertebrate Index (MMI) (Aazami *et al*., 2015) used in Iran, and the Benthic Index of Biological Integrity (Kerans & Karr, 1999) used in Europe. Most macroinvertebrate-based biomonitoring indices are applied at the different levels of taxonomic resolution and most often the accuracy of their prediction is dependent on the taxonomic sufficiency.

The study of biomonitoring is usually undertaken at the family level because of the convenience and ease of identification, availability of taxonomic keys and relevant resources. Although the family-level index can be expected to consistently separate reference sites from polluted sites, it may not detect smaller differences in water quality because of confounding sensitivities of species within a family. Species-level identifications can provide greater potential in detecting smaller differences in water quality and can result in a more reliable assessment than family-level data (Furse *et al.,* 1984; Lenat & Resh, 2001). For example, a detailed investigation on the caddisfly family Hydropsychidae has demonstrated how species differ in terms of water quality requirement, flow and food preferences, and were used in detecting subtle changes in water quality (Gordon & Wallace, 1975).

1.5.2 Taxonomic-based approach

Taxonomic-based approach to biomonitoring is a techniques that compares the taxonomic assemblages of species (e.g., taxonomic composition, species richness and abundance measures) and relate them to environmental conditions (Culp *et al.,* 2011). Taxonomic-based appraoches involve the use of several metrics such as single metrics and indices as well as multimetric indices and have been developed and used for biomonitoring programs based on the community structure of organisms including macroinvertebrates. A key factor considered in successful application of taxonomic-based approach to biomonitoring is the taxonomic sufficiency, as the taxonomic resolution can affect the accuracy of results and predictions

(Muller *et al*., 2013). Species-level identification has been advocated by previous studies because species-level resolution can identify subtle environmental changes (Monk *et al*., 2012). Within macroinvertebrate families, there exist different sensitivities of species to environmental stress and environmental requirements vary significantly within families (Lenat and Resh, 2001). Most macroinvertebrate-based biomonitoring in Africa focuses on the family-level taxonomic resolution, which can be attributed to cost and availability of adequate macroinvertebrate identification keys.

In Africa, identification of macroinvertebrates is onerous because of the problems associated with taxonomy. There is still little or no information on the identification of macroinvertebrates in Africa, where most emphasis is placed on lower taxonomic levels (Arimoro & Muller, 2010; Lakew & Moog, 2015; Arimoro *et al*., 2015). Despite the fact that identification to species-level resolution is problematic and difficult in Africa, it is important to note that the predictive power of a context-specific biomonitoring is dependent on the optimal taxonomic resolution, particularly in the use of genera-rich families such as Ephemeroptera, Plecoptera, Odonata and Trichoptera (EPOT).

In this study, Ephemeroptera, Plecoptera, Odonata and Trichoptera (EPOT) families were selected to assess the effects of sediments in the Tsitsa River and its tributaries. The choice of taxonomic resolution is often dependent on the sufficiency of identification keys and the cost. The EPOT taxa represent one of the most diverse groups of macroinvertebrates occupying all types of habitat. These macroinvertebrates have a wide array of sensitivities to stressors. Furthermore, taxa richness is a widely used parameter for analysis of macroinvertebrates data in water quality monitoring (Lenat & Barbour, 1994). Their frequent usage is attributed to their relatively easy identification (Resh, 1995). Species within the EPOT taxa display varying sensitivities to fine sediments and have been extensively used in macroinvertebrate biomonitoring approaches to assess water quality deterioration (Buendia *et al*., 2013; Turley *et al*., 2016). Therefore, the EPOT were selected as bioindicators of sediment linked stress.

1.5.3 Ephemeroptera, Plecoptera, Odonata, and Trichoptera (EPOT) as Bioindicators

The EPOT larvae are among the richest families of aquatic insects in terms of taxonomy and functional ecology. About 2500-3000 species of Ephemeroptera are identified worldwide, almost 3000 species of Plecopterans have been recorded, over 6000 species of Odonata have been recorded globally. Currently, there are about 15,000 species of Trichoptera that have been described, making Trichoptera the second most diverse monophyletic group of aquatic animals

(Ríos-Touma *et al*., 2017). Trichoptera are unevenly distributed among geographical regions with the smallest number recorded in the Afrotropican region, about 1,099 species (Morse, 2011). EPOT taxa are an important component of the freshwater ecosystems occupying almost all habitat and biotope types (stones, aquatic macrophytes, interstitial patches) of streams and rivers, including temporary streams and ponds, and deepest and shallowest parts of the ecosystems (Altermatt *et al*., 2013; Murphy *et al.,* 2015**).** The EPOT taxa represents those that can inhabit both fast-flowing and slow-flowing streams and rivers of all sizes. They are considered to have a wide range of sensitivity to deteriorating water quality (Mereta *et al.,* 2013). Their diversity, wide range of sensitivities and their presence in all habitat types make them easily available for sampling and use in biomonitoring studies.

Although the EPOT species have been used widely in freshwater biomonitoring, most of their applications rely only on taxonomic analysis i.e. community structure approach (Chi *et al*., 2006; Kefford *et al*., 2010; Degabriele, 2013). Despite the wide application of the EPOT taxa in biomonitoring studies, most biomonitoring studies involving the EPOT taxa are based on taxonomic approach, which relies on the description of community structure and pattern. The taxonomic application has been used in previous studies to assess the response of water quality to environmental changes (Arimoro & Muller, 2010). Because ecological information such as traits is measured at the individual species level and species response to environmental stress is mediated by traits, taxonomic level analysis does not pay sufficient attention to the underlying interactions between organisms and their surrounding environment. Therefore, while taxonomic-based approach has been widely used in biomonitoring, it is important to understand how macroinvertebrate species respond differently to environmental stressors.

Taxa responses are mediated by traits and therefore traits offer a potential opportunity for developing a predictive and diagnostic biomonitoring approach. Thus, in this study, both taxonomic and trait-based approaches were applied to assess the effects of sediments. This study is among the first to explicitly attempt to use taxonomic and trait-based approaches for evaluating the effects of in-stream sediments on aquatic macroinvertebrates biodiversity in South Africa.

1.5.4 Trait-based approach

Traits are attributes of organisms, such as physiological, morphological, behavioural and life-history features, that are inherent in the organisms and therefore can only be measured at the individual level without making reference to the external environment (Mcgill *et al*., 2006;

Violle *et al*., 2007). The trait-based approach (TBA) is a promising biomonitoring technique increasingly applied in functional ecology that applies species traits to assess water quality (Mathers *et al*., 2017). As ecologists attempt to understand the mechanistic relationship between species and their environment, the TBA has been seen as a promising approach because it offers the opportunity to evaluate the mediated relationship between organisms and their external environment. The TBA is thus growing in popularity across the globe and has been applied in Europe (Charvet *et al*., 2000; Kuzmanovic *et al.*, 2017), Australia (Chessman and Royal, 2004), USA (Merritt *et al*., 2002) and Africa (Odume, 2014).

The TBA is underpinned by the habitat template concept (HTC) which provides a mechanistic framework for linking traits to environmental disturbances (Southwood, 1997; Townsend and Hildrew, 1994). The HTC is based on the notion that prevailing environment characteristics select species with appropriate combinations of traits to matching the environmental requirement while eliminating those species that do not possess appropriate trait combinations for the environment (Verberk *et al*., 2013). In this regard, the environment is seen as a filter (Poff *et al.,* 2006). Consequently, in a given environment, only taxa possessing adaptive traits pass through the filter (Webb *et al.,* 2010) and such traits can be predictive and diagnostic of the prevailing stressors (Culp *et al*., 2011). For example, Statzner *et al*. (2004) observed that habitat characteristics such as fine sediment deposition act as filters for traits of organisms and shape community composition by selecting well-adapted species with an appropriate combination of traits and eliminating species that are snot well-adapted.

Most traits are affected by stressors in a predictable manner because trait response to disturbance can be contextualised (Dolédec *et al.*, 2006; Statzner *et al*., 2008). The use of traits can be diagnostic of a water quality stressor because traits can be mechanistically linked to the stressor through the underlying relational processes between the organism and its environment (Culp *et al*., 2011). For example, a study by Tullos *et al.* (2009) compared the response of trait diversities and taxonomic assemblages between 24 pairs of upstream (control) and downstream reconfigured (restored) reaches in 3 catchment land uses (urban, agricultural, rural) across the North Carolina Piedmont. The study revealed how functional-trait approaches responded better compared with taxonomic approaches. The study emphasised that the TBA could benefit the practice of river restoration when used to target restoration activities and to develop informed expectations regarding recovery following restoration activities.

The TBA should be able not only to indicate environmental stress (Gayraud *et al*., 2003), as does the taxonomic-based approach but should also discriminate between the effects of different stressors, i.e. the TBA should enhance the discriminatory capacity of biomonitoring. The potential discriminatory capacity of the TBA has been demonstrated in a recent study by Kuzmanovic *et al*. (2017), who discriminated between the effects of multiple stressors (pesticides and multiple urban land use) using TBA, in four different river system sites in Spain. Urban-related stressors selected taxa that were mainly multivoltine, which fed on deposits of organic matters. In contrast, pesticide-influenced sites selected taxa with high levels of egg protection (better egg survival), indicating a potentially higher risk for egg mortality. The potential of the TBA to add impact discriminatory capacity to biomonitoring is particularly useful because resource managers can better deploy resources at stressors with the greatest impact.

Despite the potential usefulness of the TBA, the approach is yet to gain popularity in South Africa. The approach, which is based on investigating traits as they mediate organisms and stressors, offers an opportunity to understand the processes underpinning the responses and vulnerability of aquatic organisms to stressors, and in the context of the current study, those of macroinvertebrates to sediments. Therefore, this study adopts the TBA in combination with taxonomic analysis to evaluate the responses and potential vulnerability of species of the EPOT taxa to sediments in the Tsitsa River and its tributaries.

Although the TBA holds promise in freshwater biomonitoring, it comes with several challenges, which include i) the problem of which taxonomic resolution trait should be applied, ii) detecting and quantifying trait correlations, combinations, interactions and trade-offs, iii) identification and measurement of traits, iv) trait choice and selection, and v) availability of trait information, particularly of the Afrotropical region. Some studies have shown that family level is often sufficiently able to detect environmental changes (Marshall *et al*., 2006; Mueller *et al*., 2013), while others have concluded that a higher resolution such as genus level is preferential, particularly when attempting to identify subtle differences (Doledec *et al*., 2000; Gayraud *et al*., 2003; Monk *et al*., 2012). Others such as Shemera (2017) have argued that since ecological information is often measured at the individual level, there should be no objection to the use of traits at higher taxonomic level. The importance of trait correlation and interaction have been emphasised by several authors such as Verberk *et al*. (2013) and Kuzmanovic *et al*. (2017), when using TBA. Species-environment relationships are often mediated by a combination of several trait attributes an organism possesses. Though several traits databases have been developed over the years (Charvet *et al*., 2000; Vieira *et al*., 2006; Odume *et al.,* 2018), trait information, particularly in Africa, is still limited, and the available trait data is not harmonised, this contributes to the reasons why TBA has performed poorly in the region (Odume, 2014).

In this study, the species-level taxonomic and TBA to biomonitoring was used to assess the potential vulnerability of the orders Ephemeroptera, Plecoptera, Odonata and Trichoptera (EPOT) to sediment stress.

1.6 Rationale and significance of the study

Elevated in-stream sediment remains one of the most important stressors of aquatic ecosystem functionality in South Africa. The Mzimvubu catchment where the Tsitsa River and its tributaries are situated, is prone to severe erosion, leading to in-stream sedimentation of rivers. Despite the impact of sediment on these river systems, no study has used species-level assessment (taxonomic and trait-based approach) to assess the responses and potential vulnerability of macroinvertebrates, particularly the EPOT species, to elevated sediments. The combined use of taxonomic and trait-based approaches can enable the development of tools that are not only descriptive but also diagnostic of sediment impact, and predictive of assemblage response to in-stream sediment loads. This study contributes to the science and practice of biomonitoring in South Africa through i) providing new understanding of the usefulness of EPOT at genus and species level as indicator species to assess sediment effects in South Africa water resources in particular in the Tsitsa River and its tributaries, ii) improving water quality management of rivers and providing a better understanding of impacts of anthropogenic activities on biological assemblages by using a lower taxonomic resolution i.e. genus and species, iii) the combined used of taxonomic and trait-based approaches, which could lead to incorporating a trait-based approach to existing biomonitoring tools in South Africa, with the potential to add predictive and diagnostic values to biomonitoring in South Africa.

1.7 Aim and objectives of the study

1.7.1 Aim

The aim of this study was to evaluate the community responses and vulnerability of species of the orders Ephemeroptera, Plecoptera, Odonata and Trichoptera (EPOT) to sediment stress, using taxonomic and trait-based approaches, in the studied river systems.

1.7.2 Objectives

The overall aim of the study was achieved through the following objectives:

1) To determine selected water physico-chemical and sediment characteristics of the Tsitsa River and its tributaries.
2) To assess and evaluate the taxonomic assemblage structure response of the orders Ephemeroptera, Plecoptera, Odonata and Trichoptera to a gradients of increasing sediments stress in the Tsitsa River and its tributaries.
3) To develop a trait-based approach for assessing and predicting the potential vulnerability of species of the orders Ephemeroptera, Plecoptera, Odonata and Trichoptera to sediment stress in the Tsitsa River and its tributaries.
4) To critically evaluate the complementarity of the taxonomic and trait-based approaches in relation to Ephemeroptera, Plecoptera, Odonata and Trichoptera response and vulnerability to sediment stress in the Tsitsa River and its tributaries.

The research questions that prompted the above objectives are as follows:

I) How do the Ephemeroptera, Plecoptera, Odonata and Trichoptera assemblages respond to sediment stress?

II) How can the TBA aid the development of tools for predicting the potential vulnerability of Ephemeroptera, Plecoptera, and Odonata and Trichoptera species to sediment stress?

III) Can the complementarity of taxonomical and trait-based approaches in biomonitoring provide a better and more predictive diagnosis of vulnerability and responses of EPOT species to sediments effects?

IV) What value can be derived by using taxonomic and TBA as complementary approaches?

1.8 Thesis structure

Chapter 1: This chapter contains the general introduction and literature review of the study. It also contains the rationale and significance of the study as well as the study aim and objectives.

Chapter 2: This chapter contains descriptions of the study are, materials and methods used. The chapter describes the sampling sites and sampling procedures, method and approaches used in the study, and ends with a statistical analysis.

Chapter 3: This is the first results chapter. It focuses on the taxonomic assemblage response of EPOT species to sediment stress with a particular focus on sediment particle size characterisation.

Chapter 4: This is the second results chapter. It addresses the research objective related to the use of the TBA. The chapter contains the development of using TBA for predicting and evaluating the potential vulnerability of EPOT species to sediment stress.

Chapter 5: This chapter contains a general discussion, recommendations and conclusion. The chapter discusses the results and provides a critical evaluation of the complementarity of the two approaches under study.

CHAPTER 2: STUDY AREA, GENERAL MATERIALS AND METHODS

2.1 Introduction

This chapter describes the study area and the factors influencing the delivery of sediments into the selected study sites. The selected study sites, as well as the methods, approaches and protocols used in this study, are described. The chapter ends with a description of the traits selected and a general description of the statistical analyses employed.

2.2 Study Area Description

The Mzimvubu River falls within the tertiary catchment T35A-E and has a drainage area of 19 826 km^2, with a flow length of 350 km from north to south. It is situated in the Eastern Cape Province of South Africa (Figure 2. 1). The Mzimvubu River flows mainly from the eastern escarpment of the Drakensberg Mountains near the town of Matatiele and discharges into the Indian Ocean at Port St. Johns, after passing through hills and forming tributaries with the Tina, Kinira, Mzimvubu and Mzintlava Rivers, as well as the Tsitsa River and its tributaries. The Tsitsa River catchment covers an area of 4 924 km^2 and connects to the Mzimvubu River after a flow length of approximately 200 km from northwest to southeast. The upstream of the Tsitsa River is situated in a confined channel between two steep valleys with narrow floodplains, while the majority of the study area is formed by a hilly landscape. The Tsitsa River catchment consists of tributaries such as the Qurana and Mooi Rivers that connect the Pot and Little Pot Rivers. The Mooi River forms a tributary that joins the Tsitsa River at the mid-lower reaches, to which the lower section of the Pot River and the Millstream form tributaries. The Qurana River joins the lower reaches of the Tsitsa River. The present study sites are situated in the Tsitsa, Qurana tributary, Pot and Little Pot Rivers as well as the Millstream. Therefore, the description of the study area is in terms of these river catchments and more broadly the Mzimvubu River catchment within which these rivers are situated.

2.2.1 Climate and rainfall

The climate in the Tsitsa River catchment ranges from temperate in the northern altitude to sub-tropical along the coastal belt. The catchment area falls within the former Transkei, an area that receives an annual rainfall of 700 mm to 1000 mm with peak rainfall during the summer months of November to February and an evaporation rate of 1200 mm to 1400 mm per year

(Midgeley *et al*., 1990). The spatial-temporal rainfall variability is closely associated with its macro- and meso-scale topography and its distance to the coast. The mean annual temperature range is between 6.6 ºC in winter and 20.3 ºC in summer.

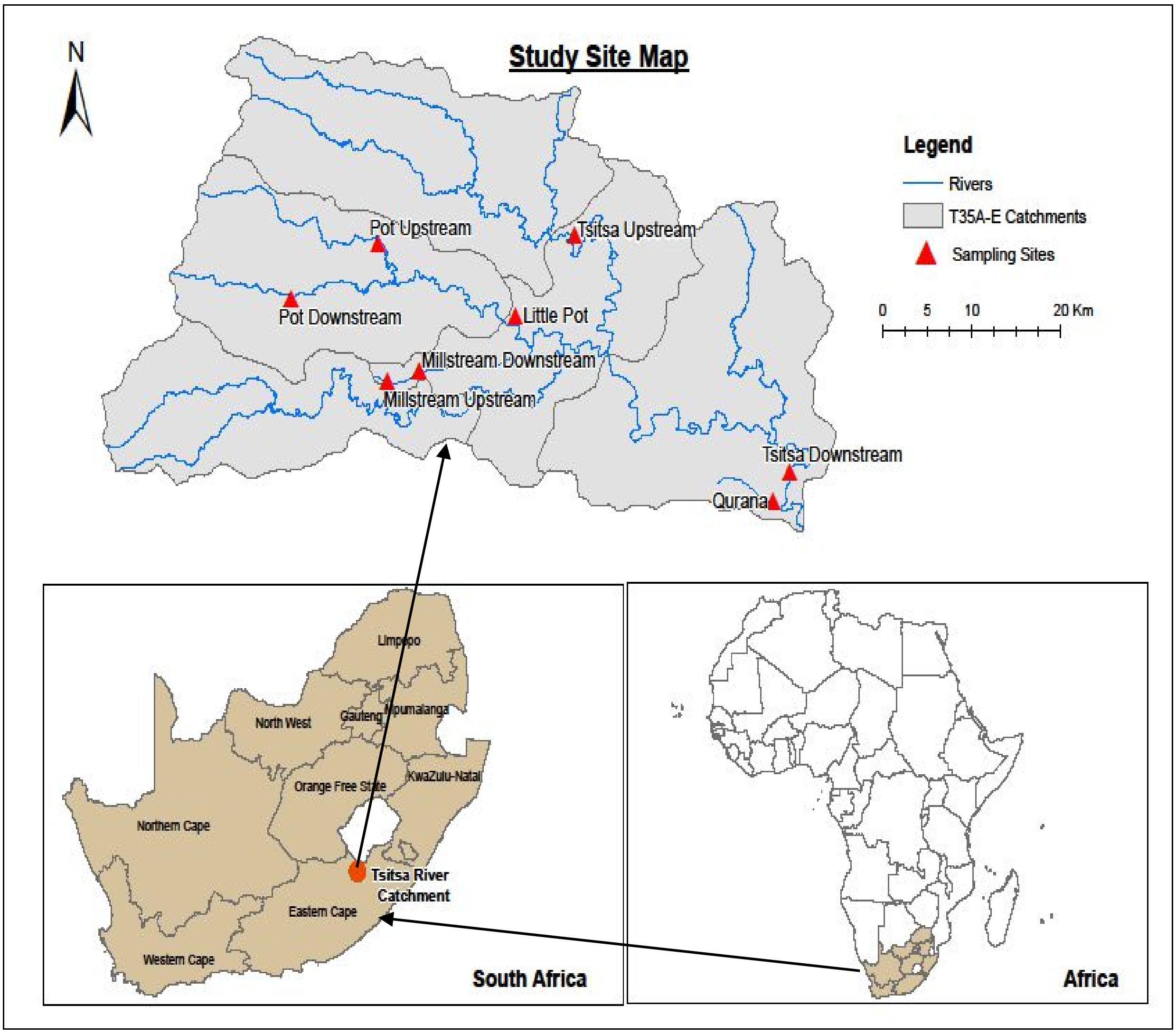


Figure 2. 1: Map of the study area showing the locations of the sampling sites in the Tsitsa, Pot, Little Pot, Millstream and Qurana Rivers. The location of the study area within South Africa is shaded dark in the Eastern Cape Province on the map of South Africa and the relative position of South Africa is marked on the map of Africa. Site 1 (Tsitsa upstream site), Site 2 (Tsitsa downstream site), Site 3 (Qurana River), Site 4 (Pot River downstream), Site 5 (Pot River upstream site, Site 6 (Little Pot River), Site 7 (Millstream upstream site) and Site 8 (Millstream downstream site).

2.2.2 Geology and soils

The soils in the catchment consist of the Karoo super-group and are primarily underlaid with highly erodible Beaufort sandstones. They are characterised by basalt material in the upper alpine zone, which is dominated by sandstone combined with shales and mudstone, and with fairly deep alluvial deposits in the lower lying areas (ERS, 2011). The alluvial soils are a combination of the parent materials from the upstream of the Mzimvubu and are composed of high organic content (ERS, 2011). The immediate floodplain areas are sandy due to their dynamic nature and constant deposits, while the wider alluvial plains have darker material reflecting greater development with clay and organic matter. Many of the alluvial areas have grey and poorly structured soil, reflecting dynamic and eroded landscapes in the catchment (ERS, 2011). The soils in the catchment are poorly drained and form shallow to moderately deep loamy soil with minimal hard rock and less prominent soil from sandy loams (Le Roux *et al.,* 2015). The central part of the catchment is dominated by duplex soils that are highly erodible, and widespread gully erosion is evident in the catchment (Le Roux & Sumner, 2013).

The Tsitsa and Qurana sub-catchment is generally known to have a high risk of soil erosion and is among the highest sediment-yield areas in South Africa (Madikizela & Dye, 2003; Le Roux *et al.,* 2015). The soils in the catchment are characterised by high erodibility (Van Tol *et al*., 2017). The high runoff and erosion in the catchment are associated with the melanic and vertic content of clay that characterises the soil in the catchment (Le Roux *et al.,* 2015). These components of the soil increase from the topsoil to the subsoil and inhibit the penetration of roots and limit plant growth. These soil properties have resulted in a large section of the catchment being affected by varying degrees of deep gullies and elevated fine sediments deposition into the surrounding rivers. For example, a recent study by Mararakanye and Le Roux (2012) indicated that about 18,000 gullies affecting an area of approximately 22,600 ha are present in the catchment.

In the low-lying regions of the Pot River, dolerites and quaternary alluvium exist in small bands and sandstone of the Claren formation dominates in the Pot and Little Pot River catchments (Gordon *et al*., 2013). All of the study sites except the Upper Pot and Little Pot are within the Molteno Sandstone. The Upper Pot and the Little Pot are in the Elliot mudstone (Gordon *et al*., 2013). Mudstone is generally more erodible than sandstone.

2.2.3 Topography, land and cover, vegetation

The landscape elevation ranges from 168 m at the catchment outlet in the southeast to 2,730 m in the northwest of the Drakensberg Mountains. Landforms are complex, ranging from the very steep (40%) mountain slopes of the Drakensberg to gently undulating foot-slopes (2%) and nearly level valley floors, (2%). The slopes are generally steep, but those in the Pot Rivers are steeper compared with the rest of the sub-catchments of the study area. The catchment is characterised by three prominent escarpment areas including the Drakensberg Mountains, the mountain ranges that separate the Highlands from the mid-slopes, and the coastal regions of the catchment. The coastal regions are dominated by valley, bush forest and Table Mountain sandstone with steep sea cliffs (Acocks, 1975).

The vegetation in the Tsitsa catchments is largely influenced by altitude and soil, as well as by livestock grazing and grassland burning. The catchment is mainly dominated by grassland including montane alpine, subalpine and alpine belts, with pockets of shrub and woodland or savannah (Flügel *et al.,* 2003; Low & Rebelo, 1998). Natural vegetation, including indigenous forest, covers approximately 3400 km^2 (70%) of the catchment area (Bäse *et al*., 2006). Most prominent vegetation in the Tsitsa catchment includes the invasive *Acacia mearnsi, Eucalyptus camaldulensis, E. viminalis*, and the *Pinus elliottii* (Maroyi, 2017) and the indigenous species including grass species such as the *Eragrostis planana, Acalypa schinzii*; and the flowering Protea trees, *Helichrysum* sp. and *Lobelias* sp., *Watsonias* sp., *Zantedeschia* sp. and *Cyrtanthus* sp. herbs and shrubs such as the *Brassica sp., Allium* sp. and *Agave americana*. These plant species are under extensive degradation as a result of land use changes, trampling by grazing livestock and other farming activities that contribute to the transport of sediments into the river channels.

2.2.4 Anthropogenic influences in the catchments of the studied river systems

The major water quality impact in the catchments of the studied river systems i.e. Tsitsa, Pot, Little Pot, Qurana Rivers and the Millstream, is excessive in-stream sediment deposition as a result of habitat degradation related to the natural soil type, poor grazing activities, cultivation of farms and fields, and forestry activities (Madikizela, 2001; Base *et al*., 2006). Agricultural activities, including particularly poor grazing practices, forestry operations and urban activities, are among the major anthropogenic influences in the Tsitsa catchment and the catchments of its tributaries (ERS, 2011). The excess sediments delivery is exacerbated by the highly unstable soils that are prone to erosion, which is evident in the extensive areas covered by gully erosion (Maroyi, 2017). Gully erosion has been implicated as the major factor causing water quality degradation in the catchment (Gordon *et al.*, 2013; Le Roux *et al.*, 2015). A recent study by Le Roux *et al.* (2015) reported that the Tsitsa catchment, including the greater Mzimvubu catchment, has approximately 12, 265 new gullies which affect an area of 3 970 m^2. The high gully erosion that is evident in the catchment affects a large proportion of the catchment total area and is the major driver of sediment transport into the river system, producing approximately 5 tonnes/hectare/year of sediments (Le Roux *et al.*, 2015). Other possible activities in the catchment that may transport sediments to the rivers include urban development, such as roads that service resource extraction or development operations or miscellaneous activities such as a bridge that services motorist and pedestrian crossing, or any other activities that disturb land surface and can generate and transport fine sediments.

The Pot and Little Pot Rivers catchments are in relatively good condition with regard to land cover and potential sediment delivery into the river systems. The Pot and the Little Pot drain a catchment that is largely managed by private owners, with well-managed cultivation and grazing patterns, making the delivery of sediments into these rivers minimal compared with that of the Tsitsa River system. The Qurana River and Millstream catchments are in almost similar conditions as the Tsitsa, although the Millstream is much better managed. The Millstream catchment is dominated by privately-owned forestry enterprises.

2.2.5 Sampling sites

The study was conducted seasonally at eight selected sampling sites over a period of one year, beginning in late winter (August 2016) and ending in late autumn (April 2017) of the following year. The selected sites are situated in the Tsitsa, Qurana, Pot and Little Pot Rivers as well as the Millstream. The eight sites were situated as follows, Site 1 (Tsitsa River upstream site), Site 2 (Tsitsa River downstream site), Site 3 (Qurana River), Site 4 (Pot River upstream site), Site 5 (Pot River downstream site), Site 6 (Little Pot River), Site 7 (Millstream upstream site) and Site 8 (Millstream downstream site). Site selection was done to reflect a gradient of sediment impact on the bases of initial visual inspection of the degree of siltation and turbidity as well as the diversity of habitat to support an array of macroinvertebrate assemblages. The initial visual assessment and turbidity measurement placed the sites in the Tsitsa River, Qurana and Millstream as being more impacted by sediments than those in the Pot and Little Pot Rivers. In addition to sediment impact consideration and habitat availability, accessibility and safety concerns were considered when selecting the sites. All the sites are situated within the Mzimvubu catchment.

Site 1 (Tsitsa River upstream site)

Site 1 is situated in the upper reaches of the Tsitsa River. The surrounding area is subjected to both private and communal land uses. There was evidence of sediment influences at the site due to visible gully erosion adjacent to the riparian areas. There are a number of homesteads around the site and the dwellers were engaged in livestock farming and field tillage, which all contribute to sediment delivery into the system. The site was selected as an example of a highly sediment-influenced site. Macroinvertebrate sampling habitats were adequate (Figure 2.2).

Dry Season	Wet Season
	

Figure 2. 2: Site 1: Tsitsa upstream site during the dry and wet seasons.

Site 2 (Tsitsa River downstream site)

Site 2 is situated between Maclear and the Qumbu district and surrounded by rural communities such as the Cekwayo, Singungweni and Ngqongweni. The site receives an influx of sediment, mainly from the upper reaches of the Tsitsa and its tributaries, and was thus selected as an example of a highly sediment-influenced site. The site is characterised by high gully erosion caused mainly by habitat modification in the riparian areas. The major occupation of the rural dwellers is subsistence agriculture, where poor grazing practices are entrenched. Macroinvertebrate sampling habitats were adequate (Figure 2. 3).

Dry Season	Wet Season
	

Figure 2. 3: Site 2: Tsitsa downstream site during the dry and wet seasons.

Site 3 (Qurana River)

Site 3 is situated on the Qurana River, which joins the lower reaches of the Tsitsa River. The site was surrounded by riparian vegetation, mostly trees and shrubs. It receives high loads of sediment from the agricultural activities in the catchment and also characterised by highly visible bank erosion. The site is characterised by a high number of channel and non-channel gullies. The site was chosen as an example of a sediment-impacted site. Macroinvertebrate sampling habitats were adequate (Figure 2.4).

Dry Season	Wet Season
	

Figure 2. 4: Site 3: Qurana tributary site during the dry and wet seasons.

Site 4 (Pot River upstream site)

Site 4 is situated at the Pot River Pass and was selected as an example of a sediment less-influenced site. The site flows through privately-owned farmland with limited cattle grazing activities. There is little evidence of gully erosion on the floodplain and was less turbid. The site has a crossing bridge that may be the major contributor of fine sediment from motorist and pedestrian traffic, though this is regarded as having only a minimal effect. In terms of sampling habitat, the site is composed of a range of diverse habitats, including stones, vegetation and sediments. Macroinvertebrate sampling habitat was thus considered adequate (Figure 2.5).

Dry Season	Wet Season
	

Figure 2. 5: Site 4: Upper Pot River site during the dry and wet seasons.

Site 5 (Pot River downstream site)

Site 5 is situated downstream of the Pot River in the district of Mount Fletcher. The site receives a mixture of water from upstream of the Pot River and flows through a privately-owned farmland. The site was selected as an example of a sediment less-influenced site. Macroinvertebrate sampling habitats were considered sufficient, with a range of sediments, stones and vegetation, although vegetation was not extensively represented (Figure 2. 6).

Dry Season	Wet Season
	

Figure 2. 6: Site 5: Pot River downstream site during the dry and wet seasons.

Site 6 (Little Pot River)

Site 6 is situated on the Little Pot River. The river at the site flows through a privately-owned farmland with limited grazing activities. The site is located on the Woodcliffe farm, with little evidence of gully erosion, and was chosen as an example of a sediment less-impacted site because of the minimal evidence of sedimentation and the clarity of the water. All macroinvertebrate sampling habitats were sufficiently represented (Figure 2. 7).

Dry season	Wet Season
	

Figure 2. 7: Site 6: Upper Little Pot River site during the dry and wet seasons.

Site 7 (Millstream upstream)

Site 7 is situated on the Millstream River. The site is a tributary of the Mooi River and receives water from a fountain that is upstream of the site. The site is approximately 5 km from the main town of Maclear. There is little evidence of gullies present at the site. The site flows through privately-owned farmland (Killarney plantation). The site was chosen as an example of a sediment-impacted site because of the agricultural activities, related to grazing and forestry, in the surrounding catchment. All macroinvertebrate sampling habitats were sufficiently represented (Figure 2.8).

Dry season	Wet season
	

Figure 2. 8: Site 7: Millstream upstream site during the dry and wet seasons.

Site 8 (Millstream downstream)

Site 8 is situated at the Millstream River where it forms a tributary and is situated on the Killarney plantation and is a few kilometres from the main centre of Maclear. The site was selected as an example of a sediment-impacted site. The major cause of sediments may be activities such as pedestrian and motorist traffic, though this is often regarded as having a minimal effect. Another possible cause of sediments might be attributed to the fact that this site receives water from the upper reaches of the river. All the sampling habitats were sufficiently represented (Figure 2.9).

Dry Season	Wet Season
	

Figure 2. 9: Site 8: Millstream downstream site during the dry and wet seasons.

The summary of geospatial information of the sampling sites is shown in Tables 2. 1
Tables 2. 1: Geospatial information and sampling site.

Site name	**Site No**	**Latitude**	**Longitude**	**Altitude (masl)**
Tsitsa upstream	1	S 30° 56' 51.5"	E 28° 27' 16.2”	126
Tsitsa downstream	2	S 31° 8' 34.69"	E 28° 40' 26.29"	887
Qurana tributary	3	S 31° 9' 29.16",	E 28° 39' 55.22"	895
Pot River upstream	4	S 30° 56'56.62"	E 28°14'1.72"	1322
Pot River downstream	5	S 31° 01’28.4”	E 28° 25’33.4”	1160
Little Pot River	6	S 30° 59’32.9”	E 28° 09’ 55”	138
Millstream upstream	7	S 31° 3' 28.04"	E 28 17' 30.91"	1413
Millstream downstream	8	S 31° 3' 6.91"	E 28° 18' 31.46"	1386

2.3 Measurement of physico-chemical variables

Water physico-chemical variables were measured at all sampling sites over the study period (begening late winter, August 2016, spring, October 2016, summer, January 2017 and autumn, April 2017) and concurrently with macroinvertebrates sampling. For each sampling event, the physico-chemical variables measured on-site included dissolved oxygen (DO), electrical conductivity (EC), turbidity, temperature and pH. Dissolved oxygen (DO), temperature and EC were measured using the multiparameter probe model H198. Turbidity was measured using the portable turbidity Orbeco-Helliage 966 Metre.

2.3.1 Collection and preservation of water samples for chemical analysis

Water samples were collected using 250 ml polyethene acid wash bottles which were then transported to the water quality laboratory of the Institute for Water Research, Rhodes University. In the laboratory, samples were preserved in a refrigerator at a temperature of 4°C until they were analysed. Preservation was in accordance with UNEP/WHO (1996) standards (Bartram *et al*., 1996). Samples were preserved for not more than 24 hours, before analysis for nitrate-nitrogen (NO_3-N), nitrite-nitrogen (NO_2-N), orthophosphate-phosphorus (PO_4-P), ammonium-nitrogen (NH_4-N), and total inorganic nitrogen (TIN) was done. Orthophosphate phosphorus and NH_4-N were analysed using Merck spectroquant® phosphate and ammonium concentration test kits, catalogue numbers 1.14752.0001 and 1.14848.0001 respectively. Analyses were in accordance with the manufacturer's instructions. Nitrate-nitrogen (NO_3-N) and NO_2-N were analysed according to APHA *et al*. (1971) on a Biotek microplate reader at 540 nm. Total inorganic nitrogen (TIN) concentration was calculated by adding the concentrations of nitrate-nitrogen, nitrite-nitrogen and ammonium-nitrogen according to Palmer *et al*. *(*2004).

2.3.2 Assessment of water quality variables in relation to Resources Water Quality Objectives (RWQOs)

The physico-chemical variables measured at the eight sampling sites were assessed in relation to the generic resource water quality objectives (RWQOs). The generic resource water quality objectives (RWQO) provide measurable objectives that ensure the balancing of use and protection of water resources. The RWQO are the water quality components of the Resource Quality Objectives. The generic RWQOs were used in this study because at the time the study was conducted, RWQOs for the studied river system were not formalised and not available.

The generic RWQOs were derived from the South African Water Quality Guidelines (SAWQGs) as set in DWAF (2006). The SAWQGs set discrete values for water quality variables called constituents that depict the change from one category of fitness for use to another. The fitness for use is a scientific judgment, involving the objective evaluation of available evidence, of how suitable the quality of the water resource is, for its intended use. It can range from water resources being completely unfit for use to being ideally fit for a specific use. The fitness for use category is determined using the Target Water Quality Range (TWQR), Chronic Effect Value (CEV) and Acute Effect Value (AEV) in the SAWQGs (Tables 2.2) (DWAF, 2006).

The SAWQGs uses the Target Water Quality range (TWQR) as the main management category by setting benchmark values or ranges for each water quality variable. The TWQR is a national management objective used to set specific ideal concentration values for a particular water variable such that it remains within no effect range. The CEV defines the concentration level of a particular variable at which there is expected to be a significant probability of chronic effects and the AEV defines the concentration level of a variable above which there is expected to be a significant acute effect. The SAWQG values were translated into generic RWQO limits as summarised in Tables 2.2 below. These limits were then used to interpret the acceptability of fitness for use in terms of ecosystem function in the studied river systems.

Tables 2.2: Generic Resources Water Quality Objectives standards for selected physico-chemical variables derived as prescribed in DWAF (2006)

Variables	**Ideal**	**Acceptable**	**Tolerable**	**Unacceptable**
EC (mSm)	30	50	85	120
DO (mg/l)	120	90	40	40
pH	7.25	8.2	0	8.4
PO_4-P (mg/l)	0.005	0.015	0.025	>0.025
NO_3-N (mg/l)	6	10	20	>20
NH_4-N (mg/l)	0.015	0.044	0.073	>0.073
TIN (mg/l)	0.5	2.5	10	>10

Linking fitness for uses to ecological categories

The RWQO takes into account the reserve, which in turn takes into account ecological categories of water resources. The ecological categories range from an unmodified natural category (Category A) to a critically modified category (Category E/F) (Tables 2. 3). The fitness for use categories were translated into ecological categories following the method prescribed in DWAF (2006) and these were then used to interpret the result of the physico-chemical variable from an ecological perspective (Tables 2. 3).

Tables 2. 3: Rules for setting and deriving fitness for use category, and translating the fitness for use into ecological categories

SAWQGs User category and effect	**Water fitness for use category**	**Ecological health category**
Upper limit of the TWQR	Ideal -100% fit for use by all users at all times	A - Natural
Average of TWR and CEV	Acceptable - slight problem encountered for limited period	B – Largely natural C – Moderately modified
Upper limit of CEV	Tolerable - moderate to severe problem for limited period	D – Seriously modified
Above the CEV	Unacceptable: unfit for intended use at all times	E/F – Critically modified

2.3.3 Sediment sampling and analysis

Settled and suspended sediments were collected using the disturbance technique (Collins and Walling, 2007; Duerdoth *et al*., 2015; Jones *et al*., 2015). To sample suspended fine sediments, an open-ended, cylindrical polyethene bucket (height 75 cm; diameter 48.5 cm) was carefully inserted into an undisturbed patch of stream bed to a depth of about 10 cm. The water column was agitated vigorously for about one minute using a wooden pole of 15 cm long, without touching the streambed. The agitated water sample was then immediated collected using 250 ml plastic bottle, while the water remained in vigorous motion. The sampled sediment was filtered through a net of 2000 µm mesh size. The filtration through a net was to remove all particles larger than 2000 µm such as cobbles, leave litters and twigs. To collect the settled fine sediment, the stream bed was disturbed vigorously to a depth of about 10 cm for one minute, to keep in suspension any subsurface fine sediments. The 250 ml of the agitated sample was quickly taken and filtered through a sieve of 2000 µm mesh size. For each sampling occasion, two sampling locations were visually identified. The samples were placed in a cooler box with ice packs and returned to the laboratory for analysis.

In the laboratory, sediment particle sizes were characterised using the Mastersizer 3000 laser diffraction particle size analyser, designed to measure particle sizes in the range of 0.02 to 2000 µm. The Mastersizer 3000 laser diffraction particle size analyser uses a blue 488.0 µm wavelength LED and red 633.8 µm wavelength He-Ne laser single-lens detection systems. Prior to sediment size distribution analysis, the samples were left to stand for 24 hours to allow the sediments to settle and part of the water solvent on the settled sediments was then gently decanted. Thereafter, the samples were oven-dried for 48 hours at a temperature of 55 ^{o}C (Figure 2. 10). The oven-dried samples were then crushed in a mortar to ensure homogeneity of the particles. About 0.3 – 0.5 g of the crushed samples was transferred into a 40 ml beaker filled up to 30 ml, and a 10 ml dispersant of sodium hexametaphosphate was then added to the beaker. The dispersant ensures that the particle sizes are evenly dispersed and the remaining organic materials within the sediments removed. A 500 ml beaker filled with tap water was placed onto the hydro EV unit of the Mastersizer spinning at 3000 rpm. The prepared sample particles were gradually added with the aid of a teaspoon onto the surface of the water in the 500 ml beaker until an obscuration range was reached. Once the obscuration range was reached, no further samples were added, but spinning continued for about 5 – 6 minutes and particle output analysed in micrometre (µm).

Figure 2. 10: Sediment samples left to settle out of solution (left photo) and evaporated samples(rightphoto).

The sediment particle size output from the Mastersizer 3000 was further analysed for particle characterisation using the GRADISTAT version 8.0 software (Blott and Pye,2001). The particle sizes were characterised as shown in Table 2. 4.

Table 2. 4: Particle size categories as used in this study (adapted from Blott and Pye, 2001)

Particle size description	**Size range (µm)**
Sand	
Very Coarse Sand	1000 – 2000
Coarse sand	<1000 – 500
Medium sand	<500 – 250
Fine sand	<250 – 125
Very fine sand	<125 – 63
Silt	
Very coarse silt	<63 – 31
Coarse silt	<31 – 16
Medium silt	<16 – 8
Fine silt	<8 – 4
Very fine silt	<4 – 2
Clay	

Clay	<2

2.4 Integrated habitat assessment of sampling sites

In freshwater ecosystems, physical habitat structure is of critical importance to the composition, diversity and abundance of resident biological communities. The Integrated Habitat Assessment System (IHAS) is an approach used in South Africa to assess the diversity and quality of the microhabitat of stream and river ecosystems. To provide a measure of the microhabitat diversity and quality at the sampling sites, an IHAS was undertaken (McMillan, 1998; Ollis *et al.,* 2006). The IHAS is based on sampling habitat and general stream characteristics such as natural and anthropogenic impacts (McMillan, 1998). The IHAS scoring is based on a hundred percent (100%) points and is divided into two sections - the sampling habitat and the stream condition.

The sampling habitat consists of 55 points and stream conditions 45 points. The sampling habitat section is sub-divided into three subsections: stones in the current (SIC) (20 points), vegetation (15 points) and other habitats (20 points). The stream condition section provides an evaluation of a site in terms of its physical characteristics and the degree of disturbance present, including estimates of aspects such as stream width, depth and velocity. The assessment was conducted at each sampling site for this study and was done by completing the IHAS assessment sheet after a visual inspection of the physical characteristics and condition of the sites.

2.5 Macroinvertebrate sampling

Macroinvertebrates were sampled concurrently with physico-chemical sampling during the study period. Macroinvertebrates were collected using a kick net (dimension 300 x 300 mm frame and 1000 µm mesh) in accordance with the South African Scoring System version 5 protocol (SASS5) (Dickens & Graham, 2002). Three distinct biotopes were sampled at each sampling event and these included stones (stones-in-current, SIC and stones-out-of-current, SOOC), vegetation (marginal and aquatic vegetation), and sediment gravel, sand and mud (GSM). The SIC included pebbles and cobbles (2 – 25 cm), and boulders greater than 25 cm situated in current that prevents the deposition of fine sediments. The SOOC included pebbles and cobbles, and boulders in pools that allow the deposition of sediments. The marginal vegetation comprised vegetation growing on the edge of the river and fringing into the river, whereas aquatic vegetation was largely submerged in the main river channel. Gravels are small stones usually less than 2 cm in diameter, and sand and mud are smaller, less than 2 mm and 0.06 mm respectively. In general, sampling the three biotopes ensured all habitats colonised by macroinvertebrates were taken into account.

During each sampling season, three replicates per site were sampled for each biotope, making a total of nine samples. Throughout the study period, a total of 36 samples were collected per site, making a total of 288 samples for the eight sampling sites. Large replicate samples were collected to permit robust statistical analysis of the data. Samples were transported to the laboratory for sorting and identification of EPOT species.

2.5.1 Macroinvertebrate sample processing and identification of Ephemeroptera, Plecoptera, Odonata and Trichoptera taxa

Sampled macroinvertebrates were preserved in 75% ethanol in plastic bottles and transported to the laboratory. In the laboratory, samples were sorted and EPOT specimens first separated from the rest of the macroinvertebrates specimens at the family level. The sorted EPOT specimens were then identified to either genus or species level depending on the taxonomic level practically possible for the specimen. The specimens were dissected for body parts, including the mouthparts, gills, and appendages, under a dissecting microscope (X10 and X40) and identification was undertaken using the keys described by De Moor *et al.* (2003). Occasionally, the gut contents were analysed. Where identification was not certain, specimens were taken to the Albany Museum in Grahamstown for specialist identification. Specimens identified with certainty were also taken to the Albany Museum for verification.

2.5.2 Taxonomic metrics used for assessing EPOT response to sediments stress

Generally, the presence of sensitive groups of macroinvertebrates, such as Ephemeroptera, Plecoptera and Trichoptera (EPT) indicate relatively un-impacted waterways (Lenat, 1993). In contrast, tolerant groups of macroinvertebrates such as the Oligochaetes and Chironomids indicate polluted waterways. Quantifying taxa richness, diversity, abundance and community patterns is commonly used for bioassessment. Certain environmental stressors may increase or decrease population sizes of certain species and these differences can be used to indicate stress level in the aquatic environment (Resh & Jackson 1993). In this study, the stressor of interest is elevated sediments. The multimetric approach combines a range of metrics and indices representing different aspects of macroinvertebrate taxonomic measures to assess river health.

Six metrics representing different taxonomic measures, including richness (number of taxa), abundance (absolute number of individuals), compositions (% relative abundance), and diversity measures were used to assess the taxonomic structural response of the EPOT taxa to sediment stress (Table 2. 5).

Table 2. 5: Macroinvertebrate taxonomic-based metrics applied to the EPOT taxa collected in the Tsitsa River and its tributaries from August 2016 to April 2017

Category	Metrics	Description
Richness measure	Mangalef index	Accounts for both individuals in a population and taxa.
Abundance measure	Absolute number of EPOT taxa individuals	Number of individual species of Ephemeroptera, Plecoptera, Odonata and Trichoptera orders.
Diversity measure	Shannon-Wener index	Takes account of the contribution of individual species to diversity and evenness, while assigning more weight to dominant species.
	Simpsons index	A measure that accounts for both richness and proportion (percent) of most common species (Goudarzian & Erfanifard, 2017).
	Pielou’s evenness	A measure that accounts for the equitability of species diversity. It represents the degree to which individuals are distributed among species with low values, indicating that one or a few species dominate, and high values indicating a relatively equal number of individuals belonging to each species (Morris et al. 2014).

2.5.3 Macroinvertebrate traits and trait selection

The traits selected for this study are relevant to the EPOT species and have a mechanistic relationship with sediment modes of stress. In selecting traits, their adaptive value in relation to the organism in the context of sediment stress was also taken into account. The traits were obtained through comprehensive review of the literature (Merritt & Cummins, 1996; Poff *et al.*, 2007; Vieira *et al.*, 2006) and from the South Africa macroinvertebrates trait databases (Odume *et al.*, 2018). Traits were assigned to each taxon using relevant literature databases and expert knowledge. The selected traits included maximum body size, locomotion, substrate attachment, body armouring, food preferences, feeding habits, biotope preferences, gill type and oviposition behaviour.

2.5.3.1 Maximum body size

Multiple biological traits (e.g. body size, life cycle, food) each described by multiple trait categories (e.g. small, intermediate, large body size), generally occur throughout a region, i.e. they could provide a 'multi-probe' biomonitoring tool for discriminating various types of human impact (e.g. Doledec *et al.*, 1999; Statzner *et al.*, 2005).

The body size measure used in this study was the length of each organism. The maximum body size of EPOT species was categorised into four trait attributes: <5 mm, 5 - 10 mm, 10 - 20 mm and >20 mm. This was done taking into consideration the maximum potential sizes of all species under the EPOT taxa. Large body size is expected to be vulnerable because a highly sedimented patch reduces the habitat available for large body species (Bonada *et al.*, 2007; Descloux *et al.*, 2014). The body size classes (<5 mm, 5 - 10 mm, 10 - 20 mm and >20 mm) employed in this study have been widely used to group macroinvertebrates into body size classes (e.g. Tomanova *et al.*, 2008). All the body size trait information was obtained from appropriate literature including the South African trait database (Tachet *et al.*, 2002; Odume *et al.,* 2018).

2.5.3.2 Locomotion

Locomotion is important because it describes the various means by which macroinvertebrates move; means of mobility can determine organisms' resilience capacity or vulnerability to sediments. Information about the locomotion of macroinvertebrate families was obtained through empirical observation and literature sources (e.g. Merritt *et al.*, 1996; Gerber &

Gabriel, 2002; Tomanova *et al*., 2008). Mobility was categorised into six trait classes including climbers, crawlers, sprawlers, swimmers, and burrowers. Traits were assigned to species using relevant literature, the SA trait database and empirical observation.

2.5.3.3 Substrate attachment

Species are attached to the substrate either permanently or temporarily while others may be free-living and the quality of such substrate can determine an organism's tolerance and sensitivity to sediments. In this study, substrate attachment was categorised into three trait attributes: permanently attached, temporarily attached, and free living. Attachment is related to sediment stress because permanently attached individuals are more likely to be buried by deposited sediments as they are unable to escape, thus increasing their likelihood of vulnerability to sediments.

2.5.3.4 Body armouring

Body armour is the protective structure a species possesses that enables it to withstand or avoid abrasion. The trait of body armouring was chosen because the species of the order EPOT comprises of individuals with soft bodies that are partially sclerotised with some species being cased or tubed. Species that build protective tubes or cases are more likely to avoid abrasion as they retract into their cases or tubes, thus making them less vulnerable than those without cases or tubes

2.5.3.5 Food preferences

Macroinvertebrates feed on different kinds of food materials, obtained through different mechanisms. The food an organism consumes determines its vulnerability. Most of the food consumed by macroinvertebrates is obtained from sediments substrate and as such, a high deposition of sediments affects the quality and quantity and the availability of food for macroinvertebrates. For example, grazers feed on periphyton and macrophytes, the high sedimentation on periphyton and macrophytes will reduce the quality of periphyton available for grazers. The food preferences for this study were obtained from relevant literature and resolved into 5 categories see Chapter 4 of this thesis.

2.5.3.6 Feeding habits

Feeding habit refers to the way in which macroinvertebrates obtain their food (Cummins and Klug, 1979). Shredders feed mainly by chewing or mining coarse detrital materials known as coarse particulate organic matter (CPOM) such as wood and non-woody materials e.g. leaves, needles, buds etc., that exist on the sediment substrate. Collectors feed mainly on the detrital fine particulate organic matter (FPOM) either by filtering food materials in transport (collector-filterers) or gathering substances deposited on sediments (collector-gatherers) (Cummins & Klug, 1979). Scrapers graze upon (i.e. shear off) food materials, particularly periphyton attached to surfaces. Predators are adapted to the capture of living prey. Feeding group has been used in many studies to assess the effect of sediment on macroinvertebrates (Rabeni *et al.*, 2005). In this study feeding habits were categorised into five attributes i.e. detritus (FPOM), detritus (CPOM), macrophytes/Algae and animal materials. The trait information for feeding group was obtained from relevant literature including (Palmer, 1991) and the South African macroinvertebrate database currently being compiled (Odume *et al.*, 2018).

2.5.3.7 Biotope preferences

The biotope preferences refer to the habitat type that macroinvertebrates inhabit, in such as stones, GSM and vegetation. Information for biotope preferences for the EPOT species was obtained from the South African macroinvertebrate database (Odume *et al.,* 2018)

2.5.3.8 Gill type

Macroinvertebrates respire using different structures such as plastron, trachea teguments, lungs and gills. However, the most common respiratory structures for aquatic macroinvertebrates is the gill. The gills come in different forms and shapes. The trait of gill type was resolved into plate-like, filamentous, lamellate and operculate gills. The gills that are exposed such as filamentous are more in contact with sediments than protected gills such as operculate gills. The trait information for gill type was obtained from relevant literature such as Tillyard (1916), Tachet *et al.* (2002), and screening and observation under the microscope.

2.5.3.9 Oviposition behaviour

Oviposition refers to the mechanism of egg-laying by macroinvertebrates. Macroinvertebrates' oviposition behaviour include endophytic and exophytic behaviours. Species exhibiting endophytic behaviour lay their eggs inside substrate structures such as tissues of plant, while those exhibiting exophytic behaviour lay eggs in open waters or on substrates. Eggs laid in the structures are less exposed to sediment stress, compared to those on surfaces of water and substrates, which make them potentially more vulnerable compared to those inside structures. Information for oviposition was obtained from the relevant literature (Corbet, 1999; Vieira *et al*., 2006; Statzner *et al*., 2007).

2.6 Statistical analysis

Data in this study were subjected to relevant univariate and multivariate statistical analyses to indicate statistical differences and to elucidate relationships and patterns. The purpose of this subsection is to briefly describe the statistical test employed in this study. In Chapters 3 and 4, the way in which each of the analyses was applied is provided.

2.6.1 Multivariate Analysis of Variance (MANOVA) and Analysis of Variance (ANOVA)

Data were first captured and prepared in an Excel spreadsheet thereafter the multivariate analysis of variance (MANOVA), a parametric statistic that compares the means between two or more samples simultaneously using multiple dependent variables, was used to compare the sites in terms of the sediment particle size distribution and the physico-chemical variables (Chapter 3). Prior to using MANOVA, the basic assumptions of normality and homogeneity of variance were investigated using the Shapiro-Wilk test and the Levene's test, respectively. When it appeared that assumptions were violated, data were transformed logarithmically or normalised if assumptions were still not met. The one-way analysis of variance (ANOVA) was undertaken to compare the sites in terms of proportions of coarse silt sediment particles as well as electrical conductivity (EC**).** When ANOVA indicated a global significant difference, a post-hoc test, the Tukey's Honesty Significant Different (HSD) test was used to indicate the sites that differed. MANOVA, ANOVA and Turkey's HSD test were conducted using the Statistica software package version 13.

2.6.2 Cluster analysis

A hierarchical cluster analysis, a multivariate statistical technique, was applied to group the sites based on turbidity values and suspended and settled sediment characteristics. The aim was to classify the sites into site groups based on their degree of similarity. The site groups were then used for further analysis, including frequency of occurrence (FROC) and indicator species in Chapters 4 and 3 respectively. The cluster analysis was also applied to analyse the similarities and dissimilarities between the sampling habitats in terms of the macroinvertebrate data for each of the sampling seasons (Chapter 3). Cluster analysis returns a hierarchical agglomerative diagram, with the x-axis indicating sites and the y-axis, the distance measure of similarity or dissimilarity. The Euclidian distance measure was used because it was deemed a more appropriate measure for non-biological variables and Bray-Curtis distance was used for biological variables. Sediment particle sizes were square root transformed prior to cluster analysis. The analysis was done using Vegan package 2.4.3 in R version 3.4.1 (Oksanen, 2017; R Core Team, 2017).

2.6.3 Kruskal-Wallis test

The Kruskal-Wallis multiple comparison test was used to investigate the significant differences between the eight sites in terms of the percent relative abundance of the EPOT vulnerability groups (Chapter 4). The Kruskal-Wallis test was also undertaken to assess the discriminatory potential of selected metrics between the sampling sites (Chapter 3). The Kruskal-Wallis test was conducted using the Statistica software package version 13.

2.6.4 Analysis of similarity (ANOSIM)

The analysis of similarity (ANOSIM) and similarity percentage (SIMPER) (Clarke, 1993) was used to compare the similarities between replicate samples within sites or seasons to those from other sites or seasons (Chapter 3). The ANOSIM produces an R-value between 0 and 1 that shows the level of similarities of replicates within a site or season with those of other sites and seasons. The Global R-value indicates the degree to which the samples are similar or dissimilar. An R-value of 1 indicates the complete separation of groups, whereas an R-value near 0 implies little or no segregation. ANOSIM was used to test whether significant differences existed between the sites in terms of the EPOT assemblages (Chapter 3).

Analysis of similarity (ANOSIM) is a multivariate non-parametric permutation procedure that detects differences based on rank similarity matrix between defined sample groups (Clarke & Warwick, 1994). In a situation where there existed a significant difference among sites and seasons, a pair-wise comparison test was done to detect sites and season that differed.

The SIMPER analysis identifies the percentage of similarity and dissimilarity contributed by each species (or factor) by disaggregating the Bray-Curtis similarities between samples. Percentage contributions of each species is influenced by the abundance of each species, the more abundant and consistently high a species is within a group, the more it contributes to the dissimilarity between groups (Clarke & Warwick, 2001). Both the ANOSIM and the SIMPER were undertaken using the Bray-Curtis distance coefficient (Bray & Curtis, 1957). ANOSIM and SIMPER were undertaken using PRIMER statistical package version 6.

2.6.5 Pearson's simple correlation

Pearson's simple correlation is used to describe the simultaneous change of random variables that are not functionally dependent on each other, while the correlation coefficient R-*value* is used as a measure of dependency (Marques de Sá & Frias, 2007). The Pearson correlation was used to evaluate the relationship between the EPOT metrics, the sediment particle sizes, turbidity, and physico-chemical variables (Chapter 3). Prior to correlation analysis, the data were transformed using a natural logarithm (log x + 1) to meet the assumption of normality. The analysis was undertaken using Statistica software version 13.

2.6.6 Pearson's point-biserial correlation

The Pearson's point-biserial correlation coefficient was computed to determine the association between EPOT species and site groups that was obtained from the cluster analysis (Chapter 4). The Pearson's point-biserial coefficient indicates the degree of a species preference for a water quality condition (De Cáceres & Legendre, 2009; De Cáceres, *et al.*, 2010). The purpose of the Pearson's point-biserial analysis was to ascertain whether taxon predicted to be more frequently associated with site groups based on their vulnerability classes were indeed so associated, based on the correlation analysis. The point-biserial correlation coefficient was used to assess the correlation between EPOT species and selected biotopes (Chapter 4). The purpose of the analysis was to determine the influence of biotopes on the assemblage structure of EPOT species and identify assemblage types based on their preferred biotopes. The statistical significance of the association was tested using the Monte Carlo permutation test with 999 permutations at *alpha* = 0.05. The Pearson's point-biserial correlation coefficient was undertaken using the Indicspecies package version 1.7.1 within the R version 3.4.1 software environment (De Cáceres, 2013; R Core Team, 2017).

2.6.6 Fourth-corner analysis

The fourth-corner test was applied to test the relationship between each trait category and sediments particles (Dray & Legendre, 2008; ter Braak *et al.*, 2012). The fourth-corner test is a multivariate analysis that provides a global picture of the traits-environment relationships. It helps to test the significance of individual trait-environment associations and reveals both positive and negative correlations of individual trait attributes to environmental variables. The statistical significance of the RLQ axes was tested using the Monte Carlo permutation test with 999 permutations at *alpha* = 0.05. RLQ and associated analyses canonical analysis (CA), principal component analysis (PCA) and Hill-Smith analysis, including the Fourth-Corner test, were performed using the ade4 package in R software version 3.4.1 (Dray & Dufour, 2007; R Core Team, 2017).

2.6.7 Ordination analyses

The RLQ analysis was applied to examine relationships between environmental variables and trait attributes of EPOT species assemblages, following Shieh *et al.* (2012) (Chapter 4). RLQ is a multivariate ordination analysis developed by Doledec *et al.* (1996) that simultaneously performs ordinations on three matrices (species abundance, environmental variables, traits). It provides an efficient means of assessing how environment variables filter species traits. Prior studies have shown that RLQ is a useful tool for evaluating trait-environment relationships (Mellado Diaz *et al*., 2008; Kummanovic *et al.,* 2017). The three tables or datasets i.e. environmental variable dataset, species dataset and trait dataset are separately analysed using correspondence analysis (CA), principal component analysis (PCA) and the Hill-Smith analysis (Hill and Smith 1976). The CA was performed on data in table L, which is the species abundance data. The R table, which is the environmental variable data and row weights (site scores), was analysed using the PCA, while the Hill-Smith analysis was performed on the Q table. The RLQ analysis was then performed on the result of the three separate analyses. In RLQ analysis, the row weights (i.e. sites and species scores) of the table L functioned as links between the table R and the table Q. Therefore, RLQ analysis was an extension of co-inertia analysis, which simultaneously took into account the information contained in the tables R, L and Q (Wesuls *et al*., 2012). The contributions to total inertia were used as a measure of relative importance of each environmental factor/trait in the RLQ analysis, to identify the most important traits and environmental factors shaping the species assemblage (Wesuls *et al*., 2012) (Chapter 4).

The non-metric multidimensional scaling (NMDS) was applied to evaluate the correlation between the sampling sites and seasons in terms of EPOT species assemblages. The Bray-Curtis distance measure (Bray & Curtis, 1957) was used because it was deemed a more appropriate measure for biological data. NMDS was undertaken using the PRIMER statistical software version 6.

Detrended correspondence analysis (DCA) (Hill and Gauch 1980) was carried out on the community of EPOT species and traits to determine the gradient length of the dataset. Determination of the data gradient length is necessary in order to choose which ordination procedure, such as redundancy (RDA) or canonical correspondence (CCA) needs to be applied. When DCA returns a gradient length of < 3 standard deviations (SD), an RDA is more appropriate; when a gradient length of > 4 standard deviations (SD) is returned, a CCA is a more appropriate test as the data are deemed linear; if DCA returns a length >3, CCA is deemed more appropriate as the data are unimodal in distribution (Leps & Smilauer, 2007). DCA was undertaken using the Vegan package version 2.4.3 in R software version 3.4.1 (Oksanen, 2017; R Core Team, 2017). Canonical correspondence (CCA) (Ter Braak & Smilauer, 2002) was used to investigate the relationship between the eight sampling sites and the EPOT species assemblages in relation to the measured selected physico-chemical water quality variables (Chapter 3). CCA was also used to evaluate the relationships between the eight sites in terms of EPOT assemblages and sediments particle distribution (Chapter 3). Furthermore, CCA was used to evaluate the correlations between the physico-chemical variables and the EPOT metrics (Chapter 3). The CCA is a direct linear ordination method, whose axes are constrained by the environmental variables in order to extract inter-related variations that are useful when gradients are short (Lepš & Šmilauer, 1999). In the CCA techniques, the species show increasing or decreasing trends (linear responses) in relation to environmental gradients. The use of CCA was generally recommended by Kleyer *et al*. (2012). CCA is a powerful tool for simplifying complex data sets, and, being a direct gradient analysis, it allows an integrated analysis of both taxa and environmental data (ter Braak & Smilauer, 2002). The statistical significance of the CCA axes was tested using the Monte Carlo permutation test with 999 permutations at *alpha* = 0.05. Canonical correspondence (CCA) was undertaken using the Vegan package version 2.4.3 within R software version 3.4.1 (Oksanen, 2017; R Core Team, 2017).

CHAPTER 3: TAXONOMIC ASSEMBLAGE RESPONSES OF EPHEMROPTERA, PLECOPTERA, ODONATA AND TRICHOPTERA (EPOT) TAXA TO SEDIMENT STRESS IN THE TSITSA RIVER AND ITS TRIBUTARIES

3.1 Introduction

The transport of sediments into aquatic ecosystems impacts on water quality and often affects macroinvertebrate diversity and assemblage structure (Mathers *et al*., 2017). The EPOT taxa are a sensitive group of macroinvertebrates and are often used as bioindicators in freshwater biomonitoring. This chapter focuses on the taxonomic assemblage response of the EPOT taxa to sediment stress, with a view to interrogating their bioindicator values. Although the EPOT group is often designated as sensitive to water quality impairment, at species or generic levels of taxonomic resolution, they could be differently sensitive and responsive to sediment stress hence in this study, species/genus level responses are investigated.

The accretion of sediments into the rivers and streams has been noted to modify macroinvertebrate assemblage structure and function (Descloux *et al.,* 2014). Macroinvertebrates, including the EPOT, can be particularly affected by alteration of habitat stability, increased turbidity, and impairment of feeding through the reduction of the food value of periphyton and the clogging of feeding sand respiratory structures, occasioned by sediment accretion (Conroy *et al*., 2016).

Furthermore, the particle size distribution of sediment is an important characteristic that can influence sediment effects on aquatic macroinvertebrates (Rosenberg & Wiens 1978; Kaller & Hartman, 2004). For example, smaller sediment particles are known to be more deleterious to macroinvertebrates because of their capacity to accumulate a high concentration of contaminants and to clog fine biological structures, and their high ion exchange capacity (Leitner *et al*., 2015).

Although most studies have focussed on sediment loads, measured in the form of total suspended solids, degree of embeddedness and turbidity, as indicators of sediment stress on macroinvertebrates (Gordon *et al*., 2013), very few studies have paid detailed attention to characterising sediment particle sizes under field conditions, and evaluating macroinvertebrate response in terms of both load, e.g. turbidity, and particle size classes. In the rivers where the current study was undertaken, a previous study by Gordon *et al.* (2013) focused on the sediment loads. Therefore, the present study evaluates the taxonomic assemblage responses of the EPOT taxa to sediment stress, by paying detailed attention to characterising the sediment particle sizes, as well as using turbidity as an indicator of sediment load. This chapter fulfils Objectives 1 and 2 in Chapter 1 section 1.7. Objective 1: *to provide information on selected physico-chemical and sediment characteristics of the Tsitsa River and its tributaries,* is achieved through the presentation of the physico-chemical and sediment particle size characteristics results, and relating them to the generic Resource Water Quality Objective (RWQOs). Objective 2: *to assess and evaluate the assemblage structure response of the orders Ephemeroptera, Plecoptera, Odonata and Trichoptera to the gradient of increasing sediments stress in the Tsitsa River and its tributaries*, is achieved through a detailed presentation of the taxonomic response of the EPOT taxa to sediment stress, as well as by identifying potential indicator taxa both for sediment stress and biotopes.

3.2 Materials and methods

3.2.1 Measurement of water physico-chemical variables and sediments

Water physico-chemical variables including dissolved oxygen (DO), electrical conductivity (EC), turbidity, temperature, pH, nitrate-nitrogen (NO_3-N), nitrite-nitrogen (NO_2-N), ammonium-nitrogen (NH_4-N), orthophosphate-phosphorus (PO_4-P) and total inorganic nitrogen (TIN) were analysed at each study site during each sampling occasion as described in Chapter 2 section 2.3**.** Sediments (settled and suspended) particles were also analysed and characterised as described in Chapter 2 section 2.3.3.

The multivariate analysis of variance (MANOVA) was used to test the significant differences ($P < 0.05$) in terms of the means of the physico-chemical variables, and sediment particle sizes across the eight sites. Prior to MANOVA, the basic assumption of normality and homogeneity of variance was tested, and normality applied when necessary, using square root transformation. The analysis was carried out as described in Chapter 2 section 2.6.1.

3.2.2 Assessment of water quality variables in relation to Resource Water Quality Objectives (RWQOs)

The physico-chemical variables measured at the eight sampling sites (Chapter 2 section 2.3.2) were analysed and interpreted seasonally using the RWQOs.

The analysis was carried out with a view to assessing the ecological status indicated by physico-chemical variables of the Tsitsa River and its tributaries. The assessment was done by deriving a set of generic ecological category boundaries for each variable using the method set out in DWAF (2006). The derived ecological category boundaries were then used to assess the ecological conditions of the Tsitsa River and its tributaries. The ecological categories range from natural i.e. A to critically modified i.e. E/F (Chapter 2 section 2.3.2 Table 2.3).

3.2.3 Cluster analysis of sites into groups

The cluster analysis was used to classify the study sites into groups in terms of settled and suspended sediment characteristics, including turbidity. The aim was to classify the sites into site groups based on their degree of similarity to each other (Chapter 2 section 2.6.2). The classification of sites into groups from the cluster analysis was based on based on the distance measure between the sites and also on based on the initial classification of sites into gradient of sediments impacts. The sites that were closely clustered together were grouped into one group and supported by the initial site selection into different impact gradient.

3.2.4 Spatial and temporary analysis of EPOT assemblage structure

The multivariate analysis of similarities (ANOSIM) was used to indicate significant differences between the sampling sites in terms of their species composition and the similarity percentage analysis (SIMPER) was used to indicate species contributing to the observed dissimilarities between the sampling sites (Chapter 2 section 2.6.4). The non-metric multidimensional scaling (NMDS) was further used to elucidate the assemblage structure of EPOT taxa at the eight sampling sites during the sampling seasons.

Influence of biotope on EPOT assemblage patterns

The EPOT species-specific biotope association was analysed using the point-biserial correlation coefficient analysis, in order to identify the assemblage patterns of EPOT species based on their preferred biotopes. The selected biotopes included stone, vegetation and GSM. The Pearson's point-biserial coefficient analysis indicates the degree of species-specific preferences for a given biotope. The statistical significance of the association was tested using the Monte Carlo permutation test with 999 permutations at alpha = 0.05 (Chapter 2 section 2.6.6).

Ephemeroptera, Plecoptera, Odonata and Trichoptera (EPOT) taxonomy metrics for assessing water quality in the studied river system

Five metrics in two categories abundance (absolute number of individuals) and diversity were used to assess the water quality in this study. The selected metrics were based on their potential to discriminate between sediment-impacted sites. The discriminatory ability of metrics was defined as the ability of individual metrics to discriminate sediment-impacted site groups from those that are less impacted (Klemm *et al*., 2002). Box plots were plotted for the site groups to evaluate the ability of each EPOT metric to discriminate between the sediment-influenced site groups 1, 2 and 3, and the less sediment-influenced site group 4. The degree of overlap of medians and interquartile ranges (IQRs) between sediment-influenced site groups and less sediment-influenced site groups was considered an indicator of the discriminatory capability of each EPOT metrics (Klemm *et al*., 2002). To establish the degree of overlap, the medians and IQRs of the sediment-influenced site groups and less sediment-influenced site groups were compared. Metrics with low discriminatory power were those with complete overlap in the IQRs of the site groups, but the medians were outside the IQRs. The metrics with high discriminatory power were those with no overlap in the IQRs between the site groups. Among the EPOT metrics only metrics that discriminated site group 1 (less sediment-influenced site group) from site group 1, 2 and 3 were retained for further evaluation. evaluated using Kruskal-Wallis test. To test for a metric's ability to detect subtle differences between site groups, the same criteria were used to assess whether metrics that discriminated less sediment-influenced site group 4 from those that were sediment-influenced (site groups 1, 2 and 3), were able to distinguish between the less sediment-influenced site groups 1, 2 and 3. Metrics that showed satisfactory discriminatory power between site group 4 and site groups 1, 2 and 3 were further examined using a Kruskal-Wallis test (Odume *et al*., 2012).

Relationships between EPOT metrics, physico-chemical variables and sediments, and EPOT metrics

The simple Pearson's correlation analysis was undertaken to assess the correlation between the selected metrics and physico-chemical as well as sediment particle size class (Chapter 2 section 2.6.5).

3.2.5 Relating the EPOT species to physico-chemical variables and sediments (settled and suspended)

The canonical correspondence analysis (CCA) was used to assess the correlation between EPOT species and sediments particle sizes, including turbidity, with a view to assessing the assemblage structure of EPOT in terms of the sediment particle distribution. Turbidity was included in this analysis because of its close relationship with sediments, being an indicator for measuring suspended sediment loads. Rare species occurring less than three times at the eight sites during the study period (August 2016 – April 2017) were removed from the CCA analysis to avoid their confounding the CCA final result. Prior to CCA, the EPOT data were subjected to detrended correspondence analysis (DCA), in order to determine the gradient length of the data. DCA revealed a gradient length of > 3.5 indicating that the unimodal assumption of CCA was met.

3.3 Results

3.3.1 Water physico-chemical variables for the eight study sites

The means, standard deviations and ranges of the physico-chemical variables analysed for all eight sampling sites during the study period are presented in (When the global multivariate MANOVA was carried out for the dataset to investigate whether the sites differed in terms of the concentrations of the physico-chemical variables, no global significant difference was observed. However, a one-way ANOVA did indicate a significant difference between the sites in terms of the EC values. The Turkey's post-hoc test revealed that the mean EC values for Site 3 (Qurana River) were significantly different from the EC values at Site 4 (Pot River upstream site), Site 5 (Pot River downstream site), and Site 6 (Little Pot River). The nutrient values across all sites were generally low and below the target water quality range (TWQR) i.e. limits below which a measurable effect on aquatic organisms would not be caused. Dissolved oxygen (DO) was above the TWQR, the range in which no measurable effects were expected in the aquatic ecosystem. The values for pH and temperature were similar across all the sites.

Table 3. 1: Means, standard deviations and ranges (in brackets) of measured physico-chemical variables at the eight sites during the four sampling seasons (August 2016 – April 2017). *P*-value is indicated for EC, being the only significant variable. (Superscript letters per variable indicate significant differences between sites, established using the Tukey's HSD test)

Variables	**Site**								***P*-value**
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	
Temp (^{0}C)	18.9 ± 0.4 (6.3 -24.0)	20.3 ± .9 (11.4 - 3)	19.8 ± 5.3 (12.4 - 2)	17.8 ± 8.1 (6.27 - 24.0)	16.7 ± 6.8 (7.4 - 23.4)	17.7 ± 6.7 (8.31- 23.4)	18.10 ± 8.58 (5.82 - 24)	21.0 ± 9.6 (6.7 - 3)	
pH	7.2 ± 0.5 (6.6 - 7.9)	7.5 ± 0.7 (6.7 - 8.1)	7.0 ± 0.9 (6.4 - 8.3)	6.5 ± 1.4 (4.5 -7.5)	7.2 ± 0.8 (6.2 - 8.2)	6.9 ± 1.5 (5.1 - 8.2)	7.63 ± 0.89 (6.50 - 8.60)	8.0 ± 0.2 (7.7 - 8.2)	
DO (mg/l)	6.58 ± 2.39 (5.1 - 109)	6.6 ±1.5 (5.3 - 8.7)	3.9 ± 2.1 (0.7 - 12.8)	10.1 ± 3.3 (6.1 -14.2)	9.7 ± 2.0 (7.94 - 12.1)	9.6 ± 3.4 (5.6 – 13.4)	11.7 ± 2.8 (3.9 - 2)	13.1 ± 3.2 (4.0 - 17.1)	
EC (mS/m)	75.25 ± 13.45^{d} (57 - 88)	74.75 ± 27.24^{d} (46 - 106)	14.75 ± 2.31^{a} (76 - 175)	53.75 ± 6.13^{b} (48 - 60)	44.75 ± 23.54^{b} (15 - 72)	47 ± 70.40^{c} (38 - 54)	61.5 ± 11.33^{d} (50 - 77)	68.75 ± 12.84^{d} (50 - 79)	**0.007**
(NO_3-N) (mg/l)	0.13 ± 0.12 (0.01 - 0.025)	1.67 ± 1.80 (0.01 - 3.86)	1.63 ± 1.71 (0.01 - 3.6)	3.6 ± 3.21 (0.17 - 7.23)	0.37 ± 0.26 (0.13 - 0.71)	2.98 ± 3.08 (0.24 - 6.13)	3.91 ± 2.90 (0.31 - 7.2)	4.75 ± 4.09 (0.2 - 10.1)	
(NO_2-N) (mg/l)	0.35 ± 0.44 (0.00 - 0.95)	0.26 ± 0.30 (0 - 0.7)	0.17 ± 0.18 (0.0025 - 0.4)	0.06 ± 0.05 (0.0025-0.11)	0.98 ± 1.75 (0.01 - 3.6)	0.14 ± 0.19 (0.032-0.43)	0.18 ± 0.23 (0.009 - 0.52)	1.60 ± 3.07 (0.001 - 6.2)	

(NH_4-N) (mg/l)	0.18 ± 0.35 (0.0025 - 0.73)	0.43 ± 0.52 (0.0025- 1.09)	0.15 ± 0.30 (0.0025 -.61)	0.15 ± 0.30 (0.0025 - 0.6)	0.19 ± 0.18 (0.003 - 0.59)	0.22 ± 0.23 (0.003 - 0.7)	0.15 ± 0.30 (0.0025 - 0.6)	0.16 ± 0.309 (0.0025- 0.62)	
(TIN) (mg/l)	0.28 ± 0.33 (0.03 - 0.749)	1.11 ± 0.52 (0.55 - 1.76)	0.54 ± 0.57 (0.02 - 1.16)	0.95 ± 0.85 (0.04 - 1.68)	0.53 ± 0.46 (0.05 - 1.13)	1.12 ± 0.96 (0.07 - 2.34)	1.06 ± 0.68 (0.077 - 1.64)	1.68 ± 1.33 (0.075 - 3.14)	
(PO_4- P) (mg/l)	0.91 ± 1.81 (0.0025- 3.63)	0.77 ± 1.54 (0.0025- 3.09)	0.77 ± 1.54 (0.003 - 3.09)	0.48 ± 0.87 (0.003 - 1.76)	0.72 ± 1.34 (0.003 - 2.73)	0.74 ± 0.41 (0.002-2.85)	0.82 ± 1.49 (0.003 - 3.05)	2.09 ± 1.41 (0.003 -3.09)	

Table 3. 2: Global MANOVA results indicating that the sites were not significantly different based on the values of the physico-chemical variables

Effect	**Multivariate Tests of Significance**					
	Test	Value	F	Effect (df)	Error (df)	p
Intercept	Wilks	0.006178	241.3158	10	15.00000	0.000000
Site	Wilks	0.014723	1.4297	70	94.28094	0.052813

3.3.2 Analysis of physico-chemical variables in relation to ecological categories

Seven physico-chemical variables, namely EC, pH, NO_3-N, NO_2-N, DO, NH_4-N and TIN were assessed for ecological health (Figure 3. 1a: to 3.1d). The results revealed that most of the sites had good water quality (ecological categories A, B or C) in terms of the concentrations of physico-chemical variables. EC results at all the sites indicated A and B categories except for Site 2 (autumn), and Site 3 (winter, spring and autumn) (Figure 3. 1a:a), that indicated E/F ecological categories. All the DO results were above the poor ecological category E/F, except for Site 3 (Qurana tributary) and Site 5 (Pot River downstream) during autumn. All the DO results generally indicated the A and B ecological categories, except for seasons at Site 2 (autumn), and Site 3 (winter, spring and autumn) (Figure 3. 1a:).

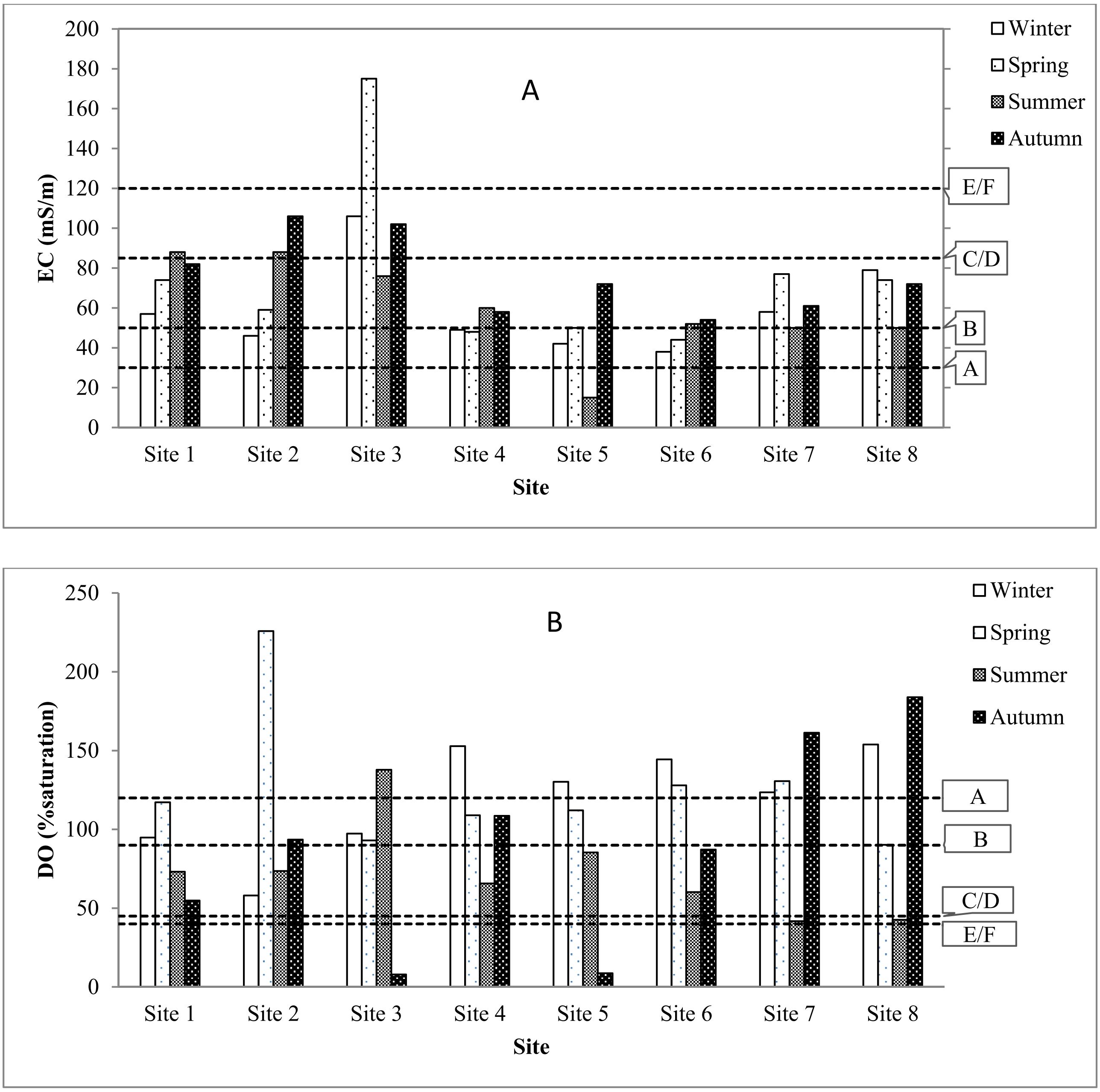


Figure 3. 1a: Ecological conditions as indicated by electrical conductivity (EC) (graph A) and dissolved oxygen (DO) (graph B) during the four sampling seasons in the studied river systems between August 2016 and April 2017. Ecological categories: A (natural), B (good), C (fair), D (poor) and E/F (very poor)

With the exception of summer at Site 8 and spring at Site 2 NO_3-N and TIN results at all the sites largely indicated ecological category B, indicative of good water quality at all sites (Figure 3.1b). During summer, PO_4-P and NH_4-N at all sites indicated ecological category E/F including PO_4-P during the winter and autumn at Site 8 and NH_4-N during winter at Site 2 (Figure 3.1c). The PO_4-P and NH_4-N values for other seasons indicated ecological category A for all sites. The pH values were within ecological categories A and B at all the sites except during spring at Site 7 (Figure 3.1d).

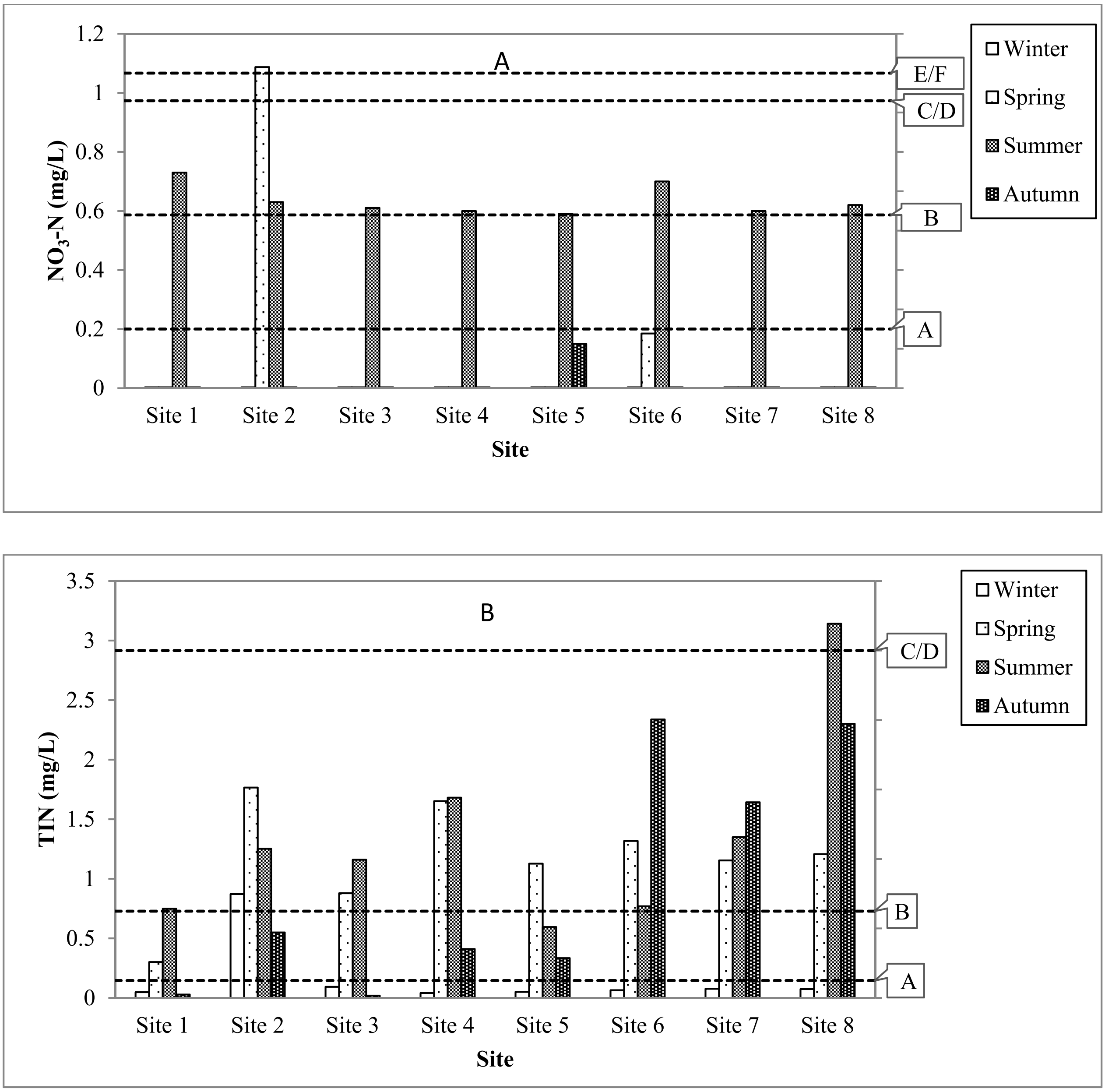
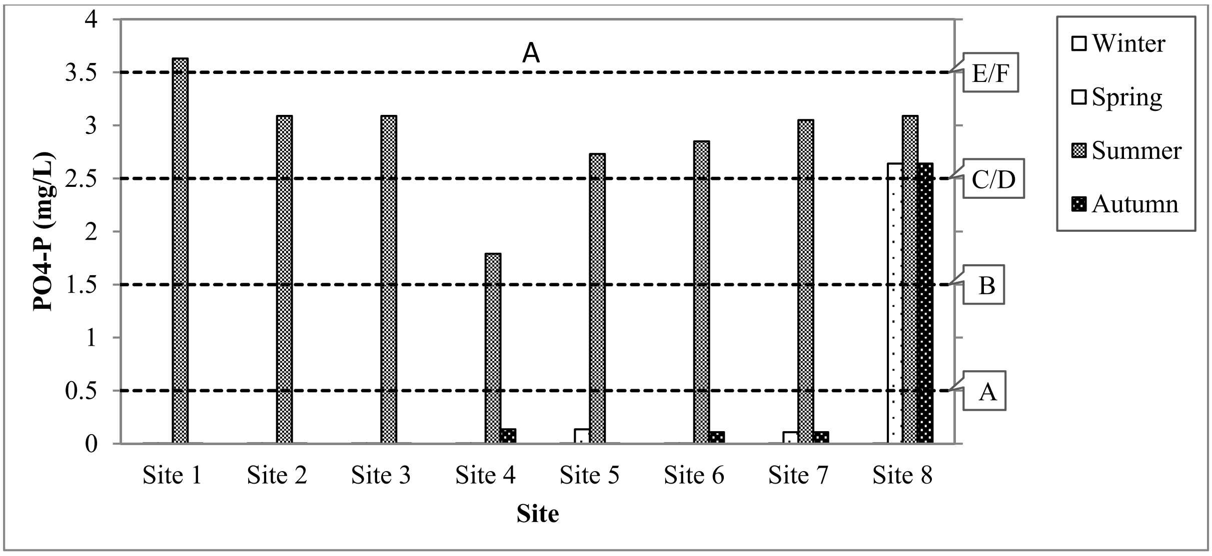


Figure 3.1b: Ecological conditions as indicated by nitrate nitrogen (NO_3-N) (graph A) and total inorganic nitrogen (TIN) (graph B) during the four sampling seasons in the studied river systems between August 2016 and April 2017. Ecological categories: A (natural), B (good), C (fair), D (poor) and E/F (very poor)



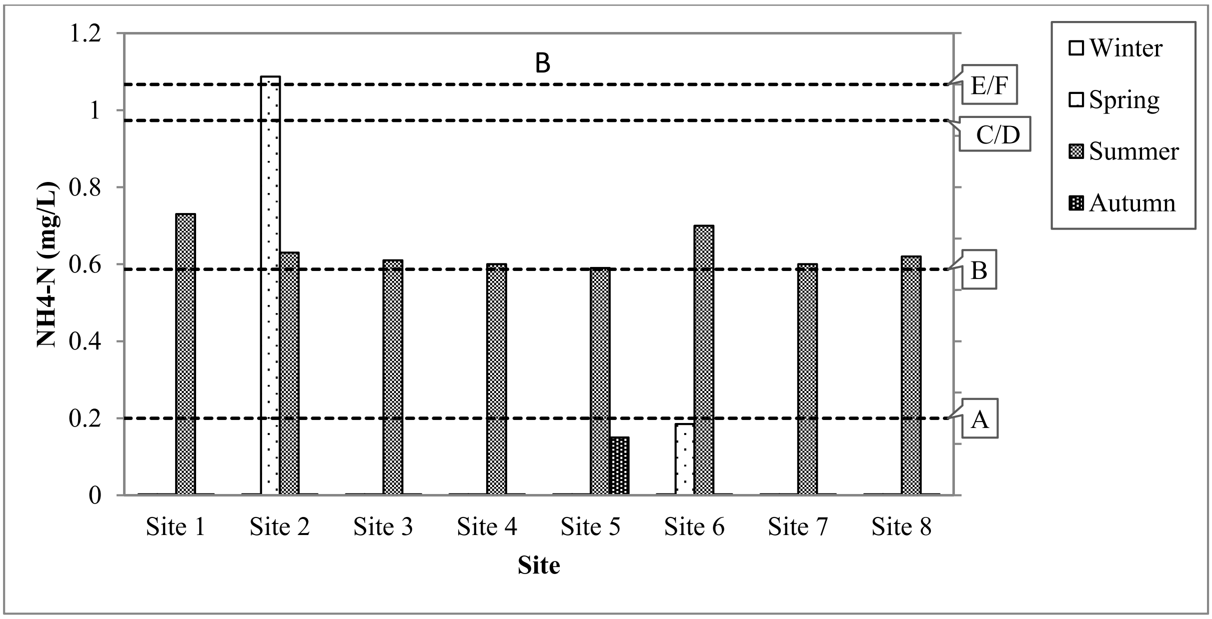


Figure 3.1c: Ecological conditions as indicated by phosphate phosphorus (PO_4-P) (graph A) and ammonia (NH_4-N) (graph B) during the four sampling seasons in the studied river systems between August 2016 and April 2017. Ecological categories: A (natural), B (good), C (fair), D (poor) and E/F (very poor)

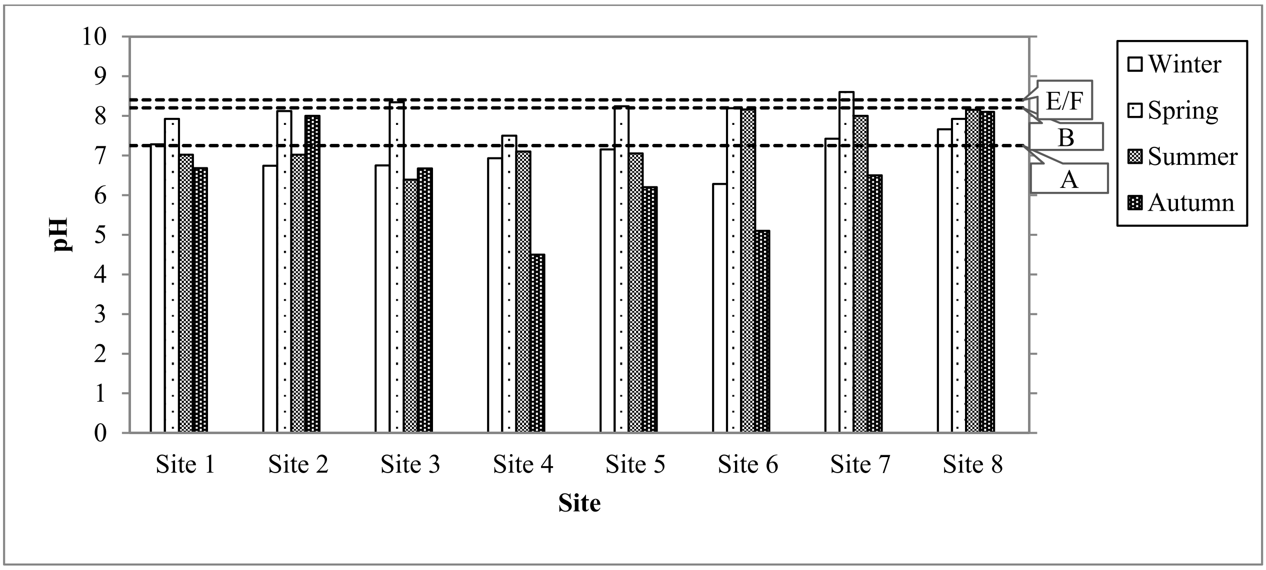


Figure 3.1d: Ecological conditions as indicated by hydrogen ion (pH) during the four sampling seasons in the studied river systems between August 2016 and April 2017. Ecological categories: A (natural), B (good), C (fair), D (poor) and E/F (very poor)

3.3.3 Analysis of settled sediments particle size distribution for the eight sites

The multivariate analysis of variance (MANOVA) that was undertaken revealed that the sites were significantly different in terms of the settled sediment particle size distribution (Table 3. 3).

Table 3. 3: Multivariate analysis of variance MANOVA results for the settled sediment particle size distribution between the sampling sites, indicating a significant difference ($P < 0.05$) between the sites during the study period (August 2016 - April 2017)

Effect	**Multivariate Tests of Significance**					
	Test	Value	F	Effect (df)	Error (df)	p
Intercept	Wilks	0.000000	7268613	10	15.00000	0.000000
Site	Wilks	0.005007	2	70	94.28094	0.000914

When the results were analysed separately for the proportion of very fine sand, fine sand, medium sand, and coarse sand particles of settled sediments, the sites also indicated global significant differences ($P < 0.05$). The proportion of coarse sand particles (<2000 – 1000) was consistently high at all sites when compared to the proportion of very fine sand (<250 – 125), fine sand particles (<500 – 250), and medium sand particles (<1000 – 500) (Figure 3. 2a:a). In terms of differences between the sites, the proportion of coarse sand particles was relatively higher at Site 5 (Pot River downstream site) while that for very fine sand particles was equally higher at Sites 5 and 6 (Pot River downstream site and Little Pot River, respectively). The proportion of medium and fine sand particles was relatively the same across all the sites (Figure 3. 2a:a).

In terms of the silt particles, the very coarse particles (<125 – 63) were proportionally higher at all the sites, except at Site 4, where the fine silt particles (<16 – 8) were proportionally higher than the rest of the silt particle sizes. The coarse silt particles (<63 – 31) were proportionally higher than the medium silt particles (<31 – 16) at all eight sites (Figure 3. 2a:a). Across the sites, the highest proportion of fine silt was recorded at Site 4, while the fine silt particles were proportionally similar at the other seven sites. The proportion of very coarse silt particles was generally higher at Sites 1, 2, 5 and 6 compared with the other sites. The medium silts were relatively stable across the sites, while the proportion of coarse silt was higher at Site 1 and lowest at Site 4 (Figure 3. 2a:).

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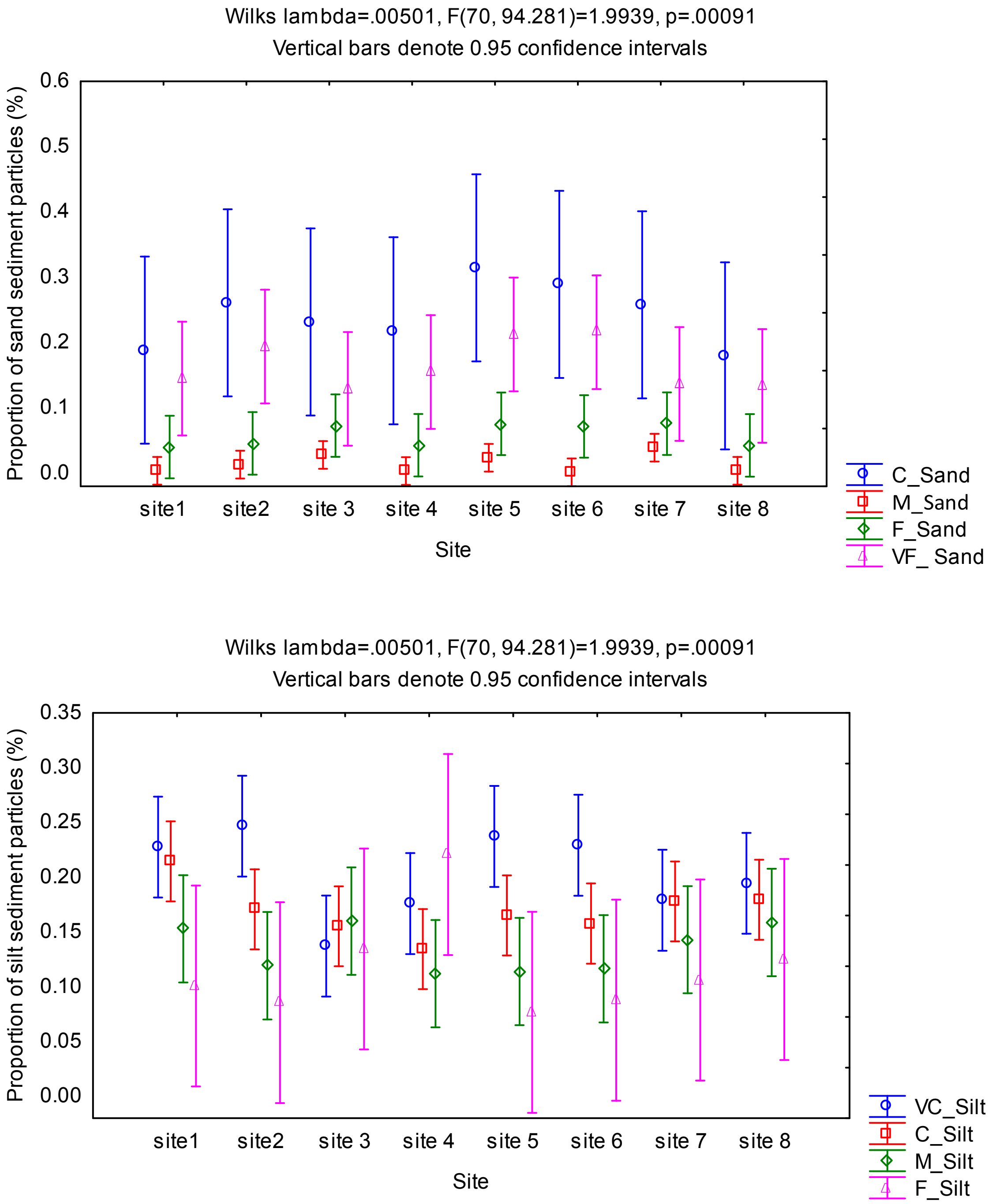

Figure 3. 2a: The proportion of settled sand and silt particle size classes across the eight sampling sites during the study period (August 2016 - April 2017). Abbreviations: C_Sand (coarse sand), M_Sand (medium sand), F_Sand (fine sand), VF_Sand (very fine sand), VC_Silt (very coarse silt), C_Silt (coarse silt), M_Silt (medium silt), F_Silt (fine silt).

When the proportion of clay (<4) and very fine silt particles (<8 – 4) was analysed together, the result indicated that the sites were still significantly different (Figure 3.2b). Both clay and very fine silt particle sizes followed a similar pattern across the sites and were highest at Sites 3 and 8 and lowest at Sites 5 (Figure 3.2b).

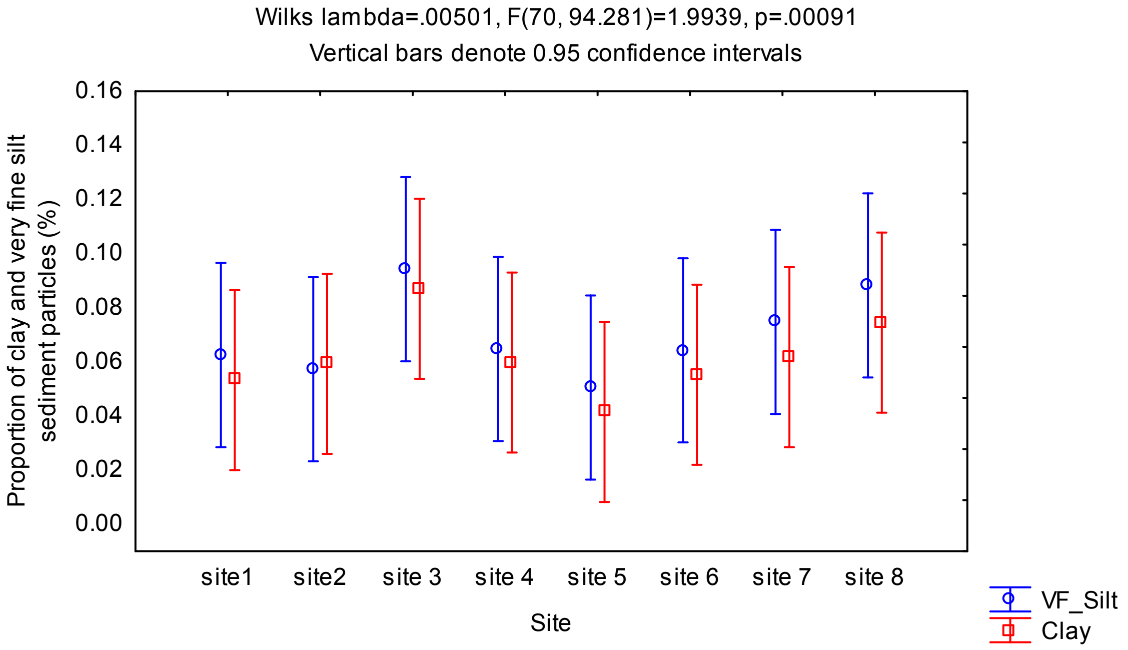

Figure 3.2b: The proportion of settled very fine silt and clay particle size distributions across the eight sampling sites during the study period (August 2016 - April 2017). Abbreviation: VF-Silt (very fine silt).

3.3.4 Analysis of suspended sediments particle size distribution and turbidity for the eight sites

The multivariate analysis of variance (MANOVA) that was undertaken to assess the differences in the sites in terms of the proportional distribution of suspended particles size classes, as well as turbidity, indicated that the sites were globally significantly different (Table 3. 4).

Table 3. 4: Multivariate analysis of variance (MANOVA) results for the suspended sediment particle size classes between the sampling sites during the study period (August 2016 - April 2017)

Effect	**Multivariate Tests of Significance**					
	Test	Value	F	Effect (df)	Error (df)	p
Intercept	Wilks	0.000000	3953290	10	15.00000	0.000000
Site	Wilks	0.008168	2	70	94.28094	0.006853

When the suspended sediment particles were analysed separately in terms of significant differences between the sites, each of the sediment particle components indicated that the sites differed based on MANOVA ($P < 0.05$). The suspended coarse sand and very fine sand particles had the highest proportional representation of the sandy component of suspended sediments across all eight sites (Figure 3. 3a). Medium sand particles had the least proportional representation across the sites, except at Site 2 where both medium particles and fine sand particles had similar or almost equal proportional representation. The coarse sand particles were highest at Site 2 (Tsitsa downstream) followed by Site 7 (Millstream upstream). The proportional representation of fine sand was almost stable across all the eight sites, while the very fine sand particles were highest at Site 6, followed by Site 7.

The percent very coarse silt and coarse silt had the highest proportional distribution across all eight sites, except at Site 2, where the medium silt particles were higher than the coarse silt particles (Figure 3. 3a). In terms of the silt particles, the fine silt particles had the lowest proportional representation across all eight sites, except at Site 3, where the proportion of fine silt particles was higher than the very coarse silt particles and coarse silt particles.

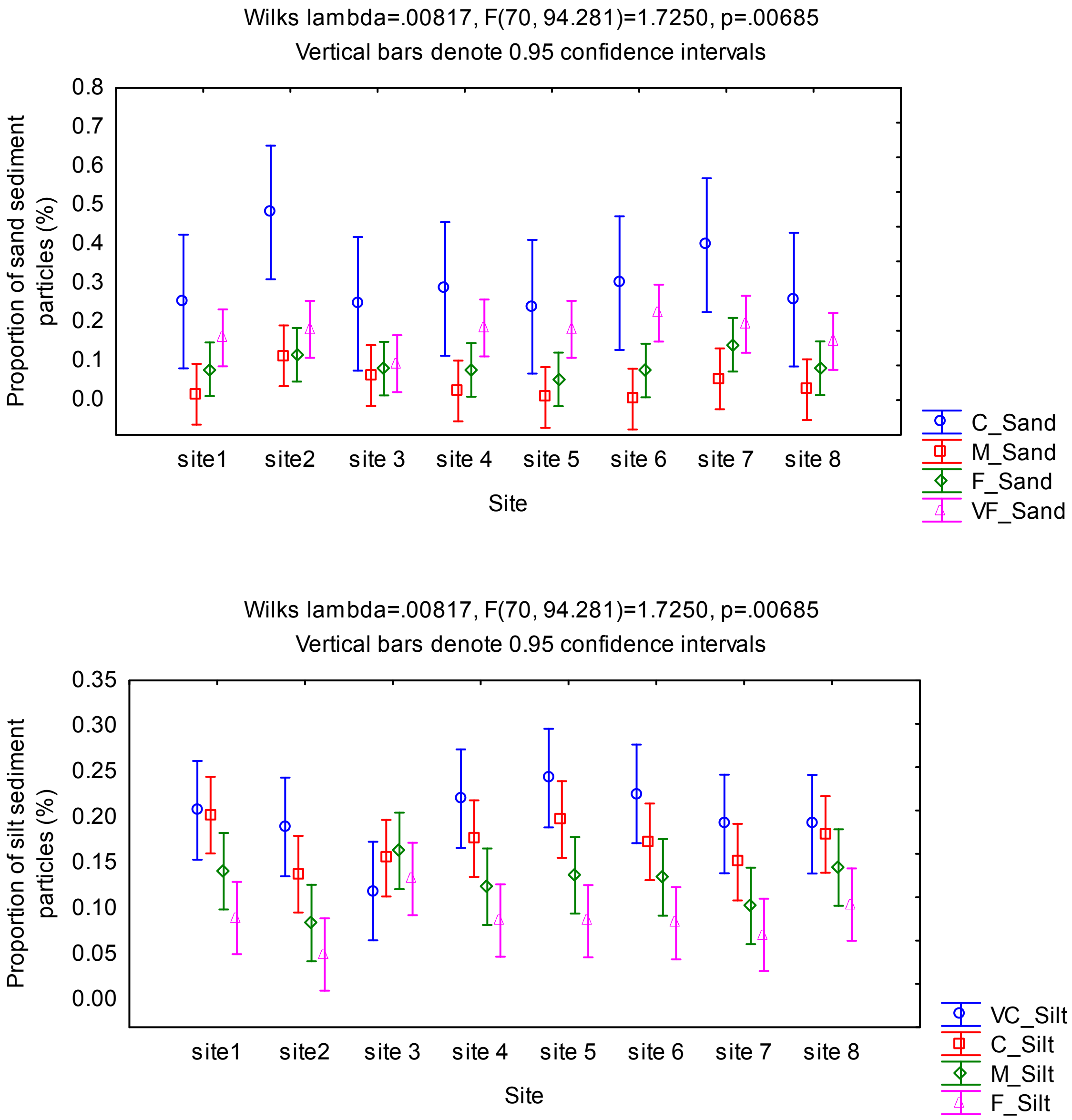

Figure 3. 3a: The proportion of suspended sand and silt particle size classes across the eight sampling sites during the study period (August 2016 - April 2017). Abbreviations: C_Sand (coarse sand), M_Sand (medium sand), F_Sand (fine sand), VF_Sand (very fine sand), VC_Silt (very coarse silt), C_Silt (coarse silt), M_Silt (medium silt), F_Silt (fine silt).

As with the settled sediment particles, the clay and very fine silt particles followed the same pattern, being highest at Site 3 and lowest at Site 2. Both clay and very fine silt particles were similar in proportional representation at Sites 1, 4, 5, 6, 7 and 8 (Figure 3.3b). Of the eight sites, the mean values of turbidity were highest at Site 1 (Tsitsa upstream site) and Site 3 (Qurana River), followed by Site 2 (Tsitsa downstream site). The lowest mean values for turbidity were recorded at Site 6 (Little Pot River), Site 4 (Pot River upstream site), Site 5 (Pot River downstream site) and Site 8 (Millstream downstream site).

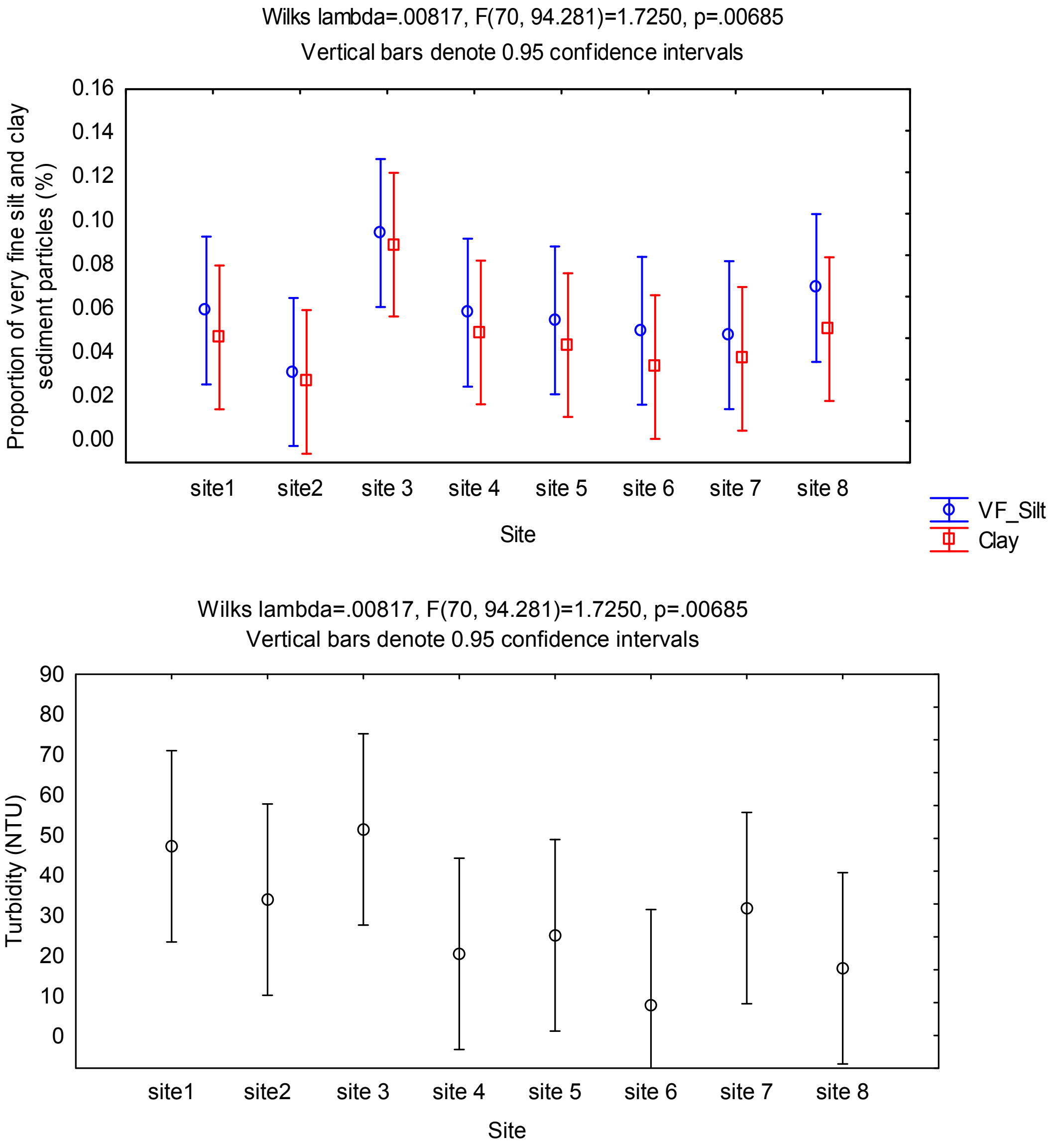

Figure 3.3b: The proportion of suspended very fine silt and clay particle size distributions as well as turbidity across the eight sampling sites during the study period. Abbreviation: VF_Silt (very fine silt).

3.3.5 Site clustering based on sediment characteristics

The cluster analysis was undertaken to assess the similarities between sites and the result revealed that four distinct site groups were formed based on the settled sediment particle size distributions (Table 3. 4). The first cluster comprised Site 1 (Tsitsa upstream site) and Site 3 (Qurana tributary). These two sites were originally designated as being highly turbid and sediment-impacted. The second cluster comprised Site 2 (Tsitsa downstream) and Site 7

(Millstream upstream). The two sites making up the second cluster were also originally designated as sediment-impacted, although their impact was deemed less than those of Sites 1 and 3. The third cluster was composed of Site 4 (Pot River upstream), Site 5 (Pot River downstream) and Site 8 (Millstream downstream). These sites together with Site 6 (Little Pot River) were designated as sites with minimal sediment impact. When the sites were clustered using the suspended sediment particle size distribution, a similar pattern as that obtained for settled sediments was observed (Table 3. 4). As with settled sediments, Sites 1 and 3 clustered together, forming the first site group, Sites 2 and 7 clustered together forming the second site group and Sites 4, 5 and 8 clustered together forming the third site group. Site 6, the Little Pot River, did not cluster with any of the sites. In summary, both settled and suspended sediment particle size distributions returned four site groups: Group 1 (Sites 1 and 3), Group 2 (Sites 2 and 7), Group 3 (Sites 4, 5 and 8) and Group 4 (Site 6). The groups formed a gradient of sedimentation in the order: Group 1> Group 2 > Groups 3 and 4.

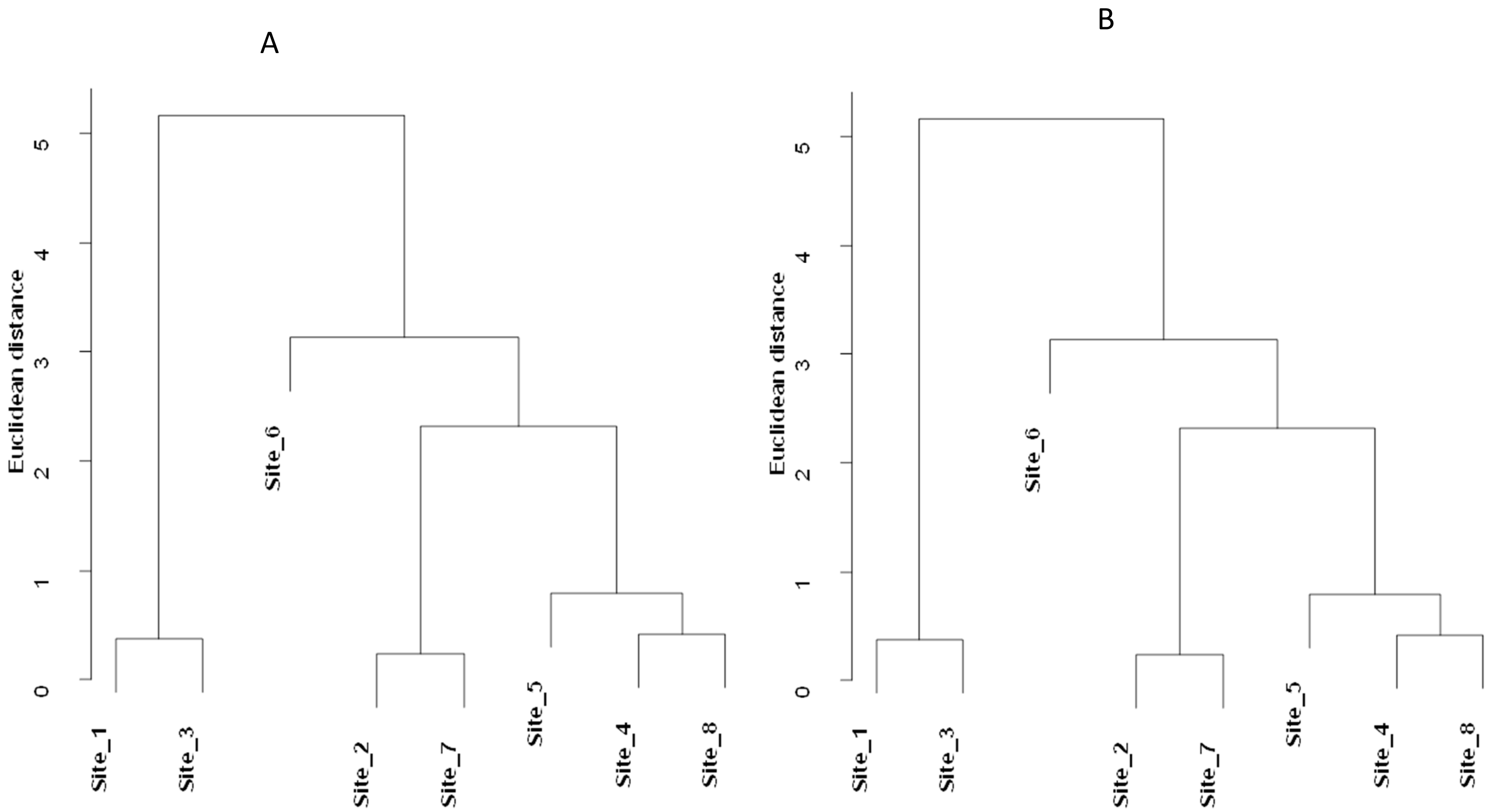

Figure 3. 4: Dendrogram showing the clustering of the sites based on settled (A) and suspended (B) sediment characteristics during the study period (August 2016 - April 2017) in the Tsitsa River and its tributaries. Four distinct site groups are identifiable: Group 1 (Sites 1 and 3) Group 2 (Sites 2 and 7), Group 3 (Sites 4, 5 and 8) and Group 4 (Site 6).

3.3.6 Spatial and temporary analysis of EPOT assemblage structure

Thirty-two taxa (species and genera) of EPOT were recorded during the study period (Appendix B1). Of the 32 taxa, 18 belong to the order Ephemeroptera, 11 taxa in Odonata, one genus in Plecoptera and two genera belong to the order Trichoptera.

At Site 1 (Tsitsa upstream), during winter, the percent relative abundance of species was dominated by *Euthraulus* sp*., Pseudocloeon* sp and *Baetis* sp. and the less dominant species were *Aeshna* sp. and *Tricorythus* sp. During spring and summer, the patterns were similar to that of winter, and in autumn, *Adenophlebia auriculata* and *Pseudocloeon glaucum* were dominant. At Site 2 (Tsitsa downstream), during winter, the relative abundance was dominated by *Pseudocloeon piscis* and *Pseudocloeon glaucum*. The relative abundance distribution of species during spring was similar to that of winter. During summer, the percent relative abundance was dominated by *Euthraulus* sp., *Pseudocloeon vinosum* and *Hydropsyche* sp. The less dominant species were *Aeshna* sp. and *Baetis* sp. The relative abundance distribution of species during autumn was similar to that of summer (Appendix B1).

At Site 3 (Qurana tributary), during winter, the percent relative abundance was dominated by *Baetis* sp. and *Euthraulus* sp. and the less dominant species were *Hydropsyche* sp. and *Pseudocloeon piscis*. During spring, *Adenophlebia auriculata* and *Pseudocloeon* sp. were dominant and the less dominant species were *Pseudagrion* sp. and *Trithermis* sp. During summer and autumn, the dominant species were *Baetis* sp. and *Pseudocloeon* sp. and the less dominant species were *Tricorythus* sp. and *Aeshna* sp.

At Site 4 (Pot River upstream), during winter, the percent relative abundance was dominated by *Euthraulus* sp. and *Pseudocloeon piscis* and the less dominant species were *Acanthiops* sp. and *Crenigomphus* sp. During spring, the dominant species were *Euthraulus* sp. and *Tricorythus* sp. and the less dominant species were *Aeshna* sp. and *Aphenicera* sp. Most abundant species during the summer were *Pseudocloeon glaucum* and *Euthraulus* sp. and the less represented species were *Afronurus* sp. and *Prosopistoma amanzamanya*. The patterns of percent relative abundance during autumn were similar to that of spring (Appendix B1).

At Site 5 (Pot River downstream), during winter, *Acanthiops tsitsa* and *Caenis* sp. had the highest percent relative abundance and the least abundant species were *Pseudagrion* sp. and *Brachytermis* sp. During summer, the dominant species were *Baetis* sp. and *Tricorythus* sp. and the less dominant species were *Afronurus* sp. and *Crenigomphus* sp. and in autumn, the relative percent abundance was similar. At Site 6 (Little Pot River), during winter, the dominant species were *Caenis* sp. and *Tricorythus* sp. and the less dominant species were *Acanthiops* sp. and *Paragomphus genei.* During summer, the highest in terms of percent relative abundance were *Baetis* sp., *Pseudocloeon piscis* and *Pseudocloeon vinosum* and the least percent relative abundance were *Crenigomphus rennei* and *Pseudagrion* sp. During autumn, *Baetis* sp. and *Crenigomphus rennei* were the most represented and the less represented were *Pseudocloeon piscis* and *Pseudocloeon glaucum* (Appendix B2).

At Site 7 (Millstream upstream), during winter, the species that were most represented in terms of percent relative abundance were *Pseudocloeon piscis* and *Enallagma* sp. The percent relative abundance patterns during spring were similar to that of winter. The *Hydropsyche* sp and *Baetis* sp. were most abundantly represented during the summer season. The percent relative abundance pattern seen during the summer season was similar to that of autumn with *Baetis* sp. well represented. At Site 8 (Millstream upstream), during winter, the dominant species were *Caenis* sp., *Pseudocloeon* sp. and *Hydropsyche* sp. and the less dominant species were *Paragomphus rennei* and Aeshna sp. During autumn, the pattern was similar to that of winter (Appendix B2).

Generally, Site 5 (Pot River downstream) and Site 6 (Little pot River) supported more species of EPOT than Sites 1 and 2 (Tsitsa up and down stream sites), Site 3 (Qurana tributary), Sites 7 and 8 (Millstream up and down stream sites) and Site 4 (Pot River downstream sites). Of the eight sites, Site 2 (Tsitsa downstream) supported the least number of species followed by Site 7 (Millstream up stream) (Figure 3. 5). In terms of seasonal variations, there was no consistent parttern in the number of EPOT species recorded per season. However, the highest EPOT species recorded in a season was during spring at Site 5 (Pot River downstream).

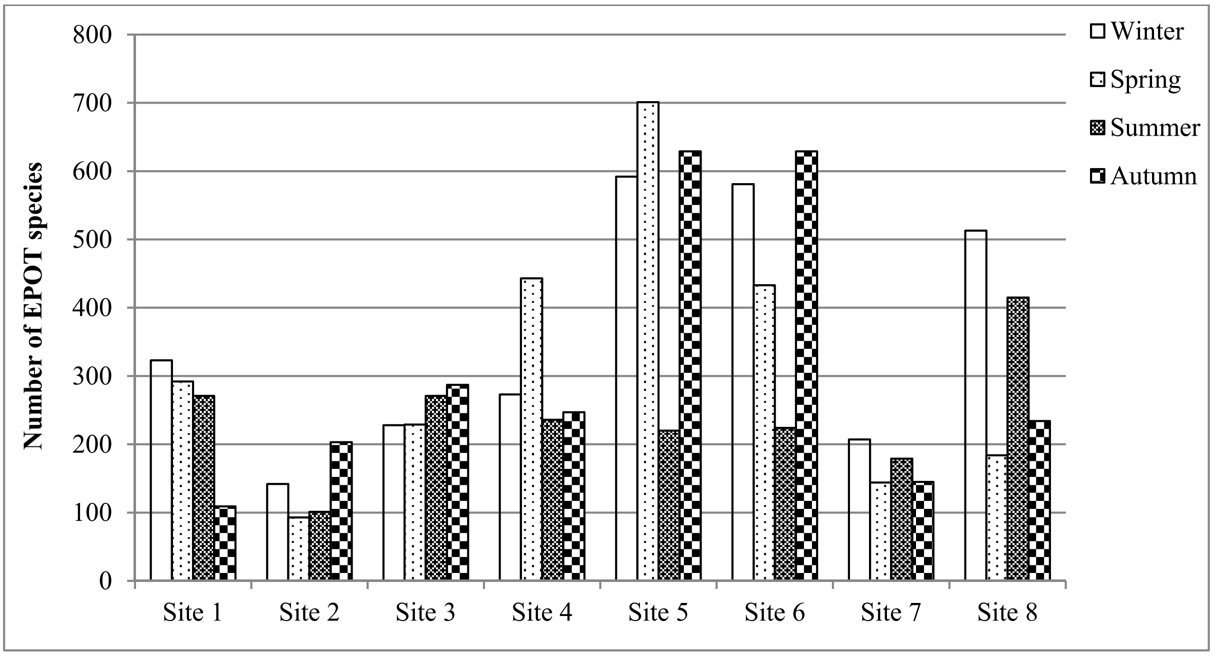

Figure 3. 5: Summary of the numbers of EPOT species sampled at the eight sampling sites in the Tsitsa River and its tributaries during the sampling seasons.

The one-way analysis of similarity (ANOSIM) and similarity percentage (SIMPER) were used to analyse the differences between the study sites in terms of EPOT assemblage structure. The ANOSIM results indicated global statistical significant differences between the sites (Global R = 0.364; $P < 0.05$). The ANOSIM pairwise permutation test indicated that of the 28 possible site pairs, 11 were significantly different in terms of species compositions and all 11 site pairs indicated a similar level of significant differences.

The SIMPER analysis revealed that the highest percent average dissimilarity was between Sites 1 and 7 at 73.07%, which was followed by percent average dissimilarity of 70.99% between Sites 3 and 7. The lowest average dissimilarity among all site pairs was between Sites 5 and 6 (44.09%), indicating a less pronounced difference between these two sites. *Euthraulus* sp., *Adenophlebia* sp., *Baeti*s sp., *Caenis* sp., *Paragomphus* sp. and *Hydropsyche* sp. were the dominant discriminating species between the sampling sites.

The ANOSIM results also indicated a global statistically significant difference between the seasons (Global R = 0.203; $P < 0.05$). The pairwise permutation test revealed that three seasonal pairs (winter and summer, winter and autumn, and spring and autumn) indicated significant differences out of the total of 6 possible season pairs (3.5). The EPOT species enabled the discrimination of the seasons from each other; winter and spring, representing the dry seasons, were largely different from summer and autumn, which represented the wet seasons.

The SIMPER result indicated that the highest percent (68.15%) average dissimilarity was between winter and autumn, which was followed by an average dissimilarity of 66.84% between spring and autumn. Out of the four seasons, the smallest percent average dissimilarity (56.10%) was between winter and spring, suggesting that the difference between these two dry seasons was less pronounced than between other seasons. *Caenis* sp., *Pseudocloeon glaucum*, *Baetis* sp., *Pseudocloeon* sp. and *Euthraulus* sp. were the main discriminating species between the seasons.

Table 3. 5: ANOSIM test showing global R statistics, pairwise test R statistics and significant level between sampling sites and seasons based on their EPOT species assemblages in the Tsitsa River and its tributaries during the study period (August 2016 - April 2017)

Global R = 0.364; *P* < 0.05		
Site pair	**R statistics**	**Significant level**
1, 6	0.281	0.029
1, 8	0.573	0.029
2, 3	0.615	0.029
3, 5	0.385	0.029
3, 6	0.729	0.029
3, 7	0.719	0.029
3, 8	0.838	0.029
4, 8	0.531	0.029
5, 8	0.615	0.029
6, 7	0.698	0.029
6, 8	0.813	0.029
Global R = 0.203; *P* < 0.05		
Season pair	**R statistics**	**Significant level**
winter, summer	0.408	0.001
winter, autumn	0.454	0.001
spring, autumn	0.28	0.002

Ordination of sites and seasons based on the EPOT assemblage structure

The non-metric multidimensional scaling (NMDS) based on the Bray-Curtis similarity measures undertaken to elucidate the assemblage patterns of EPOT species, revealed that species clustered largely by sites, more than by seasons (Figure 3. 6). In all the seasons, the EPOT species in Site 1 (Tsitsa upstream) and Site 3 (Qurana tributary) were largely separated from the other sites and clustered together to form a group. Site 7 (Millstream upstream) and Site 8 (Millstream downstream) formed a separate cluster and Site 4 (Pot River Upstream) and Site 6 (Little Pot River) were mostly clustered together. The assemblage structure of EPOT assemblage structure was useful in discriminating between the eight sites. EPOT species at Sites 1 and 3 were remarkably different from those at Sites 7 and 8, but the difference between assemblage structure at Sites 1 and 3, and those at Sites 5 and 6, were less pronounced. Sites 1, 4 and 7 were separated from the other Sites during autumn.

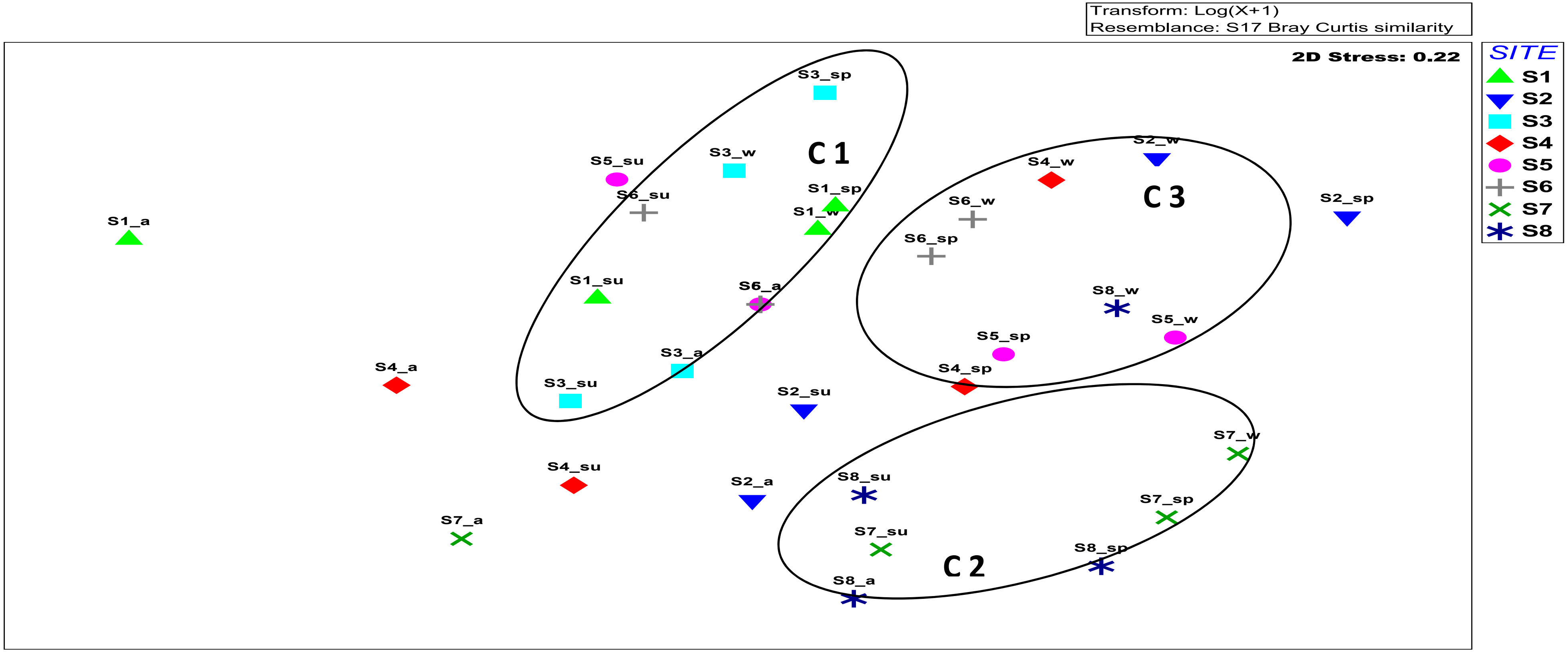

Figure 3. 6: Non-metric multidimensional scaling (NMDS) based on the Bray-Curtis similarity index, indicating how the sites and seasons were grouped together based on their similarities in terms of EPOT species abundance in the Tsitsa River and its tributaries during the study period (August 2016 – April 2017). Sites 1, 2 and 3 were clustered together in group 1; Sites 5 and 6 formed a cluster in group 2 and Sites 7 and 8 formed a cluster in group 3. (Symbols: S1, S2, S3, S4, S5, S6, S7, S8 = Site 1, Site 2, Site 3, Site 4, Site 5, Site 6, Site 7 and Site 8 respectively; wint, spring, summ and autum = winter, spring, summer, and autumn respectively; and C1 = cluster 1, C2 = Cluster 2 and C3 = Cluster 3).

3.3.7 Associations between biotopes and EPOT species in the studied river systems

The Pearson's point-biserial correlation coefficient was undertaken to evaluate the association between biotopes and the EPOT species. Five EPOT assemblage types were identified based on their associations with the biotopes (Table 3.6). The first assemblage type with the highest number of species was associated with stone biotope and consist of 14 species, of these, nine species indicated significant associations with the stone biotope ($P < 0.05$) and these species included *Euthraulus* sp., *Hydropsyche* sp., *Aeshna* sp., *Afronurus* sp., *Aphenicera* sp., and *Tricorythus* sp. (Table 3.6). The second assemblage type preferred the vegetation biotope, and consisted of seven species, of which three, namely *Pseudagrion* sp., *Phylomacromia* sp., and *Pseudagrion spernatum,* were significantly associated with vegetation. The third assemblage type consisted of four species i.e. *Paragomphus genei*, *Crenigomphus* sp., *Trithermis* sp. and *Crenigomphus rennei,* none of which indicated a significant association with the GSM biotope. The fourth assemblage type consisted of five species i.e. *Pseudocloeon* sp., *Brachytermis* sp., *Adenophlebia* sp., *Acanthiops* sp. and *Oligoneuropsis lawrencei* that were associated with both stone and vegetation biotopes. Out of the 5 species, only *Pseudocloeon* sp. indicated a significant association with stone and vegetation biotopes. The last assemblage type consisted of 2 species i.e. *Pseudocloeon glaucum* and *Acanthiops tsitsa* that were associated with both stone and GSM biotopes. Only *Pseudocloeon glaucum* was significantly associated with stone and GSM biotopes.

Table 3. 6: EPOT species preferences for biotopes: stone, vegetation (Veg.), and gravel sand and mud (GSM) in the Tsitsa River and its tributaries during the study period (August 2016 - April 2017). The numbers 1 and 0 under the biotope columns indicate species preferring or not preferring a particular biotope, respectively. Code (biotope preference code) indicates species preferring the stone (1), vegetation biotope (2), GSM (3), both the stone and the vegetation (4), and both the stone and the GSM biotopes (5). A boldface value under the *P-value* indicates significant association ($P < 0.05$).

Taxa	**Stone**	**Veg**	**GSM**	**Code**	**Point-biserial coefficient**	***P*-value**
Euthraulus sp.	1	0	0	1	0.42	**0.001**
Hydropsyche sp.	1	0	0	1	0.299	**0.001**
Aeshna sp.	1	0	0	1	0.298	**0.001**

Afronurus sp.	1	0	0	1	0.274	**0.001**
Aphenicera sp.	1	0	0	1	0.261	**0.001**
Tricorythus sp.	1	0	0	1	0.252	**0.001**
Adenophlebia auriculata	1	0	0	1	0.231	**0.002**
Cheumatosyche sp.	1	0	0	1	0.202	**0.004**
Baetis sp.	1	0	0	1	0.183	**0.01**
Elassoneuria sp.	1	0	0	1	0.151	0.119
Oligoneuropsis sp.	1	0	0	1	0.144	0.119
Afronurus bernardi	1	0	0	1	0.115	0.14
Prosopistoma amamzamanya	1	0	0	1	0.087	1.000
Pseudocloeon piscis	1	0	0	1	0.08	0.422
Pseudagrion sp.	0	1	0	2	0.385	**0.001**
Phylomacromia sp.	0	1	0	2	0.26	**0.001**
Pseudagrion spernatum	0	1	0	2	0.244	**0.001**
Pseudocloeon vinosum	0	1	0	2	0.162	**0.019**
Enallagma sp.	0	1	0	2	0.138	0.072
Caenis sp.	0	1	0	2	0.124	0.108
Paragomphus sp.	0	1	0	2	0.054	0.859
Paragomphus genei	0	0	1	3	0.14	0.072
Crenigomphus sp.	0	0	1	3	0.135	0.071
Trithermis sp.	0	0	1	3	0.063	1.000
Crenigomphus renei	0	0	1	3	0.053	0.687
Pseudocloeon sp.	1	1	0	4	0.155	**0.031**
Brachytermis sp.	1	1	0	4	0.114	0.225
Adenophlebia sp.	1	1	0	4	0.109	0.172
Acanthiops sp.	1	1	0	4	0.096	0.273
Oligoneuropsis lawrencei	1	1	0	4	0.066	0.785
Pseudocloeon glaucum	1	0	1	5	0.161	**0.024**
Acanthiops tsitsa	1	0	1	5	0.043	0.759

3.3.8 Ephemeroptera, Plecoptera, Odonata and Trichoptera (EPOT) metrics for assessing water quality in the studied river system

The box plot was used to evaluate the discriminatory ability of selected metrics between the site groups derived from the cluster analysis. The result showed that except for evenness and Margalef's species richness index, all the selected metrics were considered to have a satisfactory discriminatory ability, and were able to distinguish between the site groups (Figure 3.7a and 3.7b). The metrics that showed satisfactory discriminatory ability between the site groups were further investigated using the Kruskal-Wallis test ($P < 0.05$). The Kruskal-Wallis test revealed that out of the four metrics that were considered to show satisfactory discriminatory ability, Simpson index, EPOT abundance and Shannon index indicated a significant difference between the site groups ($P < 0.05$).

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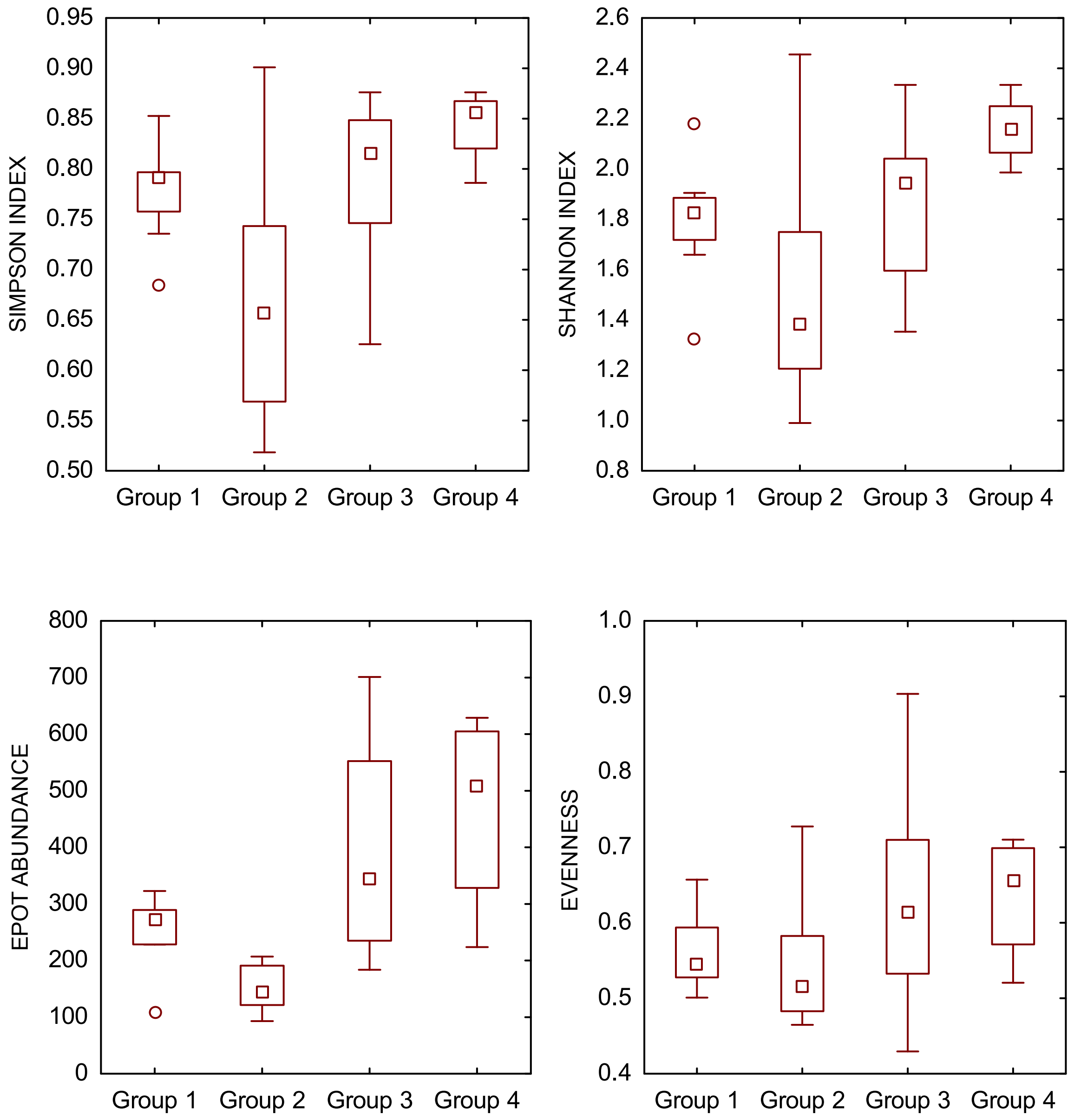

Figure 3. 7a: Medians (small squares) and quartiles (rectangles) for Simpson index, Shannon index, EPOT abundance and evenness for four site groups in the Tsitsa River and its tributaries between August 2016 and April 2017. Range bars = maximum and minimum non-outlier numbers, circles = outliers.

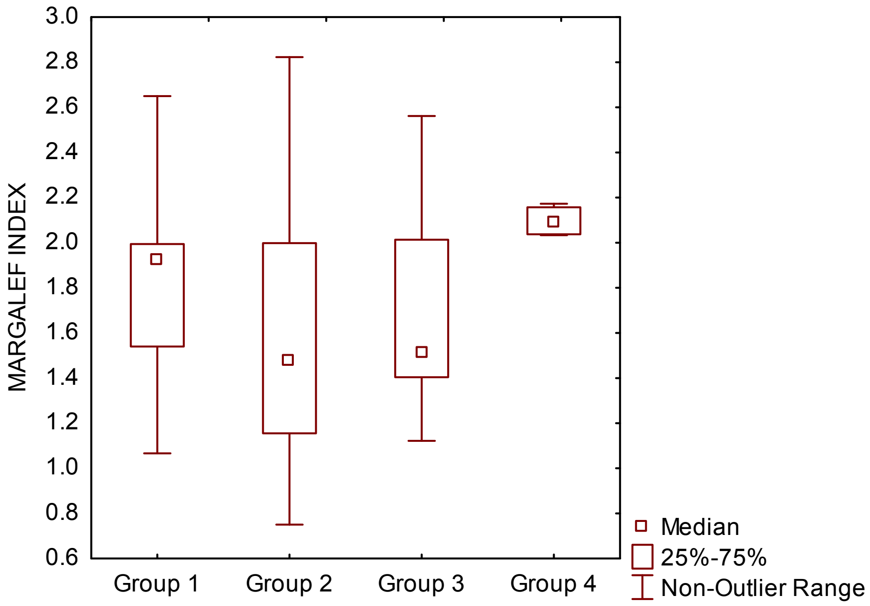

Figure 3.7b**:** Medians (small squares) and quartiles (rectangles) for Margalef's richness index for the study period for four site groups in the Tsitsa River and its tributaries between August 2016 and April 2017. Range bars = maximum and minimum non-outlier numbers.

Correlation between physico-chemical variables, sediments particle size classes and the EPOT metrics

The simple correlation analysis was undertaken to evaluate the correlation between the selected metrics, water physico-chemical variables and sediment particle classes. The result revealed that apart from the Margalef's species richness index, the metrics did not show statistically significant correlation with any of the physico-chemical variables (Table 3.7). The result revealed that the only significant correlation was between TIN and Margalef's species richness index that indicated significant correlation. The result further showed that all metrics indicated none significant negative association with EC and turbidity except Margalef's species richness index that indicated none significant positive association with EC. Except for evenness, all the metrics indicated none significant positive correlation with TIN (Table 3.7).

Table 3. 7: Pearson's simple correlation coefficients between physico-chemical variables and selected EPOT metrics.

Variables	**EPOT abundance**	**Simpson index**	**Shannon index**	**Evenness index**	**Margalef's species richness index**
EC	-0.28	-0.25	-0.19	-0.28	0.05
DO	0.13	-0.09	-0.09	-0.27	-0.02
pH	0.11	0.06	0.04	0.09	-0.08
Temp	-0.18	0.09	0.17	-0.12	**0.35***
NO_3 -N	0.16	-0.16	-0.05	-0.13	-0.06
NO_2-N	-0.07	0.15	0.14	0.03	0.14
PO_4-P	-0.03	0.27	0.3	0.14	0.28
NH_4-N	-0.18	0.21	0.2	0.06	0.23
TIN	0.1	0.05	0.13	-0.07	0.13
TUR	-0.32	-0.23	-0.23	-0.33	-0.05

* = significant at $P < 0.05$

The correlations between EPOT summary metrics and sediment particle class (settled and suspended) sizes were assessed using the simple correlation analysis and presented in Table 3.8. The correlation coefficients between the metrics and settled particle size classes showed that all metrics indicated none significant negative correlation with the proportion of medium sand. The result also revealed that all metrics indicated significant positive correlations with the proportion of coarse silt, medium silt and very fine silt, except evenness and EPOT abundance that indicated none positive correlations. All metrics indicated no significant negative correlation with the proportion of sand and fine sand, except for EPOT abundance metrics that indicated a none positive correlation and Margalef's species richness index and Shannon index that indicated significant positive correlation (Table 3.8).

The correlation result between EPOT metrics and suspended sediment particle size classes revealed that all the metrics indicated a none significant negative correlation with the proportion of sand, medium sand, fine sand, and very fine sand, except EPOT abundance that indicated none positive correlation with these sediment size classes and evenness that indicated significant negative correlation with sand and fine sand. The result also revealed that the metrics indicated a none positive significant correlation with the proportion of medium silt, fine silt, very fine silt and clay, except EPOT abundance that indicated a none positive association with these sediment size classes, and Shannon index and evenness that indicated positive significant correlation with medium silt and fine silt. Margalef's species richness index and evenness indicated a significant positive association with the proportion of very fine silt (Table 3.8).

Table 3. 8: Pearson's simple correlation coefficients between EPOT metrics and settled and suspended sediment particle characteristics. Boldface indicates a significant difference.

Variable	**EPOT abundance**	**Simpson index**	**Shannon index**	**Evenness index**	**Margalef's species richness index**
	Settled sediment particle size classes				
Coarse sand	0.02	-0.29	**-0.38***	-0.05	**-0.50****
Medium sand	-0.11	-0.24	-0.3	-0.1	-0.29
Fine sand	0.06	-0.31	**-0.39***	-0.06	**-0.48****
Very fine sand	0.07	-0.19	-0.28	0.01	**-0.44***
Very coarse silt	0.16	0.1	-0.04	0.07	-0.22
Coarse silt	0.15	**0.40***	**0.38***	0.05	**0.41***
Medium silt	0.05	**0.38***	**0.45****	0.08	**0.56****
Fine silt	-0.19	-0.18	-0.07	-0.1	0.06
Very fine silt	0.01	0.3	**0.40***	0.11	**0.51****
Clay	-0.05	0.29	**0.39***	0.12	**0.49****
	Suspended sediment particle size classes				
Coarse sand	0.09	-0.24	-0.27	**-0.39***	-0.18
Medium sand	-0.16	-0.21	-0.22	-0.28	-0.09

Fine sand	0.11	-0.23	-0.24	**-0.35***	-0.13
Very fine sand	0.33	-0.13	-0.16	-0.18	-0.23
Very coarse silt	0.13	-0.1	-0.16	0.01	-0.32
Coarse silt	0.03	0.15	0.12	0.24	-0.02
Medium silt	-0.05	0.32	**0.36***	**0.39***	0.29
Fine silt	-0.15	0.29	**0.36***	**0.40***	0.35
Very fine silt	-0.18	0.27	0.34	**0.37***	**0.35***
Clay	-0.28	0.18	0.24	0.26	0.32

* = significant at $P < 0.05$; ** = significant at $P < 0.01$

3.3.9 Relating EPOT species with sediment particle size characteristics including turbidity

The multivariate canonical correspondence analysis (CCA) was undertaken to assess the relationships between EPOT species and sediment particles size characteristics, including turbidity. The analysis was carried out with a view to assessing the influences of settled and suspended sediment particle characteristics including turbidity on the assemblage structure of EPOT species and the site groups derived from the cluster analysis. The CCA analysis was conducted separately for 1) EPOT species and settled sediment characteristics, and 2) EPOT species and suspended sediments characteristics including turbidity.

The CCA analysis undertaken on settled sediment and EPOT species revealed that the first three axes accounted for 45.80% cumulative variance. The first CCA axis explained 20.52% variance with an eigenvalue 0.14, the second axis explained 14.68% variance with an eigenvalue of 0.10 and the third axis accounted for 10.60% variance with an eigenvalue of 0.07 (Table 3.9). The Monte Carlo (1000 permutations) showed significant correlations between the first axis and the settled sediments particles (Table 3.9).

The CCA ordination plot on the first 2 axes indicated a strong positive correlation between the proportion of clay, very fine silt, medium silt, and coarse silt for site group 1 during summer and autumn, and site group 3 during summer. These proportions of suspended sediments particle sizes strongly favoured the positive correlation of *Baetis* sp., *Aeshna* sp., *Paragomphus* sp., *Cheumatosyche* sp. and *Pseudocloeon glaucum.* The result also indicated a strong positive correlation between the proportion of very coarse silt, fine sand, very fine sand and sand settled sediment classes with site groups 2 and 3 during winter and spring. The settled sediment proportion (very coarse silt, fine sand, very fine sand and sand) also strongly favored the positive correlation of *Hydropsyche* sp., *Caenis* sp., *Pseudocloeon* sp., *Pseudocloeon piscis* and *Phylomacroma* sp. and these were negatively correlated with the finer proportion of sediments (very coarse silt, fine sand, very fine sand and sand) for site group 1, classified as a sediment-impacted site group (Figure 3. 8a).

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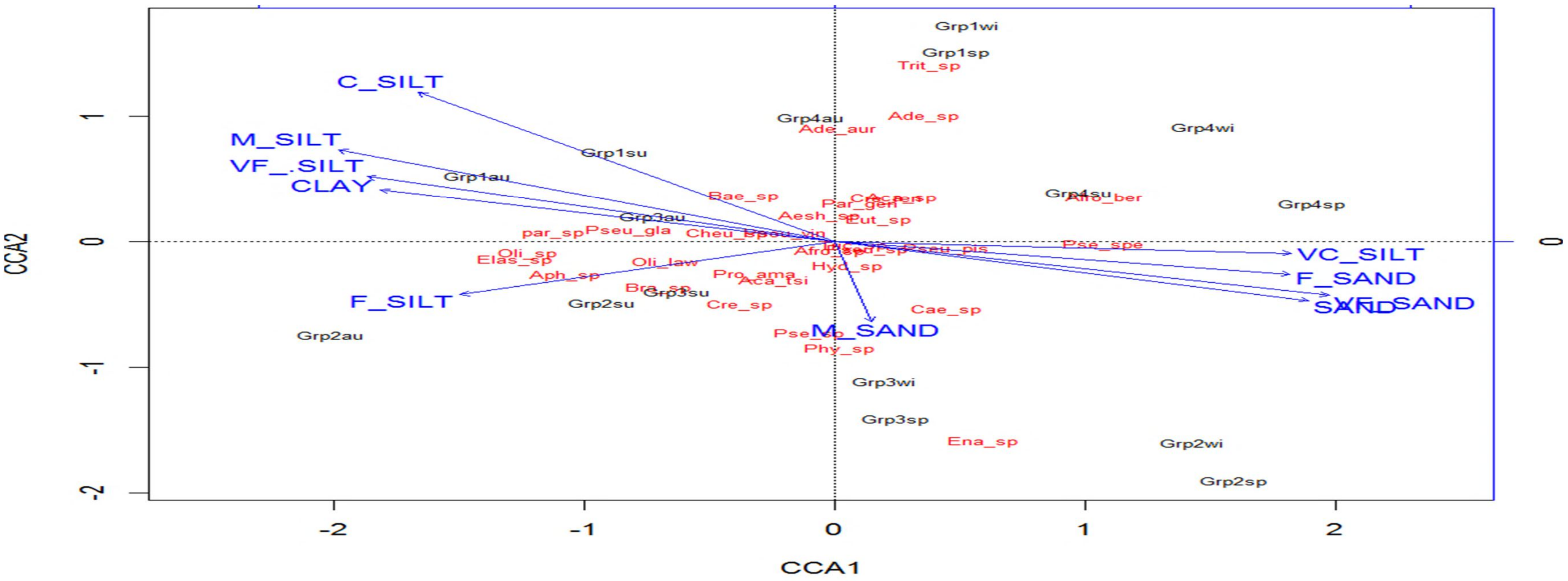

Figure 3. 8a: CCA ordination showing the correlation between settled sediment particle size classes and the EPOT species during the study period (August 2016 – April 2017) across the site groups. Abbreviations: Aca_sp (*Acanthiops* sp.), Aca_tsi (*Acanthiops tsitsa*), Ade_sp (*Adenophlebia* sp.), Ade_aur (*Adenophlebia auriculata*), Afr_sp (*Afronurus* sp.), Afro_ber (*Afronurus bernardi*), Bae_sp (*Baetis* sp.), Cae_sp (*Caenis* sp.), Eut_sp (*Euthraulus* sp.), Pseu_pis (*Pseudocloeon piscis*), Pse_gla (*Pseudocloeon glaucum*), Pse_sp (*Pseudocloeon* sp.), Pse_vin (*Pseudocloeon vinosum*), Tri_sp (*Tricorythus* sp.), Aph_sp (*Aphenicera* sp.), Aes_sp (*Aeshna* sp.), Bra_sp (*Brachytermis* sp.), Cre_sp (*Crenigomphus* sp.), Cre_ren (*Crenigomphus rennei*), Par_gen (*Paragomphus genei*), Phy_sp (*Phylomacromia* sp.), Pse_sp (*Pseudagrion spernatum*), Pse_sp (*Pseudagrion* sp.), Che_sp (*Cheumatosyche* sp.) and Hyd_sp (*Hydropsyche* sp.). Site groups: Grp1 (group 1), Grp2 (group 2), Grp3 (group 3), Grp4 (group 4). Seasons: wi (winter), sp (spring), su (summer) and au (autumn). Settled sediment classes: M_SAND (medium sand), F_SAND (fine sand), VF_SAND (very fine sand), VC_SAND (very coarse sand), C_SAND (coarse sand), M_SILT (medium silt), F_SILT (fine silt), and VF_SILT (very fine silt).

The CCA ordination undertaken on turbidity and suspended sediments and EPOT species revealed that the first three axes explained 48.40% cumulative variance. The first axis with eigenvalue 0.14 explained 20.69% variance, the second axis with eigenvalue 0.12 explained 17.35% variance and the third axis with eigenvalue 0.07 explained 10.35% variance (Table 3.9). The Monte Carlo (1000 permutations) showed significant correlations between the first two axes and suspended sediment particle characteristics including turbidity (Table 3.9).

The CCA ordination plot revealed that the proportion of very coarse silt, very fine sand, and sand were strongly positively associated with *Pseudocloeon piscis*, *Afronurus bernardi*, *Caenis* sp., *Pseudocloeon spernatum*, *Tricorythus* sp. and *Hydropsyche* sp. These species indicated strong positive correlation with site group 2 during winter and spring, and site group 4 during summer; they also indicated strong negative correlation with the finer proportion of suspended sediments characteristics (clay, very fine silt, fine silt and medium silt) and turbidity. The proportion of clay, very fine silt, fine silt and medium silt, and turbidity strongly favoured the assemblage of *Paragomphus genei*, *Cheumatosyche* sp, *Baetis* sp. *Aeshna* sp. and *Pseudocloeon glaucum* with strong positive correlation with site group 1 during summer and autumn and site group 3 during autumn. The proportion of medium sand indicated strong positive correlation with site groups 2 and 3 during summer and autumn and indicated a positive correlation with the assemblage of *Brachytermis* sp., *Aphenicera* sp., and *Acanthiops* sp. (Figure 3.8b).

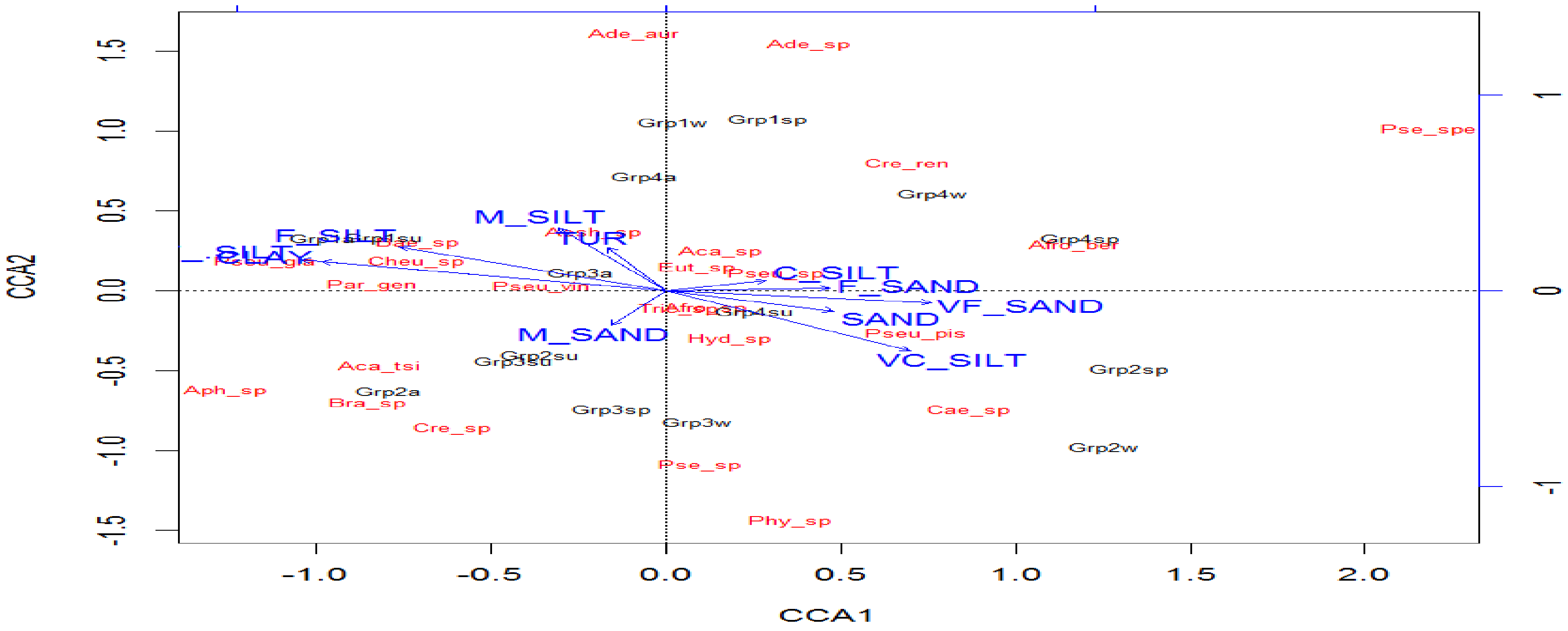

Figure 3.8b: CCA ordination showing the correlation between suspended sediment and turbidity particles and the EPOT species during the study period (August 2016 – April 2017) across the site groups. Abbreviations: Aca_sp (*Acanthiops* sp.), Aca_tsi (*Acanthiops Tsitsa*), Ade_sp (*Adenophlebia* sp.), Ade_aur (*Adenophlebia auriculata*), Afr_sp (*Afronurus sp*.), Afro_ber (*Afronurus bernardi*), Bae_sp (*Baetis* sp.), Cae_sp (*Caenis* sp.), Eut_sp (*Euthraulus* sp.), Pseu_pis (*Pseudocloeon piscis*), Pse_gla (*Pseudocloeon glaucum*), Pse_sp (*Pseudocloeon* sp.), Pse_vin (*Pseudocloeon vinosum*), Tri_sp (*Tricorythus* sp.), Aph_sp (*Aphenicera* sp.), Aes_sp (*Aeshna* sp.), Bra_sp (*Brachytermis s*p.), Cre_sp (*Crenigomphus* sp.), Cre_ren (*Crenigomphus rennei*), Par_gen (*Paragomphus genei*), Phy_sp (*Phylomacromia* sp.), Pse_sp (*Pseudagrion spernatum*), Pse_sp (*Pseudagrion* sp.), Che_sp (*Cheumatosyche* sp.) and Hyd_sp (*Hydropsyche* sp.). Site group: Grp1 (group 1), Grp2 (group 2), Grp3 (group 3), Grp4 (group 4). Season: w (winter), sp (spring), su (summer) and a (autumn). Suspended sediment classes: M_SAND (medium sand), F_SAND (fine sand), VF_SAND (very fine sand), VC_SAND (very coarse sand), C_SAND (coarse sand), M_SILT (medium silt), F_SILT (fine silt), VF_SILT (very fine silt), TUR (turbidity).

Table 3. 9: CCA summary statistics showing eigenvalues, percent variance explained and the *P* and *F* scores for the first three ordination axes. Boldface values indicate axes that show *P* and *F* significant difference.

CCA properties	**Settled sediments**			**Suspended sediments and turbidity**		
	Axis 1	**Axis 2**	**Axis 3**	**Axis 1**	**Axis 2**	**Axis 3**
Eigenvalues	0.14	0.10	0.07	0.14	0.12	0.07
% variance explained	20.52	14.68	0.11	20.69	17.35	10.35
% cumulative variance explained	20.52	35.2	45.8	20.69	38.05	48.39
P-value	**0.045**	0.090	0.173	**0.010**	**0.018**	0.088
F-score	3.2	2.29	1.66	4.422	3.71	2.21

3.4 Discussions

3.4.1 Physico-chemical variables

The physico-chemical characteristics of water are important factors that determine the abundance, diversity and distribution of aquatic biota including macroinvertebrates (Arimoro. 2011; Berger *et al*., 2017). The measurement of physico-chemical variables is essential for monitoring water quality, as it permits direct assessment of the status of aquatic ecosystems that are exposed to deleterious anthropogenic factors. In the present study, the physical-chemical constituents, except for EC, of the sites were not statistically significantly different between the sites (Table 3. 1). The EC concentrations were highest at Site 3 (Qurana tributary) and indicated the E/F ecological category for all seasons except summer at this site. However, EC values for all other sites indicated the B and C/D ecological categories, which were indicative of good and fair water quality conditions (Table 3. 1). The higher concentration of EC at Site 3 during the dry season relative to other seasons could be due to low flow, reduced dilution during the dry season and the geology of the river channel that characterises the catchment (Grobler *et al*., 1987).

The Tsitsa River and its tributaries are important resources for both domestic use and agricultural activities. The values of the water physico-chemical variable were generally low, revealing that the major impact on water quality in the river systems might be attributed to other factors such as elevated sediments. However, the concentration of nutrients such as NH_4-N and PO_4-P was generally higher at all sites during summer, compared to other seasons. The concentration of nutrients variables for all sites were closely within the target water quality limits and indicated ecological category A, which was indicative of the natural ecological condition.

The low concentrations of nutrients at all sites can be an indication of low inorganic nutrients input into the rivers from the surrounding catchment, or nutrient uptake by phytoplankton and aquatic macrophytes as well as microbial activities. In a study by Madikizela and Dye (2001), the authors noted that due to the low nutrient concentrations, the source of nutrients into the river systems might be due to natural processes such as decaying plants and animal materials. Natural nutrient sources in rivers are typical of rural, undisturbed catchments in South Africa. In addition, other studies such as Madikizela & Dye (2010) and Gordon *et al.* (2013), have reported relatively low nutrient concentrations in the Tsitsa River and its tributaries, including the greater Mzimvubu catchment, corroborating the findings of the present study.

The DO concentrations recorded in the study were generally high at all the sites, indicating largely natural conditions as per the ecological category derived through the generic RWQOs (DWAF, 2006). The relatively high concentrations of DO recorded at all sites may be due to the high photosynthetic rate of phytoplankton and other aquatic macrophytes (Shirma and Rathore, 2000; Ravindra *et al*., 2003) or an indication of low input of organic sediments into the studied river systems. Madikizela & Dye (2010) and Gordon *et al.* (2013) also reported high DO concentrations in the studied river systems, suggesting that sediments inputs have not led to the DO depletion in the river systems. The implication might be that sediment inputs in the catchment are largely inorganic. Concentrations of pH and temperature values did not indicate statistical significance among the sampling sites. The pH and temperature values reported in this study were within the optimal range for most aquatic macroinvertebrates.

3.4.2 Analysis of sediment particle size classes

The sediment particle sizes are a fundamental aspect of stream substrate and can influence the density and assemblage structure of macroinvertebrates (Henley *et al*., 2000; Sutherland *et al*., 2012). Sediment results showed that the proportion of very fine sand, fine sand, medium sand and coarse sand particles, the sites were still statistically significantly different from each other. The proportions of coarse sand particles were consistently higher than the proportions of fine and medium sand particles at all sites (Figure 3.2a). Different catchment anthropogenic activities such as agriculture, sand mining and silviculture are associated with the transport of varying classes of sediment particles into the streams and river ecosystems. A recent study by Alberto *et al*. (2016), who investigated stream sediment load input from agriculture in Prince Edward Island, Canada, has demonstrated the importance of agricultural activities in delivering sediments to river systems.

The high proportions of coarse sand at all sites might be attributed to subsistence agricultural activities in the river systems. At Sites 5 and 6 the proportions of coarse sand and very fine sand dominated the particle sizes. The predominance of fine sand may be attributed to relatively scarce cultivation and grazing activities at these sites. Studies have shown that agricultural activities contribute smaller particles of sediments to rivers and streams (Lamberti & Berg, 1995). Fine sediment particles can affect stream ecosystems with particular impact on macroinvertebrates. Their degrees of impacts are however dependent on the particle classes and properties of particles and other confounding factors such as level of exposure and presence of multiple stressors.

Finer particles (<63 µm) are known to have more deleterious effects on organisms partly because of their ion exchange potentials and accumulation on delicate structures of organisms (Naden et al. 2016). However, a previous study by Zweig & Rabeni (2001), who investigated the response of macroinvertebrates to sediment particle in four streams in Missouri, Ozark Highlands, observed that macroinvertebrate taxon richness significantly declined and EPT richness was negatively affected by fine sand sediments. Therefore, the increased accumulation of the proportion of fine sand and coarse sand particles at most sites could have a negative impact on the stream and river ecosystems and macroinvertebrate assemblages.

The proportion of very coarse silt was consistently higher than the proportions of fine silt, medium silt and coarse silt at all sites except Site 3 (Qurana River). The proportion of coarse silt was highest at Site 4 (Pot River downstream). When the proportions of clay and very fine silt were analysed, the result revealed similar patterns across the sites with their lowest values at Site 5 (Pot River downstream) and their highest values at Site 3 (Qurana River) and Site 8 (Millstream downstream). The high accumulation of finer particles of settled sediments such as the proportion of clay and very fine silt at Site 3 (Qurana River), may affect the distribution of macroinvertebrates at this site more than other sites. A previous study by Kaller and Hartman (2004), who studied the effects of fine sediments (<0.125) from stream-side roads on macroinvertebrates in 7 Appalachian streams, showed a negative association of EPOT taxa to sediments. It is therefore clear that fine sediment within the class range of silt can affect macroinvertebrates in rivers and streams. The delivery of fine silt particles into riverine systems has been attributed to runoffs from gravel roads (Bilby *et al.,* 1989), and this could be a possible explanation of the high proportion of coarse silt at Site 3 (Qurana River).

When suspended sediment particles were analysed, the proportions of very fine sand, fine sand and coarse sand showed a similar pattern to that of settled sediment particles. The proportion of coarse sand was higher, followed by the proportion of very fine sand, when compared to that of fine sand and medium sand across the sites. Similar to that of settled sediment particles, the proportion of very coarse silt was higher than the proportions of fine silt and medium silt, with its lowest value at Site 3 (Qurana River) and highest value at Site 5 (Pot River downstream). In addition, the proportion of clay and very fine silt were highest at Site 3 (Qurana River) and lowest at Site 2 (Tsitsa River downstream).

3.4.3 Spatial and temporal analysis of EPOT community assemblage structure

Macroinvertebrates are known to exhibit a varying degree of sensitivity to environmental water quality deterioration (Baroldi *et al*., 2016). Thus, assessing the diversity of macroinvertebrate assemblages as biological indicators of water quality is increasingly used to complement traditional physico-chemical measurements. In the present study, thirty-two EPOT taxa, identified to species or genus level, were recorded. The highest number of individual EPOT species recorded was at Site 5 during spring, while the least number of individuals recorded was at Site 2 during spring. The patterns of EPOT assemblages at Site 1 (Tsitsa upstream) and Site 3 (Qurana River) were relatively similar in terms of their percent relative abundances across the four seasons. The most commonly represented EPOT species included *Pseudocloeon* sp., *Baetis* sp., and *Hydropsyche* sp., occurring during most of the sampling seasons expect a few seasons at some sites.

When the difference in the sites was analysed using the non-metric multi-dimensional scaling (NMDS) in terms of EPOT assemblages structure, three site clusters were identified. Site 1 (Tsitsa upstream) and Site 3 (Qurana River) were closely clustered together in the first cluster. These two sites were initially selected to have high sediment impact and were further grouped together into site group 1 by the cluster analysis, which represented the highly sediment-influenced site group. Cluster 2 consisted of Site 7 (Millstream upstream) and Site 8 (Millstream downstream), while Site 4 (Pot River upstream), Site 5 (Pot River downstream) and Site 6 (Little Pot River) formed Cluster 3. The sites in Cluster 2 consisted of sites that had been classified into the site groups 2 and 3, the less sediment-influenced site groups. The third cluster consisted of sites classified into site groups 3 and 4, the less sediment-influenced site groups. The assemblage structures of EPOT species largely enabled the discrimination of the sampling sites according to their degree of sediment stress, except at Site 2 (Tsitsa downstream), which was largely separated from the three site groups.

3.4.4 Association between EPOT species and biotopes

The biophysical characteristics of aquatic systems, such as biotope, play an important role in shaping the diversity and assemblage structure of species (Wood & Armitage, 1997; Lamouroux *et al*., 2004; Laini *et al.,* 2014). Aquatic ecosystems with diverse and complex habitat patches tend to support more organisms than systems with less complex habitat. In this study, five EPOT groups were identified based on their preferred biotopes. Stone biotope supported the highest number of species compared with the other biotope groups. Nine species out of the 14 species associated with stone biotope indicated statistical significant assications with the stone biotope (Table 3.6). The findings of this study are similar to Odume *et al*. (2015), who reported that the stone biotope supported more species of macroinvertebrates than other biotopes such as vegetation and sediments (GSM). The stone biotope is known to exhibit a morphologically and structurally complex habitat and has the potential to support diversities of organism compared to less complex biotopes.

It has been recognised that various factors such as individual species traits, food availability and food preference, habitat diversity, stream hydraulics and provision of refugia play a major role in structuring species assemblages (Lamouroux *et al*., 2004). For example, *Pseudocloeon* sp., *Brachytermis* sp., *Adenophlebia* sp., and *Oligoneuropsis lawrencei* preferred the stone and vegetation biotope (Table 3.6). These species are mostly grazers and collector-gatherers that feed on detritus (FPOM) and aquatic macrophytes and prefer high water-depth. This study, therefore, provided more insight into EPOT species preferences to different biotopes by providing information on the preferred biotopes of EPOT species, and also indicated that EPOT species showed more preference for the stone biotope (Table 3.6).

3.4.5 Relating EPOT species with sediment particle characteristics and turbidity

This study revealed that EPOT species assemblages responded to changes in the water quality in the studied river systems. Canonical correspondence analysis (CCA) separated the less impacted site groups from the highly impacted site groups. The CCA ordination also showed that EPOT species were significantly associated with settled and suspended sediment characteristics as indicated by the first and second axes.

The CCA ordination revealed that in terms of settled sediment particles, the proportions of clay, very fine silt, medium silt and coarse silt indicated strong positive correlations with site groups 1 and 3. Sediment tolerant EPOT species such as *Paragomphus* sp., *Aeshna* sp. and *Baetis* sp. were positively correlated with these site groups. Site groups 3 and 4 during winter and spring were positively correlated with the proportions of very coarse silt, very fine sand, fine sand, medium sand and coarse sand. These proportions of sediment particles favoured the assemblages of EPOT species-sensitive taxa such as *Hydropsyche* sp., *Pseudocloeon* sp. and *Pseudocloeon piscis.* The result generally revealed that EPOT species-tolerant taxa were positively correlated with the proportions of clay, very fine silt, medium silt and coarse silt at site groups 1, 2 and 3. These sediment particles were negatively associated with sediment-sensitive EPOT species such as *Hydropsyche* sp., *Pseudocloeon* sp., *Pseudocloeon piscis,* and *Pseudocloeon* sp. Kaller & Hartman (2004) reported a negative correlation of EPOT taxa richness to fine sediments particles <0.125 mm, similar to the silt particles classes sample in this study. Their sampling occurred in spring and autumn over two years in seven Appalachian streams in the USA. However, this present study further revealed that all sediment particles are important in explaining the CCA ordination results and species such as *Paragomphus* sp., *Baetis* sp. and *Aeshna* sp. appeared to be good indicators of sediment-influenced site groups.

In terms of suspended sediments, the CCA ordination indicated similar results to that of settled sediment results, and also indicated that EPOT species assemblages were significantly influenced by sediment particle characteristics. However, turbidity contributed to the factors influencing EPOT species assemblage structures at site groups 1, 2 and 3, indicating positive associations with these site groups. Previous studies by Martin & Neely (2001) and Donohue & Irvine (2003), have also indicated significant effects of sediment loads on the assemblage structure of macroinvertebrates in Typha Wetland, Michigan, USA and Lake Tanganyika, East Africa.

In general, EPOT species assemblages in the Tsitsa River and its tributaries appeared to be good indicators of sediment-influenced river systems, consisting of species with varying degrees of sensitivities to sediment stress. They can be said to exhibit a species-specific response to fine sediment particles. Relyea *et al*. (2000) and Buendia *et al.* (2013) also reported a species-specific response to fine sediments.

3.4.6 Ephemeroptera, Plecoptera, Odonata, and Trichoptera (EPOT) metrics

Diversity metrics have been increasingly implemented in ecology to assess and identify ecological impacts and evaluate the integrity of aquatic ecosystems (Pires et al., 2000). In this study, five EPOT metrics were applied to discriminate between site groups obtained from the cluster analysis (Figure 3.4). Of the five metrics, EPOT abundance, Simpson, and Shannon index enabled the satisfactory discrimination (i.e. clearly separating highly sediment-influenced site group 1 from less sediment-influenced site group 4) between site groups (Figure 3.7). However, the Pearson's simple correlation revealed that except for Margalef's species richness index, all the metrics did not indicate statistically significant correlation with the physico-chemical variables. The none significant association between physico-chemical variables and EPOT metrics, apart from Margalef's richness index, might be an indication that water quality conditions did not have much influence on EPOT metrics. Another possible explanation could be that physico-chemical variables presence in the river systems might not have led to water quality deterioration. Therefore, it could be implied that water quality deterioration in the studied river systems could be attributed to other influences such as sediment stress.

The Pearson's correlation analysis revealed that all the metrics, except EPOT abundance, were significantly correlated with sediment particles. However, settled sediment had the highest significant correlations with EPOT metrics. Shannon's index decreased with increase in the proportion of settled sand and fine sand sediments, but increased with increase in the proportion of settled coarse silt and medium silt. Previous studies have assessed the sensitivity of taxonomic metrics to fine sediment stress (Angradi, 1999; Zweig & Rabeni, 2001) and have reported both positive and negative correlations between taxonomic metrics and sediments. Angradi (1999) found weak correlation between taxonomic metrics and deposited sediments, while Zweig & Rabeni (2001) reported a strong significant relationship between them.

The decrease in Shannon's index is consistent with findings by Buendia *et al.* (2013), who reported a decrease in Shannon's index as fine sediments increased in an undisturbed catchment in Isabena, in the northeast of Spain. Doeser (2016) also reported a negative association of Shannon's index with sand particles. However, evenness did not show any significant association with settled sediments. Similar results were reported by Kilgour *et al.* (2004) and Buendia *et al.* (2013), who also indicated that evenness did not significantly correlate with settled sediment particles.

In terms of suspended sediment particles, Shannon's index including Margalef's richness index also indicated significant sensitivity to suspended sediment particles. Shannon's index indicated a positive significant correlation with the proportion of fine silt and medium silt sediment particles. In contrast to settled sediments, evenness indicated a strong sensitivity to suspended sediments particles; this indicates a strong significant positive correlation with the proportion of very fine silt, fine silt and medium silt. In further contrast to settled sediments, Margalef's richness index indicated weak correlation with suspended sediment particles. Margalef's index was positively significantly correlated with only very fine silt. Gordon *et al.* (2013) reported similar findings, where Margalef's richness index indicated no significant correlation with sediment load.

The present study revealed that EPOT metrics responded either positively or negatively to increasing fine sediment particles. The Shannon's index and Margalef's richness index, followed by evenness, were the most sensitive to sediment particles. However, these sensitivities differed with sediment particle size classes, as well as with settled or suspended sediments particles. For example, Shannon's index and Margalef's richness index indicated significant negative correlations with proportions of fine sand and coarse sand settled sediment particles but indicated positive significant correlations with proportions of clay, very fine silt, fine silt and medium silt. In addition, evenness indicated a none significant correlation with settled sediments but indicated a strong significant correlation with suspended sediment particles.

3.5 Conclusion

The results of this study revealed that sediment input into the studied river system is the primary stressor of water quality. This is supported by the fact that the majority of the water quality variables measured during the study largely indicated either an ecological category A or B for the sites. However, the sites were distinct based on sediment loads, measured in the form of turbidity, and sediment particle characteristics. Based on the sediment particle sizes, four distinct site groups were observed: Site group 1 (Tsitsa River upstream and Qurana tributary), site group 2 (Tsitsa River downstream and Millstream upstream), site group 3 (Pot River, both upstream and downstream, and Millstream downstream) and site group 4 (Little Pot River). These groups clearly formed a gradient of decreasing sediment influence in the order site group 1> site group 2> site groups 3 and 4. The taxonomic assemblage response of the EPOT revealed that the groups have species that are both tolerant and sensitive to sediment stress. For example, species such as *Paragomphus* sp., *Aeshna* sp., and *Baetis* sp. mostly associated with site groups 1 and 2, which were more sediment-influenced than site group 3 and 4. This finding stresses the importance of species-level identification in biomonitoring, as species of the orders EPOT have traditionally been regarded as sensitive to water quality impairment. Furthermore, the taxonomic assemblage analysis revealed that species of the EPOT in the studied river systems showed a differential preference for the sampled biotopes. Most species preferred the stone biotope, as opposed to the vegetation and GSM biotopes. It is therefore important that when undertaking biomonitoring studies with the EPOT group, comparable biotope groups are sampled at sites intended for comparison.

CHAPTER 4: DEVELOPING A TRAIT-BASED APPROACH FOR EVALUATING AND PREDICTING THE VULNERABILITY OF EPHEMEROPTERA, PLECOPTERA, ODONATA AND TRICHOPTERA (EPOT) TAXA TO SEDIMENT STRESS

4.1 Introduction

Sediment stress can affect aquatic organisms through a variety of means and species are impacted to different degrees. The influence of sediment stress on aquatic organisms is complex and is a function of interacting factors including duration of exposures, particles distributions, sediment load, geochemical composition of particles and sources of the sediments, as well as the susceptibility of the resident organisms (Murphy *et al*., 2015; Odume *et al*., 2018). For example, a previous study showed that long-term exposure of only sensitive macroinvertebrates to sediment particles had sub-lethal effects at a concentration of 36 mg/l and lethal effects at 58 mg/l (Gordon & Palmer, 2015). The study further showed that freshwater macroinvertebrates were tolerant at short-term exposure (<24 hours). Sediments effects on macroinvertebrates could be direct e.g. increased drift of some macroinvertebrates species, substrate modifications and clogging of interstitial spaces or burial and clogging of feeding and respiratory apparatus (Jones *et al*., 2012). Sediment stress on macroinvertebrates could also be indirect e.g. changes in the physical and chemical condition of streams, or reduction of primary productivity and dissolved oxygen (Lenat, 1981; Osterlin *et al*., 2010).

Therefore, evaluating and understanding the responses of species assemblages to in-stream sediment stress through an explicit consideration of macroinvertebrate traits, is critical because traits mediate the relationship between organisms and their external environment. That is, the types of traits possessed by an organism determine whether the organism is able to adapt and thrive in the presence of a particular stressor.

Traits of an organism play an important role in defining the relationships with and potential vulnerability of a species to its environment (Poff, 2006). Traits may provide a mechanistic understanding of species-environment relationships, as they reflect the organism's adaptive potential in a specific environmental context (Verberk *et al*. , 2013; Piliere *et al*., 2016; Odume *et al.,* 2018). Therefore, an explicit analysis of the trait an organism possesses can provide clues as to whether an organism is potentially vulnerable to a particular stressor or not. The response of an organism to its environment is underlain by the interactions between an organism and its environment factors; therefore, such interactions can determine whether a species could be vulnerable or tolerant to specific environmental stressors (Odume *et al.,* 2018). Thus, a good understanding of traits and their relationship to specific environmental stressors could help in interpreting and condensing trait information on a number of species into a few potential vulnerability groups (Verberk *et al*., 2013).

The habitat template concept (HTC) and habitat filter concept (HFC) (Southwood, 1977; Townsend, 1994) provide a sound basis for linking traits to environment attributes. The trait-based approach (TBA) to biomonitoring is based on sound theoretical ecology, which takes into account the relationships between traits and the organism's environment. The TBA is increasingly being applied in biomonitoring studies and most applications of the TBA predict the response of individual traits to environmental conditions (Tomanova *et al*., 2008). It is however argued that environmental stressors do not filter species on the basis of separate traits but on a set of co-adaptive combinations of traits. Analysing species' responses using the TBA by taking account of trait combination, trade-off and spin-off recognises the value of trait interactions in shaping biotic assemblages (Verberk *et al*., 2013). In this study, the potential vulnerability of the EPOT species to sediment stress is investigated using the TBA. The approach developed in this study explicitly takes into account, trait combination and interactions as argued by Verberk *et al*. (2013).

Therefore, this chapter addresses Objective three as stated in (Chapter 1 section 1.7) of this thesis: *to develop a trait-based tool for assessing and predicting the potential vulnerability of species of the order Ephemeroptera, Plecoptera, Odonata and Trichoptera to sediment stress in the Tsitsa River and its tributaries.* The above overall objective is achieved by addressing the following specific sub-objectives in this chapter: i) develop a trait-based approach for classifying EPOT species into vulnerability classes in relation to sediment stress ii) use the developed trait-based approach to predict the assemblage responses of EPOT species to sediment stress in the studied river systems iii) compare trait-based predictions with taxonomic-based analysis using the Pearson's point-biserial correlation and iv) evaluate the value of analysing the responses of individual trait attributes compared with the approach advocated in this study.

4.2 Materials and methods

4.2.1 Developing a trait-based approach for assessing the potential vulnerability of EPOT taxa to sediment stress

The TBA developed in this study followed six steps in accordance with Odume *et al*. (2018): i) reviewing the literature for reported sediment modes of stress on macroinvertebrates, including the EPOT taxa, ii) selecting traits that are mechanistically linked to the sediments modes of stress, iii) identifying vulnerable trait attributes for each trait category per species, iv) identifying non-redundant vulnerable trait attributes per species for each selected trait category, where applicable, v) quantifying the measure of functional trait diversity (FTD) per trait category for each species, and vi) classifying species into four vulnerability classes based on the combination of trait attributes possessed, FTD and non-redundant vulnerable trait attributes.

Reviewing sediments mode of stress on EPOT

The literature was reviewed extensively to identify documented mechanisms by which sediments influence macroinvertebrates. Sediment modes of stress could be through direct or indirect mechanisms and are summarised in (Table 4. 1).

Table 4. 1: A summary of sediment modes of stress on EPOT taxa after extensive literature review (adapted from Odume *et al*., 2018)

Sediments mode of stress	**Notes**
Clogging	Inputs of excessive fine sediments into the aquatic environment can lead to clogging of delicate organs such as the gills and filter feeding apparatus. Gill clogging can potentially lead to respiratory impairment and if sediments remain in increased deposition over a long time, may lead to organism's death (Jones *et al*., 2012). Similarly, clogging of filter feeding apparatus could impede feeding, reducing feeding and energy efficiency. This is particularly the case for some mayflies and caddisflies that have net-like feeding and respiratory structures (Jones *et al.,* 2012).
Physical abrasion	Increased sediment input, particularly moving at high velocity in riverine ecosystems, are likely to cause abrasion of soft, fleshy and exposed body parts (Wilkes *et al*., 2017). The movement of suspended particles in water columns renders such soft-bodied and exposed species, particularly the Stonefly species that swim in open water, vulnerable to sediment stress. The tube-building organisms, or taxa that are able to retract into hidings or whose body are partly or fully sclerotized, are likely to be less vulnerable to the abrasive effect of moving sediment particles (Kurtak, 1978).
Burial	Increased and sustained deposition of sediment on the streambed can lead to the burial of less motile and attached macroinvertebrate (Jones *et al*., 2012; Bona *et al*., 2016). Slow moving taxa are also vulnerable if they cannot keep pace with the rate of sediment accretion (Conroy *et al*., 2017). This is particularly the case for most burrowing and crawling mayfly and dragonfly species. Further, as a direct consequence of burial, the chemical environment of microhabitats can also be impacted by oxygen reduction and other chemical processes. Therefore, taxa that are attached either permanently or temporarily or sedentary in nature, are particularly vulnerable to burial and this effect may be amplified if such taxa

	are also sensitive to oxygen depletion in their microhabitats. Heptageniidae nymph that is particularly sensitive to low dissolved oxygen concentration can be vulnerable (Gaufin *et al.,* 1974).
Substrate modification	Gradual and sustained accretion of fine sediments can alter the stability of the streambed, modify substrate surfaces and fill up interstitial spaces, thereby impacting on those taxa with a preference for stable substrates such as stones and vegetation (Wilkes *et al*., 2017). Substrate modification can also impact the chemical conditions of microhabitats, affecting organisms that are sensitive to changes in physico-chemical conditions in their microhabitats.
Physico-chemical effects (Increased turbidity oxygen and depletion)	A major physico-chemical stress by sediments on macroinvertebrates is through the depletion of dissolved oxygen and increased turbidity (Billota & Brazier, 2008). Sediment delivery from catchments rich in organic materials is likely to stimulate microbial activities that can cause the depletion of DO level. Increased sediment loads can also impact on the vertical distribution of oxygen, influencing the depth to which organisms may burrow (Jones *et al*., 2012). Turbidity impacts on visibility, with implications for predators that rely on visual clarity to search for prey. The potential effects of elevated sediments deposition on food availability and quality are complex and involve a range of interacting factors. For example, increased sediment deposition may cover the surfaces of substrates, reducing the growth of periphyton and the overall quality of the available food resources (Buendia *et al*., 2013; Wilkes *et al*., 2017), which may impact on grazers. Reduced light penetration can also impact the growth of periphyton. On the other hand, depending on the accretion rate, increased sediment can increase particulate organic matter, important food resources for the filter feeders, which may lead to the blossoming of filter-feeding macroinvertebrates (Jones *et al*., 2012). However, it is important to note that sustained deposition of sediment over an extended period may result in negative effects on filter-feeders as earlier described. Shredders can be negatively affected as sediments accumulate over leaf litters (Buendia *et al.,* 2013; Descloux *et al*., 2014).

Trait selection and analysis

Based on the identified sediment modes of stress on macroinvertebrates, trait categories that were deemed mechanistically linked to these modes of stress were selected. A total of 10 trait categories were selected and resolved into 32 trait attributes (Table 4. 2). Selected traits include those that are related to life-history (i.e. oviposition behaviour), morphology and physiology (e.g. body form, maximum body size, and respiration), behaviour (e.g. locomotion), and ecological preferences (e.g. substrate attachment, biotope preferences, feeding habits and food preferences). In addition to mechanistic linkage with sediment modes of stress, in selecting traits for this study, other factors were also considered, and these include the ease of trait measurement/observation and availability of trait information. The traits information was obtained from the newly compiled South African Macroinvertebrate Database (Odume *et al.,* 2018), as well as from the literature and expert consultation at the Albany Museum in Grahamstown, South Africa.

Table 4. 2: Traits and trait attributes selected for the development of the TBA for assessing potential vulnerability of EPOT taxa in the studied rivers systems

Trait category	Trait attribute	Code
Gill type (A)	Filamentous gill	A1
	Plate-like gill	A2
	Lamellate gill	A3
	Operculate gill	A4
Locomotion (B)	Burrowers	B1
	Crawlers	B2
	Sprawlers	B3
	Clingers/climbers	B4
	Swimmers	B5
Body armouring (C)	Soft-bodied and exposed	C1

	Sclerotized	C2
	Cased/tubed	C3
Food preference (D)	Detritus (FPOM)	D1
	Detritus(CPOM)	D2
	Macrophytes/Algae	D3
	Animal Materials	D4
Biotope preferences (E)	Stone	E1
	Sediments (gravel, sand, and mud)	E2
	Vegetation	E3
Feeding habits (F)	Filter feeders	F1
	Grazers/scrapers	F2
	Shredders	F3
	Predators	F4
Substrate attachment (G)	Temporarily attached	G1
	Permanently attached	G2
	Free-living	G3
Oviposition behaviour (H)	Exophytic	H1
	Endophytic	H2
Maximum body size (I)	<5 mm	I1
	>5 - 10 mm	I2
	>10 - 20 mm	I3
	>20 mm	I4

Identifying vulnerable trait attributes

For each trait category, attributes likely to confer vulnerability of species in the context of sediment stress were identified and termed "*vulnerable trait attributes*" (Table 4. 3) (*Odume et al.,* 2018). In the context of this study, vulnerable trait attributes are described as trait features an organism possesses that can make the organism vulnerable to a particular environmental stressor such as sediments. Therefore, it is important to note that trait attributes which are termed vulnerable are context specific, only relating to the stressor of interest. This implies that trait attributes classified potentially vulnerable to sediments might be tolerant to another stressor. The response of a given trait to a particular stressor can be known, because traits respond in a given way under a specific environmental context. Therefore, accurate prediction of an organism's response can be known by understanding the relationship between traits and its environment under a given stressor of interest (Lancaster *et al.,* 2009; Hawkins *et al.,* 2010). In the context of sediment stress, 17 vulnerable trait attributes were identified based on their predicted response to sediment stress. Expert ecological knowledge in the field of aquatic ecology was also consulted (Odume *et al.,* 2018). The rationale for identifying vulnerable trait attributes for each trait category and subsequent classification of species into vulnerability classes is dependent on the trait functional redundancy and diversity, and the numbers of vulnerable trait attributes possessed by an organism (Odume *et al.,* 2018). Therefore, organisms possessing greater combinations of vulnerable trait attributes are expected to be more vulnerable than those possessing fewer combinations of vulnerable trait attributes. Another factor considered was that a given sediment mode of stress can influence an organism in multiple ways. For example, burial can affect an organism by depleting oxygen availability, reducing the habitat availability, and greatly reducing access to food, and this contributes to the complexity of developing an approach for assessing the potential vulnerability of EPOT species to sediments stress.

Table 4. 3: A summary of trait category, trait attributes, identified vulnerable trait attributes and sediment modes of stress, as well as a brief rationale for linking sediment modes of stress to each trait category

Trait category	**Trait attribute**	**Mode of sediment stress**	**Vulnerable trait attribute**	**Rationale**
Gill type (A)	Filamentous gill (A1), Plate-like (A2), Lamellate gill (A3), and Operculate gill (A4)	Physical abrasion and clogging	Filamentous gill (A1), Plate-like gill (A2), and Lamellate gill (A3)	Sediment accretion in riverine systems causes fine sediments particles to accumulate in respiratory structures such as gills. Such accumulation can affect the optimal functioning of gills through physical damage and scouring due to clogging (Hynes, 1970; Jones *et al*., 2012). The effects of sediments on the gills are likely to be more severe for gills that are unprotected compared with protected gills. Therefore, unprotected gills such as filamentous and lamellate gills were deemed more vulnerable compared with operculate gills that are protected (Corbin & Goonan, 2010).

Locomotion (B)	Burrower (B1), Crawlers (B2), Sprawlers (B3), Clingers/climbers (B4), and Swimmers (B5)	Substrate modification, burial, and change in water chemistry	Burrower (B1) and Crawlers (B2)	Mode of organism's movement can determine its ability to escape from impending danger or threat. Elevated sediments modify river substrates and water chemistry, and may cause burial through the accumulation of sediment particles on the substrate. This increased accumulation leads to substrata instability, covering of substrate and reduced oxygen content in interstitial spaces, which can affect organisms that depend primarily on streambed and other substrata (Odume *et al.,* 2018). Less motile taxa such as burrowers and crawlers EPOT species have been predicted in the literature to be more vulnerable to sediment effect through burial, change in water chemistry and substrate modification (Wilkes *et al.,* 2017). The effect of burial can, therefore, be less pronounced in EPOT species that can actively move and escape from elevated sediment deposition.

Body armouring (C)	Soft-bodied and exposed (C1), sclerotized (C2), and cased/tubed (C3)	Physical abrasion	Soft-bodied and exposed (C1)	In lotic systems, sediment particles are transported in water current; moving sediment particles can have a scouring effect on soft-bodied and exposed species. The effects are likely to be aggravated if deposited sediments are moving at a sustained high velocity. Such effects are likely to be more pronounced for species without body protection apparatus such as sclerites, cases or tubes. Therefore, species with no protection are likely to be more vulnerable to physical abrasion compared with those with body protection (Odume *et al.,* 2018). The orders EPOT comprise mostly of species with soft and unprotected body surfaces that are constantly in contact with sediments, and are thus expected to be vulnerable to sediments particles. The EPOT species such as the Leptocerids that construct cases are therefore expected to be tolerant to sediments.

Food preferences (D)	Detritus (FPOM) (D1), Detritus (CPOM) (D2), Macrophytes/algae (D3), and Animal materials (E4	Burial, increased turbidity, change in water chemistry	Detritus (FPOM) (D1), Detritus (CPOM) (D2), and Macrophytes/algae (D3)	The increased deposition of sediments can lead to accumulation on the streambed, which can cause the covering of primary producers such as periphyton and macrophytes. Burial can affect the food quality and availability by increased deposition of periphyton. Therefore, species that depend on *periphyton* and macrophyte*s* as their primary source of food are likely to be more affected. In addition, accumulation of sediments in interstitial spaces of stream habitat can lead to changes in water chemistry and increased turbidity (Odume *et al.,* 2018). An increase in turbidity can reduce the light penetration available for photosynthesis thereby leading to anoxic conditions (Graham, 1990). Reduced photosynthetic rate can affect the organic content of streams and thereby reduce the detrital materials available as food for organisms.

Biotope preference (E)	Stone (E1), Vegetation (E2), and Sediments (gravel, sand and mud) (E3)	Change in water chemistry, burial and substrate modification	Stone (E1) and Vegetation (E2)	Gradual and sustained sediment accretion modifies the stability of substrates and the chemistry of micro-habitats, and fills up interstitial spaces between stones, making taxa with a preference for more stable substrates such as stone and vegetation more likely to be vulnerable compared with those with a natural preference for sediments and an open water column (Doretto *et al*., 2017).
Feeding habits (F)	Filter feeder (F1), Grazers/scrapers (F2), Shredders (F3), and Predators (F4)	Clogging and burial	Filter feeder (F1), Grazers/scrapers (F2), and Shredders (F3)	Deposition of sediment can cover food sources such as algae, impacting on the availability and quality of food for grazers (Graham, 1990). Further, constant accretion of sediment into stream systems can lead to the clogging of filter-feeding apparatus of filter feeders. Filter feeders, shredders and grazers are therefore likely to be vulnerable to sediment accretion. In addition, clogging causes the loss of habitat, reduces the permeability of dissolved oxygen, and other chemical processes (Archaimbault *et al.,* 2010). These affects species preferring

				stone habitat patches.b Most predatory species are active and are able to escavate theselves from sediments, thereby displaying less vulnerability to burial.
Substrate attachment mode (G)	Temporarily attached (G1), Permanently Attached (G2), and Free-living (G3)	Substrate modification and burial	Temporarily attached (G1)	Sediment accretion can lead to accumulation of sediments over substrates, which may cause the burial of both substrates and attached organisms (Wood *et al*., 2005). Species that are either permanently attached or temporarily attached with potential for less mobility are more likely to be buried compared with free-living species that are active swimmers (Jones *et al.,* 2012). Increased sediments accretion of substrates can lead to unstable substrate. The temporary and permanent attached organisms that depend on stable substrate are vulnerable

				to substrate modification (Wood & Armitage, 1997). Most EPOT species requires specific type of substrate for attachment, thus clogged can lead to drift (Connolly & Pearson, 2004).
Oviposition behaviours(H)	Exophytic oviposition (H1) and Endophytic oviposition (H2).	Burial, clogging, and substrate modification	Exophytic oviposition (H1)	Egg stage is a crucial and delicate stage of EPOT life cycles because it determines the survival of taxa. Most taxa deposit eggs on or in water, which end up on substrates and are said to exhibit exophytic behaviour. Others deposit eggs in plant materials i.e. those that exhibit endophytic behaviour. Eggs deposited on highly sedimented substrates are likely to be affected by clogging and abrasion. Sediment accretion can cause instability of substrate materials and reduced oxygen availability. Eggs deposited exophytically on sedimented substrates can be more vulnerable than those deposited endophytically in plant materials.

Maximum body size (I)	<5 mm (I1), >5-10 mm (I2), >10-20 mm (I3), and >20 mm (I4).	Burial and substrate modification	<5 mm (I1) and >5-10 mm (I2)	A small body size is assumed to offer an adaptive advantage in sediment polluted habitats through an association with short lifecycles (Townsend & Hildrew, 1994). The reduction of pore spaces in heavily sedimented and clogged substrates potentially favours taxa with small body sizes (Gayraud & Philippe, 2003; Xu *et al*., 2012), as they are able to penetrate into the clogged substrata (Buendia *et al*., 2013). Therefore, in a sediment-stressed system, a small body is likely to confer resilience on the species compared with a larger body size.

Functionally redundant trait attributes

In functional ecology, the concept of functional redundancy is used to describe a situation in which two or more species perform a similar function or role in a community or the ecosystem (Schemera *et al.,* 2017). Functional redundancy is a key determinant of the stability or persistence of ecosystem function, as multiple species are able to perform a similar function in a way that their role in the ecosystem is interchangeable and replaceable. Therefore, the ecosystem with a high functional redundancy is functionally more stable and resilient compared with those of low redundancy. In developing the proposed TBA, the concept of functional redundancy was applied to describe vulnerable trait attributes that are functionally redundant with non-vulnerable trait attributes per trait category per species. For example, in terms of trait mobility, crawling is identified as a vulnerable trait attribute, whereas swimming is not. However, many EPOT species crawl and swim making these two traits functionally redundant; thus, crawling would be described as a functionally redundant vulnerable trait attribute. On the other hand, when no other trait attribute for a trait category can perform the function of the vulnerable trait attribute, such a trait attribute is referred to as a functionally non-redundant vulnerable trait attribute (Odume *et al.,* 2018). For example, in substrate attachment, resolved into temporarily attached, permanently attached and swimmers, permanently attached is a functionally non-redundant attribute for those species that are attached to the substrate throughout their life period. For species such as *Adenophlebia* sp. and *Brachytermis* sp. that can both swim and become attached temporarily, the trait attribute *temporarily attached* would be termed functionally redundant. Therefore, the potential impact of functionally non-redundant vulnerable trait attributes was weighted higher on the potential overall vulnerability of the species. This approach was developed by Odume *et al*. (2018) to classify the South African macroinvertebrates into vulnerability classes. The rationale for using functionally redundant trait attributes to classify EPOT species as vulnerable, is that the more functionally non-redundant vulnerable trait attributes an EPOT possesses, the higher the likelihood of such species' vulnerability to sediment stress.

Functional trait diversity (FTD) (plasticity)

The concept of functional diversity is generally used to describe what organisms do in communities or ecosystems and is measured and assessed using various methodologies (Petchey & Gaston 2006; Schemera *et al*., 2017). Functional diversity enhances resource use and niche complementarity, and it is therefore a critical determinant of biodiversity persistent in an ecosystem (Odume *et al.,* 2018). A functionally diverse assemblage is predicted to be more stable and resilient to disturbances than a functionally homogenous assemblage (Petchey and Gaston, 2006).

Functional diversity (FD) is generally viewed as one of the key parameters which underpin the functioning of ecosystems and communities (Tilman *et al.,* 1997; Petchey and Gaston, 2006). Its quantification has attracted growing interest both in aquatic (Bady *et al.,* 2005) and terrestrial ecology (Mason *et al*., 2003; Petchey *et al*., 2007), and is defined as the values or range of values for existing functional traits in a community capable of affecting ecosystem functioning, such as primary production, stability, nutrient cycles and the provision of ecosystem services (Cardinale *et al*., 2012).

In this study, the concept is applied here for each taxon per trait category to qualitatively describe the range of trait diversity that exists for a species per selected trait category. The rationale is that the EPOT taxa are comprised of many species and genera, each with a range of different sensitivities to stressors, environmental requirement, behaviour and tolerance. In addition, within some taxa, different aquatic life stages differ in their environmental requirements and are therefore functionally diverse. The implication is that a species with a wider range of functional diversity is more likely to withstand perturbation than those with a narrower range of functional diversity. For example, in the genus *Baetis*, all the feeding modes are represented and is, therefore, more likely to withstand food scarcity, compared with a specialist feeding genera such as the *Hydropsyche* and *Aphenicera* genera, that feed on detrital materials and smaller organisms. Therefore, in developing the approach, the concept of functional trait diversity (FTD) is applied to scale the perceived functional diversity of each species per selected trait category (Odume *et al.,* 2018).

In this study, FTD scaling of EPOT species was done with the help of the South African macroinvertebrate trait database. The method was developed for South African macroinvertebrates by Odume *et al*. (2018), where a scale of 1 - 5 was chosen, where 1 indicates low FTD for the trait category for a given genus or species, 3 indicates moderate FTD and 5 indicates high FTD. For example, a genus whose members are shredders, predators, grazers/scrapers, collector/gatherers and filter feeders will be awarded an FTD of 5 for the trait category *feeding habit*; and a genus whose members feed only by shredding and grazing will be awarded an FTD of 1 for the same trait category (Appendix B).

Description and classification of EPOT species into vulnerability classes

The potential vulnerability of EPOT species to sediment stress was calculated following the approach used by Odume *et al.* (2018) and was based on the vulnerable trait attributes possessed, non-redundant vulnerable trait attributes and FTD per trait category **(**Appendix B**).** The vulnerability score for each species was calculated as shown in Equation 1:

$$\text{Taxon vulnerability score} = \mathrm{N} + \sum_{\mathrm{n1}}^{\mathrm{nth}} \left(\mathrm{n} * \frac{1}{\mathrm{FTD}}\right) + 2\,(NR) \text{ .... Equation 1}$$

where N = total number of vulnerable trait attributes possessed, n = vulnerable trait attribute per trait category, FTD = functional trait diversity per trait category, NR = total numbers of non-functionally redundant vulnerable trait attributes possessed. A weighting of 2 was assigned to the NR to reflect the greater impact on the potential vulnerability of a species due to the non-redundancy of the factors making up the NR. The inverse of FTD was taken because it was assumed that the greater the FTD, the more resilient or tolerant the species. Using the calculated taxa vulnerability scores, the EPOT species were classified into the four vulnerability classes A, B, C and D using percentile distribution values, as seen in Table 4. 4.

Table 4. 4: Classification of EPOT species into potential vulnerability classes based on the percentile distribution of calculated vulnerability scores

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

Predicting the responses of EPOT species assemblage structure to sediment stress using the vulnerability classes and species frequency of occurrences

The EPOT assemblage predictions were made using two metric measures i) the percent relative abundance of the vulnerability classes (i.e. highly vulnerable, vulnerable, tolerant and highly tolerant) and ii) the frequency of occurrence (FROC) for species across site groups.

Predictions using the percent relative abundance of the vulnerability classes

Using percent relative abundance data for the four classes, the following predictions were made:

i) The percent relative abundance of the highly vulnerable and vulnerable classes (i.e. vulnerability classes A and B) would decrease compared with the percent relative abundance of the tolerant and highly tolerant classes (i.e. classes C and D) at sites with higher sediments influences, i.e. at Site 1 (Tsitsa upstream site), Site 2 (Tsitsa downstream site), Site 3 (Qurana tributary), and Sites 7 and 8 (Millstream upstream and downstream sites).

ii) The percent relative abundance of the highly vulnerable and vulnerable classes would recover compared with the tolerant and highly tolerant classes at the less sediment-impacted sites e.g. Site 4 (Pot River upstream site), Site 5 (Pot River downstream site) and Site 6 (Little Pot River).

iii) The potential effects of sediments on the highly vulnerable and vulnerable classes should be more pronounced during the wet season than the dry season due to a potential increase in sediment delivery into the systems during the wet season.

Predictions using the frequency of taxon occurrence (FROC)

Predictions using FROC were made for each of the 32 species per site group during the dry and wet season. As detailed in Chapter 3 section 3.3.5, site group 1 comprises Sites 1 and 3, site group 2 comprises Sites 2 and 7, site group 3 comprises Sites 4, 5 and 8, while site group 4 comprises only Site 6.

The frequency of occurrence for each species per site group for the dry seasons (winter and spring) and the wet seasons (summer and autumn) was calculated by determining the percent number of times a species occurred in all replicate samples over the study period. Biotope replicate samples were calculated by pooling each biotope replicate for the four seasons per site to form a single replicate sample. For example, all stone 1, vegetation 1 and GSM 1 biotope replicates for all the seasons per site were pooled together to form replicate 1.

Percent FROC was calculated using Equation 2 as shown below.

$$\%\,\mathrm{FROC} = \frac{n}{\mathrm{N}} * 100\% \quad \text{.. Equation 2}$$

where n = number of times a species occurred for a site group in all replicate samples over the study period; N = total number of replicate samples for a site group over the study period.

The percent FROC calculated for individual species was scaled from 1-5 as shown in (Table 4. 5).

Based on the FROC scaling the following predictions were made:

i) Species belonging to the highly vulnerable and vulnerable classes (i.e. A and B) were predicted to occur at a lower FROC of between 1-3 and species belonging to the tolerant and highly tolerant classes (i.e. C and D) were predicted to occur at a higher FROC of between 3-5 for the more sediment-influenced site groups 1 and 2.

ii) Species belonging to the highly vulnerable and vulnerable classes (A and B) were predicted to recover to a FROC of between 3 and 5 at the less sediment-influenced site groups 3 and 4. No prediction at these site groups was made for taxa belonging to the tolerant and highly tolerant classes, as these taxa are likely to occur across all site groups.

Table 4. 5: Showing the frequency of occurrence scale and scores for FROC rating

FROC (%)	**FROC scaling**
0	0
>0 —10	1
>10 — 25	2
>25 — 50	3
>50 — 75	4
>75 — 100	5

4.3 Statistical and data analyses

4.3.1 Evaluating the relative abundance response of the EPOT vulnerability classes to sediment stress

The percent relative abundance of EPOT vulnerability classes were assessed across the eight sites with a view to comparing them across the sites. The Box plot was used to visualise the distribution of the percent relative abundance of the vulnerability classes for the sites. The Kruskal-Wallis multiple comparison tests were further used to test for statistically significant differences between the vulnerability classes (A – D) per site, and per season.

4.3.2 Association between EPOT species and the site groups

The Pearson's point-biserial correlation was used to investigate the association between EPOT species assemblages and site groups. Groups of sites obtained from the cluster analysis in Chapter 3 section 2.6.6 were used for the correlation analysis. The main objective was to evaluate site-taxon correlation and determine the degree of correspondence between these associations and the taxa vulnerability classification developed in this study. The analysis aimed to evaluate the site statistical associations of EPOT species classified into different vulnerability classes with the core purpose of checking whether the vulnerable species are associated with the less sediment-impacted site classes or not. Therefore, the analysis could help to investigate the predictive power of the trait-based classification of EPOT to vulnerability classes. The Pearson point-biserial analysis was undertaken using the ADE4 statistical package for R version 3.4.1 (R Core Team, 2017), as described in Chapter 2 section 2.6.6.

4.3.3 Association between sediment characteristics and individual trait attributes

The RLQ analysis was undertaken to examine the relationship between environmental variables and individual trait attributes of EPOT species assemblages, following Shieh *et al.* (2012). The RLQ and Fourth-corner analyses were done with a view to evaluating how EPOT trait attributes are associated with sites and sediment characteristics. The RLQ analysis was undertaken to assess the correspondence between the predicted trait response and its statistical association with the site groups and sediment particle classes. The RLQ and associated analyses (CA, Hill-Smith, and PCA) were undertaken using the ADE4 package for R version 3.4.1 (Dray & Dafour, 2007; R Core Team, 2017), as described in Chapter 2 section 2.6.7.

The significance of the relationship between environmental variables and trait composition of EPOT assemblages was tested using the Monte-Carlo permutation test (999 random permutations) of rows of the matrices of traits and environmental variables. The Fourth-corner test was further used to test the significance of the relationship between each individual trait attribute and environmental variables (Dray & Lengendre, 2008; ter Braak *et al.,* 2012).

4.4 Results

4.4.1 EPOT assemblage predictive responses to sediment stress in the Tsitsa River and its tributaries

Based on the approach described in the methods section, the EPOT species were assigned to four vulnerability classes: highly vulnerable (A), vulnerable (B), tolerant (C) and highly tolerant (D). Of the 32 species recorded in this study, eight species each were designated as highly vulnerable and vulnerable, nine species were tolerant, while seven species were designated highly tolerant (Table 4. 6). The highly vulnerable species included *Cheumatosyche* sp., *Hydropsyche* sp., and *Baetis* sp., while the vulnerable species included *Ellassoneuria* sp., *Acanthiops tsitsa* and *Aphenicera* sp. *Adenophlebia auriculata*, *Euthraulus* sp., and *Pseudagrion* sp. were designated tolerant while *Caenis* sp., *Enallagma* sp. and *Crenigomphus* sp. were designated highly tolerant to sediment stress (Table 4. 6).

Table 4. 6: EPOT species classification into the vulnerability classes

Highly vulnerable (A)	**Vulnerable (B)**
Tricorythus sp.	*Elassoneuria* sp.
Cheumatosyche sp.	*Acanthiops tsitsa*
Baetis sp.	*Aphenicera* sp.
Pseudocloeon vinosum	*Acanthiops* sp.
Pseudocloeon sp.	*Oligoneuropsis* sp.
Hydropsyche sp.	*Pseudocloeon glaucum*
Phylomacromia sp.	*Prosopsitoma amanzamanya*
Adenophlebia sp.	*Oligoneuropsis lawrencei*
Resilient/tolerant (C)	**Highly resilient/tolerant (D)**
Adenophlebia auriculata	*Caenis* sp.

Euthraulus sp.	*Enallagma* sp.
Pseudagrion sp.	*Crenigomphus* sp.
Trithermis sp.	*Paragomphus* sp.
Aeshna sp.	*Crenigomphus* renei
Pseudocloeon piscis	*Paragomphus genei*
Afronurus bernadi	*Afronurus* sp.
Brachytemis sp.	
Pseudagrion spernatum	

The EPOT species assemblage response was predicted based on the relative abundance of the vulnerability classes and FROC of the individual species. It was predicted that the percent relative abundance of the highly vulnerable (A) and vulnerable (B) classes would decrease at sediment-impacted sites i.e. Site 1 (Tsitsa upstream site), Site 2 (Tsitsa downstream), Sites 7 and 8 (Millstream upstream and downstream sites) and Site 3 (Qurana tributary). The percent relative abundance of the highly vulnerable and vulnerable classes would then recover at the less impacted sites i.e. Site 4 (Pot River upstream site), Site 5 (Pot River downstream site) and Site 6 (Little Pot River).

The percent relative abundance of EPOT species assemblage of the highly vulnerable class was relatively high at all sites, which might be attributed to the abundance of *Baetis* and *Pseudocloeon* genera in the classes at all sites. During the dry season, the percent relative abundance of the vulnerable classes at the Tsitsa sites (upstream and downstream sites) decreased when compared to the tolerant (C) and highly tolerant (D) classes (Figure 4.1a).

At Site 3 (Qurana), during the dry season, the percent relative abundance of the vulnerable (B) classes decreased relative to the tolerant (C) and highly tolerant (D) classes, though the highly tolerant (D) class was relatively low at this site compared to the tolerant class (C) (Figure 4.1a). The percent relative abundance of EPOT species assemblage for all the vulnerability classes at site 3 (Qurana) was generally higher during the dry season than during the wet season.

At Site 4 (Pot River upstream), the highly vulnerable (A) and vulnerable (B) classes decreased relative to the tolerant (C) and the highly tolerant (D) classes. However, the highly vulnerable (A) and vulnerable (B) classes seemed to increase when compared to the highly vulnerable (A) and vulnerable (B) classes at Site 1 (Tsitsa upstream site), Site 2 (Tsitsa downstream site) and

Site 3 (Qurana River) (Figure 4.1a). At Site 5 (Pot River downstream), Site 6 (Little Pot River) and Sites 7 and 8 *(*Millstream upstream and downstream sites*)* during the dry season, the percent relative abundance of the vulnerable class (B) decreased consistently when compared to the tolerant (C) and highly tolerant (D) classes (Figure 4.1b).

During the wet season at Sites 1 and 2 (Tsitsa upstream and downstream sites), percent relative abundance of the vulnerable class decreased when compared to the percent relative abundances of tolerant (C) and highly tolerant (D) classes at these sites (Figure 4.1c). The predictions largely followed the observed data at Sites 1 and 2, except for the highly vulnerable class that was consistently high at all sites.

Similar to the dry season, at Site 3 (Qurana tributary) during the wet season, the percent relative abundance of the vulnerable (B) classes decreased relative to the tolerant (C) and highly tolerant (D) classes, though the highly tolerant (D) class was relatively low at this site compared to the tolerant class (C) (Figure 4.1c). Generally, the percent relative abundances of EPOT species assemblage for all the vulnerability classes during the wet season was lower than that of the dry season at Site 3. At Site 4 (Pot River upstream) during the wet season, the percent relative abundance of the vulnerable class decreased when compared to the percent relative abundance of tolerant and highly tolerant classes during this season.

At Site 5 (Pot River downstream) and Site 6 (Little Pot River) during the wet season, the percent relative abundance of the vulnerable class showed a slight decrease when compared to that of tolerant and highly tolerant classes (Figure 4.1d). However, the percent relative abundance of the vulnerable class was seen to increase when compared to Site 1 (Tsitsa upstream), Site 2 (Tsitsa downstream), Site 3 (Qurana River), Site 7 (Millstream upstream) and Site 8 (Millstream downstream).

Similar to the percent relative abundance at Sites 7 and 8 (Millstream upstream and downstream sites) during the dry season, the percent relative abundance of the vulnerable class decreased when compared to the percent relative abundance of tolerant and highly tolerant classes during the wet season (Figure 4.1d). The prediction made was true at Sites 7 and 8 during both the dry and wet seasons, except for the highly vulnerable class that was high at these sites.

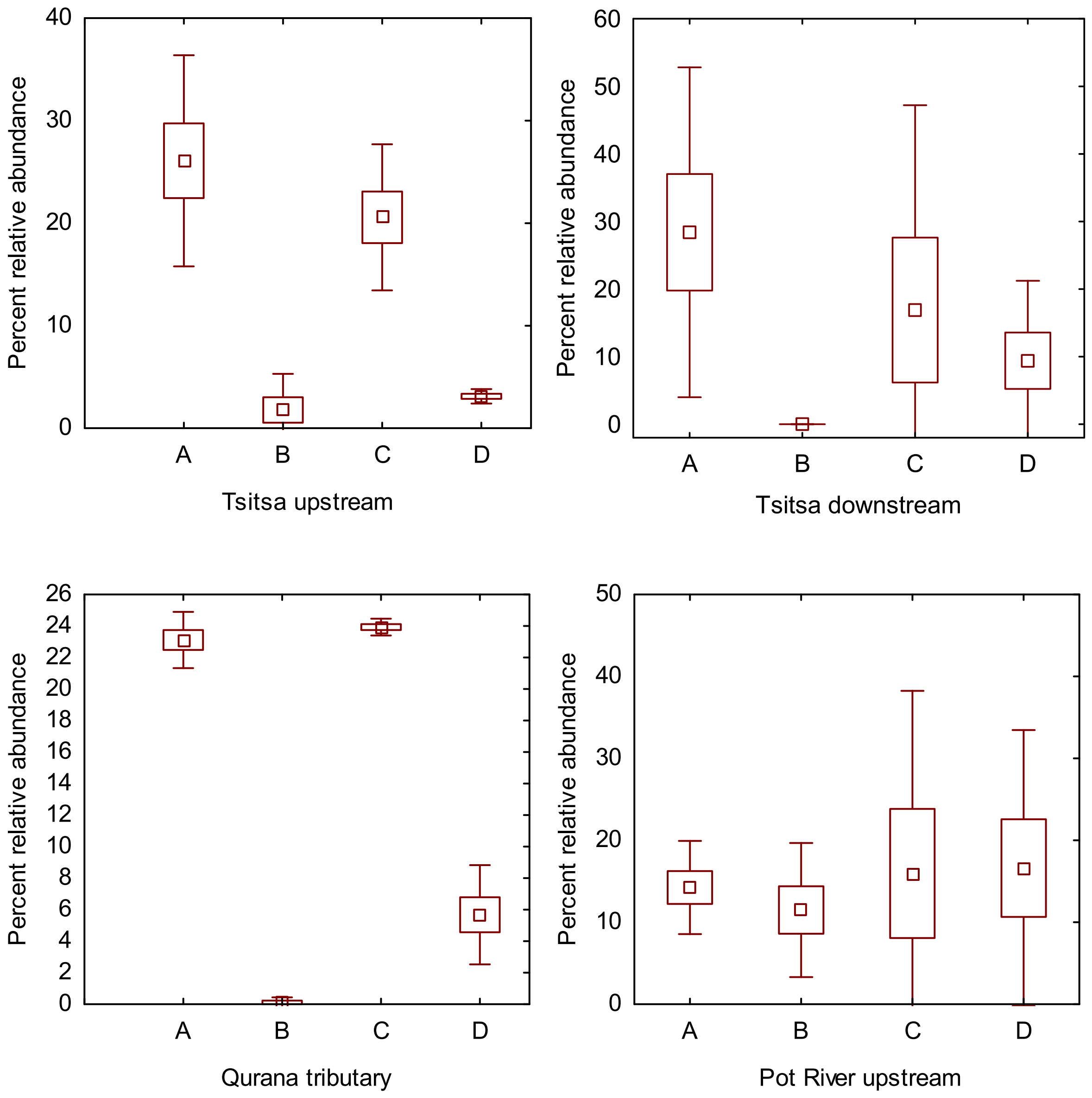

Figure 4 1a: Percent relative abundance of EPOT vulnerability classes at the Tsitsa upstream and downstream sites (Sites 1 and 2), Qurana tributary (Site 3) and Pot River upstream (Site 4) during the dry season. Abbreviation: Classes: A = Highly vulnerable B = Vulnerable, C = Tolerant and D = Highly tolerant.

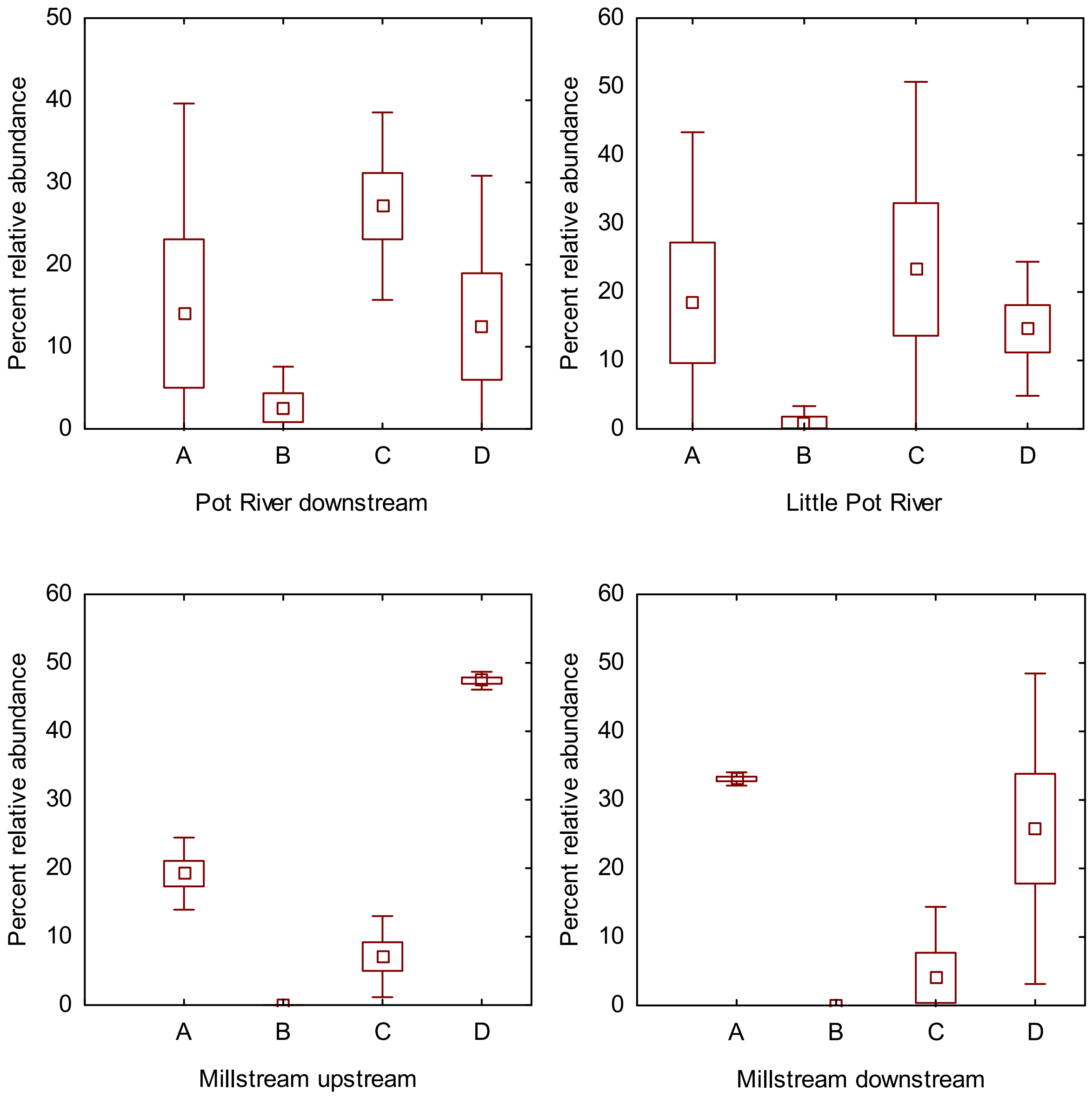

Figure 4 1b: Percent relative abundance of EPOT vulnerability classes at the Pot River downstream (Site 5), Little Pot River (Site 6) and Millstream upstream and downstream sites (Sites 7 and 8) during the dry season. Abbreviation: Classes: A = Highly vulnerable B = Vulnerable, C = Tolerant and D = Highly tolerant.

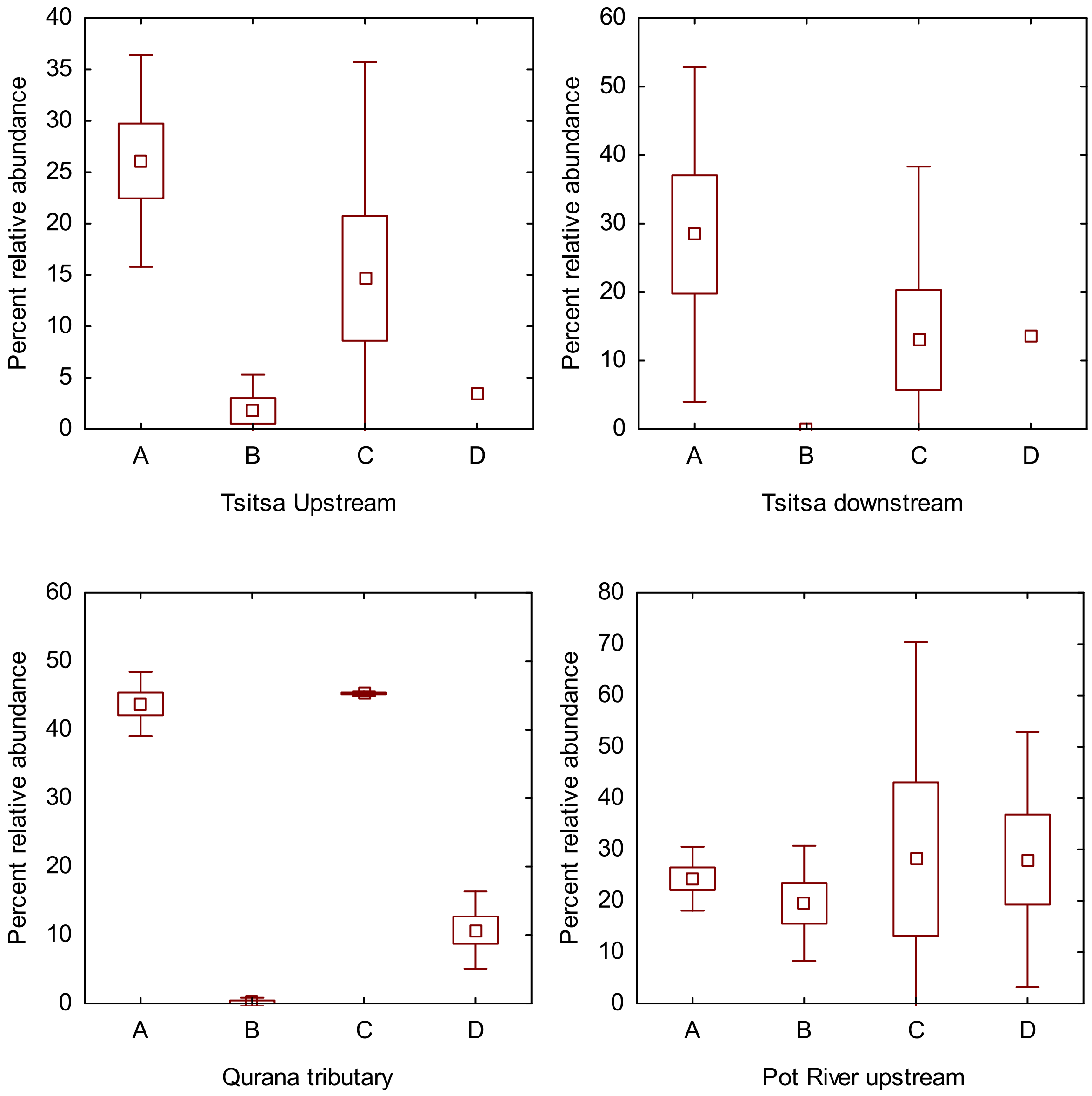

Figure 4 1c: Percent relative abundance of EPOT vulnerability classes at the Tsitsa upstream and downstream sites (Sites 1 and 2), Qurana tributary (Site 3) and Pot River upstream (Site 4) during the wet season. Abbreviation: Classes: A = Highly vulnerable B = Vulnerable, C = Tolerant and D = Highly tolerant.

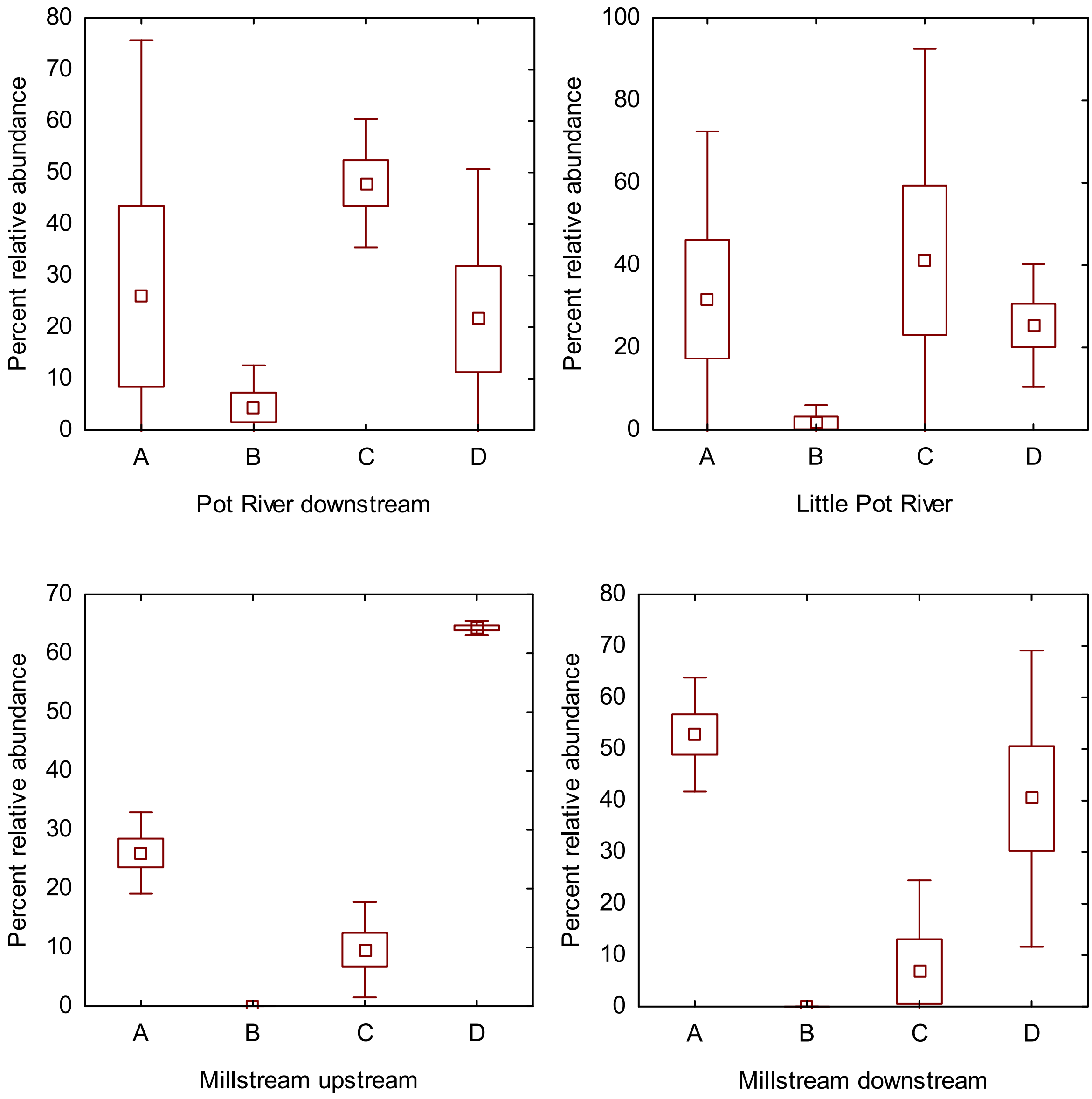

Figure 4 1d: Percent relative abundance of EPOT vulnerability classes at the Pot River downstream (Site 5), Little Pot River (Site 6) and Millstream upstream and downstream sites (Sites 7 and 8) during the wet season. Abbreviation: Classes: A = Highly vulnerable B = Vulnerable, C = Tolerant and D = Highly tolerant.

4.4.2 EPOT assemblage frequency of occurrence – predicted and observed

Predictions were made in terms of the potential frequency of occurrence (FROC) of the EPOT species across the site groups. The rationale is that the FROC of species designated as either highly vulnerable or vulnerable would decrease at site groups 1 and 2 (i.e. more sediment-influenced site groups) compared with site groups 3 and 4 (i.e. less sediment-influenced site groups).

Based on the predictions made, the highest correspondence was at site group 2 with 61.29%, which was followed by site group 1 with 58.06% during the dry season and the lowest observed percent correspondence was 35.7% at site group 3 during the dry season. Site group 4, which was less influenced by sediment stress, was observed to have 50% correspondence between observed and predicted FROC for both the dry and wet seasons (Table 4. 7). The predictions were mainly accurate for the FROC of the highly vulnerable and vulnerable species at site groups 1 and 2, while the tolerant and highly tolerant species had the majority of the incorrect predictions at site groups 3 and 4 during the wet season.

Table 4. 7: Observed and predicted frequency of occurrences for the individual species across the site groups during the dry and wet seasons. Abbreviations: Obs. = Observed, Pred. = Predicted; VC = vulnerability class, A = Highly vulnerable, B = Vulnerable, C = Tolerant and D = Highly tolerant

Species	**Dry season**						**Wet season**								
	Site Group 1		**Site Group 2**		**Site Group 3**		**Site Group 1**		**Site Group 2**		**Site Group 3**		**Site Group 4**		
	Obs.	Pred.	Obs.	Pred.	Obs.	Pred.	Obs.	Pred.	Obs.	Pred.	Obs.	Pred.	Obs.	Pred.	VC
Acanthiops tsitsa	0	0-3	0	0-3	3	3-5	2	0-3	2	0-3	4	3-5	0	3-5	A
Adenophlebia sp.	2	0-3	0	0-3	0	3-5	1	0-3	0	0-3	0	3-5	1	3-5	B
Adenophlebia auriculata	2	0-3	0	0-3	0	3-5	2	0-3	0	0-3	0	3-5	3	3-5	B
Afronurus sp.	2	3-5	2	3-5	3		2	3-5	2	3-5	2		3		D
Afronurus bernardi	0	3-5	0	3-5	0		0	3-5	0	3-5	0		4		D
Baetis sp.	5	0-3	0	0-3	3	3-5	4	0-3	4	0-3	5	3-5	4	3-5	A
Caenis sp.	3	3-5	5	3-5	5		3	3-5	4	3-5	4		5		D

Ellassoneuria sp.	0	0-3	0	0-3	0	3-5	0	0-3	2	0-3	0	3-5	0	3-5	B
Euthraulus sp.	4	3-5	3	3-5	4		3	3-5	2	3-5	2		5		C
Oligoneuropsis sp.	0	0-3	0	0-3	0	3-5	0	0-3	2	0-3	0	3-5	0	3-5	B
Oligoneuropsis lawrencei	0	0-3	0	0-3	0	3-5	0	0-3	2	0-3	1	3-5	0	3-5	V
Pseudocloeon piscis	5	3-5	4	3-5	3		3	3-5	1	3-5	2		4		C
Pseudocloeon glaucum	2	0-3	0	0-3	0	3-5	4	0-3	4	0-3	4	3-5	3	3-5	B
Pseudocloeon sp.	5	0-3	5	0-3	5	3-5	4	0-3	2	0-3	2	3-5	4	3-5	A
Pseudocloeon vinosum	2	0-3	3	0-3	0	3-5	3	0-3	3	0-3	2	3-5	4	3-5	A
Prosopistoma amamzamanya	0	3-5	0	3-5	1		0	3-5	0	3-5	1		0		C
Tricorythus sp.	1	0-3	0	0-3	3	3-5	0	0-3	3	0-3	4	3-5	5	3-5	A
Aphenicera sp.	0	0-3	0	0-3	0	3-5	0	0-3	3	0-3	2	3-5	0	3-5	B
Aeshna sp.	1	3-5	0	3-5	4		1	3-5	2	3-5	2		4		C
Brachytermis sp.	0	3-5	0	3-5	0		1	3-5	2	3-5	0		0		C
Crenigomphus sp.	1	3-5	0	3-5	0		0	3-5	2	3-5	2		0		D

Crenigomphus renei	3	3-5	3	3-5	0		0	3-5	2	3-5	2		4		D
Enallagma sp.	0	3-5	1	3-5	0		0	3-5	0	3-5	0		0		C
Paragomphus genei	3	3-5	0	3-5	3		3	3-5	0	3-5	0		3		D
Paragomphus sp.	0	3-5	0	3-5	0		0	3-5	0	3-5	0		0		D
Phylomacromia sp.	3	3-5	2	3-5	3		3	3-5	0	3-5	2		0		A
Pseudagrion spernatum	3	3-5	3	3-5	1		0	3-5	0	3-5	0		3		C
Pseudagrion sp.	0	3-5	3	3-5	0		0	3-5	4	3-5	2		1		C
Trithermis sp.	1	3-5	0	3-5	0		0	3-5	0	3-5	0		0		D
Cheumatosyche sp.	3	0-3	0	0-3	1	3-5	4	0-3	2	0-3	4	3-5	1	3-5	A
Hydropsyche sp.	5	0-3	2	0-3	4	3-5	3	0-3	5	0-3	4	3-5	5	3-5	A
% Correspondence. Ob and Pred	**58.06**		**61.29**		**35.7**		**48**		**41.9**			**42.9**		**50**	

4.4.3 Comparing statistically-based associations of the EPOT species, and species vulnerability classification, with the site groups

The Pearson's point-biserial correlation analysis was used to assess the association between EPOT species and site groups obtained from the cluster analysis. The result of the analysis revealed that the EPOT species formed eight assemblage types based on their associations with site groups and the combination of site groups (Table 4.8).

Site group 1 had one species i.e. *Paragomphus genei*, and that was significantly associated with this site group. Two species, *Elassoneuria* sp. and *Oligoneuropsis lawrencei,* indicated a none significant association with site group 2. None of the species that indicated statistical associations with site groups 1 and 2 were classified as highly vulnerable (A) or vulnerable (B). The only EPOT species that indicated a strong significant association with these groups was classified as a highly tolerant (D) species.

Site group 3 had eight associatedted species and none indicated a significant association. Site group 4 had nine associated species, of which six species, including *Afronurus Bernard*, *Crenigomphus rennei*, *Aeshna* sp., *Adenophlebia auriculata, Adenophlebia* sp. and *Afronurus* sp., were significantly associated with the site group. Of the six species that indicated a statistical association with site group 4, three were classified into the highly tolerant class (D), two classified as tolerant (C) and one classified as highly vulnerable (A).

Site groups 1 and 3 had four species, which included *Acanthiops tsitsa*, *Cheumatosyche* sp., *Pseudocloeon glaucum*, and *Phylomacromia* sp., indicating a significant association with them except for *pylomacromia sp.* that indicated none significant association. The result also indicated that of these four species, three were classified as highly vulnerable (A) and one vulnerable (B).

Three species were associated with site groups 3 and 4, but only one, *Tricorythus* sp., was significantly associated, and this species was classified as highly vulnerable (A). Site groups 1, 3 and 4, had three species, *Euthraulus* sp., *Baetis* sp., and *Pseudocloeon* sp. indicating an association with them. Of these species, only *Euthraulus* sp. indicated a significant association with the site groups and was classified into the tolerant class (C).

In general, the results revealed that most species classified as highly vulnerable (A) and vulnerable (B) tended to indicate a statistical association with less sediment-influenced site groups 3 and 4. These site groups appeared to accommodate more species than the sediment influenced site groups 1 and 2, and only one species indicated a statistical association with site group 1 (Table 4. 8).

Table 4. 8: Pearson's point-biserial correlation analysis showing EPOT species association with site groups and their vulnerability class during the study period (August 2016 – April 2017). Site group 1 (Sites 1 and 3), Site group 2 (Sites 2 and 7), Site group 3 (Sites 4, 5 and 8), Site group 4 (Site 6). Boldface indicates significant associations at $P < 0.05$. Abbreviation: VC = vulnerability classes. Code represents site group preference code, which indicates species preferring site group 1 (1), site group 2 (2), site group 3 (3), site group 4 (4), both site groups 1 and 3 (5), site groups 2, 3, and 4 (6), both site groups 3 and 4 (7), and site groups 1, 3 and 4 (8).

Taxa	**Site group 1**	**Site group 2**	**Site group 3**	**Site group 4**	**Code**	**Point-biserial Coefficient**	***P*-value**	**VC**
Paragomphus genei	1	0	0	0	1	0.338	**0.017**	D
Ellassoneuria sp.	0	1	0	0	2	0.259	0.145	B
Oligoneuropsis sp.	0	1	0	0	2	0.226	0.246	B
Crenigomphus sp.	0	0	1	0	3	0.266	0.071	D
Hydropsyche sp.	0	0	1	0	3	0.227	0.207	A
Oligoneuropsis lawrencei	0	0	1	0	3	0.211	0.258	B
Brachytermis sp.	0	0	1	0	3	0.209	0.276	C
Enallagma sp.	0	0	1	0	3	0.2	0.297	D
Trithermis sp.	0	0	1	0	3	0.192	0.381	C
Paragomphus sp.	0	0	1	0	3	0.171	0.666	D
Prosopistoma amamzamanya	0	0	1	0	3	0.169	0.703	B
Afronurus bernardi	0	0	0	1	4	0.435	**0.001**	C
Crenigomphus rennei	0	0	0	1	4	0.352	**0.001**	D
Aeshna sp.	0	0	0	1	4	0.338	**0.017**	C

Adenophlebia auriculata	0	0	0	1	4	0.336	**0.018**	C
Adenophlebia sp.	0	0	0	1	4	0.3	**0.012**	A
Afronurus sp.	0	0	0	1	4	0.299	**0.033**	D
Pseudocloeon piscis	0	0	0	1	4	0.285	0.054	C
Pseudagrion spernatum	0	0	0	1	4	0.284	0.051	C
Pseudocloeon vinosum	0	0	0	1	4	0.102	0.841	A
Acanthiops tsitsa	1	0	1	0	5	0.417	**0.001**	A
Cheumatosyche sp.	1	0	1	0	5	0.318	**0.03**	A
Pseudocloeon glaucum	1	0	1	0	5	0.295	**0.047**	B
Phylomacromia sp.	1	0	1	0	5	0.261	**0.084**	A
Pseudagrion sp.	0	1	1	1	6	0.349	**0.016**	C
Aphenicera sp.	0	1	1	1	6	0.28	**0.048**	B
Tricorythus sp.	0	0	1	1	7	0.468	**0.001**	A
Acanthiops sp.	0	0	1	1	7	0.274	0.057	B
Caenis sp.	0	0	1	1	7	0.192	0.353	D
Euthraulus sp.	1	0	1	1	8	0.323	**0.023**	C
Baetis sp.	1	0	1	1	8	0.184	0.427	A
Pseudocloeon sp.	1	0	1	1	8	0.116	0.776	A

4.4.4 Correlating trait attributes, sites, species and sediment characteristics, including turbidity, using the RLQ analysis during dry season

For settled sediments, during the dry season, the first two axes of the RLQ analysis explained 78.87% cumulative variance. The first axis with eigenvalue 0.51 explained 41.15% variance and the second axis with eigenvalue 0.46 explained 37.72% variance between settled sediments, species and trait attributes**.** The Monte-Carlo test was undertaken to evaluate the significance of the association between trait attributes and settled sediments during the dry season and revealed that there was a significant association between traits and settled sediment characteristics ($P < 0.05$).

Because the RLQ analysis represented the partial ordination of the environmental characteristics (i) the species abundances, and (ii) the species traits, the proportions of the variance contributed by each matrix in the final RLQ analysis were compared to those resulting from their separate analyses. The first RLQ axis with an eigenvalue of 0.51, covariance of 0.71 and correlation of 0.24 accounted for 92.5% variance in the R settled sediment dataset analysis. For example, the R (92.5%) variance is calculated by dividing the inertia contributed by the R separate analysis to the combined RLQ analysis with the eigenvalue of the R separate analysis (i.e. 4.3268/4.6756 = 0.9254). The first axis similarly accounted for 48.87% variance in the L species dataset analysis and 28.68% variance in the Q trait dataset analysis. Similarly, the second axis with an eigenvalue of 0.711 accounted for 97.04% in the separate analysis of the R settled sediment dataset, 37.71% in the separate analysis if the L species dataset and 61.20% in the separate analysis of the Q trait dataset (Table 4.9). The result showed that the separate R analysis for the settled sediment dataset contributed the highest to the variance in the first and second axes of the combined analysis.

The ordination result of the combined RLQ analysis showed that during the dry season, the proportion of clay, very fine silt, medium silt and coarse silt were clustered together and were positively associated with site group 1 (Figure 4.2). These proportions of settled sediment particles largely represented the proportion of finest sediments and were positively associated with traits such as shredding, large body size, crawling and substrate attachment. These proportions of settled sediments i.e. clay, very fine silt, medium silt and coarse silt also indicated a negative association with sediment-sensitive traits such as filter feeding, grazing/scraping and filamentous gill. Species that were closely associated with these particle size classes included both the sediment-sensitive and tolerant species such as *Cheumatosyche* sp., *Aphenicera* sp., *Trithermis* sp. and *Crenigomphus* sp.

The proportions of very coarse silt, coarse sand and sand were clustered and associated with the less sediment-influenced site groups 3 and 4. These proportions of settled particles classes were positively associated with traits such as filamentous gill, stone biotope, grazing and scraping, and detritus (CPOM). Species such as *Trichorythus* sp., *Afronurus* sp., *Euthraulus* sp., *Ellassoneuria* sp. and *Caenis* sp. were positively associated with these proportions of settled sediments. Finally, the result also showed that fine silt was positively associated with site group 3 and favoured species such as *Enallagma* sp., *Paragomphus* sp., *Paragomphus genei*, *Crenigomphus* sp., *Prosopistoma amanzmanya* and *Pseudagrion* sp.

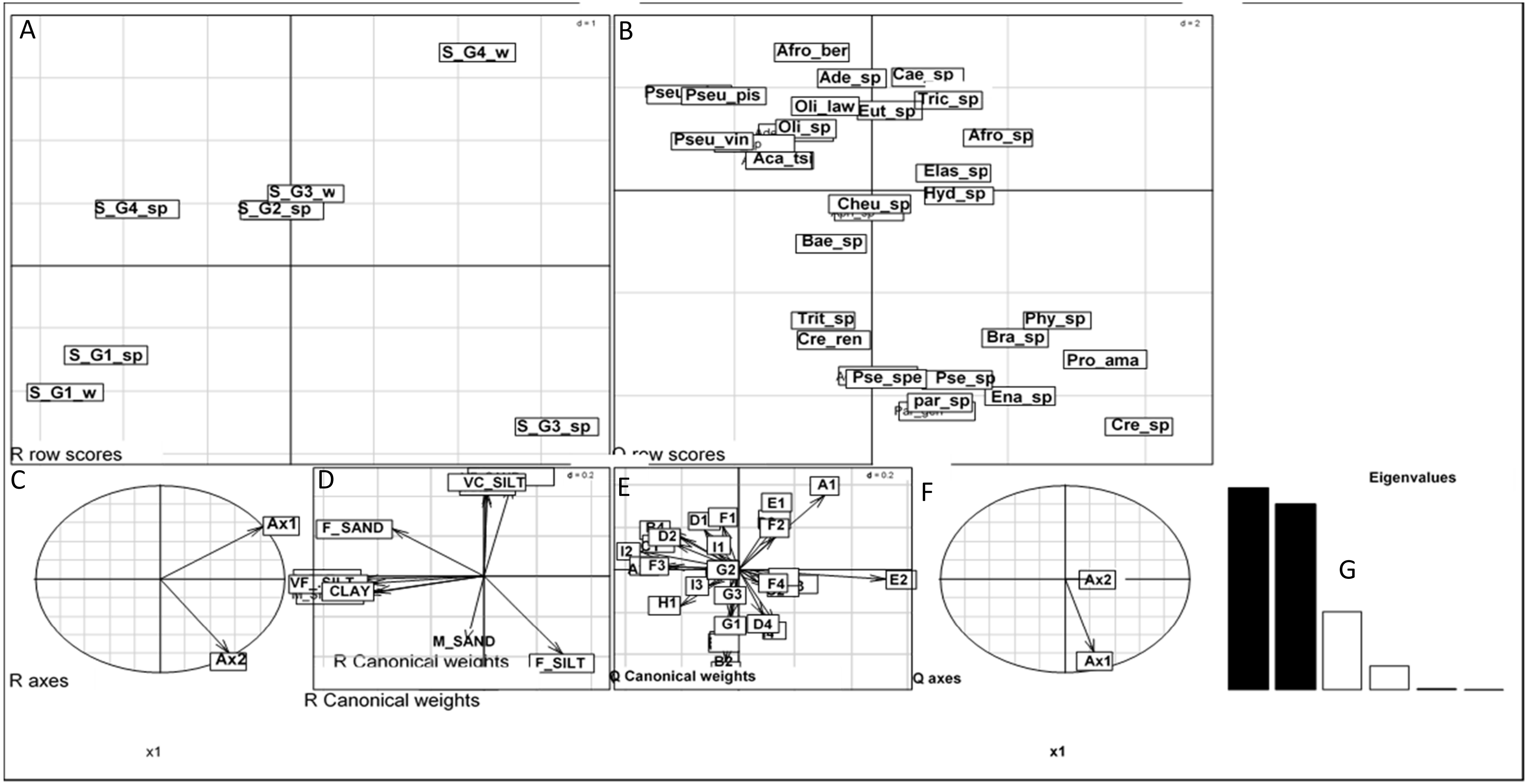

Figure 4 2: RLQ ordination diagram of the first two axes showing the results of the association of the settled sediment particles, species and trait attributes during the dry season (August 2016 – April 2017) in the Tsitsa River and its tributaries. A shows the weight of the sampling sites, B weights of species, C is the principal RLQ axes of settled sediments particles, D shows the correlations of settled particles with RLQ axes, E shows the correlations of the traits attributes with RLQ 1 and 2, F shows the principal RLQ axes of trait attributes and G is a histogram of eigenvalues. Abbreviations: Trait attributes: filamentous gill (A1), plate-like gill (A2), lamellate gill (A3), operculate gill (A3), burrowers (B1), crawlers (B2), sprawlers (B3), clingers/climbers (B4), swimmers (B4), soft-bodied and exposed (C1), sclerotized (C2), cased/tubed (C3), detritus (FPOM) (D1), detritus (CPOM) (D2), macrophytes/Algae (D3), animal materials (D4), stone (E1), sediments (gravel, sand, and mud) (E2), vegetation (E3), filter feeders (F1), grazers/scrapers (F2), shredders (F3), predators (F4), temporarily attached (G1), permanently Attached (G2), free-living (G3), exophytic (H1), endophytic (H2), <5 mm (I1), >5 - 10 mm (I2), >10 - 20 mm (I3), >20 mm (I4).

The RLQ analysis result that was undertaken to assess the association between species, traits and suspended sediment characteristics including turbidity during the dry season revealed that the first two axes explained 89.33% cumulative variance. The first axis with an eigenvalue 1.22 accounted for 63.44% variance and the second axis with eigenvalue 1.11 accounted for 25% variance (Table 4.9). The Monte-Carlo test revealed no significant association between trait attributes and suspended sediment characteristics including turbidity ($P > 0.05$).

The RLQ analysis was compared to the result of the separate analysis showed that the first axis with eigenvalue of 1.22, correlation of 0.23 accounted for 92.70% of the variance in the separate analysis of the R dataset, 46.97% variance in the separate analysis of the L dataset and 66.46% variance in the separate analysis of the Q dataset. Similarly, the second axis accounted for 98.51%, 52.94% and 63.38% variance in the separate analysis of the R, L and Q datasets respectively (Table 4.9)

The ordination plots showed that the proportions of clay, very fine silt, fine silt and medium silt were associated with site groups 1 and 3 and favoured the positive association of most mayfly species such as *Adenophlebia auriculata*, *Afronurus* sp., *Acanthiops tsitsa* and *Baetis* sp. (Figure 4.3). Trait attributes such as the burrowing, temporarily attached, detritus (CPOM) and plate-like gill were positively associated with these sediment particles.

The proportion of fine sand, medium sand and coarse sand representing the larger particles were positively associated with site group 1 and trait attributes such as lamellate gill, exophytic oviposition and large body-sized traits (>10 – 10 and >20 mm). Species that were favoured by these proportions of sands include most Odonate species including *Crenigomphus* sp., *Enallagma* sp., *Paragomphus* sp., *Trithermis* sp. and *Aeshna* sp. The proportion of medium silt and coarse silt were positively associated with site group 4 and traits such as small body-sized (<5 mm) species, filamentous gill, detritus (FPOM) and macrophytes/algae feeding species. Corresponding species that indicated a strong positive association with these proportions of suspended sediments include *Oligoneuropsis lawrencei*, *Ellassoneuria* sp. and *Euthraulus* sp. (Figure 4.3).

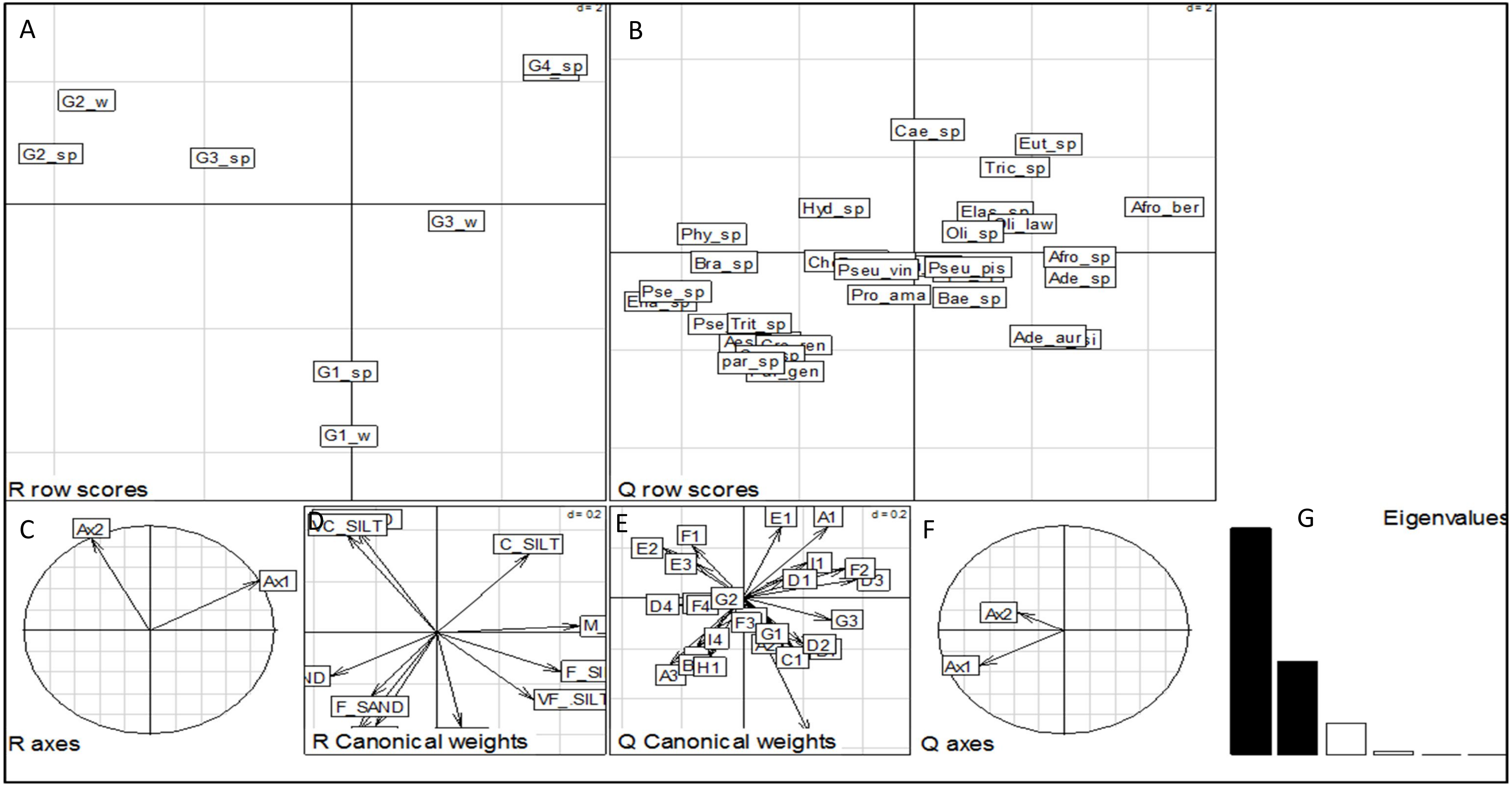

Figure 4 3: RLQ ordination diagram of the first two axes showing the results of the association of the **suspended** sediment particles and turbidity, species and trait attributes during the dry season (August 2016 – April 2017) in the Tsitsa River and its tributaries. A shows the weight of the sampling sites, B weights of species, C is the principal RLQ 1 and 2 of suspended sediments particles and turbidity, D shows the correlations of suspended particles and turbidity with RLQ axes, E shows the correlations of the traits attributes with RLQ axes, F shows the principal RLQ axes of trait attributes and G is a histogram of eigenvalues. Abbreviations: Trait attributes: filamentous gill (A1), plate-like gill (A2), lamellate gill (A3), operculate gill (A3), burrowers (B1), crawlers (B2), sprawlers (B3), clingers/climbers (B4), swimmers (B5), soft-bodied and exposed (C1), sclerotized (C2), cased/tubed (C3), detritus (FPOM) (D1), detritus (CPOM) (D2), macrophytes/Algae (D3), animal materials (D4), stone (E1), sediments (gravel, sand, and mud) (E2), vegetation (E3), filter feeders (F1), grazers/scrapers (F2), shredders (F3), predators (F4), temporarily attached (G1), permanently Attached (G2), free-living (G3), exophytic (H1), endophytic (H2), <5 mm (I1), >5 - 10 mm (I2), >10 - 20 mm (I3), >20 mm (I4).

Table 4.9: Properties of the multivariate RLQ analysis of the correlation of traits, EPOT species, settled sediments and suspended sediments characteristics including turbidity, accounted for by the first two axes in the Tsitsa River and its tributaries during the dry season of the study period (August 2016 – April 2017)

RLQ properties	**Dry season**			
	Settled sediments		**Suspended sediments**	
	Axis 1	Axis 2	Axis 1	Axis 2
Separate independent analysis				
Variance (R/CA) %	46.76	37.41	47.65	36.70
Eigenvalue	4.68	3.74	5.25	4.04
Variance (L/H-SM) %	30.39	22.29	30.39	22.29
Eigenvalue	0.25	0.18	0.25	0.18
Variance (Q/PCA) %	25.63	14.61	25.63	14.61
Eigenvalue	6.92	3.94	6.92	3.94
Combined RLQ analysis				
Variance (RLQ) %	41.15	37.71	63.44	25.89
Eigenvalue	0.51	0.46	1.22	0.499
Covariance	0.71	0.68	1.11	0.71
Correlation	0.24	0.16	0.23	0.23
Variance (R/RLQ) %	92.54	97.03	92.70	98.51
Variance (L/RLQ) %	48.72	37.71	46.98	52.94
Variance (Q/RLQ) %	28.68	61.20	66.46	63.38

4.4.5 Correlating trait attributes, sites, species and sediment characteristics including turbidity using the RLQ analysis during the wet season

The result of the RLQ analysis on species, trait attributes and settled sediments during the wet season revealed that the first two axes explained 98.47% cumulative variance of the dataset. The first axis with eigenvalue 1.36 accounted for 91.51% variance and the second axis accounted for 6.95% of the dataset (Table 4.10). The Monte-Carlo test revealed that there was a significant difference in the associations between the traits and settled sediments during the wet season ($P < 0.05$).

When the combined RLQ analysis was compared to the separate analysis of the species, traits and settled sediment dataset, the result showed that the first RLQ axis with an eigenvalue of 1.36, covariance of 1.17 and correlation of 0.214 accounted for 92.33% variance in the R (settled sediment dataset) analysis, 43.04% variance in the L (species dataset) and 6% variance in the Q (trait dataset) analysis (Table 4.10). Because the first axis of the RLQ analysis explained most of the variation in the analysis, the second axis will not be discussed further.

The ordination plot revealed that the proportion of very fine sand, fine sand and coarse sand were positively associated with site group 4 during summer. These proportions of sediments favoured the positive association of trait attributes such as soft-bodied and exposed, grazing and scraping, burrowers and temporarily attached. These traits were represented by species such as *Adenophlebia auriculata*, *Trichorythus* sp. and *Caenis* sp. The proportions of clay, very fine silt, fine silt, medium silt and coarse silt were associated with site groups 1, 2 and 4 with associated trait attributes such as filter feeding, crawling and lamellate gill. These particles favoured species such as *Cheumatosyche* sp., *Pseudocloeon* sp. and *Pseudocloeon vinosum* (Figure 4.4).

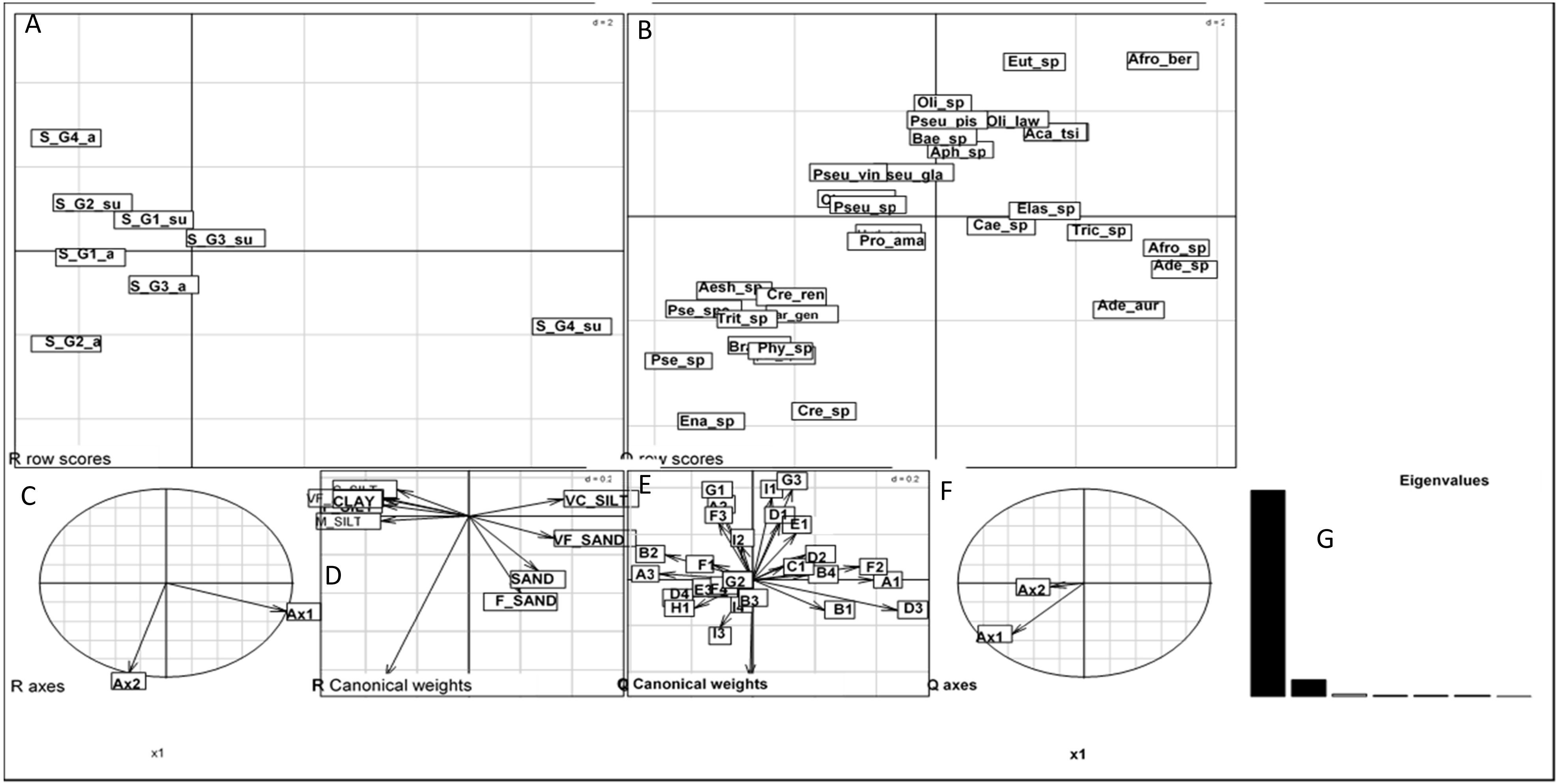

Figure 4 4: RLQ ordination diagram of the first two axes showing results of the association of the **settled** sediment particles, species and trait attributes during the wet season (August 2016 – April 2017) in the Tsitsa River and its tributaries. A shows the weight of the sampling sites, B the weights of species, C the principal RLQ 1 and 2 of settled sediments particles, D the correlations of settled particles with RLQ axes, E the correlations of the traits attributes with RLQ axes, F the principal RLQ axes of trait attributes and G is a histogram of eigenvalues. Abbreviations: Trait attributes: filamentous gill (A1), plate-like gill (A2), lamellate gill (A3), operculate gill (A3), burrowers (B1), crawlers (B2), sprawlers (B3), clingers/climbers (B4), swimmers (B4), soft-bodied and exposed (C1), sclerotized (C2), cased/tubed (C3), detritus (FPOM) (D1), detritus (CPOM) (D2), macrophytes/Algae (D3), animal materials (D4), stone (E1), sediments (gravel, sand, and mud) (E2), vegetation (E3), filter feeders (F1), grazers/scrapers (F2), shredders (F3), predators (F4), temporarily attached (G1), permanently Attached (G2), free-living (G3), exophytic (H1), endophytic (H2), <5 mm (I1), >5 - 10 mm (I2), >10 - 20 mm (I3), >20 mm (I4).

The RLQ analysis result of the test that was undertaken on species, trait attributes and suspended sediment including turbidity during the wet season, revealed that the first two axes explained 86.30% cumulative variance of the dataset. The first axis with eigenvalue 0.39 accounted for 64.99% variance and the second axis with eigenvalue 0.13 accounted for 21.31% variance in the dataset (Table 4. 10).

When the combined RLQ analysis was compared to the separate analyses of R, L and Q datasets, the result showed that the first axis with eigenvalue 0.39, covariance 0.63 and correlation of 0.21 accounted for 92.11%, 50% and 22.63% variance in the first axis of the separate R, L and Q datasets. Similarly, the second axis of the combined RLQ analysis accounted for 96.69%, 38.14% and 33.01% variance in the second axis of the separate R, L and Q datasets (Table 4. 10). The Monte-Carlo test of significance revealed a significant association between trait attributes and suspended sediments during the wet season ($P < 0.05$).

The ordination plot revealed that site group 1 during summer and autumn was clustered and showed a positive association with the proportions of very fine silt, fine silt and medium silt including turbidity. These proportions of suspended sediments were positively correlated with clinging and climbing, animal material feeders, sprawlers and large body (>20 mm). Species that indicated a positive association with these proportions of sediments included *Phylomacromia* sp., *Paragomphus* sp., *Crenigomphus* sp., and *Enallagma* sp. (Figure 4.2d). The proportion of very fine sand was associated with site group 4 and favoured the association of species such as *Caenis* sp., *Pseudocloeon glaucum* and *Oligoneuropsis lawrencei*. These species were represented by trait attributes such as the stone biotope, filamentous gill and exophytic oviposition. Trait attributes such as filter feeding, soft-bodied and exposed, and temporarily attached, were negatively correlated with most of the particles such as fine sand, medium sand and coarse sand except the proportions fine sand, medium sand and turbidity with strong positive association. Species such as *Hydropsyche* sp., *Cheumatosyche* sp. and *Aphenicera* sp. were representative species that were associated with the proportions of fine sand, medium sand, and coarse sand (Figure 4.5).

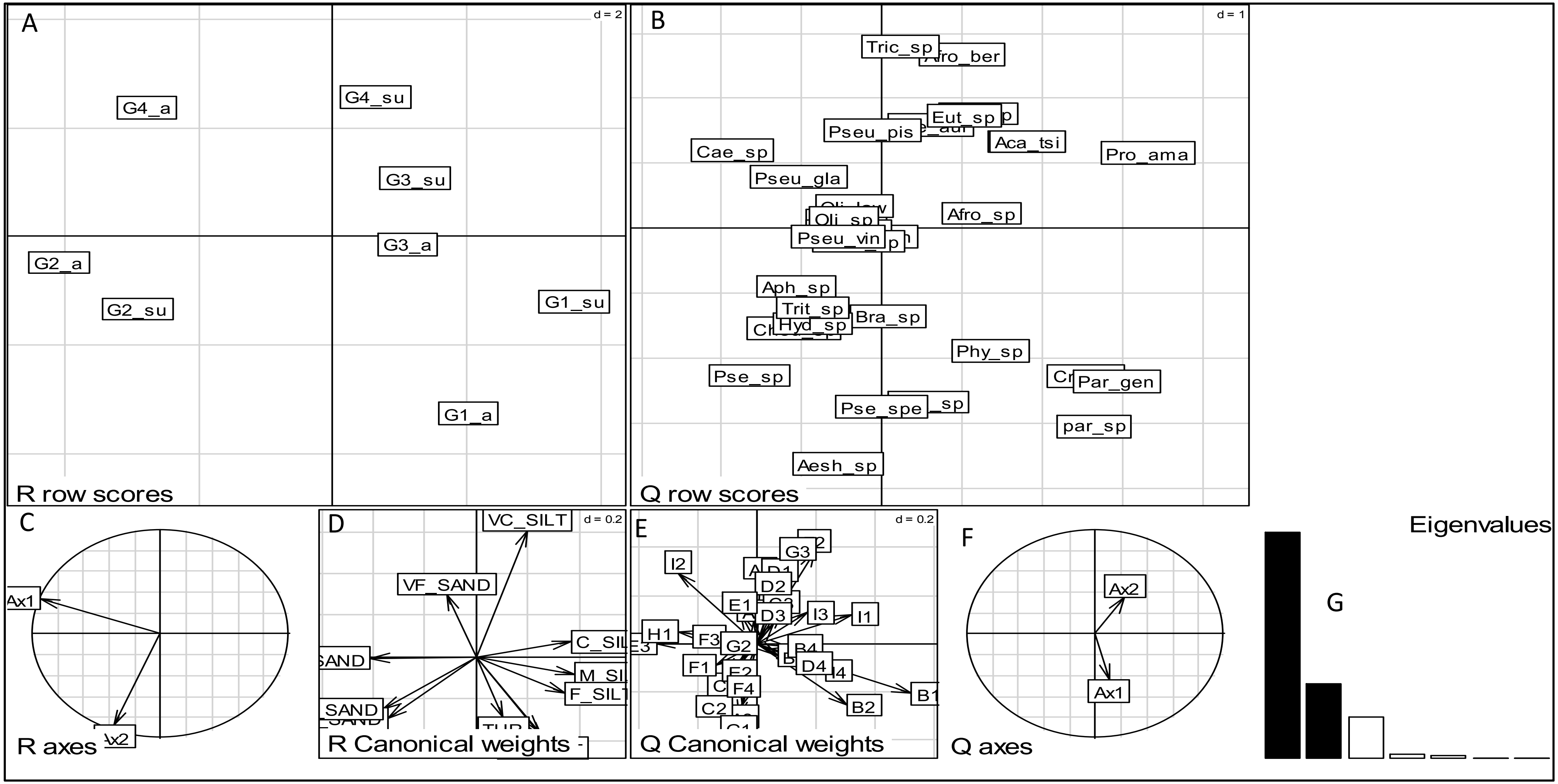

Figure 4 5: RLQ ordination diagram of the first two axes showing results of the association of the **suspended** sediment particles and turbidity, species and trait attributes during the wet season (August 2016 – April 2017) in the Tsitsa River and its tributaries. A shows the weight of the sampling sites, B the weights of species, C is the principal RLQ 1 and 2 of suspended sediments particles including turbidity, D shows the correlations of suspended particles and turbidity with RLQ axes, E shows the correlations of the traits attributes with RLQ axes, F shows the principal RLQ axes of trait attributes and G is a histogram of eigenvalues. Abbreviations: Trait attributes: filamentous gill (A1), plate-like gill (A2), lamellate gill (A3), operculate gill (A3), burrowers (B1), crawlers (B2), sprawlers (B3), clingers/climbers (B3), swimmers (B4), soft-bodied and exposed (C1), sclerotized (C2), cased/tubed (C4), detritus (FPOM) (D1), detritus (CPOM) (D2), macrophytes/Algae (D3), animal materials (D4), stone (E1), sediments (gravel, sand, and mud) (E2), vegetation (E3), filter feeders (F1), grazers/scrapers (F2), shredders (F3), predators (F4), temporarily attached (G1), permanently Attached (G2), free-living (G3), exophytic (H1), endophytic (H2), <5 mm (I1), >5 - 10 mm (I2), >10 - 20 mm (I3), >20 mm (I4).

Table 4. 10: Properties of the multivariate RLQ analysis of the correlation of traits, EPOT species, settled sediments and suspended sediments characteristics including turbidity accounted for by the first two axes, in the Tsitsa River and its tributaries during the wet season of the study period (August 2016 – April 2017).

RLQ properties	**Wet season**			
	Settled sediments		**Suspended sediments**	
	Axis 1	Axis 2	Axis 1	Axis 2
Separate independent analysis				
Variance (R/CA) %	83	13.2	64.99	21.31
Eigenvalue	8.3	1.32	6.6	2.84
Variance (L/H-SM) %	30	22.3	27.38	23.2
Eigenvalue	0.3	0.18	0.18	0.16
Variance (Q/PCA) %	26	40.2	22.19	14.3
Eigenvalue	6.9	.94	6.21	4
Combined RLQ analysis				
Variance (RLQ) %	92	6.85	64.99	21.31
Eigenvalue	1.4	0.1	0.39	0.13
Covariance	1.2	0.32	0.62	0.36
Correlation	0.2	0.14	0.21	0.13
Variance (R/RLQ) %	92	99.7	92.11	96.69
Variance (L/RLQ) %	43	31.8	50	33.01
Variance (Q/RLQ) %	56	62.7	22.63	38.14

4.4.6 The correlation of individual traits category to environmental variables

The RLQ provides a global picture of the traits-environment relationships but the Fourth-corner analysis can be used to test the significance of individual trait-environment associations. The Fourth-corner analysis provides a means of testing the association between the individual trait attributes and environmental variables through the link provided by the species abundance.

The Fourth-corner test undertaken to assess the significance of the association between the individual trait attributes and settled sediments characteristics indicated a total of nine significant associations. Of the nine significant associations, lamellate gill indicated three significant negative associations with the proportion of coarse sand, fine sand and very fine sand and four significant positive associations with the proportions of coarse silt, medium silt, very fine silt and clay. Filamentous gill indicated significant negative associations with the proportions of medium silt and very coarse silt (Figure 4.6).

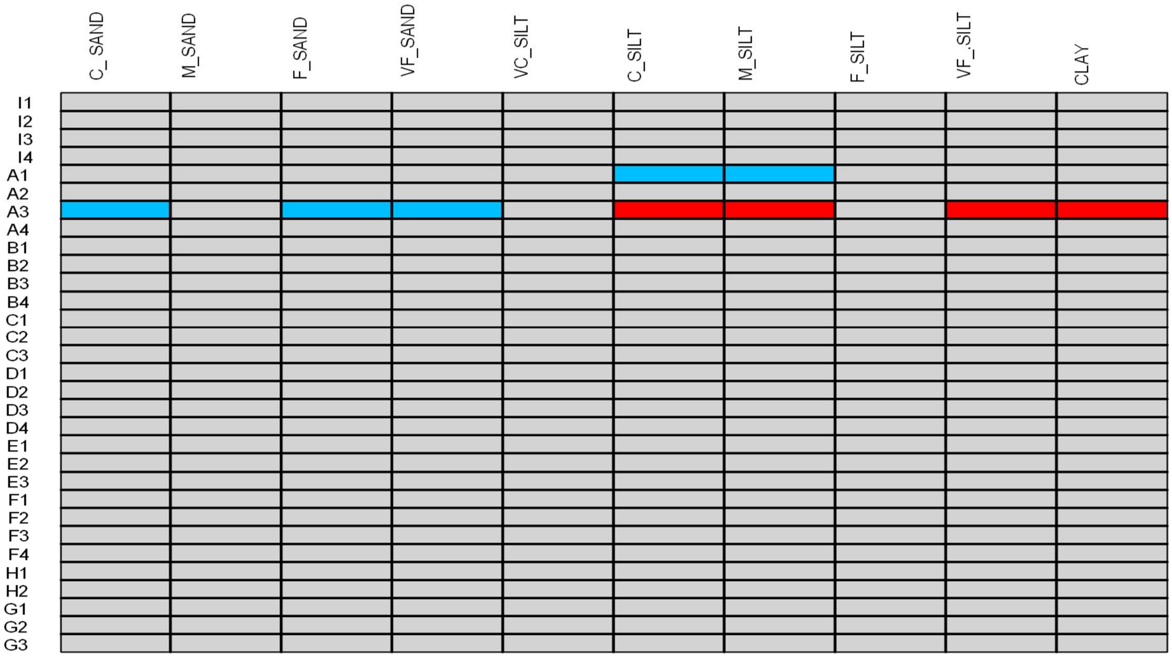

Figure 4 6: Results of the Fourth-corner analysis performed using taxon abundance, trait attributes and settled sediment classes during the study period (August 2016 – April 2017) in the Tsitsa River and its tributaries. Significant positive associations are represented by red cells, and significant negative associations correspond to the blue cells. Non-significant associations are in grey. The settled sediment particles are along the horizontal axis and the trait attributes on the vertical axis. Abbreviations: Trait attributes: filamentous gill (A1), plate-like gill (A2), lamellate gill (A3), operculate gill (A3), burrowers (B1), crawlers (B2), sprawlers (B3), clingers/climbers (B3), swimmers (B4), soft-bodied and exposed (C1), sclerotized (C2), cased/tubed (C4), detritus (FPOM) (D1), detritus (CPOM) (D2), macrophytes/Algae (D3), animal materials (D4), stone (E1), sediments (gravel, sand, and mud) (E2), vegetation (E3), filter feeders (F1), grazers/scrapers (F2), shredders (F3), predators (F4), temporarily attached (G1), permanently Attached (G2), free-living (G3), exophytic (H1), endophytic (H2), <5 mm (I1), >5 - 10 mm (I2), >10 - 20 mm (I3), >20 mm (I4).

The Fourth-corner test between the individual trait categories and suspended sediment particles detected nine significant associations ($P < 0.05$). Burrowers were the most significantly associated trait attribute with four positive associations and three negative associations. Burrowers were seen to be positively associated with the proportion of suspended particles such as clay, very fine silt, fine silt and medium silt and negatively associated with proportions of larger particles including very fine sand, fine sand and coarse sand (Figure 4.3b). The small body size (<5 mm) was the only trait attribute that was positively associated with turbidity, indicating a positive significant association (Figure 4.7).

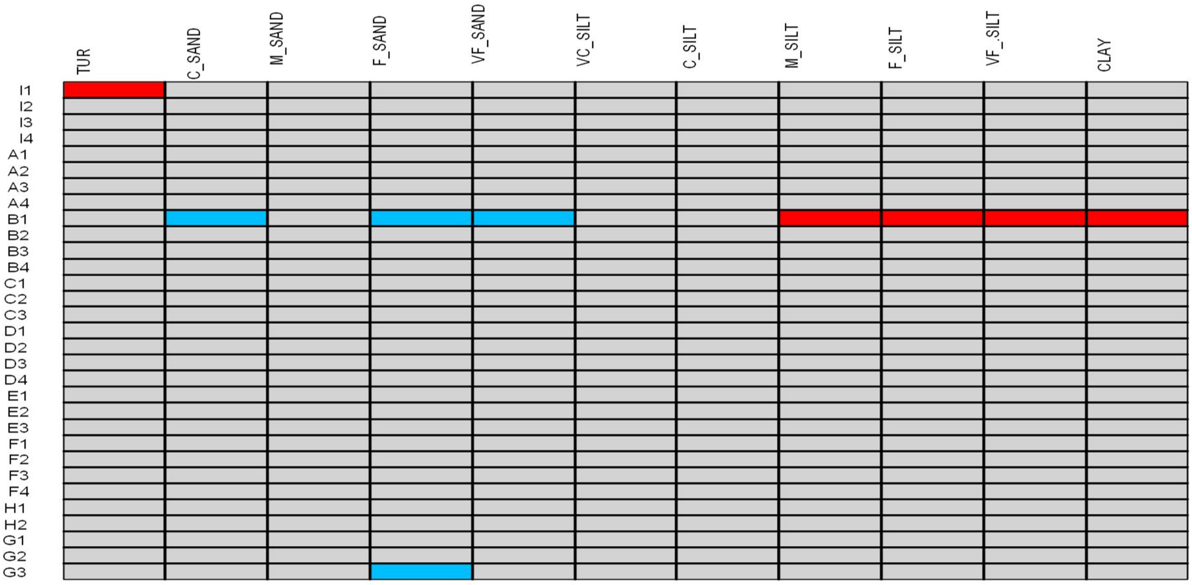

Figure 4 7: Results of the Fourth-corner analysis performed using taxon abundance, trait attributes and suspended sediment particle classes including turbidity during the study period (August 2016 – April 2017) in the Tsitsa River and its tributaries. Significant positive associations are represented by red cells, and significant negative associations correspond to the blue cells. Non-significant associations are in grey. The settled sediment particles are along the horizontal axis and the trait attributes on the vertical axis. Abbreviations: Trait attributes: Trait attributes: filamentous gill (A1), plate-like gill (A2), lamellate gill (A3), operculate gill (A3), burrowers (B1), crawlers (B2), sprawlers (B3), clingers/climbers (B3), swimmers (B4), soft-bodied and exposed (C1), sclerotized (C2), cased/tubed (C4), detritus (FPOM) (D1), detritus (CPOM) (D2), macrophytes/Algae (D3), animal materials (D4), stone (E1), sediments (gravel, sand, and mud) (E2), vegetation (E3), filter feeders (F1), grazers/scrapers (F2), shredders (F3), predators (F4), temporarily attached (G1), permanently Attached (G2), free-living (G3), exophytic (H1), endophytic (H2), <5 mm (I1), >5 - 10 mm (I2), >10 - 20 mm (I3), >20 mm (I4).

4.5 Discussion

4.5.1 The trait-based approach as a diagnostic and predictive tool for assessing species response to in-stream sediment stress

Traits play an important role in species-environment interactions, and hence help to determine the organism's adaptive potential in a specific environmental context. Because species' response to environmental pressures is context-specific, traits can also help to determine the potential vulnerability of species to a given stressor. In this study, a novel trait-based approach was developed, which allowed for the classification of the EPOT species into potential vulnerability classes based on the combination of trait attributes possessed. The study further evaluated the analysis of individual trait response to sediment stress.

The trait-based approach was useful in explaining the vulnerability of the EPOT species; it enabled the prediction of species' responses to sediment stress. For example, the result of the trait-based analysis showed that species possessing vulnerable trait attributes such as filter feeders, soft-bodied and filamentous gills were mostly grouped into the highly vulnerable and vulnerable classes and were associated mostly with site groups that were highly sediment-influenced. Based on the prediction made for the EPOT species that the percent relative abundance of the highly vulnerable and vulnerable classes would decrease in sediment-impacted sites, the results were largely in congruence with the predictions, where the highly vulnerable class decreased in percent relative abundance in comparison to the tolerant and highly tolerant classes at the sediment-influenced sites. The result also revealed that those species in the highly vulnerable and vulnerable classes were largely associated with the less sediment-influenced Site 4 (Pot River upstream), Site 5 (Pot River downstream) and Site 6 (Little Pot River), where they were observed to increase in percent relative abundance compared to the sediment-influenced Site 1 (Tsitsa upstream), Site 2 (Tsitsa downstream) and Site 3 (Qurana tributary). However, an unanticipated finding was that the percent relative abundance of the highly vulnerable class was observed to be consistently high at all sites for two seasons. A plausible explanation for this might be the presence of *Baetis* and *Pseudocloeon* genera that were observed to be high at almost all the sites. Another possible explanation might be provided by the short period of data collection, which was undertaken over only four seasons.

In terms of the frequency of occurrence (FROC) prediction, there was a correspondence of more than 50% between the observed and predicted data at sediment-influenced site groups 1 and 2. These predictions were mainly accurate for the highly vulnerable and vulnerable classes indicating that the FROCs of the majority of species classified under them were accurate. Furthermore, the Pearson's point-biserial correlation analysis result indicated that most of the species classified as being either highly vulnerable or vulnerable to sediment stress, constituted the majority of taxa that were statistically associated with the less sediment-impacted site groups 3 and 4. The correspondence between the statistically-based association and the prediction made based on the approach developed in this study gives credence to the species classification into vulnerability groups. None of the species classified as highly vulnerable or vulnerable were associated with the highly sediment-influenced site groups 1 and 2, except for *Hydropsyche* sp., *Ellassoneuria* sp. and *Oligoneuropsis* sp., which in fact did not indicate a statistically significant association. These results are in accordance with other studies which have indicated that modified habitats may show a shift in the structure of traits, as those taxa with sensitive traits are filtered out (Buendia *et al*., 2013; Feio & Dolédec, 2012).

The trait-based tool/approach used in this study provided a mechanistic and adaptive basis for explaining the potential vulnerability of species to a given environmental stressor. Thus, the TBA provided a means of predicting the potential vulnerability of the EPOT species to the sediment stress in the studied river systems, with potential for its wider application in other river systems in South Africa. It is, however, important to note that, due to the complexities associated with species responses to sediment stress the limited trait information used in the current study, the approach developed would need further refinement investigation to improve its predictive power.

4.5.2 Ephemeroptera, Plecoptera, Odonata, Trichoptera (EPOT) traits responses to sediment stress

In this study, the RLQ ordination analysis was used to assess the association of individual trait attributes to sediment particle characteristics. The findings of this study revealed that the studied sites were largely grouped together by sediment particle characteristics, species were associated with sites groups based on the trait attributes possessed. For example, during the dry season, based on settled sediment particles, the less impacted site groups were clustered together, the proportions of coarse silt, very coarse silt, fine silt and medium silt sediements were associated with these site groups. A possible association of these particle sizes could be that the larger fine classes of sediments contributed less to sediment stress in the studied river systems. Trait attributes associated with these particles sizes i.e. coarse silt, very coarse silt, fine s, medium s coarse s, included filter feeding, stone biotope, filamentous gill small body size. The corresponding species that were positively associated included *Oligoneuropsis lawrencei*, *Pseudocloeon* sp., *Ellassoneuria* sp., *Hydropsyche* sp. *Caenis* sp.

The highly impacted site group 1 was positively associated with proportions of clay, very fine silt, fine silt coarse silt. These particles favoured the assemblages of trait attributes such as shredding, large body size, exophytic oviposition operculate gill. The corresponding species that were positively associated included *Baetis* sp., *Trithermis* sp. *Crenigomphus* sp. These findings show that in terms of the settled sediments characteristics, species that were classified as highly vulnerable or vulnerable to sediment stress were supported by the analysis of the individual trait attributes, sites species.

Similar to settled sediments, finer suspended sediments particles were also positively associated with site group 1, though the majority of trait attributes species positively associated with the site group were those classified as highly vulnerable or vulnerable. However, the correlation of *Baetis* sp. to the proportion of clay, very fine silt fine silt is in accordance with previous studies that found *Baetis* sp. to be sediment tolerant (Reylea *et al*., 2000; Buendia *et al*., 2013).

In general, the positive correlation of filter feeders, stone biotope, filamentous gill to the proportion of larger sediment particles (coarse silt, very coarse silt, fine sand and coarse sand) and negative correlation to finer particles (clay, very fine silt and fine silt) supports the argument that finer particles of sediment are more deleterious to species than larger particles (Kaller & Hartman, 2004). For example, in a recent study by Conroy *et al*. (2017), who investigated the response of macroinvertebrate species to burial by sediment particles in Mail, Europe, showed that finer sediment fractions are potentially most harmful to aquatic biota.

Therefore, the associations of these trait attributes with the proportions of very coarse silt, fine sand, medium sand coarse sand were expected, as filter feeders, filamentous gill species preferring the stone biotope are affected by clogging of feeding respiratory structures (Jones *et al*., 2012). The positive association of *Baetis* sp., *Trithermis* sp. and *Crenigomphus* sp. to site group 1 that was correlated to the finer proportions of sediments such as clay, very fine silt, fine silt of sediments, can be explained by the corresponding association of the resilient traits such as predators, large-body size operculate gill, and is in accordance with a previous study by Buendia *et al*. (2013). The correlation between large-body size the highly sediment-impacted sites was not expected, as generally in high sediment areas smaller-bodied species with faster life cycles are favoured (Townsend & Hildrew, 1994), because of their potential for faster recolonisation (Smook & Milner, 2002). However, larger invertebrates are able to move within areas of deposited sediments allowing large-bodied species to be able to escape increased sediment deposition (Lamourox *et al*., 2004).

During the wet season, settled sediment particles did not differentiate between the site groups, except for site group 4 during summer, which was separated from the other site groups. Site group 4 was associated with very fine sand, fine sand coarse sand, which were dominated by trait attributes such as burrowing, macrophytes, filamentous gill. These trait attributes were represented by *Caenis* sp., *Tricorythus* sp. and *Adenophlebia* sp.

The result of the suspended sediment particles and turbidity analysis during the wet season separated the sediment-impacted site groups 1 and 2 from the other sites groups. Site group 1 was also positively associated with clay, very fine silt, fine silt, silt and turbidity. Clingers/climbers, large body size, burrowing, crawling and predators represented by *Paragomphus* sp., *Crenigomphus* sp. and *Phylomacroma* sp. were the majority of species positively associated with these particles site group. This result supported the prediction made that climbing, crawling and large body size would be resilient to sediment influences (Shieh *et al*., 2012). Descloux *et al*. (2013), who investigated the effect of sediment colmation on macroinvertebrates in the Rhone river catchment in France, indicated that the traits crawling, plus propensity for attachment, were tolerant to increasing sediment deposition.

In general, changes in habitat characteristics in the Tsitsa River and its tributaries appeared to select for certain trait attributes that reflected changes in the ability of the EPOT species to withstand disturbances, as only the traits conferring resilience and resistance were largely selected in the impacted sites. These findings are in accordance with previous studies, which indicated that impacted habitat might affect trait structure, as those species with sensitive trait attributes are filtered out (Péru & Dolédec, 2010). In the studied river systems, the proportion of sediment particle sizes that was mostly associated with the highly-impacted sites was the finer proportion of settled and suspended sediments. The representation of predators, clingers/climbers, active swimmers, large body size and predators at the sediment-influenced sites, supported the prediction and description of traits made in this study. Filter feeders, filamentous gill, stone biotope and FPOM were the most negatively associated trait attributes at the sediment-influenced sites. This study, therefore, shows that the traits can exhibit varying responses to sediment size classes provide further insight into traits' response to sediment stress. The majority of the species classified as sediments tolerant were found to possess one or more of the trait attributes that are resilient to sediments, whereas those described as being vulnerable and highly vulnerable possessed the majority of the vulnerable trait attributes, which were further elucidated by the Fourth-corner analysis.

4.6 Conclusion

The findings of this study showed that environmental gradient acts as a filter on EPOT species trait composition. The trait-based approach developed in this chapter proved useful in classifying species into vulnerability classes and in predicting the potential occurrences of species in the context of sediment stress. The percent relative abundance of the vulnerable class, when compared to the highly tolerant and tolerant classes, decreased at the sediment-influenced sites. The trait-based approach developed in this chapter and the association of species to sediments was useful in diagnosing the ways in which sediment stress structures both species and trait assemblages. The promising results obtained in this study should serve as an impetus for further research on additional traits and the responses of traits to other environmental stressors in South Africa, particularly since the TBA is yet to gain popularity in Africa, including South Africa.

CHAPTER 5: GENERAL DISCUSSION, CONCLUSION, RECOMMENDATIONS

5.1 Introduction

In-stream sediment transport deposition are important processes of streams riverine systems critical for structuring aquatic ecosystems (Owens *et al*., 2005). However, ecological degradation of stream ecosystems occurs due to altered sediment delivery dynamics from anthropogenic activities. The impact of sediments on aquatic systems and biota is exacerbated in catchments that drain rural and peri-urban communities whose major means of livelihood is poorly managed agricultural practices. Previous studies have reported that the major water quality stressor in the riverine systems where this study was undertaken is excessive sediment deposition (Madikizela & Dye, 2010). However, relatively few available studies have examined the effects of elevated sediment characteristics on the assemblage structure of aquatic organisms and these few studies have not explicitly used a predictive trait-based approach.

In this study, the taxonomical and trait-based approaches were used to assess the responses and potential vulnerability of the order EPOT to sediment stress. This chapter therefore reviews the findings of this thesis, relates them to the overall aim and objectives of this thesis, draws conclusions to the research hypothesis and questions posed by the study. The chapter provides a comparative explanation of the findings of this study, the implications and significance in relation to taxonomic and trait-based biomonitoring approaches in South Africa. Finally, the chapter gives insight into the limitations of the methods and approaches employed in this study, concludes by making recommendations for future studies.

5.2 Taxonomic-based biomonitoring approach for assessing EPOT responses to sediment stress

5.2.1 Species-level assessment of sediment stress in the Tsitsa River its tributaries

The result of the species-taxonomic assessment of sediment stress on the assemblage structure of EPOT in the Tsitsa River its tributaries was presented in Chapter 3 of this thesis. Sediment particle distribution is a primary driver of macroinvertebrate assemblage structure distribution in aquatic ecosystems (Sutherl *et al*., 2010). For example, in this study, the taxonomic assessment of EPOT taxa indicated that sediment stress influenced the assemblage structure and distribution of EPOT species across the eight sampling sites. The results indicated that the majority of the EPOT species were recorded at the less sediment-influenced Site 4 (Pot River upstream) and Site 5 (Pot River downstream). Most of the sediment-sensitive species that occurred at Site 4 (Pot River upstream) and Site 6 (Little Pot River) were less represented at Site 1 (Tsitsa River upstream), Site 2 (Tsista River downstream) and Site 3 (Qurana River) (Appendix B1). The fewer occurrences of EPOT species at the sediment-influenced sites are an indication that elevated sediment input has adverse effects on the assemblage structure of EPOT at these sites. For example, the presence of species such as *Oligoneuriopsis* sp., *Prosopistoma amanzamanya* and *Tricorythus* sp. at Sites 4, 5 and 6, their absence from Sites 3 and 8, and their rarity at Site 2, suggest that Site 2 is less impacted than Sites 3 and 8. These differences in assemblages structure of EPOT are further supported by the ANOSIM analysis result, where Sites 3 and 8 indicated a statistically significant difference (Chapter 3 Table 3.5). A previous study by Schletterern & Fureder (2009), who investigated the ecology and distribution of European *Prosopistoma* sp. in the Volga, Daugava, Rhine Rivers, also concluded that the occurrence of *Prosopistoma* sp. is a typical component of a reference site. Therefore, the occurrence of one species of *Prosopistoma* sp. at Site 4 (Pot River upstream) might be attributed to good water quality at this site with little impact of sediment.

The EPOT species assemblages in the studied river systems also showed seasonal differences, with more species being recorded during winter and spring compared to summer autumn (Chapter 3 Figure 3.5). The high number of species recorded during winter and spring suggests that the assemblages of EPOT during winter and spring are more similar than that of summer and autumn. This result is also supported by the NMD result, which indicated statistically significant differences between winter autumn as well as between spring autumn (Appendix B1). A previous study by Madikizela & Dye (2010), who investigated the community composition and distribution of macroinvertebrates in the Mzimvubu River catchment in Eastern Cape, South Africa, where the Tsitsa River and its tributaries are situated, also reported more similarity between spring and autumn than summer in terms of macroinvertebrate assemblages. The low occurrence of EPOT during spring and autumn can be attributed to the a likely abrasive effect of suspended sediment during high flows in summer. Another possible explanation might be inaccessibility of sampling habitat during high water depth, particularly in summer. However, the rarest species such as *Prosopistoma amanzamanya*, *Aphenicera* sp., *Elasoneuria* sp. and *Oligoneuriopsis lawrencei* recorded in the study period, occurred mostly during spring and summer. Their occurrence during these two seasons might be explained by their preference for shallow to high depth fast-flowing water (Barber-James & Lugo-Ortiz, 2003; Yam, 2015). A previous study by Schletterern & Fureder (2009) also reported the presence of *Prosopistoma* sp. during spring summer in fast-flowing water in the Volga, Daugava Rhine Rivers.

Furthermore, the result of the ANOSIM and SIMPER analyses of EPOT assemblage structures, enabled the discrimination of sites and seasons in the studied river systems. The ANOSIM result indicated a global statistical significant difference between the sites in terms of EPOT assemblages. The EPOT assemblages enabled the discrimination of Site 1 from Site 6, where the two sites indicated a statistically significant difference. The species level assessment also indicated a statistically significant difference between Sites 2 and 8 (Appendix B1 and B2). The result was supported by the NMDS analysis that clustered Sites 1 and 3 in one cluster Sites 5 and 6 in a different cluster. The result emphasises the importance of taxonomic assessment of water quality deterioration at species-level to enable the discrimination of subtle differences between sites. The EPOT are functionally taxonomically diverse groups of macroinvertebrates occupying almost all habitats, with a wide range of sensitivities to environmental conditions.

5.2.2 Species responses of the order EPOT to sediment stress

The taxonomic assessment of the EPOT responses to sediment particle characteristics using the CCA ordination revealed that their responses are species-specific and species belonging to the same family can display varying responses to sediment stress. For instance, species such as *Paragomphus* sp., *Aeshna* sp., *Baetis* sp. and *Cheumatosyche* sp. were strongly positively associated with the highly sediment-influenced site groups 1 and 3, while species such as *Afronurus* sp., *Pseudocloeon* sp. and *Paragomphusgenei* positively associated with the less sediment-influenced site group 4.

The proportions of settled sediments that positively influenced the assemblages of the species at site groups 1 and 3 were clay, very fine silt, medium silt and coarse silt. The proportion of medium silt, very coarse silt, fine silt, very fine silt and coarse silt favoured the assemblages of species such as *Hydropyche* sp., *Phylomacromia* sp., *Enallagma* sp. and *Pseudocloeon piscis* at site group 3 during winter and spring. The association of finer proportions of settled sediment particles such as clay, very fine silt, medium silt and coarse silt at site groups 1 and 3 indicates that finer sediment particles were responsible for the high sediment influence at these site groups.

Furthermore, the taxonomic analysis of EPOT species' responses to sediment indicates that species such as *Paragomphus* sp., *Aeshna* sp., *Baetis* sp. and *Pseudocloeon* sp. can be indicators of sediment-influenced sites, and species such as *Afronurus* sp., *Adenophlebia* sp. and *Euthraulus* sp., that indicated negative associations with all sediment particles, can be good indicators of less sediment-influenced sites. This finding is similar to the findings of Buendia *et al.* (2013) and Conroy *et al.* (2017), who indicated that species within the order EPOT responded in different ways to sediments and can be important taxa to consider in assessing fine sediment impact. For example, *Euthraulus* sp. and *Afronurus* sp. have been reported to be sensitive to sediments (Hubler *et al.*, 2016) while *Baetis* sp. and *Caenis* sp. *Adenopphlebia* sp. appeared to be tolerant (Relyea *et al.*, 2000; Turley *et al.*, 2016). Similarly, in this study, *Baetis* sp. exhibited a positive association with increasing proportions of fine sediment particles, in contrast, *Adenophlebia* sp. indicated a negative response to sediment particle characteristics. The findings are generally consistent with a number of previous studies (e.g. Bo *et al.*, 2007; Bryce *et al.*, 2010), who suggested that for the most part, finer sediment proportions are most harmful to organisms and sensitive species tend to negatively associate with them.

The CCA results, that tested association between turbidity and suspended sediment, EPOT taxa, were largely similar to the findings of settled sediments, with some exceptions. Turbidity was closely associated with silt and clay proportions with the same species being associated, although *Oligoneuropsis lawrencei*, *Aphenicera* sp. and *Prosopistoma amazanmanya* were positively associated. Similar to settled sediments above, *Caenis* sp., *Hydropsyche* sp., *Euthraulus* sp., *Pseudocloeon piscis* and *Afronurus* sp. were positively associated with the proportions of very coarse silt sediments, except that species *Hydropsyche* sp., *Aeshna* sp. and *Crenigomphus* sp. were positively correlated with turbidity.

The differences observed in the responses of EPOT species to settled and suspended sediments might be due to the transport of sediments particles in water columns, as some species may be impaired by moving particles while others might utilise the moving particles carried in biofilms of fine sediment particles. The implication, therefore, might be that species can respond differently to particles of settled and suspended sediments. The negative correlation of species such *Baetis* sp. and *Afronurus* sp. with the proportion of s particles is in accordance with a previous study by Wood *et al.* (2005), that reported increased drift in *Baetis* sp. *Ecdynurus* sp. to s particles. The CCA result showed that *Baetis* sp. and *Afronurus* sp., including Odonate species, were positively associated with proportions of clay silt. These buttress the fact that sediments can affect the assemblage structure of EPOT species and that their responses are species-specific.

5.3 Trait-based biomonitoring approach for assessing EPOT responses to sediment stress

5.3.1 Trait-based assessment of sediment stress in Tsitsa River its tributaries

Traits play an important role in mediating between species and environmental variability, and are thus assumed to determine the adaptational potentials of species to environmental stress (Usseglio-polatera *et al.*, 2000). Therefore, trait response pattern may help to suggest the stressor type impacting the aquatic system. When the response pattern is known, it could allow biomonitoring approaches to become more impact-diagnostic and predictive of assemblage response to a specific stressor (Odume *et al.,* 2018). This is because traits are assumed to capture the causal mechanisms that structure biological assemblages. In this study, a trait-based approach was developed for assessing the responsee and potential vulnerability of the EPOT taxa to sediment stress in the Tsitsa River and its tributaries.

Based on the TBA developed, EPOT taxa were classified into four vulnerability classes, which included highly vulnerable (A), vulnerable (B), tolerant (C) and highly tolerant (D). This approach enabled the grouping of taxa with a similar combination of co-adaptive trait attributes that responded to sediment stress. For instance, filter feeding EPOT species that have filamentous gills and are soft-bodied were classified as highly vulnerable (A) or vulnerable (B). Predictions were made based on the TBA developed for this study. It was predicted that the percent relative abundance of the highly vulnerable and vulnerable classes should decrease at sites that were potentially more influenced by sediments. The result was largely in congruence with the predictions. The result revealed that the percent relative abundance of the vulnerable class decreased in the sediment-influenced Site 1 (Tsista upstream), Site 2 (Tsitsa downstream), Site 3 (Qurana River) and Sites 7 and 8 (Millstream upstream and downstream), compared to the tolerant and highly tolerant classes (Chapter 4 Figure 4.1). The predictions were not in congruence with the highly vulnerable class (A), which was consistently high at all sites in terms of percent relative abundances. However, in terms of the frequency of occurrence (FROC) prediction, there was a correspondence of more than 50% between the observed and predicted data at sediment-influenced site groups 1 and 2. These predictions were mainly accurate for the highly vulnerable and vulnerable classes, indicating that the FROC of the majority of species classified under these classes was reliable. Furthermore, the statistical association of EPOT species was tested using the Pearson's point biserial associations the result indicated that few taxa were associated with the sediment-influenced site groups. The only taxa that indicated a statistically significant association with sediment-influenced site group 1 was *Paragomphus* sp. and this species was classified as highly tolerant (D) based on the newly developed trait-based approach. Filter feeding EPOT species that have filamentous gills and are soft-bodied were not significantly associated with sediment-influenced site groups (Chapter 4 Table 4.8). Rabeni *et al.* (2005) also investigated the response of macroinvertebrate functional groups to fine sediments and found filter feeding species to be the most sensitive of all functional groups examined. The findings of this study were consistent with the habitat template concept (HTC) habitat filtering concept (HFC) (Southwood, 1977; Townsend & Hildrew, 1994; Poff, 1997), which predicted that resilient traits would correlate with highly impacted sites. The statistical associations of most EPOT species with site groups that were less sediment-influenced provided further insight into the predictive power of the trait-based approach in evaluating the assemblage response of the EPOT to sediment stress.

5.3.2 Individual trait responses to sediment particle classes in the Tsitsa River its tributaries

The RLQ combined analysis was effective in isolating the traits and corresponding species that were correlated with sediment characteristics. The RLQ analysis was used to examine whether different trait attributes within a species display different environmental responses to sediment particle classes. For example, trait attributes such as filter feeding, filamentous gills, burrowing detritus (FPOM) displayed different responses to settled sediment particle classes. Filter feeding and detritus (FPOM) were positively associated with the proportion of fine silt and negatively associated with site group 4 at spring. Filamentous gill and stone biotope were positively associated with the largest proportions of sediment particles such as very coarse silt coarse sediment, with positive association with site group 4 during winter, while burrowers were associated with proportions of fine silt and clay at site group 1. The varying responses of trait attributes that characterised species could imply that, in assessing species vulnerability to a particular stressor, analysing single trait attributes' response alone might be misleading. This is because it is not the individual traits in isolation that determines the adaptive potential of the organism, but the combination of the entire traits possessed. Therefore, analysing species' responses to environmental stressors by taking into account the co-adaptive response of the combination of trait attributes could provide a more meaningful assessment of responses of species to an environmental stressor. For example, species such as *Hydropsyche* sp. possessing combinations of trait attributes such as filter feeding, filamentous gill, and soft and exposed body classified as highly vulnerable, was classified based on the overall combined co-adaptive responses of the individual trait attributes to sediment stress.

The result also revealed that the responses of a given trait attribute were not consistent between species, nor between vulnerability groups. For example, clinging/climbing species such as *Afronurus* sp. and *Euthraulus* sp. indicated a statistical association with site groups 1 and 4, but the single trait attribute of clinging indicated a different response, with a positive association with site group 1. This implies that environmental responses of individual trait attributes were not consistent across species or vulnerability groups, further implying that the environmental response observed for a given trait may be mediated by other traits, which may have correlative or trade-off effects, particularly as traits occur in syndrome (Poff *et al*., 2006; Menezes *et al*., 2016). Furthermore, shredding and temporarily attached species such as *Adenophlebia* sp. classified as highly vulnerable, indicated a statistically significant association with the less sediment-impacted site group 4 (Chapter 4 Table 4.8). When analysed singly, they were strongly associated with the sediment-influenced site group 1. This finding indicates that the responses of *Adenophlebia* sp. were markedly influenced by the responses of other trait attributes which form suits of traits that were used in the classification system developed in this study, or other traits that were not analysed in the present study. The findings are in congruence with a recent study by Piliere *et al*. (2016), who investigated the importance of interrelationships for understanding environmental responses of streams' macroinvertebrates using data obtained from 609 streams across Ohio, USA. The study revealed that trait attributes such as streamlined body shape poor female diversity of the *Heptageniidae* family, classified as sensitive, responded differently to agricultural impact.

In general, the findings of the study revealed that responses of individual trait attributes to a particle stressor were not consistent between species. In addition, the responses of trait attributes can differ with sediment stress, season, the presence of other trait attributes particle size characteristics. For instance, the Fourth-corner analysis revealed that lamellate gill responded negatively to the proportion of coarse silt, fine sand, and very fine sand but positively to coarse silt, medium silt, very fine silt and clay (Chapter 4 Figure 4.6). Similarly, the trait attribute such as burrower responded negatively to coarse sanda, fine sand, and avery fine sand positively to medium silt, fine silt, very fine silt and clay (Chapter 4 Figure 4.7).

5.4 Evaluating the complementarity of the taxonomic trait-based biomonitoring approaches for assessing EPOT responses to sediment stress

Various biomonitoring approaches have been extensively applied in South Africa in managing freshwater ecosystems, yielding exciting results. Traditionally, biomonitoring studies use the taxonomic composition of macroinvertebrate assemblages to detect water quality deterioration (Culp *et al*., 2011). This approach assumes the presence of a species that possesses the appropriate adaptive attributes needed to survive environmental conditions at a site (Culp *et al*., 2011). Trait-based approaches define an organism's attributes and relate them to an environmental condition. In this study, the taxonomic and novel trait-based approach at species level were used to evaluate the responses of the orders EPOT to sediment stress. Because the trait-based approach is relatively new in South Africa and has gained little popularity and TBA tend to offer predictive and diagnostic potentials, it is imperative to do a comparative evaluation of the two approaches in predicting the response and vulnerability of EPOT to sediments.

A major shortcoming of the taxonomic approach analysis is the poor causal relationship with stressors (Menezese *et al*., 2010). The poor link between species the environment can affect the predictive and diagnostic potentials of the taxonomic approach. The trait-based approach developed in this study was useful in classifying the species into potential vulnerability classes in predicting EPOT species' response to sediment stress. For example, filter feeding EPOT species that have filamentous gills, soft exposed body, that graze on detritus (FPOM), indicated little or no associations with the sediment-influenced Sites 1 and 2 (Tsitsa upstream downstream), Site 3 (Qurana tributary) Sites 7 and 8 (Millstream upstream and downstream) during dry and wet seasons. However, in the taxonomic analysis, filter feeding and filamentous gill breathing species occurred both at the sediment-influenced and non-influenced sites. The occurrence of species possessing these trait attributes i.e. filter feeding fillemntous gill buttresses the poorer discriminatory ability of taxonomic analysis compared to trait-based approach, where the TBA was more helpful in discriminating between the impacted sites and the un-impacted sites. In addition, the higher percent relative abundance of filter feeding and filamentous gill breathing species at Sites 1 and 2 (Tsista upstream downstream) Site 8 (Millstream downstream), compared to Site 3 (Qurana River) and Site 7 (Millstream upstream), suggests that the latter three sites are less impacted than Site 3 (Qurana River) and Site 7 (Millstream upstream). Thus, the decrease in the percent relative abundance of filter feeding anda filamentous gill breathing species that were classified as vulnerable (B) at Sites 3 and 7, suggests that sediment input was a major biotic stressor at these sites. The trait-based approach therefore has the potential to increase the predictive discriminatory power of biomonitoring of water quality impairment. When the objective is to discriminate between impacted sites or between references sites, a trait-based approach can be useful. Startzner *et al.* (2005) have shown that the TBA can predict the anticipated changes across reference sites more accurately than the taxonomic approach precisely because the TBA pays attention to the underlying processes of interractions between the species and the environment. Charvet *et al.* (1998), in a study to compare the traditional approaches i.e. diversity, biotic indicese and community structure with the TBA for two sites being upstream and downstream impacted by effluent from a waste water treatment plant in France, revealed that the TBA performed better in separating the upstream anda downstream sites. In this study, the TBA that was developed responded predictably to sediment impacts, provided an adaptive and mechanistic basis for interpreting EPOT species assemblage responses to sediment stress in the eight sampling sites. In general, the TBA developed in this was helpful in providing more subtle discrimination of the study sites and classification of the EPOT species into vulnerability classes. Similalry, the taxonomic approach proved useful in providing the taxonomic identity of the EPOT species providing insight to their assemblage structure across the selected study sites. The taxonomic approach was particularly important stage towards the TBA as it provided the identity of species. Therefore, the complementary use of the taxonomic and trait-based approach enabled a more robust assessment of EPOT species response to sediment stress in the studied river system.

The TBA proved useful in predicting EPOT species' responses to sediment stress and in discriminating between sites in the studied river systems. However, the TBA does not necessarily replace the traditional taxonomic approach but can act as a complementary tool (Odume *et al.*, 2014). Similarly, Doledec *et al.* (1999) highlighted that although an ideal biomonitoring tool needs to be generic in terms of geographical application, specific in terms of stressor diagnosis, derived from sound theoretical ecological concepts, which are possible to obtain through application of a TBA, more research is needed to integrate the TBA into biomonitoring techniques in South Africa. Therefore, in this study, the TBA showed the potential to be integrated into biomonitoring of sediment stress, alongside the taxonomic-based approach in South Africa.

5.5 Management implications

The need for sustainable water quality management for the benefit of all users the protection of the aquatic ecosystems in South Africa, has long been recognised, which has led to the promulgation of the National Water Act 36 of 1998 (NWA). The management of water resources in South Africa attempts to maintain a balance between the use of water resources and atheir protection. The management approach allows for sustainable use of water resources the protection of aquatic ecosystems, to enable the maintenance of aquatic ecosystem health. Protecting the aquatic ecosystem provides for protection of both instream and riparian aquatic biota (macroinvertebrates, fish, and vegetation) and physico-chemical components of the aquatic ecosystems (Palmer *et al*., 2004). To ensure the efficient management of water quality aquatic ecosystems, the NWA provides for the ecological Reserve, a component of the IWRM, which incorporates macroinvertebrates' bioassessment as well as physico-chemical measurement aspects of ecotoxicology in the determination of ecosystem health of water resources in South Africa (Palmer *et al*., 2004; DWS, 2013). Assessment of macroinvertebrates assemblages provides an integrated assessment of aquatic ecosystem integrity because biota are subjected to anthropogenic influences from water users in the environment which integrate their effects over time (Bonada *et al*., 2006; 2007).

In this study, macroinvertebrates-based biomonitoring methods, which included EPOT taxonomic and trait-based approaches, were applied to assess the effect of sediments in the Tsitsa River and its tributaries. The combined use of the taxonomic and trait-based approach enabled the discrimination between selected sites in the studied river systems, based on the responses anda vulnerability of EPOT to sediment stress. The result of the study revealed that, of the eight sampling sites, Sites 1 and 3, grouped into site group 1, were the most sediment-influenced, while site group 4, comprised of only Site 6, was the least sediment-influenced. The taxonomic assessment result showed that Site 6 supported more EPOT species, while the major species associated with Site 1 were the sediment-tolerant species such as *Aeshna* sp., *Paragomphus* sp and *Baetis* sp. The trait-based approach developed in the study was able to classify EPOT species based on their sensitivity to sediment stress, which further enabled the discriminations of the sampling sites. The two complementary approaches enabled the efficient assessment of sediment stress in the Tsitsa River provided more insight into individual species' sensitivity to sediment particle characteristics.

The assessment of increased sediment in rivers is usually a difficult task. Therefore, the integration of the trait-based approach into biomonitoring approaches in South Africa, presents an aimpact-diagnostic potentials of predicting biotic responses to environmental stressors. An ecologically relevant biomonitoring approach needs to be predictive and diagnostic, be able to determine sources and levels of impairment. The complementary use of the approaches enables managers faced with limited resources, to identify key species that are sensitive to sediments, which may aid to direct and inform areas where intervention measures need to be deployed.

5.6 Conclusions

This study has demonstrated that the ecological conditions indicated by water quality variables in the Tsitsa River and its tributaries were between the A and B ecological categories, which are indicative of good water quality conditions. The ecological categories suggest that the major water quality problem in this studied riverine system is sediments. The ecological categories as indicated by the water quality variables helped to meet objective one of this thesis by providing water physico-chemical characteristics of the Tsista River and its tributaries. The taxonomic-based biomonitoring was helpful in classifying sites according to their levels of sediment, with species that are sensitive being associated with less sediment-impacted sites. The taxonomic-based appraoch was useful in addressing the second objective of this thesis as indicated by the varying responses of EPOT species to a gradient of sediment impact. The results of the trait-based approach developed in this study (Chapter 4) were helpful in meeting the third objectives of this thesis and clearly classifying the EPOT species according to their potential degree of vulnerability to sediment stress. The trait-based approach further enabled the distinct discrimination of the sampling sites on the basis of sediment stress, which highlighted the need to integrate the TBA into routine biomonitoring programmes in South Africa. The predictive responses of the EPOT species provided by the TBA, complemented the taxonomic-based biomonitoring.

5.7 Limitations of the study

This study has demonstrated that the complementarity of the taxonomic and trait-based approaches to biomonitoring can improve the predictive potentials of existing biomonitoring tools in South Africa. However, before these approaches can become fully useful, a full understing of the environmental variables responsible for the distribution of traits is necessary. A major limitation observed in the study is the lack of adequate identification keys for species-level identification. This challenge is more problematic in the study region, where little is known of Afro-tropical macroinvertebrate species; trait information available for the study was thus limited.

5.8 Recommedantion For Further Research and Integration of traits into water resource management

The following recommendation are made for future studies and for the integration of traits into water resource management in South Africa

- For the full potential of the TBA to be realised in South Africa, more life-history research is needed to make critical trait information particularly those related to reproduction avaliable.
- More research is needed on the complementary use of taxonomic and trait-based responses in multiple stressor context and in systems experiencing diverse stresses
- In South Africa, the integration of traits into Resource Quality Objectives would add diognistic and predictive value to the biological components of the RQOs narratives.

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APPENDICES

Appendix A: Showing means. Standard deviations and ranges of settled and suspended sediment classes for the eight sampling sites over the study period (August 2016 – April 2017) in the Tsitsa River and its tributaries

Variables	Site 1	Site 2	Site 3	Site 4	Site 5	Site 6	Site 7	Site 8	*P*-values
			Settled sediment classes						
S	0.19 ± 0.02 (0.16 - 0.21)	0.26 ± 0.106 (0.11 - 0.33)	0.23 ± 0.02 (0.02 - 0.42)	0.22 ± 0.13 (0.125 - 0.41)	0.315 ± 0.11 (0.21 - 0.41)	0.29 ± 0.16 (0.57 - 0.38)	0.26 ± 0.19 (0.89 - 0.44)	0.18 ± 0.095 (0.01 - 0.26)	
Medium s	0.003 ± 0.01(0.0 - 0.01)	0.01 ± 0.03 (0.00 - 0.05)	0.03 ± 0.03 (0.00 - 0.05)	0.004 ± 0.01 (0.00 - 0.01)	0.02 ± 0.02 (0.01 - 0.04)	0.002 ± 0.002 (0.0 - 0.004)	0.04 ± 0.04 (0.001 - 0.07)	0.003 ± 0.004 (0.00 - 0.01)	
Fine s	0.04 ± 0.03 (0.01 - 0.07)	0.05 ± 0.03 (0.01 - 0.06)	0.07 ± 0.07 (0.001 - 0.14)	0.04 ± 0.040 (0.02 - 0.10)	0.08 ± 0.033 (0.04 - 0.1)	0.07 ± 0.047 (0.01 - 0.12)	0.08 ± 0.07 (0.01 - 0.14)	0.04 ± 0.03 (0.01 - 0.07)	
Very fine s	0.15 ± 0.03 (0.12 - 0.18)	0.19 ± 0.09 (0.101 - 0.27)	0.13 ± 0.110 (0.02 - 0.22)	0.16 ± 0.01 (0.08 - 0.30)	0.21 ± 0.05 (0.16 - 0.26)	0.22 ± 0.11 (0.05 - 0.29)	0.14 ± 0.08 (0.06 - 0.21)	0.13 ± 0.07 (0.07 - 0.19)	

Very coarse silt	0.23 ± 0.01 (0.22 - 0.24)	0.25 ± 0.04a (0.2 - 0.28)	0.14 ± 0.05b (0.08 - 0.18)	0.18 ± 0.08 (0.08 - 0.26)	0.24 ± 0.02 (0.22 - 0.25)	0.23 ± 0.06 (0.17 - 0.28)	0.18 ± 0.03 (0.13 - 0.11)	0.19 ± 0.02 (0.18 - 0.21)	$P < 0.05$
Coarse silt	0.21 ± 0.03 (0.17 - 0.24)	0.17 ± 0.02 (0.15 - 0.20)	0.16 ± 0.04 (0.12 - 0.19)	0.13 ± 0.06 (0.05 - 0.19)	0.17 ± 0.02 (0.15 - 0.19)	0.16 ± 0.03 (0.14 - 0.21)	0.18 ± 0.04 (0.14 - 0.21)	0.18 ± 0.02 (0.17 - 0.20)	
Medium Silt	0.15 ± 0.01 (0.14 - 0.17)	0.12 ±0.03 (0 .0.09 - 0.16)	0.16 ± 0.08 (0.93 - 0.24)	0.11 ± 0.07 (0.04 - 0.18)	0.11 ± 0.03 (0.09 - 0.14)	0.12 ± 0.05 (0.09 - 0.18)	0.14 ± 0.05 (0.01 - 0.10)	0.16 ± 0.03 (0.13 - 0.2)	
Fine silt	0.1 ± 0.01 (0.09 - 0.11)	0.09 ± 0.03 (0.06 - 0.13)	0.14 ± 0.07 (0.08 - 0.2)	0.22 ± 0.23 (0.06 - 0.56)	0.08 ± 0.30 (0.05 - 0.11)	0.09 ± 0.05 (0.06 - 0.02)	0.11 ± 0.04 (0.07 - 0.16)	0.13 ± 0.03 (0.01 - 0.16)	
Very fine silt	0.06 ± 0.01 (0.06 - 0.07)	0.057 ± 0.02 (0.04 -0.09)	0.09 ± 0.05 (0.05 - 0.15)	0.07 ± 0.04 (0.02 - 0.11)	0.05 ± 0.02 (0.03 - 0.07)	0.06 ± 0.04 (04 - 0.12)	0.08 ± 0.04 (0.04 - 0.11)	0.09 ± 0.03 (0.06 - 0.12)	
Clay	0.05 ± 0.01 (0.04 - 0.07)	0.06 ± 0.02 (0.05 - 0.09)	0.09 ± 0.04 (0.05 - 0.14)	0.06 ± 0.04 (0.02 - 0.11)	0.04 ± 0.02 (0.02 - 0.06)	0.06 ± 0.04 0.03 - 0.11)	0.06 ± 0.04 (0.02 - 0.1)	0.08 ± 0.03 (0.05 - 0.11)	
			Suspended sediments classes						
S	0.25 ± 0.1 (0.1-0.32)	0.48 ± 0.03 (0.28 - 0.81)	0.25 ± 0.23 (0.02 -0.46)	0.29 ± 0.11 (0.14 - 0.04)	0.24 ± 0.11 (0.13 -0.33)	0.3 ± 0.08 (0.2 - 0.4)	0.4 ± 0.02	0.26 ± 0.16	

							(0.12 - 0.53)	(0.08 - 0.39)	
Medium s	0.01 ± 0.02 (0.0 - 0.05)	0.11 ± 0.02 (0.004 - 0.4)	0.06 ± 0.07 (0.0 - 0.04)	0.02 ± 0.02 (0.0 - 0.01)	0.01 ± 0.01 (0.0 - 0.012)	0.002 ± 0.001(0.0 - 0.003)	0.05 ± 0.04 (0.01 - 0.01)	0.03 ± 0.03 (0.0 - 0.4)	
Fine s	0.08 ± 0.05 (0.01 - 0.12)	0.12 ± 0.1 (0.06 - 0.27)	0.08 ± 0.08 (0.004 - 0.15)	0.08 ± 0 .04 (0.01 - 0.1)	0.05 ± 0.03 (0.02 - 0.08)	0.08 ± 0.04 (0.02 - 0.12)	0.14 ± 0.07 (0.02 – 0.2)	0.08 ± 0.06 (0.01 - 0.13)	
Very fine s	0.16 ± 0.05 (0.01 - 0.11)	0.18 ± 0.09 (0.05 - 0.24)	0.09 ± 0.07 (0.02 - 0.15)	0.18 ± 0.07 (0.13 - 0.24)	0.18 ± 0.08 (0.11 - 0.25)	0.22 ± 0.05 (0.18 - 0.29)	0.19 ± 0.09 (0.09 - 0.27)	0.15 ± 0.07 (0.07 - 0.2)	
Very coarse silt	0.21 ± 0.02 (0.19 - 0.24)	0.19 ± 0.12 (0.06 - 0.29)	0.12 ± 0.04 (0.06 - 0.15)	0.22 ± 0.05 (0.15 - 0.25)	0.24 ± 0.04 (0.22 - 0.28)	0.23 ± 0.04 (0.21 - 0.21)	0.19 ± 0.02 (0.17 -0.21)	0.19 ± 0.01 (0.18 - 0.2)	$P < 0.05$
Coarse silt	0.2 ± 0.03 (0.16 - 0.23)	0.14 ± 0.08 (0.05 -0.21)	0.16 ± 0.04 (0.12 - 0.22)	0.18 ± 0.07 (0.17 - 0.19)	0.11 ± 0.02 (0.18 - 0.21)	0.17 ± 0.03 (0.12 - 0.11)	0.15 ± 0.04 (0.13 - 0.21)	0.18 ± 0.03 (0.16 - 0.21)	
Medium Silt	0.14 ± 0.01 (0.12 - 0.15)	0.08 ± 0.04 (0.03 - 0.12)	0.16 ± 0.07 (0.1 - 0.23)	0.12 ± 0.03 (0.01 - 0.15)	0.14 ± 0.03 (0.11 - 0.17)	0.13 ± 0.03 (0.01 - 0.15)	0.1 ± 0.04 (0.08 - 0.16)	0.15 ± 0.05 (0.12 - 0.23)	
Fine silt	0.09 ± 0.02 (0.07 - 0.11)	0.05 ± 0.02 (0.02 - 0.07)	0.13 ± 0.07 (0.07 - 0.21)	0.09 ±0.04 (0.06 - 0.13)	0.07 ± 0.04 (0.05 - 0.12)	0.08 ± 0.01 (0.07 - 0.01)	0.07 ± 0.04 (0.04 - 0.13)	0.1 ± 0.04 (0.07 - 0.15)	
Very fine silt	0.06 ± 0.03 (0.04 - 0.09)	0.03 ± 0.02 (0.014 - 0.06)	0.09 ± 0.06 (0.05 - 0.17)	0.06 ± 0.03 (0.03 - 0.01)	0.06 ± 0.03 (0.03 - 0.08)	0.05 ± 0.01 (0.04 - 0.06)	0.05 ± 0.04 (0.02 - 0.01)	0.07 ± 0.03 (0.05 - 0.01)	
Clay	0.05 ± 0.03 (0.02 - 0.08)	0.03 ± 0.02 (0.01 - 0.05)	0.09 ± 0.06 (0.03 - 0.16)	0.05 ± 0.03 (0.02 - 0.08)	0.04 ± 0.03 (0.02 - 0.07)	0.03 ± 0.01 (0.02 - 0.05)	0.04 ± 0.03 (0.01 - 0.08)	0.051 ± 0.03 (0.03 - 0.08)	

Appendix B1: Percent relative abundance and distribution of EPOT species collected seasonally during the study period (August 2016 - April 2017) at Site 2 (Tsitsa upstream), Site 2 (Tsitsa Downstream), Site 3 (Qurana tributary), Site 4 (Pot River upstream) in the Tsitsa River and its tributaries. Abbreviations: Wn = winter, Sp = spring, Su = summer and Au = autumn.

Taxon	**Site 1**				**Site 2**				**Site 3**				**Site 4**			
	Wn	Sp	Su	Au	Wn	Sp	Su	Au	Wn	Sp	Su	Au	Wn	Sp	Su	Au
Acanthiops sp.	6.2												7.9			7.5
Acanthiops tsitsa		1.2						0.3			4.2	12.3		1.6	1.6	
Adenophlebia sp.	4.7		0.8							5.1	11.5					
Adenophlebia auriculata				13.1					12.5	32.4		3.1				
Afronurus sp.		1.9	4.3	1.1	2.2		3	0.3	0.5				14.8		1	0.7
Afronurus bernardi																
Baetis sp.	14.3	16	8.5				3	9.3	35.7		33.2	24.8		0.5	15.2	8.5
Caenis sp.					5.7	16.7	8	2.2				0.5	11.5	9.2	8	
Elassoneuria sp.								2.8				1.3				
Euthraulus sp.	38.3	28	46.9	2.2	5.8		38.8		25.5	5.4	14.9	11.4	19.9	44.6	14.5	1.5
Oligoneuriopsis sp.								2.8				1.8				
Oligoneuria lawrencei							4								7.7	
Pseudocloeon piscis	7.1	6.4			45.7	4.6		6.6	2.7	6.8	0.4		24.4			
Pseudocloeon glaucum				34.3				7.7		0.6	4				26.3	1.5

Pseudocloeon sp.	17.5	32	18.9		31.9	69	3	6.4	7.5	26.1	2.5	14.8	8.9	12.7		
Pseudocloeon vinosum		4.3		9.9			2	2		1.7		1.3				1.4
Prosopistoma amamzamanya															0.5	
Tricorythus sp.	0.2							8.2				0.2		21.9	9.2	36.6
Order Plecoptera																
Aphenicera sp.							9	9.6			0.4	0.5		0.1	3.9	3.4
Order Odonata																
Aeshna sp.	1.3		0.4				1		8.2	1.7	0.8	6.9	9.9	1.3		0.7
Brachytermis sp.			0.8					0.6							0.5	0.7
Crenigomphus sp.		0.4						1.9			2.2		3.6	0.9	1.5	
Crenigomphus renei	5.2	4.6			2.5	4	2.5			7.7						
Enallagma sp.																
Paragomphus genei	0.3		11.2	4.4					7			4.7				
Paragomphus sp.											0.4					3.4
Phylomacromia sp.					6.2											
Pseudagrion spernatum		4.3			0.7	5.7										

Pseudagrion sp.							5	17.3		0.6		0.9			0.5	3.4
Trithermis sp.		0.4								1.1						
Order Trichoptera																
Cheumatosyche sp.	1		6.5							1.8	7.6	7.6			1	12.9
Hydropsyche sp.	3.9	1.2	1.6				11.9	13.7	0.5			7.8		7.2		
Total number of individuals	**323**	**292**	**271**	**109**	**142**	**93**	**101**	**203**	**228**	**229**	**271**	**287**	**273**	**443**	**236**	**247**

Appendix B2: Percent relative abundance and distribution of EPOT species collected seasonally during the study period (August 2016 - April 2017) at Site 5 (Pot River downstream), Site 6 (Upper Little Pot), Site 7 (Millstream (Upstream) and Site 8 (Millstream downstream) in the Tsitsa River and its tributaries. Abbreviations: Wn = winter, Sp = spring, Su = summer and Au = autumn.

Taxon	**Site 5**				**Site 6**				**Site 7**				**Site 8**			
	Wn	Sp	Su	Au	Wn	Sp	Su	Au	Wn	Sp	Su	Au	Wn	Sp	Su	Au
Acanthiops sp.					0.2	3.3	9.6	4.3								
Acanthiops tsitsa	25	17.4		8.9							1.8				18.6	0.5
Adenophlebia sp.					14.9											
Adenophlebia auriculata					3.2			7.7								
Afronurus sp.	8	3.2	0.3	1.4	5.6			4.2								

Afronurus bernardi					1.7	6.6	3.4									
Baetis sp.			22.4	16		1.6	18.5	21.1			58.4	49.4		3.9	19.4	4.3
Caenis sp.	25.7	8.5	13.3	6.9	19.4	17.4	6.5	0.8	72.3	63.3	12.9		25.6	39.4	14.9	
Elassoneuria sp.																
Euthraulus sp.		34.7	8.4		14.3	4.9	7.7	3.5		0.7			3.9			
Oligoneuriopsis sp.																
Oligoneuria lawrencei																
Pseudocloeon piscis	6.4	0.2			4.7	4.2	13.7	0.5	3.4				6.8		0.7	0.9
Pseudocloeon glaucum						0.1		0.8			2.1	6.6			5	12.5
Pseudocloeon sp.	14.3	5.9		9.5	9	6.8		4.8	5.5	5.8	1.8		33.7	2.8	4.7	7.7
Pseudocloeon vinosum			18.2			0.5	25.9	1.7		3.6		2.6			6.4	
Prosopistoma amamzamanya																
Tricorythus sp.	1.5	3.8	19.7	12.7	18.9	4.4	8.2	9.8			0.6					
Order Plecoptera																
Aphenicera sp.																
Order Odonata																
Aeshna sp.	2.5	3.1	0.5	4.9	1.3	1.4		11.4				2.6	3.2	0.6		1.4

Brachytermis sp.	0.6										0.8				
Crenigomphus sp.	2.4		0.3	2.3							1.7				0.2
Crenigomphus renei			1.9	5.9	0.8	3.2	0.5	16.3							
Enallagma sp.										2.9				9.8		
Paragomphus genei		5.9			0.5		3.6						0.1			
Paragomphus sp.			1.5													
Phylomacromia sp.													3.2	3.9	1.6	13.4
Pseudagrion spernatum			1.1			5.2										
Pseudagrion sp.	1.3	3.6		5.8			1		4	12.2	1.2	2.5				
Trithermis sp.																
Order Trichoptera																
Cheumatosyche sp.	13.6			15.1				7.7				14		5.6	26.3	
Hydropsyche sp.		14.6	3.3	12.3	5.9	4.7	1.7	5.6	15.2	11.5	21.3	2.5	24.2	34.8	3.1	59.6
Total number of individuals	**592**	**701**	**220**	**629**	**581**	**433**	**224**	**629**	**207**	**144**	**179**	**145**	**513**	**184**	**415**	**234**