Diversity of zooplankton and aquatic macroinvertebrates in the Ntsikeni Nature Reserve

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List of Abbreviations

A	April
Ag	Silver
Al	Aluminium
ANOSIM	Analysis of similarities
ANOVA	Analysis of variance
As	Arsenic
Au	Gold
Av. Abund	Average abundance
Av. Sim	Average similarity
B	Boron
Ba	Barium
Be	Beryllium
Bi	Bismuth
Ca	Calcium
Cd	Cadmium
Co	Cobalt
Contrib%	Contribution percentage
Cr	Chromium
CSIR	Council for Scientific and Industrial Research
Cu	Copper
Cum.%	Cumulative percentage
d	Margalef's species richness
D	December
DWA	Department of Water Affairs, South Africa
DWAF	Department of Water Affairs and Forestry, South Africa
EC	Electrical conductivity
EKZN	Ezemvelo KwaZulu-Natal Wildlife
EPA	Environmental Protection Agency
Fe	Iron
GPI	Gabhisa Planning and Investments
GSM	Gravel, sand and mud
H'	Shannon diversity index
Hg	Mercury
HGM	Hydrogeomorphic

ICP-MS	Inductively coupled plasma mass spectrophotometry
J	July
J’	Pielou’s evenness index
K	Potassium
KZN	KwaZulu-Natal
Mg	Magnesium
Mn	Manganese
Mo	Molybdenum
N	Total individuals
Na	Sodium
NBF	Neutrally buffered formalin
NFEPA	National Freshwater Ecosystems Priority Areas
Ni	Nickel
NMDS	Non-metric multidimensional scaling
NR	Ntsikeni Nature Reserve
O_2	Dissolved oxygen
P	Phosphorus
Pb	Lead
PCA	Principle component analysis
Pd	Palladium
Pt	Platinum
Rb	Rubidium
RDA	Redundancy analysis
S	Total taxa
SANBI	South African National Biodiversity Institute
Sb	Antimony
Se	Selenium
Sim/SD	Similarity/standard deviation
SIMPER	Similarity percentage
SQG	Sediment Quality Guidelines
Sr	Strontium
TDS	Total dissolved solids
Th	Thorium
Ti	Titanium
Tl	Thallium

TWQR	Target Water Quality Range
U	Uranium
USEPA	United States Environmental Protection Agency
USFWS	United States Fish and Wildlife Service
V	Vanadium
WRC	Water Research Commission
Zn	Zinc

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Abstract

The Ntsikeni Nature Reserve is one of 23 wetlands in South Africa that is designated as a Ramsar Wetland of International Importance. The Ntsikeni wetland complex comprises an area of 1070 ha, located within a provincial nature reserve of 9200 ha. This reserve has an altitude in excess of 1800 m, thus making the Ntsikeni wetland complex one of the largest high-altitude wetlands to have obtained protective status under the Ramsar Convention. However, the available aquatic biodiversity information is scanty with very little known apart from the bird diversity of the wetland complex. Therefore, the aim of this research project was to establish the diversity, community structure, and distribution of zooplankton and aquatic macroinvertebrates at the Ntsikeni Nature Reserve.

Samples were collected from 10 selected sites located throughout the Ntsikeni Nature Reserve during three seasonal surveys in the winter month of July 2015, the summer month of December 2015, and the autumn month of April 2016. Water and sediment samples were collected *in situ* and transported back to the laboratory for further analyses. Water samples were analysed to determine nutrient and metal concentrations using the Spectroquant® Pharo 300 and an Agilent 7500ce inductively coupled plasma mass spectrophotometer (ICP-MS) respectively. Sediment analyses were conducted to determine grain size percentages and metal concentrations using accepted techniques. Zooplankton were sampled using a plankton net with a mesh size of 50 µm, while aquatic macroinvertebrates were sampled using standard sweep nets measuring 30 cm x 30 cm with a mesh size of 1 mm. All collected zooplankton and macroinvertebrates were identified to the lowest taxonomic level possible.

Water quality variables such as dissolved oxygen, electrical conductivity (EC), and temperature were found to be responsible for macroinvertebrate variation. However, water quality variables had no significant influence on zooplankton community structure. Arsenic (As), cadmium (Cd), chromium (Cr), copper (Cu), lead (Pb), mercury (Hg), nickel (Ni), silver (Ag) and zinc (Zn) concentrations in the sediment were compared to the Sediment Quality Guidelines (SQG) as provided by the US Environmental Protection Agency (USEPA). The metal concentrations fell within accepted guidelines, however, these sediment quality variables were located in areas with the least macroinvertebrates.

Seasonality originally appeared to have an effect on both zooplankton and macroinvertebrate community structures. During the winter survey in July 13 zooplankton taxa and 84 macroinvertebrate taxa were found. During the summer survey in December 18 zooplankton taxa and 95 macroinvertebrate taxa were found. During the autumn survey in April 20 zooplankton taxa and 94 macroinvertebrate taxa were found. However, an analysis of similarities (ANOSIM) revealed that seasonality had no significant influence on zooplankton or aquatic macroinvertebrate community structures of the Ntsikeni Nature Reserve.

In total, 25 zooplankton taxa and 129 macroinvertebrate taxa were identified. When the Ntsikeni wetland complex was compared to other water bodies, both nationally and internationally, it was established that the Ntsikeni Nature Reserve contained a high variety of aquatic organisms. Statistical analyses of the combined data revealed the area's biodiversity, dominant genera, and overall connection between abiotic and biotic factors. Overall, the Ntsikeni wetland complex contains a rich biodiversity of both zooplankton and aquatic macroinvertebrates. The research of this project has assisted in updating the available aquatic biodiversity information for the Ntsikeni Nature Reserve.

Keywords: Ramsar, Ntsikeni Nature Reserve, wetland, water quality, sediment, zooplankton, macroinvertebrates.

Chapter 1 – Introduction

1.1 Overview

1.1.1 Wetland definition

The word “wetlands” can be used to describe many forms of aquatic ecosystems including riverine floodplains, tree-covered swamps, high-altitude rain-pools, and even saline lakes (Dallas and Day, 2004). Ponds, lakes, rivers, marshes, swamps, and bogs are also listed as wetlands; in short, any open water areas that are shallow and either intermittently covered or saturated (Matthews, 1993).

In South Africa, wetlands are defined by the National Water Act (no 36 of 1998) as “land which is transitional between terrestrial and aquatic systems where the water table is usually at or near the surface, or the land is periodically covered with shallow water, and which land in normal circumstances supports or would support vegetation typically adapted to life in saturated soil.” This definition was derived from the United States Fish and Wildlife Service (USFWS) Classification System for Wetlands and Deepwater Habitats in the USA (Cowardin *et al.,* 1979).

The Ramsar Convention defines a wetland as: “areas of marsh, fen, peatland or water, whether natural or artificial, permanent or temporary, with water that is static or flowing, fresh, brackish or salt, including areas of marine water, the depth of which at low tide does not exceed six metres” (Matthews, 1993, pg. 38; Duguid *et al.,* 2005, pg. 50). Furthermore, “wetlands may incorporate riparian and coastal zones adjacent to the wetlands, and islands or bodies of marine water deeper than six metres at low tide lying within the wetlands” (Duguid *et al.,* 2005, pg. p1).

The condition of a wetland and where it can be located is determined by:

- the hydrology of the area – the distribution of water over both time and space;
- the geomorphology – which refers to the shape of the land;
- the season to season presence/absence of water;
- the availability of depressions or basins for water to accumulate;
- the depth of the water when it does accumulate (Dallas and Day, 2004).

Wetlands are important ecosystems that comprise abiotic characteristics of an area, such as climate, geology, and water, together with biotic communities suited to prevailing environmental conditions of an area (Macfarlane *et al.,* 2009). These ecosystems form an important part of hydrological systems in that their presence decrease run-off within a catchment. Wetlands also perform various hydrological functions such as purification of surface water, groundwater recharge, flood attenuation, and erosion control (Bird, 2010). This ensures the longer residence time of water within the catchment, thereby causing biotic communities to become dependent on it (Dixon and Wood, 2003). Wetlands accumulate suspended materials, thus they are referred to as deposition systems and often called “sinks” (Dallas and Day, 2004). Wetlands act as natural filters that regulate both the quantity and quality of the water in the ecosystem. These ecosystems have a vast capacity for storing water that can trap sediments, protect shorelines, offset floods, recharge groundwater, oxygenate water, recycle nutrients, cleanse polluted waters, and eventually release purified water back into the system (Breedt and Dippenaar, 2013; Hiestermann and Rivers-Moore, 2015). It is for this reason that wetlands are often called the “kidneys of the landscape” (Hiestermann and Rivers-Moore, 2015). Wetlands are highly productive ecosystems that support unique fauna and flora thereby providing suitable habitat for fish, reptiles, invertebrates, plants, amphibians, birds, and mammals (Breedt and Dippenaar, 2013; Hiestermann and Rivers-Moore, 2015).

1.1.2 Wetland degradation

All over the world, wetlands are considered to be one of the most endangered habitat types; South Africa itself has also experienced considerable loss of wetlands and their benefits (Bowd *et al.,* 2006a). South Africa is a country that experiences comparatively high temperatures and variable seasonal rainfall over much of the country (Dallas and Day, 2004). In general, rainfall in South Africa is unreliable and unpredictable (Breedt and Dippenaar, 2013). Throughout most of the provinces in South Africa permanent bodies of standing fresh water are scarce to virtually non-existent (Dallas and Day, 2004).

Mankind has, for centuries, viewed wetlands as places that need to be drained, converted or developed (Matthews, 1993). Wetlands across South Africa, that have not been concreted or drained, only make up a total of 2.4 % of the country's total surface area (Nel and Driver, 2012); a percentage that can be converted into 2.93 million ha of a total 122.1 million ha. Of all South Africa's wetlands, 65 % are threatened, of which 48 % are critically endangered (Nel and Driver, 2012; Cowden *et al*., 2014). Since wetlands are water resources that contribute to agriculture, industry, and domestic uses, these ecosystems should not be destroyed (Breedt and Dippenaar, 2013).

The most common threat to wetlands in South Africa is that of urbanisation. As cities and towns expand, it is often into, and at the cost of, ecological sensitive areas such as wetlands (Breedt and Dippenaar, 2013; Cowden *et al*., 2014). Wetlands are also impacted by upstream and downstream impoundments, water abstraction, artificial drainage, cultivation, and over-grazing (Cowden *et al*., 2014). Habitat loss, ecosystem disruption, fragmentation, and global warming all contribute to loss of wetlands, not only in South Africa, but globally (Hiestermann and Rivers-Moore, 2015). Through this degradation of wetlands, some have disappeared altogether leading to undesirable consequences such as loss of groundwater reserves, constant need for irrigation, shoreline destruction, and flash floods (Matthews, 1993).

1.1.3 Ramsar background

Whether it is intentional or accidental, wetlands are being negatively impacted due to human activities (Breedt and Dippenaar, 2013). It was due to the dissappearance of waterfowl and fish species, which resulted from the loss of wetlands, that the need was realised for international agreements (Matthews, 1993). When draining and land reclamation were at their peak in certain areas, technical experts gathered at a number of international conferences and meetings, and in so doing drafted the Ramsar Convention for Wetlands of International Importance (Koester, 1989). It was clear that this convention had to be international since the circulation of water in the atmosphere is not confined to any boundary. In addition, fish that hatch in one country often travel to another as adults and migratory water birds require many wetlands across numerous countries to rest, feed, and breed in (Matthews, 1993).

The Ramsar Convention had its beginnings in 1971 when the representatives of 18 nations signed a treaty in the small town of Ramsar, Iran on the 3rd of February (Matthews, 1993). According to Matthews (1993), the Ramsar Convention is the first among a number of modern instrumental tools which aim to conserve natural resources on a global scale. Koester (1989, pg. XI) stated that "the Ramsar Convention is the oldest of the global nature conservation treaties, and the only one to deal with a particular ecosystem type."

The Ramsar Convention establishes that wetlands across the globe should be selected based on their international significance with regards to limnology, zoology, botany, ecology or hydrology (Ramsar, 2017). The Ramsar List (Ramsar, 2017, pg. 1) states that its vision is: "to develop and maintain an international network of wetlands which are important for the conservation of global biological diversity and for sustaining human life through the ecological and hydrological functions they perform."

The Ramsar Convention has set out a total of nine criteria, along with accompanying guidelines, to assist contracting parties in identifying their priority sites for designation (Ramsar Convention Secretariat, 2010). These criteria were adopted by the 4th, 6^{th,} and 7th Meetings of the Conference of the Contracting Parties, to aid as a guide to implement Article 2.1 on the designation of Ramsar sites (Duguid *et al.,* 2005). These criteria, according to the Ramsar Convention Secretariat (2010, pg. 29), are divided into Group A of the criteria (which refers to "sites containing representative, rare, or unique wetland types") and Group B of the criteria (which refers to "sites of international importance for conserving biodiversity"). Group B can be further divided into:

- "criteria based on species and ecological communities,
- specific criteria based on waterbirds,
- specific criteria based on fish, and
- specific criteria based on other taxa" (Ramsar Convention Secretariat, 2010, pg. 29).

South Africa has 23 wetlands that have been designated as Ramsar Wetlands of International Importance and this protects an area of approximately 557 028 ha (Ramsar, 2017). South Africa originally joined the Ramsar Convention in March of 1975 when Barberspan (North West Province) and De Hoop Vlei (Western Cape Province) were designated as Ramsar Wetlands of International Importance (Ramsar, 2017). Since that time South Africa has methodically been adding more and more wetlands to the list of Ramsar wetlands across the country. The most recent wetland to be added to the Ramsar Convention — bringing South Africa's total number of Ramsar wetlands to 23 — is the Bot-Kleinmond Estuarine System located in the Western Cape Province (Ramsar, 2017).

1.1.4 The Ntsikeni Nature Reserve

The province of KwaZulu-Natal (KZN) experiences large annual variations in both temperature and rainfall due to the Drakensberg escarpment marking the western boundary of the province and the warm Mozambique current in the east (Hiestermann and Rivers-Moore, 2015). It is estimated that approximately 5 % of the KZN province is covered in wetlands comprising a total area in excess of 42 000 km². This is due to the topography, varied geology, high mean annual precipitation, relatively low potential evapotranspiration, infiltration, percolation, interflow, and streamflow that all occur in the area (Hiestermann and Rivers-Moore, 2015). However, of all the wetlands in the KZN province, more than half have been modified (Bowd *et al*., 2006b).

Within this province lies the Ntsikeni Nature Reserve (Figure 1), located within the Umzimkhulu catchment on the Lubhukwini River (Nxele, 2007; Blackmore, 2010). The Ntsikeni Nature Reserve as a whole was designated as a Ramsar Wetland of International Importance in February 2010 (Ramsar, 2010). The Ntsikeni Nature Reserve has a total area of 9200 ha (Blackmore, 2010; EKZN, 2016;), however the wetland complex within the reserve itself only comprises an area of 1070 ha (Kotze, 2003; Blackmore, 2010). The wetland complex within the Ntsikeni Nature Reserve is one of the largest high-altitude wetland complexes in South Africa, with its extent ranging in altitude from 1580 to 2321 m asl (Blackmore, 2010).

The Ntsikeni wetland complex acts as a connection between aquatic and terrestrial environments; thereby creating a place for organisms from these environments to meet and interact (Breedt and Dippenaar, 2013). The Ntsikeni wetland complex performs valuable stream-flow regulations, provisioning services, cultural services, and biodiversity support functions (Blackmore, 2010; Hiestermann and Rivers-Moore, 2015). The wetland complex provides a key source of sustainable potable water for the rural communities living downstream, as well as plant species that are high in fibre which are ideal for weaving and other provisioning services such as wood collection (Blackmore, 2010).

According to the criteria provided by the Ramsar Convention, the Ntsikeni Nature Reserve adheres to criteria 1, 2, and 6 (Blackmore, 2010). Criterion 1 falls under Group A of the criteria for the designation of Wetlands of International Importance, while criteria 2 and 6 both fall under Group B of the criteria (Ramsar Convention Secretariat, 2010). The Ntsikeni Nature Reserve adheres to criterion 1 in that it “contains a representative, rare or unique example of a natural or near-natural wetland type found within the appropriate biogeographic region” (Ramsar Convention Secretariat, 2010, pg. 28). The Ntsikeni wetland complex is a good example of a large high-altitude wetland that is in good condition and is completely contained within the boundaries of the Ntsikeni Nature Reserve. It is also a site found in a wetland-rich area of approximately 100 km² that has sustained low levels of hydrological impact and modification to its ecological character (Kotze, 2003; Blackmore, 2010).

Criterion 2 falls under Group B’s “criteria based on species and ecological communities” subdivision (Ramsar Convention Secretariat, 2010, pg. 29) The reserve adheres to criterion 2 in that it “supports vulnerable, endangered, or critically endangered species or threatened ecological communities” (Ramsar Convention Secretariat, 2010, pg. 30). The Ntsikeni Nature Reserve is an important breeding site for the endangered Wattled Crane (*Grus bugeranus*) that has been classified as vulnerable, and is also likely to support the endangered Long-toed Tree Frog (*Leptopelis xenodactylus)* as well as other Red Data species (Blackmore, 2010).

Criterion 6 falls under Group B's "specific criteria based on waterbirds" subdivision (Ramsar Convention Secretariat, 2010, pg. 29). The Ntsikeni Nature Reserve adheres to criterion 6 in that it "regularly supports 1 % of the individuals in a population of one species or subspecies of waterbird" (Ramsar Convention Secretariat, 2010, pg. 35). Blackmore (2010) indicates that at the time of its designation, the Ntsikeni Nature Reserve supported two or three pairs of breeding Wattled Crane out of 68 active breeding pairs found in South Africa; thus indicating that the wetland complex supported 3 – 4 % of the country's breeding population.

1.1.5 Macroinvertebrates as bio-indicators

In order to manage water resources accurately, the monitoring of various components of aquatic ecosystems, such as water quality and biotic communities, must be an integral part of the process (Malherbe *et al.,* 2010). Ecosystem health is quantified by using biological indicators, such as algae, macroinvertebrates, and fish that can reflect the quality of the water in which they live (Dallas and Day, 2004). Macroinvertebrates are particularly useful as indicators of river health due to their well-known status of having different sensitivities to pollution and being adapted to living in certain environmental conditions (Ferreira *et al.,* 2009; Wolmarans *et al.,* 2014). Studies have indicated that aquatic macroinvertebrates have potential to act as bio-assessment tools in wetlands as well (Bird, 2010). They are known to retain and break down organic material, recycle minerals and nutrients, and also contribute to energy processing through different trophic levels (Malherbe *et al.,* 2010). Environmental factors that have been found to affect macroinvertebrate composition include water colour, degree of eutrophication, salinity, and temperature changes (Davis *et al.,* 1993; Walmsley, 2000; Kemp *et al.,* 2014). In addition, it has also been found that vegetated biotopes have higher diversity and abundance than do open-water biotopes (Bird *et al.,* 2014).

Aquatic macroinvertebrate assemblage data has proven to be more effective at distinguishing levels of eutrophication in wetlands than has water chemistry tests (Davis *et al.,* 1993). Chemical water quality monitoring on its own is insufficient since it does not account for higher level effects of chemicals on biota, nor does it account for temporal or longitudinal changes (Malherbe *et al.,* 2010).

1.2 Problem statement

In 2013 a Water Research Commission (WRC) workshop about wetland management and research needs in South Africa indicated that there is a general lack of information regarding the aquatic biodiversity of South Africa's Ramsar sites, including that of the Ntsikeni Nature Reserve. To the authur's knowledge, the last study conducted in the Ntsikeni Nature Reserve, with regards to aquatic biodiversity, was done in 2010 as part of the process for designating the Ntsikeni Nature Reserve as a Ramsar site (Blackmore, 2010).

According to the Ramsar Convention Secretariat (2010), contracting parties that have designated a Ramsar Site are urged to revise the data provided in the Information Sheet on Ramsar Wetlands at least every six years. Since its designation as a Ramsar Wetland of International Importance in February 2010 (Ramsar, 2010), the Ntsikeni Nature Reserve has received no further update on its aquatic biodiversity information. The few aquatic macroinvertebrates that have been found at the Ntsikeni Nature Reserve have only been identified to family level (Blackmore, 2010), thus indicating the gap in available research. As far as can be determined no information is available regarding the zooplankton biodiversity of the Ntsikeni Nature Reserve.

This research has provided information by determining overall water quality and sediment composition within the Ntsikeni wetland complex. Also, both the macroinvertebrate and zooplankton biodiversity have been updated to a lower taxanomic level.

1.3 Aims and Objectives

Aim: This research aims to establish the community structure as well as distribution of zooplankton and aquatic macroinvertebrates of the Ntsikeni Nature Reserve.

Objectives:

- Seasonal sampling and assessment of water quality at selected sites in the Ntsikeni Nature Reserve.
- Seasonal sampling and assessment of the sediment quality at selected sites in the Ntsikeni Nature Reserve.

- Seasonal sampling of zooplankton and aquatic macroinvertebrates to determine the biodiversity of the Ntsikeni Nature Reserve.
- Relating water and sediment quality to the zooplankton and aquatic macroinvertebrate community structure of the Ntsikeni wetland complex using statistical analyses.

1.4 Hypotheses

Based on the objectives, two hypotheses were formulated for the purpose of this study. These are:

1. Water and sediment quality has an influence on the aquatic macroinvertebrate community structure of the Ntsikeni Nature Reserve's wetland complex.
2. The zooplankton and aquatic macroinvertebrate community structure of the Ntsikeni Nature Reserve will be influenced by season.

With regards to the influence of seasonal changes on zooplankton and macroinvertebrates it is expected that changes in temperature, water flow, and spatial distribution (habitat) changes may occur.

1.5 Thesis Structure

A general introduction chapter which includes a literature review along with a general study area chapter has been compiled. These two chapters are then followed by three stand-alone data chapters each containing their own introduction, methods, results, discussion, and conclusion sections. A final conclusion chapter was included to tie up the results of this study. The overall thesis structure is as follows:

Chapter 1 (Introduction) provides an overview of wetlands and Ramsar sites. A problem statement, hypotheses, aims, and objectives are all given to explain the rationale of the study at the Ntsikeni Nature Reserve.

Chapter 2 (Study Area and Site Selection) provides a historic review of the reserve, background data available for the wetland complex, and sites classifications using Ollis *et al.,* (2013).

Chapter 3 (Water and Sediment) presents the water quality and sediment quality of the Ntsikeni Nature Reserve. The materials and methods are laid out, and the results are given and discussed.

Chapter 4 (Zooplankton) lists all zooplankton taxa and abundances found throughout the Ntsikeni Nature Reserve during the three surveys (July/winter 2015, December/summer 2015 and April/autumn 2016). Both univariate and multivariate analyses i.e. Margalef's species richness (d), Shannon diversity index (H'), Pielou's evenness index (J'), distributional k-dominance plots, hierarchical clustering, non-metric multidimensional scaling (NMDS) and redundancy analysis (RDA) were applied to the data collected.

Chapter 5 (Macroinvertebrates) lists all macroinvertebrate taxa collected and identified at the Ntsikeni Nature Reserve during the three surveys (July/winter 2015, December/summer 2015 and April/autumn 2016). The same univariate and multivariate analyses used in Chapter 4 were used to illustrate and assess the spatial and temporal patterns in biodiversity and community structure of macroinvertebrates.

Chapter 6 (Conclusion and Recommendations) indicates whether the hypotheses formulated in the first chapter are indeed accepted or rejected based on the information gathered and analyses compiled in the previous chapters. Recommendations are also given so as to allow for more effective studies at the Ntsikeni Nature Reserve to be conducted in the future.

Chapter 2 – Study Area and Site Selection

2.1 Overview

South Africa receives an average annual rainfall of 450 mm, while the world average is 860 mm annually (Breedt and Dippenaar, 2013). According to de Villiers and de Wit (2010) South Africa is a dry country with 97.8 % of the country being classified as either arid or semi-arid. Approximately 65 % of the country receives less than 500 mm of rainfall annually, and 21 % of the country receives less than 200 mm of rain annually (Breedt and Dippenaar, 2013). The KwaZulu-Natal (KZN) Province is considered to have a sub-tropical climate, which experiences summer rainfall between 900 – 1200 mm per annum (Fairbanks and Benn, 2000). KwaZulu-Natal is responsible for 28.5 % of South Africa's national mean annual runoff (Rivers-Moore and Goodman, 2010), thereby highlighting the importance of freshwater conservation in the province.

The Ntsikeni Nature Reserve has been identified as a critical node for protected area expansion as part of both national and provincial protected area expansion strategies (EKZN, 2016). The Ntsikeni Nature Reserve contains a palustrine emergent wetland complex which is situated in a valley-bottom and dominated by a combination of both sedges and grasses (Blackmore, 2010). The Ntsikeni wetland complex, which has been identified as one of the KZN Province's 28 priority wetlands, falls within an area that is very rich in wetlands (Kotze, 2003). Within this wetland-rich area, the Ntsikeni wetland complex is the only wetland to have received protected status, and is only one of eight Ramsar wetlands of international importance to exist within the KZN Province (Kotze, 2003; Ramsar, 2017).

The Ntsikeni Nature Reserve, located between the towns of Underberg and Kokstad (Blackmore, 2010; EKZN, 2016), was originally part of the northern area of the Eastern Cape Province under the management of the Mzimkhulu district (Nxele, 2007; EKZN, 2016). In 2006, with the re-demarcation, the Ntsikeni Nature Reserve was transferred from the Eastern Cape Province to the KZN Province and so became the responsibility of Ezemvelo KZN Wildlife (Nxele, 2007; EKZN, 2016).

Ezemvelo KZN Wildlife is committed to maintaining the ecological character of the reserve and to ensure the wise use of the wetland complex therein (EKZN, 2016). This is done in accordance with Ramsar requirements, relevant legislation, and the already existing management policies of Ezemvelo KZN Wildlife (EKZN, 2016).

The Ntsikeni Nature Reserve (coordinates: 30°8'S; 29°28'E) (Kotze, 2003; Ramsar, 2010) consists of an area of 9200 ha (Kotze, 2003; Nxele, 2007; Blackmore, 2010; EKZN, 2016; Ramsar, 2017). It falls within South Africa's summer rainfall region which is characterised by a mean annual precipitation of 911 mm, a mean maximum temperature of 17.4°C, and a mean minimum temperature of 9.5°C (Blackmore, 2010; EKZN, 2016). Ntsikeni was named from the eastern boundary Ntsikeni mountain range called "iNtsikeni" by the Zulu people (EKZN, 2016). The word "intsika" in isiZulu means "the pillar" referring to the structure used in traditional huts to lend support to the roof structure. It is due to its resemblance to this "intsika", and that the mountain stands out above the landscape, that Ntsikeni Mountain obtained its name (EKZN, 2016).

The Ntsikeni wetland complex is underlain by sandstone and mudstone of the Tarkastad formation and the Molteno Formation (Karoo Supergroup) along with some Adelaide mudrock and sandstone (Kotze, 2003; Blackmore, 2010; EKZN, 2016). Intrusive dolerites of the Jurassic Age can also be found (EKZN, 2016). The entire protected area is bounded by mountainous peaks that are capped with Karoo Dolerite (Blackmore, 2010). The wetland complex is of natural origin due to dolerite dykes and a major dolerite sill at the outlet of the wetland complex (Kotze, 2003). The Ntsikeni wetland complex is characterised by a central broad flat valley-bottom which is comprised of alluvial sediments which rise up into undulating grasslands (Blackmore, 2010).

The Ntsikeni Nature Reserve is an area that conserves a representative portion of the Drakensberg Foothill Moist Grassland (EKZN, 2016). The permanently saturated marsh areas of the wetland complex are dominated by *Carex acutiformis* (Kotze, 2003). The hummocked sedge meadow is dominated by a mixture of grass and sedge species such as *Aristida junciformis* and *Bulbostylis schoenoides* (Kotze, 2003).

The remaining areas, such as seasonally waterlogged zones, wet grassland transitional zones, and surrounding non-wetland areas, are all dominated by a mixture of grass species (Kotze, 2003; Blackmore, 2010). Although the reserve is largely clear of alien plants, a small proportion of the wetland's catchment (< 1 %) is occupied by invasive tree species such as *Acacia* spp. and *Eucalyptus* spp. (Kotze, 2003; Blackmore, 2010). As part of a rehabilitation program, invasive trees are being cleared (Nxele, 2007).

The land comprising the Ntsikeni Nature Reserve used to be privately owned via deeds dated 1880 (EKZN, 2016). In 1950 the government bought up farms that were recognised then as “important water catchment areas” (Blackmore, 2010, pg. 4). These farms were Clairmont 45, Longridge 47, Killarney 48, Mount Pleasant 49, Abergeldy 50, Glengyle 51, Rokeby Park 52, and Milton 53 (Blackmore, 2010; EKZN, 2016). According to EKZN (2016), Ntsikeni was designated a reserve through the Government Notice No.145 of 1978 contained in the Transkei Government Gazette No.53 dated 1 September 1978.

Although the land on which Ntsikeni is situated was purchased in 1950 by the government (Blackmore, 2010; EKZN, 2016), the tenants of the previous landowners were allowed to continue living on the reserve (EKZN, 2016). However, due to mismanagement, by 1999 more than 200 people were illegally living in 96 homesteads with several thousand head of livestock grazing throughout the reserve (EKZN, 2016). During 1999 and 2000 all inhabitants voluntarily vacated and were relocated outside the Ntsikeni Nature Reserve and thus, in April 2000, rehabilitation of the Ntsikeni wetland complex began (Schuyt, 2005; EKZN, 2016).

Once rehabilitation began it was found that a number of abandoned drainage channels in key areas of the wetland complex existed (Blackmore, 2010). According to Schuyt (2005), as part of the rehabilitation project, the following measures were implemented: the existing canals were blocked by building concrete weirs and earthwork structures; canals were dug to aid in the dispersal of water; gabion structures were built to stabilise erosion; firebreaks were burnt on a yearly basis to prevent the wetland complex itself from burning; and alien invasive plants that obstructed water flow into the wetland complex were cleared.

Kotze (2003) stated that all drains collectively did not have a significant impact on the overall hydrological integrity of the Ntsikeni wetland complex. Blackmore (2010, pg. 7) stated that the Ntsikeni wetland’s “hydrological processes are intact and functioning” as part of Ntsikeni’s designation as a new Ramsar site.

The rehabilitation project of April 2000 began with 68 workers being employed to help with the rehabilitation activities (Schuyt, 2005). The local communities surrounding the Ntsikeni Nature Reserve always viewed the area as a source of water, but with the advent of the rehabilitation project the people began to view the wetland complex from

a socio-economic perspective. A questionnaire by Nxele (2007) indicated that the rehabilitation project was of great socio-economic value for contract workers and other stakeholders, such as community members. Surrounding communities now view the wetland complex as a provider of job opportunities for the local people, a means of poverty relief, and as a means to bring the people and the reserve closer together (Nxele, 2007).

The Ntsikeni Nature Reserve's natural beauty, ecological value, and presence of several flagship species, such as the Wattled Crane, allow for great tourism potential (Kotze, 2003; EKZN, 2016). In addition, the Ntsikeni wetland complex is one of KZN's priority wetlands (EKZN, 2016). Tourism experiences include, but are not limited to, game viewing, birding, horse riding trails, hiking trails, mountain bike trails, education for schools and higher institutions, scouting, hunting, family adventures, and crafting (GPI, 2008). Birding and/or game viewing has been identified as the core tourism product of the Ntsikeni Nature Reserve, and environmental education programmes as the secondary tourism product (GPI, 2008). Tourism developments can increase benefits that the Reserve provides to surrounding communities (Kotze, 2003), thereby contributing to socio-economic development (EKZN, 2016).

2.2 Site classification

In order to accurately assess the invertebrate biota of a wetland a sufficient number of biotopes need to be selected in order to provide a representative sample of the wetland (Bird *et al*., 2014). In wetlands, the distribution of macroinvertebrates seem to be primarily affected by vegetation types and abundance (Bird *et al*., 2014).

Across the Ntsikeni wetland complex (Figure 1) a total of ten sites were selected and surveyed with site NR6 being further sub-divided into two parts, A and B. These ten sites were selected across a wide area so as to provide a representative sample of the wetland's overall aquatic biodiversity. Three surveys were conducted during the time period of the research project. The first being in July (winter) 2015; the second in December (summer) 2015; and the last survey in April (autumn) 2016. The data from these three surveys combined accounted for temporal and spatial variations as well as changes in the macroinvertebrate community structures.

Sites NR1 through to NR7 (Figure 1) are all connected by the main fluvial wetland system, with site NR7 being the point were the water exits at the northern part of the reserve. Site NR 8 flows north too, yet is located within a separate subcatchment within the wetland complex. Sites NR9 and NR10 are both located in a subcatchment on the southern part of the reserve. However, unlike sites NR1 to NR7, sites NR9 and NR10 flow in a southern direction.

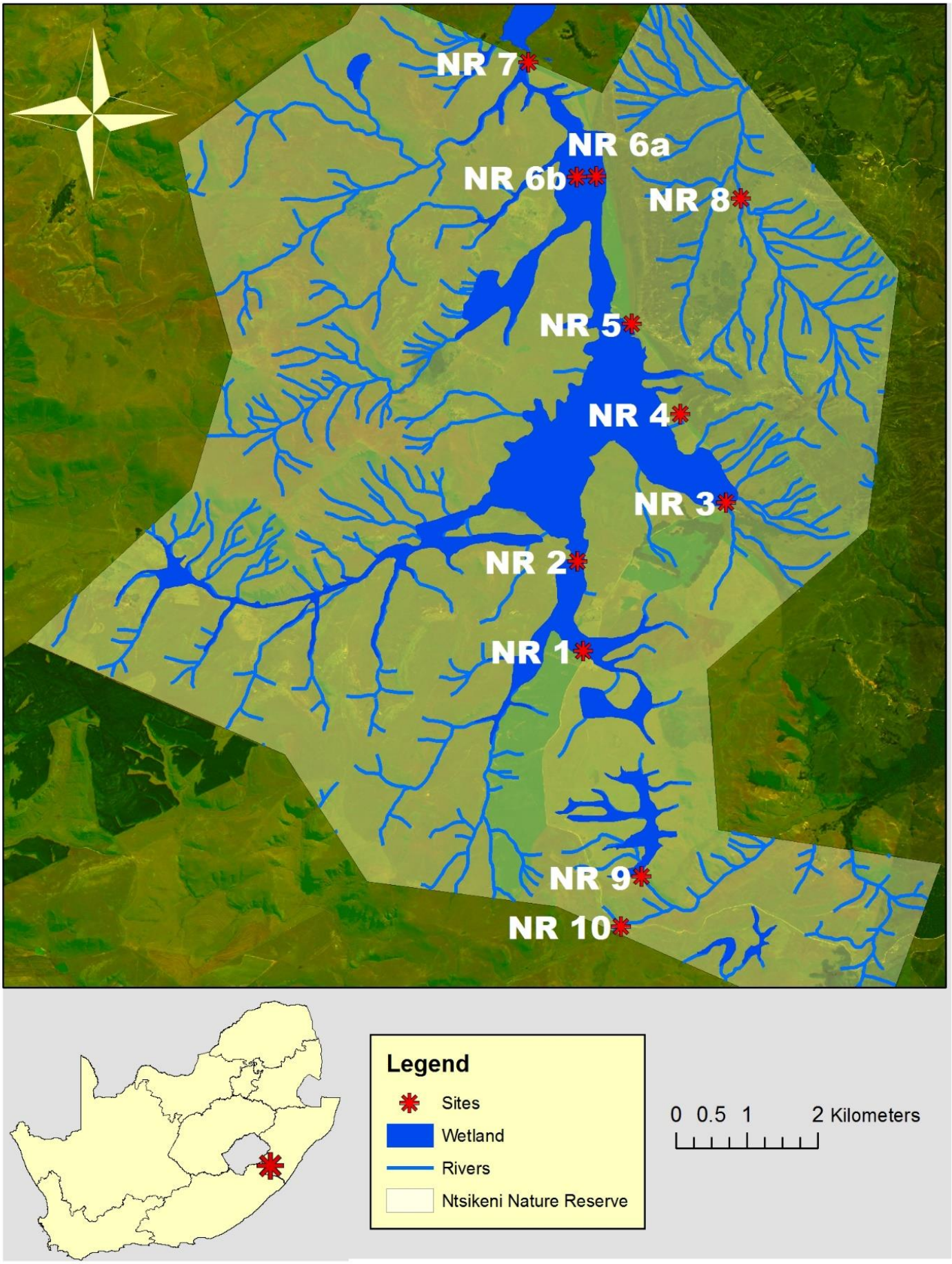

Figure 1: Map of the Ntsikeni Nature Reserve (NR) showing the Ntsikeni wetland complex and the location of selected sampling sites (NR1 – NR10).

Classification is a process that enables humans to organise and understand complex and variable objects, systems or ideas and is a process that is used inherently in ecology (Ollis *et al.,* 2015). Ollis *et al.* (2013, pg. 1) developed a process entitled the "Classification System for Wetlands and other Aquatic Ecosystems in South Africa" which was specifically developed for wetlands and other inland aquatic ecosystems in South Africa (Ollis *et al.,* 2013; 2015). The inland component of the Classification System is comprised of a six-tiered structure which progresses from Systems at the broadest spatial scale (Level 1) down to six descriptors at Level 6 (Ollis *et al.,* 2013). Brief descriptions of each Level are given bellow as laid out by Ollis *et al.* (2013).

Level 1 can be classified as Inland Systems, Estuarine Systems, or Marine Systems. An Inland System is defined as, "an aquatic ecosystem with no existing connection to the ocean. These ecosystems are characterised by the complete absence of marine exchange and/or tidal influence" (Ollis *et al.,* 2013, pg. 2).

At Level 2 of the Classification System, Ollis *et al.* (2013) suggested two optional spatial frameworks. Namely (1) Department of Water Affairs (DWA) Ecoregions and (2) National Freshwater Ecosystem Priority Areas (NFEPA) WetVeg Groups. With regards to DWA Ecoregions, there are 31 Ecoregions across South Africa to choose from. With regards to the NFEPA Wet Veg Groups, there are currently 133 groups to choose from. To which ecoregion and group the Ntsikeni Nature Reserve belongs was identified using maps provided by Ollis *et al.* (2013).

At Level 3 of the Classification System for Inland Systems (Ollis *et al.,* 2013) four Landscape Units have been provided based on the landscape setting within which an aquatic ecosystem is situated. These four Landscape Units are valley floor, slope, plain, and bench. With bench being further subdivided into hilltop, saddle, and shelf (Level 3B).

Level 4 of the Classification System allows for identification of the Hydrogeomorphic (HGM) Units of an inland aquatic ecosystem. At Level 4A seven primary HGM Types are recognised for Inland Systems. These seven HGM Types are: river, channelled valley-bottom wetland, unchannelled valley-bottom wetland, floodplain wetland, depression, seep, and wetland flat. These primary HGM Types (refered to as Level 4A units) can be further subdivided from Level 4B down to Level 4C. However, in some

cases, as with channelled valley-bottom wetlands and unchannelled valley-bottom wetlands, further subdivision is not applicable.

Level 5 (Hydrological Regime) of the Classification System describes the behaviour of water within the system and, for wetlands, in the underlying soil as well. Non-river Inland Systems can be categorised into the period of inundation (Level 5A), saturation (Level 5B), and depth class of permanently inundated systems (Level 5C).

Level 6 of the Classification System provides several descriptors for identifying the structural, chemical, or biological characterisation of Inland Systems. These descriptors are listed non-hierarchical in relation to one another. These descriptors are: natural/artificial, salinity, pH, substratum type, vegetation cover, and geology.

With regards to the Ntsikeni wetland complex, marginal vegetation proved to be the dominant biotope and was thus sampled. Stones areas were also sampled, but were limited in distribution and availability. Based on the structure of sites throughout the wetland complex, the Ntsikeni wetland complex was classified from Level 1 to Level 6 using accepted criteria as laid out in Ollis *et al.* (2013). Level 1, 2, 3, 5, and 6 are discussed within the text while Level 4 is presented in table format.

Level 1: Since the Ntsikeni Nature Reserve rangs in altitude from 1580 to 2321 m asl (Blackmore, 2010) the reserve has no connection to the open ocean whatsoever. Therefore the Ntsikeni Nature Reserve is classified at Level 1 as an Inland System with a high level of confidence.

Level 2: The relevant DWA Level 1 Ecoregion is the South Eastern Uplands Ecoregion, while the relevant NFEPA WetVeg Group is the Sub-Escarpment Grassland Group 5. Level 2 is classified with a high level of confidence.

Level 3: The landscape unit setting of the wetland is classified as a “valley floor” at Level 3. This was done so with a high level of confidence.

Table 1: Summary of results of the application of Level 4 of the Classification System developed by Ollis *et al.* (2013) to the Ntsikeni wetland complex. All Level 4C criteria were found to be not applicable, therefore, Level 4C is excluded from this table. Confidence rating of the classification of each site at each level is given in brackets.

Level 4: Hydrogeomorphic (HGM) Unit			
Site No.	**4A**	**4B**	**Photo showing structure of sites**
NR1	Unchannelled valley-bottom wetland (high)	n/a	
NR2	Channelled valley-bottom wetland (high)	n/a	

Table 1 (continued):

NR3	Unchannelled valley-bottom wetland (high)	n/a	
NR4	Unchannelled valley-bottom wetland (high)	n/a	
NR5	Unchannelled valley-bottom wetland (high)	n/a	

Table 1 (continued):

NR6a	River (high)	Lowland river (medium)	
NR6b	Floodplain (high)	Floodplain depression (medium)	
NR7	River (high)	Transitional (medium)	

Table 1 (continued):

NR8	River (high)	Upper foothills (medium)		
NR9	Unchannelled valley-bottom wetland (high)	n/a		
NR10	Unchannelled valley-bottom wetland (high)	n/a		

Level 5: Sites NR2, NR3, NR5, NR6a, NR6b, NR7, NR8, and NR9 are classified as permanently inundated systems (Level 5A) since surface water was found at each survey. All of these sites have a depth listed as littoral (Level 5C) which is defined as < 2 m maximum depth at the average annual low-water level (Ollis *et al*., 2013). Sites NR1, NR4, and NR10 are classified as seasonally inundated systems (Level 5A) since little to no surface water was found during the autumn survey. However, the influence of South Africa's drought during this time period could possibly have been responsible. The saturation periodicity is classified as being permanently saturated (Level 5B), possibly due to subsurface flow. Permanently saturated is defined as "where all the spaces between the soil particles are filled with water throughout the year, in most years" (Ollis *et al*., 2013, pg. 43). All sites classified under Level 5 are done so with a low level of confidence due to limited experience.

Level 6: The Ntsikeni wetland complex is classified as a natural aquatic ecosystem with fresh water since total dissolved solids (TDS) concentrations and electrical conductivity (EC) ranges measured less than < 3 g/l and < 500 mS/m respectively (Ollis *et al*., 2013). With regards to pH, all sites were found to range between circum-neutral and alkaline since changes in pH were observed during different surveys.

Sites NR1, NR2, NR3, NR4, NR5, NR6b, and NR10 had silt (mud) substratum types. Site NR6a contained both bedrock and silt, with silt found on the downstream side and bedrock on the upstream side of the site. Site NR7 (Table 1) was composed of bedrock (i.e. each rock larger than 30 cm in diameter). Site NR8 (Table 1) was comprised of bedrock, boulders, cobbles, and pebbles. Site NR9 (Table 1) had an accumulation of boulders, but only silt was found at the actual sampling area.

All sites sampled were vegetated (Table 1) (Level 6A). Sites contained both aquatic and herbaceous vegetation forms (Level 6B), as well as both floating and submerged aquatic vegetation (Level 6C). Floating aquatic vegetation is defined as "plants that have their foliage and flowers lying on the water surface" (Ollis *et al*., 2013, pg. 57). According to Ollis *et al.* (2013, pg. 57), submerged aquatic vegetation is defined as "plants occurring in water that are rooted in the underlying substratum and have their foliage below the water surface". Floating attached (rooted) vegetation was mostly found at the sites (Level 6D). All substrate classification descriptors of Level 6 were classified with a medium level of confidence.

Chapter 3 – Water and Sediment

3.1 Introduction

Wetlands are accumulating systems where both sediment and water collect (Dallas and Day, 2004; Malan and Day, 2012). Wetlands, being systems that function as sinks (Malan and Day, 2012), are a place where water can be filtered (Greenfield *et al*., 2007) thereby allowing for pollutants to build-up (Dallas and Day, 2004). This filtering of water affects downstream water quality by reducing turbidity, suspended solids, nutrients, and contaminants from the surface waters (Johnston, 1991; Greenfield *et al*., 2007; van As *et al.,* 2012).

Water is without a doubt the most valuable natural resource since it forms the basis for life to exist on Earth (Griffiths *et al.,* 2015). Without water no organism can live since most animals consist of 50 – 65 % water (van As *et al.,* 2012) with most aquatic organisms' mass composed of 80 – 90 % water (Griffiths *et al.,* 2015). Sediment was reduced from solid rock by the process of mineral alteration into particles which are then transported by a fluid, such as water (Strahler and Strahler, 2005). Since water is the primary medium responsible for the transport of materials into and out of a water body, its velocity and the size of the particles are what determines the amount of sediment entering a wetland (Johnston, 1991).

Water quality variables have an effect on aquatic organisms, whether beneficial or detrimental; these variables consist of both physical (e.g. temperature and turbidity) and chemical (e.g. pH, dissolved oxygen, and conductivity) attributes (Dallas and Day, 2004). With this in mind, monitoring ecosystems, such as wetlands, is an essential part of managing water resources (Malherbe *et al.,* 2010). This is particularly important since wetlands are naturally more variable in terms of water chemistry than are rivers (Malan and Day, 2012).

According to available literature (Kotze, 2003; Blackmore, 2010), the water quality of the Ntsikeni wetland complex appears to be good based on the facts that: the catchment experiences low levels of human activity; sensitive macroinvertebrate taxa (indicative of good quality water) have been found within the wetland complex; and, anecdotal evidence exists of high clarity of the water within the complex.

The sediments of The Ntsikeni wetland complex are primarily mineral, of the Katspruit form, but some organic-rich soils occur in the lower-lying, permanently saturated areas (Kotze, 2003; Blackmore, 2010; EKZN, 2016). However, these organic-rich soils are generally weakly developed, thus resulting in peat being uncommon within the wetland complex (Kotze, 2003; Blackmore, 2010). The dominant soils found within the Ntsikeni Nature Reserve are classified as Mispah, which is mostly comprised of lithosols, which are soils that contain rock fragments on hard or weathering rock and may be prone to erosion (EKZN, 2016).

South Africa is a water scarce country (Malherbe *et al*., 2015), however, due to the KZN Povince's sub-tropical climate (Fairbanks and Benn, 2000) the Ntsikeni Nature Reserve contributes a mean annual runoff of 22 x 10^6 m^3 (Kotze, 2003; EKZN, 2016). Even though Ntsikeni's water quality "appears to be good" (Kotze, 2003, pg. 3; Blackmore, 2010, pg. 4), there are no quantitative data on water and sediment quality of this Ramsar site. The aim of this chapter is to determine the characteristics of the water and sediment quality of the Ntsikeni wetland complex during the three seasons sampled. These data will then be used to explain the community structure and distribution of zooplankton and aquatic macroinvertebrates in following chapters.

3.2 Materials and Methods

The sampling surveys took place in July (winter) 2015, December (summer) 2015, and April (autumn) 2016 to determine seasonal variability in water and sediment. During each survey, water and sediment samples were collected from the sites that were described in Chapter 2.

3.2.1 Water quality

Electrical conductivity (EC), oxygen saturation (%), oxygen content (mg/l), total dissolved solids (TDS), temperature (°C), and pH were recorded *in situ* using pre-calibrated Extech DO600 handheld water quality meters. Water samples were collected in 500 ml polyethylene bottles and frozen immediately for transport to the laboratory. Laboratory analyses consisted of ammonium, chloride, nitrate, nitrite, sulfate, phosphate, and alkalinity. The defrosted samples were analysed using the methodologies set out by the Spectroquant® Pharo 300 and accompanying manual (UV/VIS Spectrophotometer). The following test kits were used to determine the concentrations: ammonium (1.14752.0001); chloride (1.14897.0001); nitrate (1.09713.0001); nitrite (1.14776.0001); sulfate (1.14791.0001); orthophosphate (1.14848.0001); and alkalinity (1.11109.0001) (methods adapted from Vlok *et al.,* 2013).

For metal analyses, 50 ml water was filtered through a 0.45 µm cellulose nitrate filter to remove the suspended particles. The filtered water was analysed for the dissolved metal faction using an Agilent 7500ce inductively coupled plasma mass spectrophotometer (ICP-MS) (methods adapted from Greenfield *et al.,* 2012).

3.2.2 Sediment

Sediment samples were collected *in situ* by filling 500 ml polyethylene, wide-mouthed bottles with a representative fine sediment sample (top 5 cm) from each site. All sediment analysed was based on a single sample collection per survey per site. Each sample was frozen until the analysis could be completed. Once defrosted in the laboratory the sediment was placed in small glass jars and dried for three days at 70 °C.

A fixed mass of sediment was measured off and placed in a Clear Edge Test sieve system. This system consists of six fitted sieves of the following sizes: > 4000 µm (gravel/pebbles); > 2000 µm (very coarse sand/very fine pebbles); > 500 µm (coarse

sand); > 212 µm (medium sand); > 53 µm (very fine sand); and < 53 µm (clay and silt/mud) (USEPA, 1991; Liefferink *et al.,* 2014; Malherbe *et al*., 2015). The sieves and sediment were shaken using a King Test VB 200 300 Sieve Shaker for 20 min. After shaking, the sediment collected in each sieve was weighed and the percentage of the original mass was calculated (USEPA, 1991; Malherbe *et al.*, 2015).

Dried sediment was also used for metal determination. A mass of 0.2 g sediment was weighed and placed in teflon vessels along with 10 ml 65 % nitric acid (Kartal *et al*., 2006). The vessels were then placed in a Milestone Ethos Easy Microwave Digestion System and digested for 30 min at a temperature of 200 °C followed by a 10 min cooling period (methods adapted from Kartal *et al*., 2006 and Malherbe *et al.*, 2015). After digestion, each sediment sample was diluted with 1 % nitric acid to a volume of 50 ml. Thereafter, each sediment sample was filtered through a 0.45 µm cellulose nitrate filter to remove suspended particles. The filtered sediment samples were analysed for metal concentrations using standard ICP-MS techniques (Agilent 7500ce). All solutions were made up using MilliQ Ultrapure water and certified reference materials were used to verify the recovery.

3.2.3 Statistical analyses

The spatial and temporal differences of both water quality, i.e. *in situ*, nutrient and metal concentrations, as well as sediment metal concentrations, were determined using one-way analyses of variance (ANOVA). Data were tested for normality using the Kolmogorov-Smirnov test with $p < 0.05$. If $p < 0.05$, Tukey's *post hoc* statistical analysis test was used to determine significant difference; if $p > 0.05$, the Kruskal-Wallis *post hoc* test was performed (methods adapted from de Klerk *et al.,* 2012). These analyses were completed on Graphpad Prism version 6.

The spatial and temporal character of the water quality, i.e. *in situ*, as well as the nutrient and metal concentrations, were analysed using principle component analyses (PCA) in Canoco version 5 (Malherbe *et al.*, 2015). A PCA plot is used to group variables into factors that explain the correlations among a large number of interrelated quantitative variables (Kartal *et al*., 2006). The plot can summarise many variables by a few factors which can then be interpreted according to the variables' meaning (Kartal *et al*., 2006). In order to determine the degree of variability explained by seasons, a RDA plot was constructed using surveys as explanatory variables.

All sediment data, consisting of grain size and metal concentrations, were used to construct a RDA using Canoco version 5. A RDA plot is a weighted summation ordination method that is used to determine differences in composition at study sites (Malherbe *et al.,* 2010). The RDA (a constrained counterpart of PCA) can provide a clear summary of the underlying structure of the data set which allows the researcher to focus on the variance that is of particular interest (van den Brink *et al*., 2003).

3.3 Results

3.3.1 Water quality

The detailed water quality data are presented in Tables A1 – A2 in Appendix A. These data were used to construct the spatial and temporal graphs showing mean and standard error of metal concentrations within the wetland complex. Furthermore, the data was used to construct a PCA bi-plot and RDA tri-plot representing the spatial and temporal variability in the water quality of the Ntsikeni wetland complex. The spatial variation of metal concentrations throughout the wetland complex was calculated by using the different surveys (July, December, April) as replicates to determine the mean and standard error of each site (Figures 2 – 7). As mentioned under the statistical analyses section, if $p < 0.05$ it is considered to be significant (de Klerk *et al.,* 2012). No significant variables were found between the spatial data of dissolved metal concentrations (i.e. $p > 0.05$) (Figures 2 – 5).

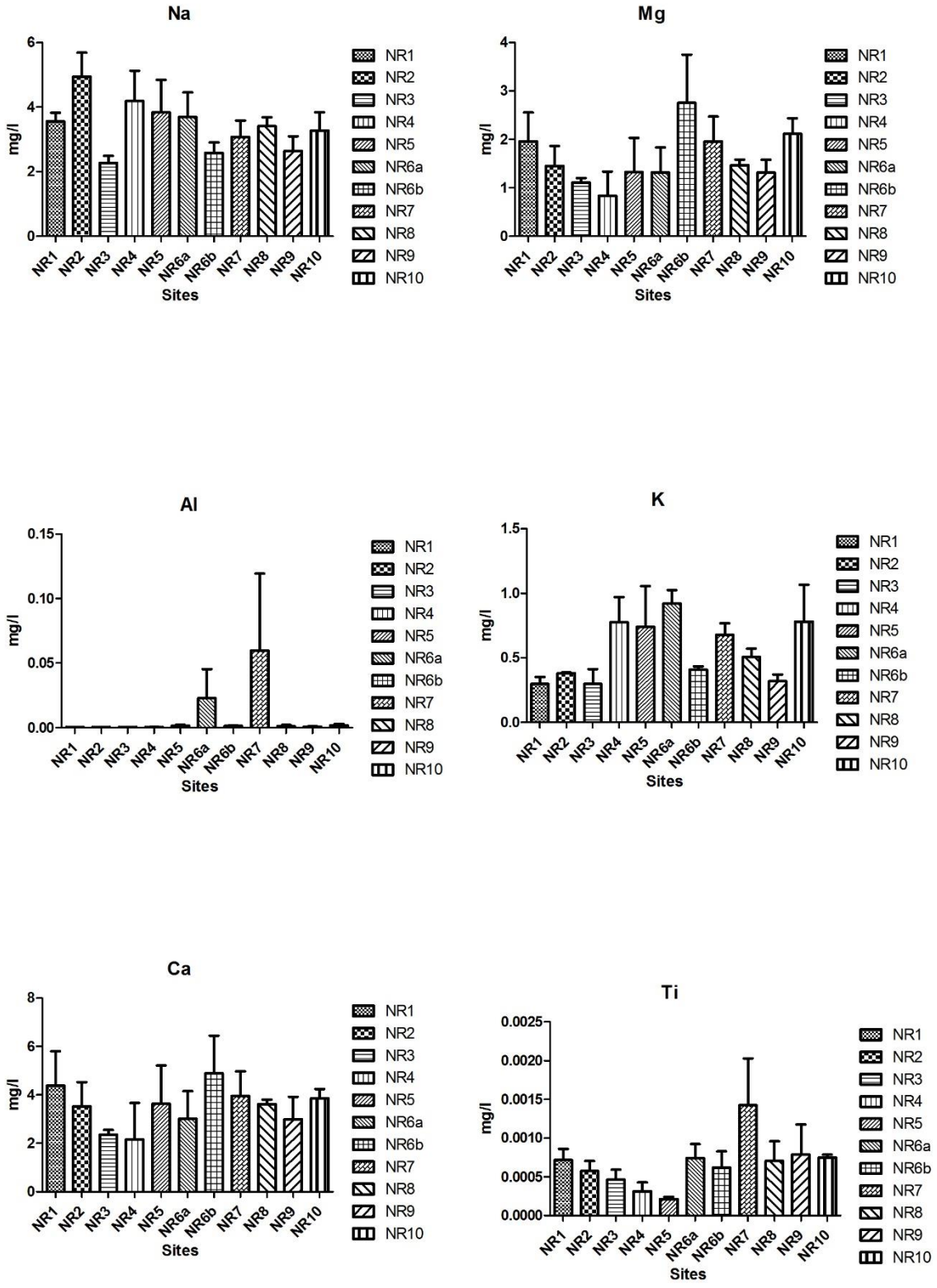

Figure 2: Spatial variation of sodium (Na), magnesium (Mg), aluminium (Al), potassium (K), calcium (Ca), and titanium (Ti) concentrations in the water (mg/l) across the Ntsikeni Nature Reserve's sampling sites (NR1 – NR10). Bars indicate mean concentrations using seasonal surveys as replicates; error bars indicate standard error.

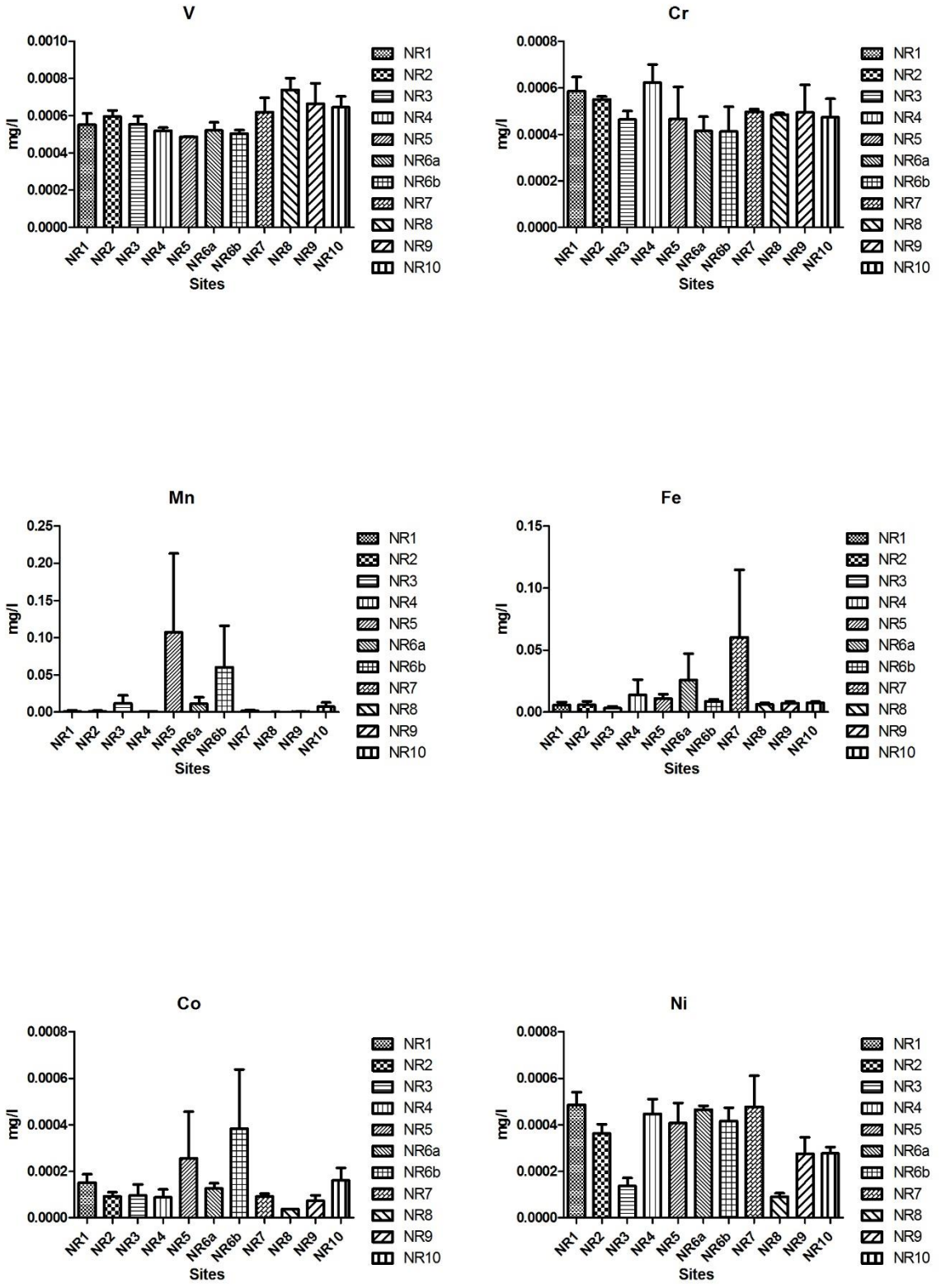

Figure 3: Spatial variation of vanadium (V), chromium (Cr), manganese (Mn), iron (Fe), cobalt (Co), and nickel (Ni) concentrations in the water (mg/l) across the Ntsikeni Nature Reserve's sampling sites (NR1 – NR10). Bars indicate mean concentrations using seasonal surveys as replicates; error bars indicate standard error.

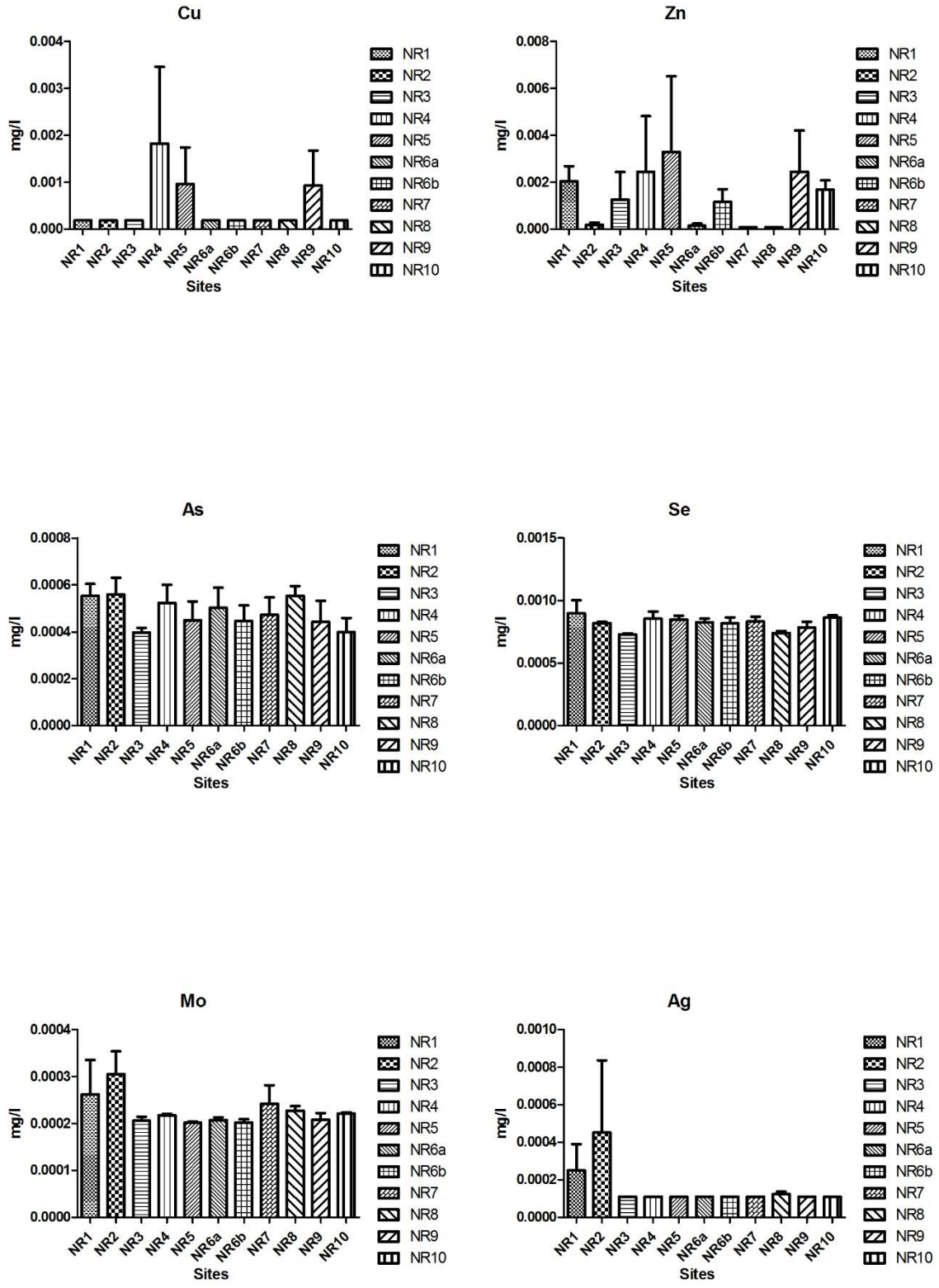

Figure 4: Spatial variation of copper (Cu), zinc (Zn), arsenic (As), selenium (Se), molybdenum (Mo), and silver (Ag) concentrations in the water (mg/l) across the Ntsikeni Nature Reserve's sampling sites (NR1 – NR10). Bars indicate mean concentrations using seasonal surveys as replicates; error bars indicate standard error.

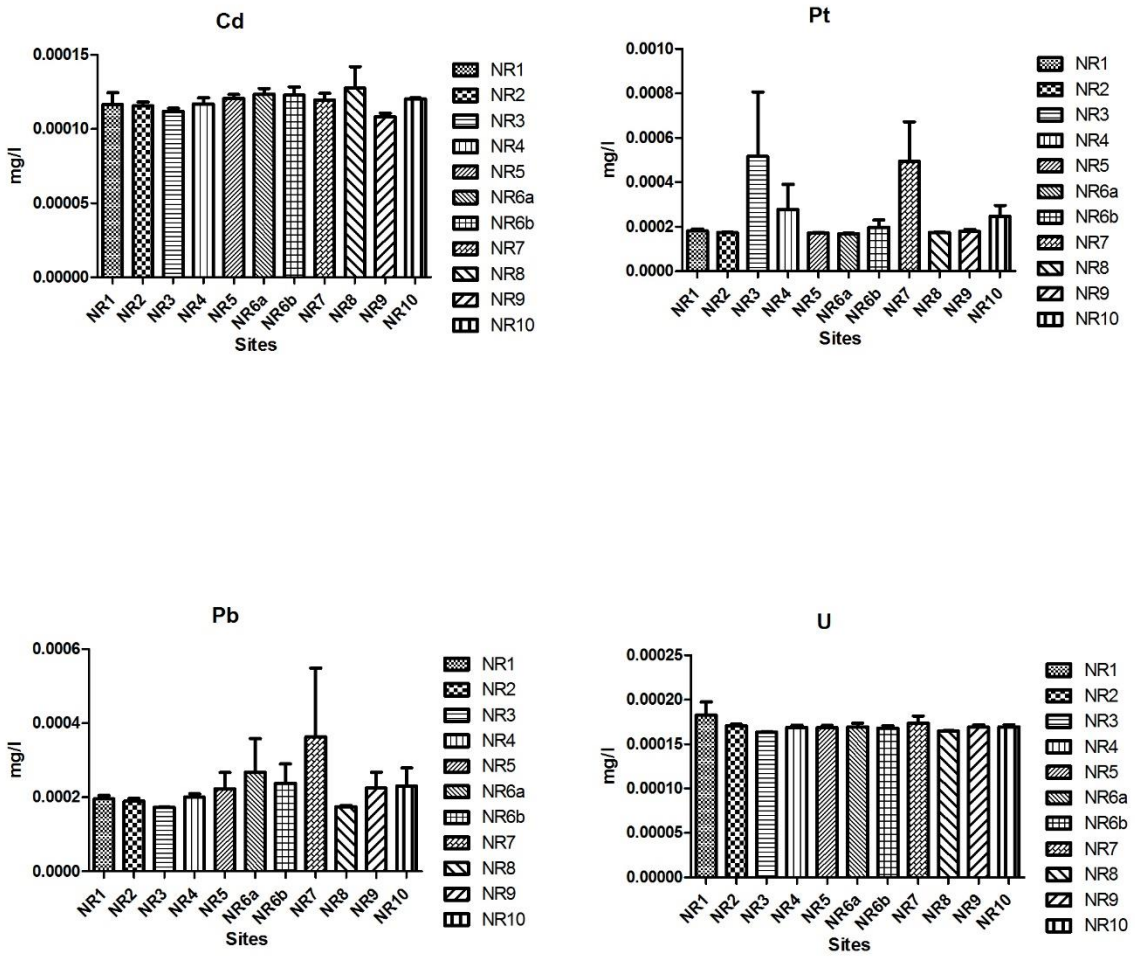

Figure 5: Spatial variation of cadmium (Cd), platinum (Pt), lead (Pb), and uranium (U) concentrations in the water (mg/l) across the Ntsikeni Nature Reserve's sampling sites (NR1 – NR10). Bars indicate mean concentrations using seasonal surveys as replicates; error bars indicate standard error.

By using ANOVA, the spatial variation of both physico-chemical and nutrient concentrations throughout the wetland complex was calculated using the different surveys (July, December, April) as replicates to determine the mean and standard error of each site (Figures 6 – 7). Once again, no significant differences were found to exist on a spatial scale between the different sites (Figures 6 – 7).

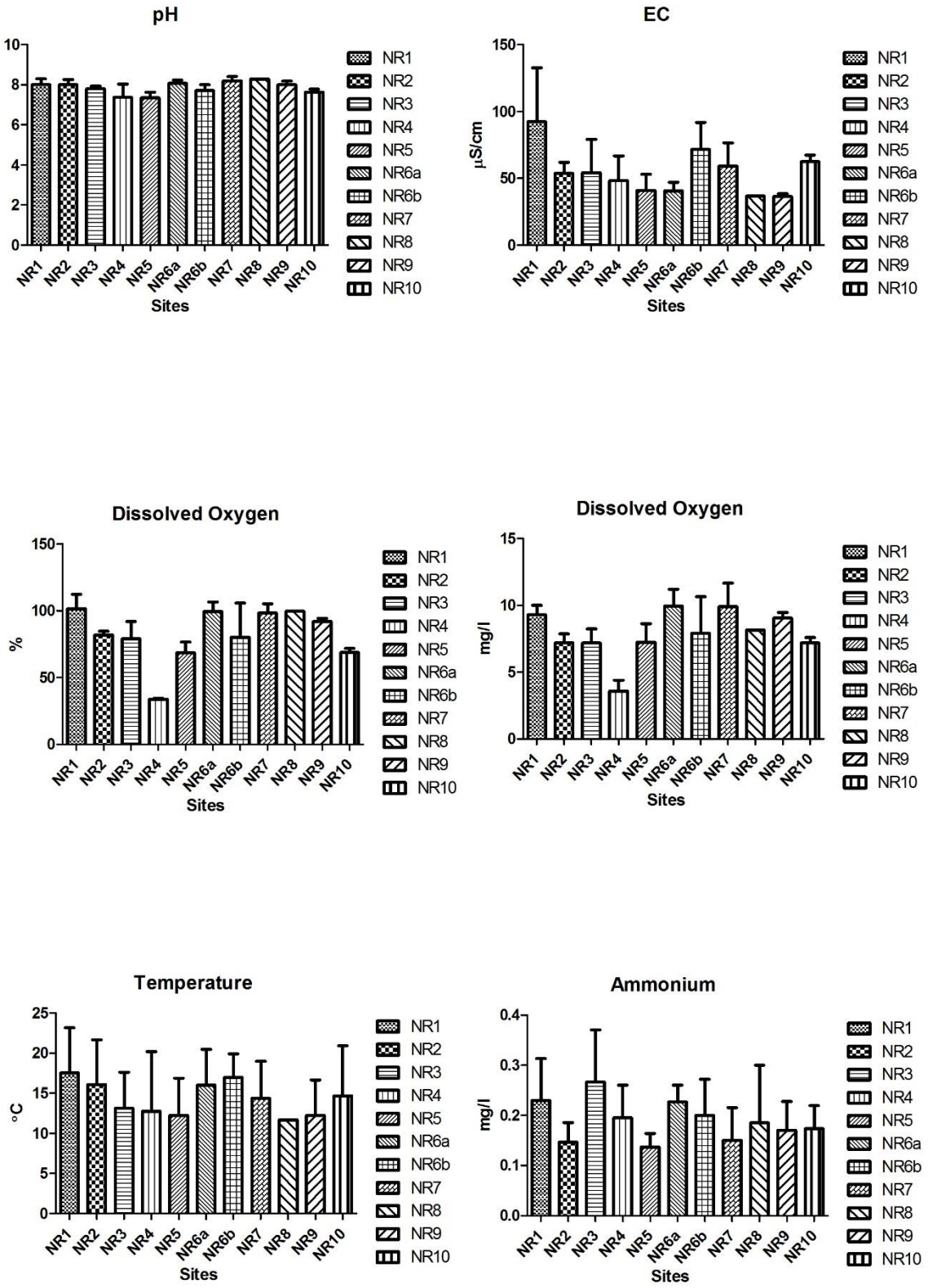

Figure 6: Spatial variation of physico-chemical and nutrient concentrations in the water across the Ntsikeni Nature Reserve's sampling sites (NR1 – NR10). Bars indicate mean values using seasonal surveys as replicates; error bars indicate standard error. EC – electrical conductivity.

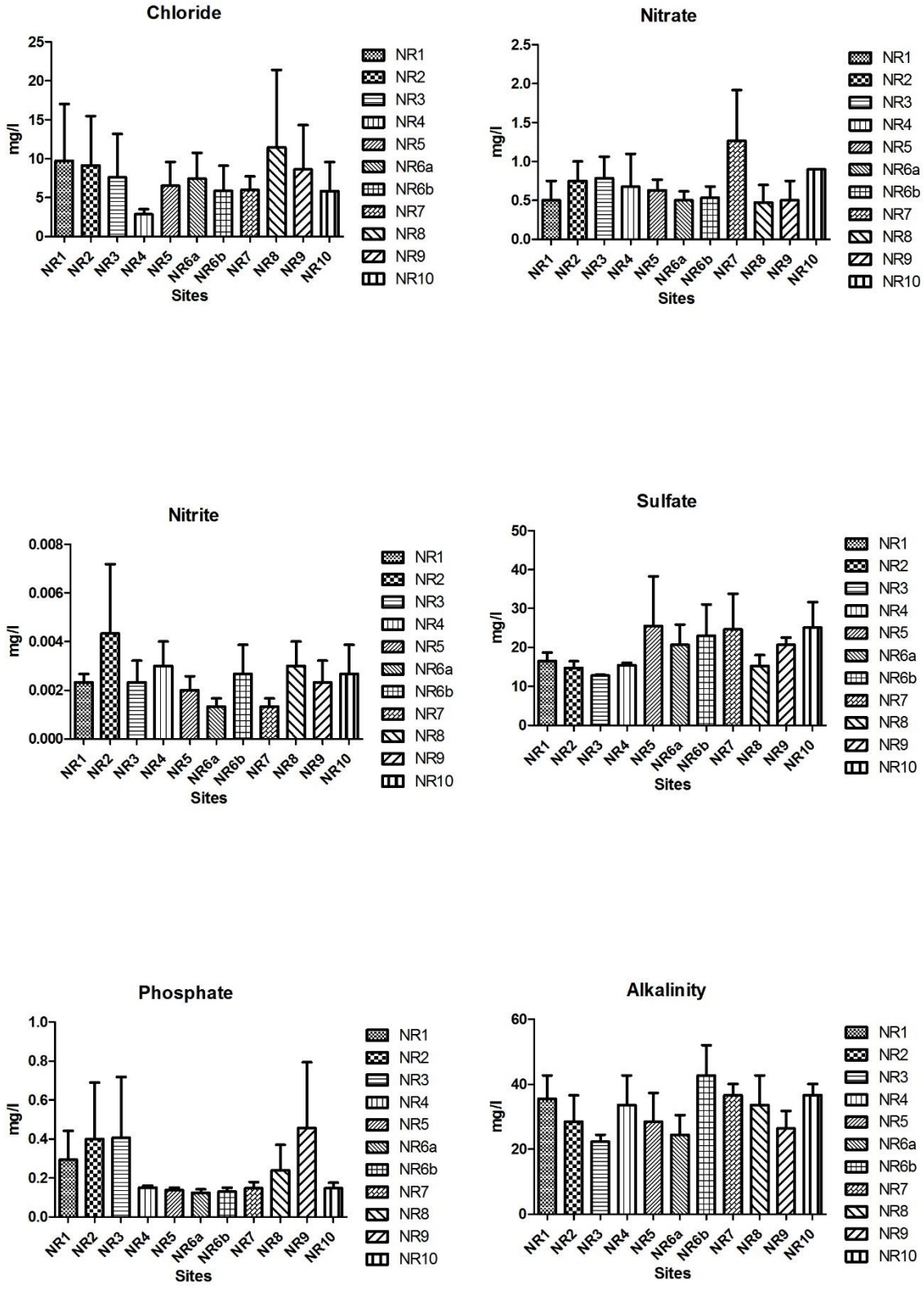

Figure 7: Spatial variation of nutrient concentrations in the water (mg/l) across the Ntsikeni Nature Reserve's sampling sites (NR1 – NR10). Bars indicate mean concentrations using seasonal surveys as replicates; error bars indicate standard error.

Water quality data of the Ntsikeni wetland complex was used to determine if there were significant differences between sites (spatial). In addition, further ANOVA tests were performed to determine if significant differences existed between different seasons (temporal). The temporal variation of metal concentrations, physico-chemicals, and nutrients throughout the wetland complex was calculated by using the different sites (NR1 – NR10) as replicates to determine the mean and standard error of each survey (Figures 8 – 13). With regards to the temporal variation, certain variables showed significant differences to exist during different surveys.

Sodium (Na), magnesium (Mg), aluminium (Al), calcium (Ca), chromium (Cr), manganese (Mn), arsenic (As), selenium (Se), and lead (Pb) showed significant differences ($p < 0.05$) between the winter (July) and summer (December) surveys (Figures 8 – 11). Sodium (Na), Mg, Ca, Cr, cobalt (Co), As, Se, Pb, and uranium (U) indicated significant differences between the winter (July) and autumn (April) surveys (Figures 8 – 11). Only Mn indicated significant difference between the summer (December) and autumn (April) survey (Figure 9). No other metal dissolved in the water indicated significant differences during the rainy months (December and April).

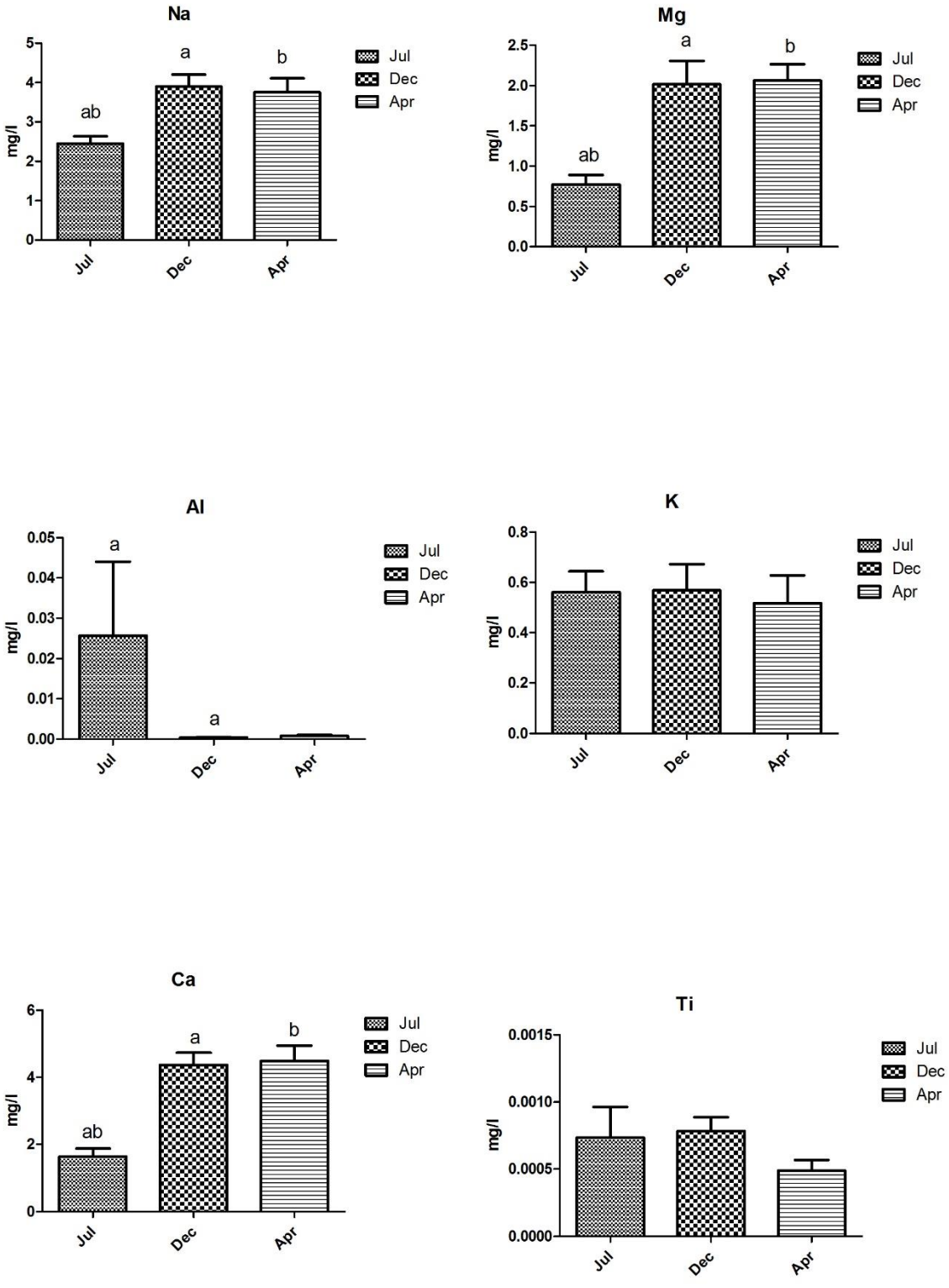

Figure 8: Temporal variations (July/winter, December/summer, and April/autumn) of sodium (Na), magnesium (Mg), aluminium (Al), potassium (K), calcium (Ca), and titanium (Ti) concentrations dissolved in the water (mg/l). Bars indicate mean concentrations using different sites as replicates; error bars indicate standard error. Bars with common superscript differ significantly ($p < 0.05$).

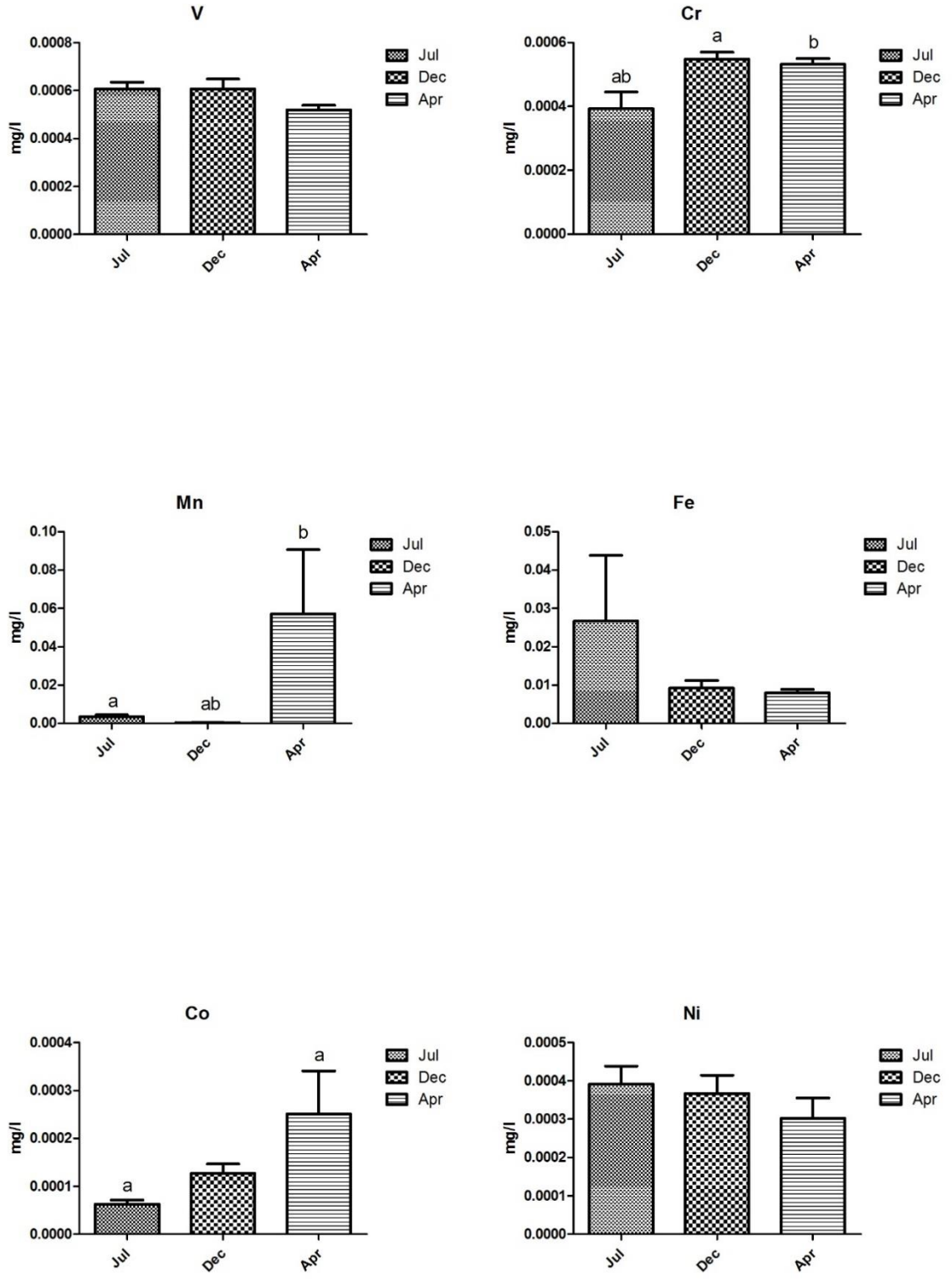

Figure 9: Temporal variations (July/winter, December/summer, and April/autumn) of vanadium (V), chromium (Cr), manganese (Mn), iron (Fe), cobalt (Co), and nickel (Ni) concentrations dissolved in the water (mg/l). Bars indicate mean concentrations using different sites as replicates; error bars indicate standard error. Bars with common superscript differ significantly ($p < 0.05$).

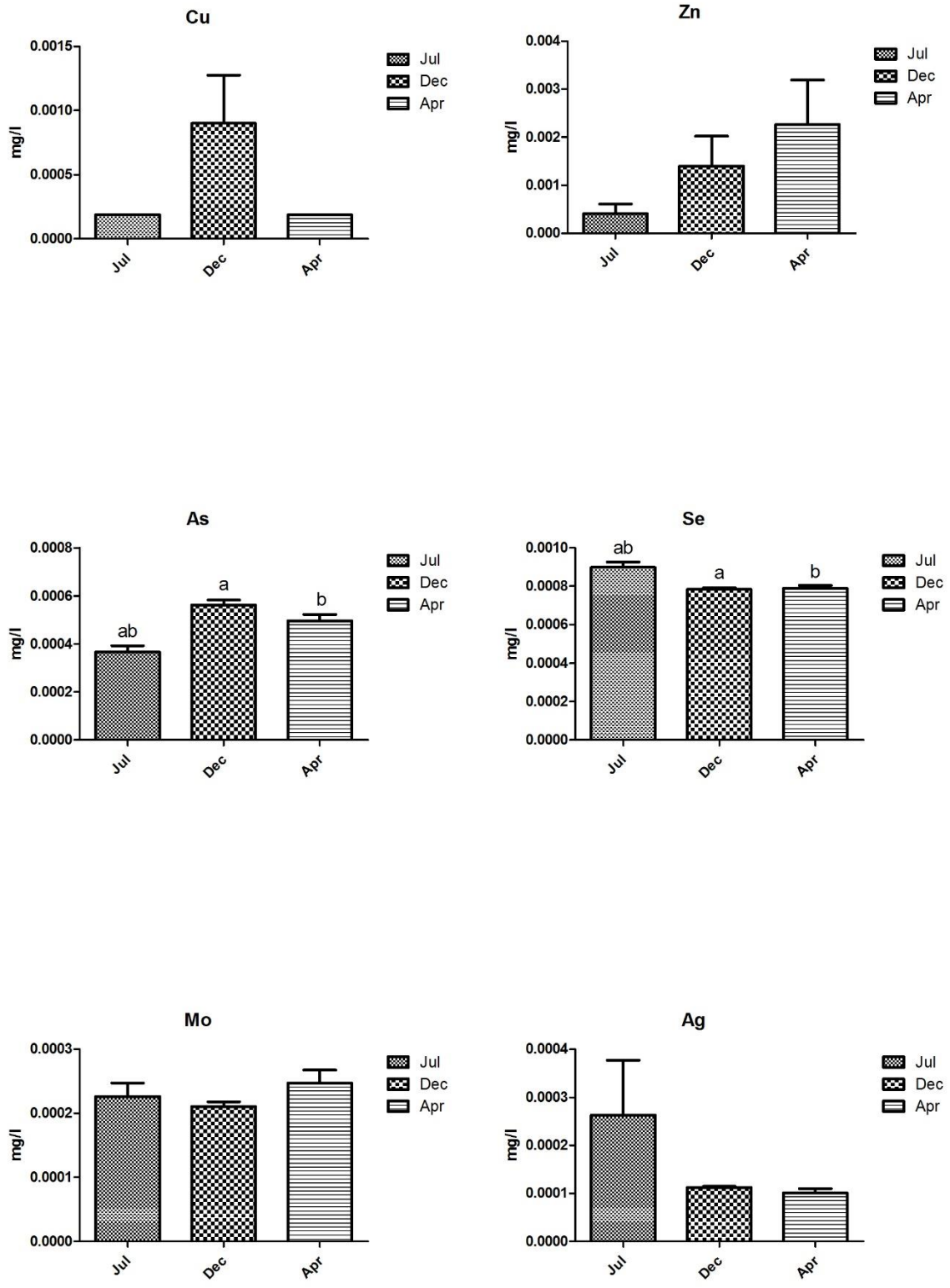

Figure 10: Temporal variations (July/winter, December/summer, and April/autumn) of copper (Cu), zinc (Zn), arsenic (As), selenium (Se), molybdenum (Mo), and silver (Ag) concentrations dissolved in the water (mg/l). Bars indicate mean concentrations using different sites as replicates; error bars indicate standard error. Bars with common superscript differ significantly ($p < 0.05$).

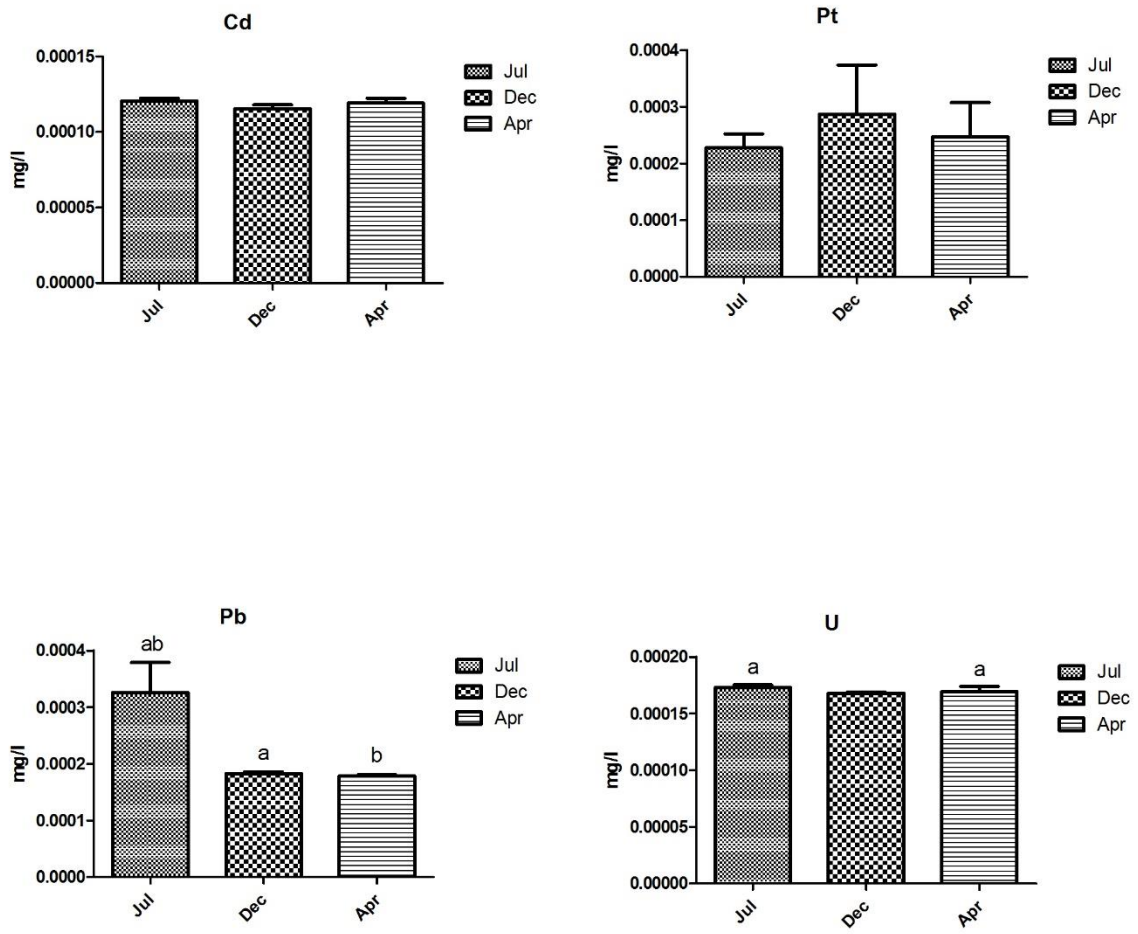

Figure 11: Temporal variations (July/winter, December/summer, and April/autumn) of cadmium (Cd), platinum (Pt), lead (Pb), and uranium (U) concentrations dissolved in the water (mg/l). Bars indicate mean concentrations using different sites as replicates; error bars indicate standard error. Bars with common superscript differ significantly ($p < 0.05$).

The temporal variations were calculated for both physico-chemical and nutrient concentrations across the different seasons (Figures 12 – 13). With regards to the July (winter) and December (summer) surveys dissolved oxygen (mg/l), temperature, and alkalinity were the only variables to indicate significant differences ($p < 0.05$) (Figures 12 – 13). Ammonium, chloride, nitrate, phosphate, and alkalinity all indicated significant differences between the July (winter) and April (autumn) survey (Figures 12 – 13). Between the December (summer) and April (autumn) surveys temperature, ammonium, chloride, nitrite, sulfate, and phosphate indicated significant differences in values (Figures 12 – 13).

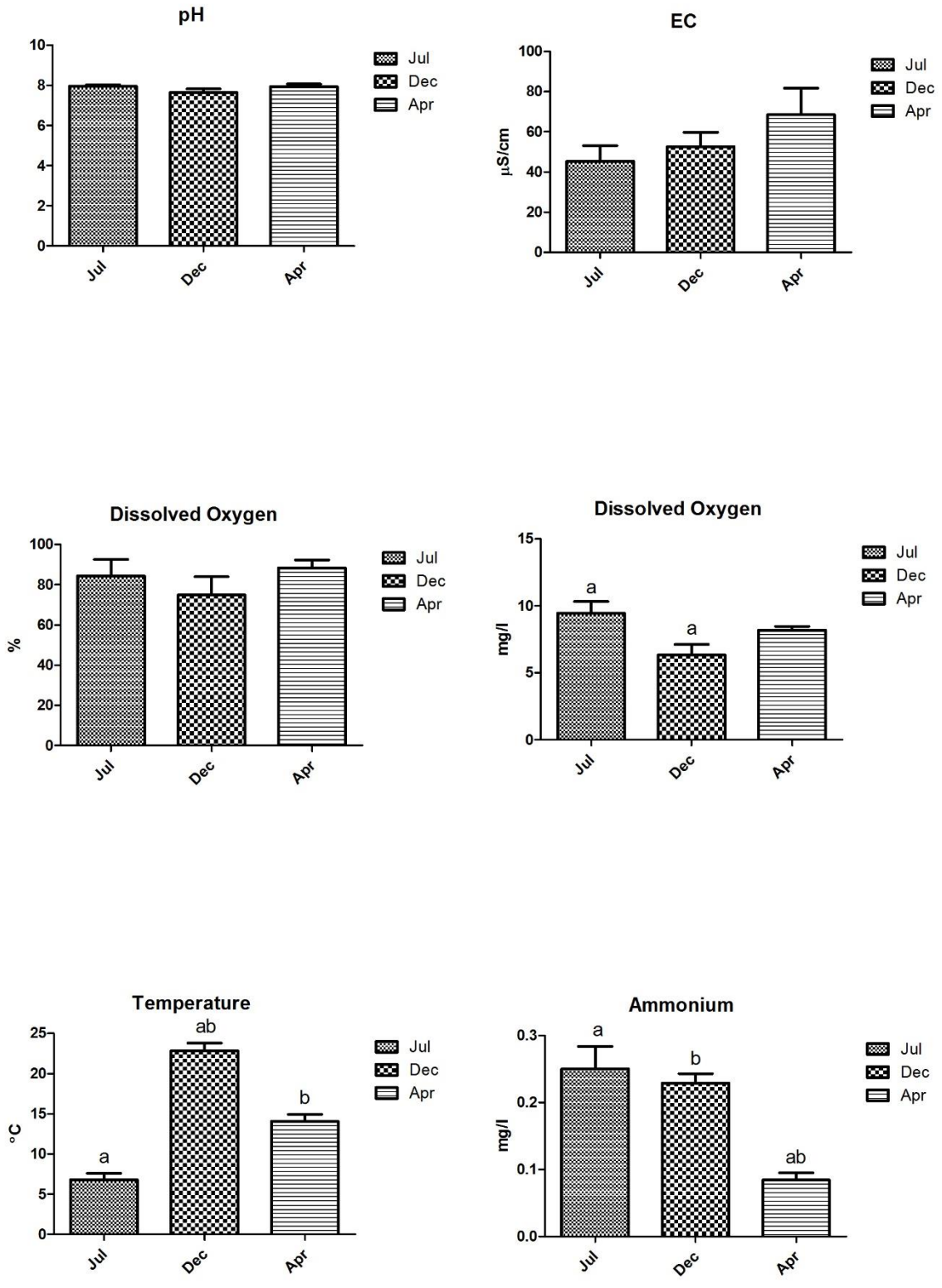

Figure 12: Temporal variation (July/winter, December/summer, and April/autumn) of physico-chemical and nutrient concentrations in the water. Bars indicate mean concentration using different sites as replicates; error bars indicate standard error. Bars with common superscript differ significantly ($p < 0.05$). EC – electrical conductivity.

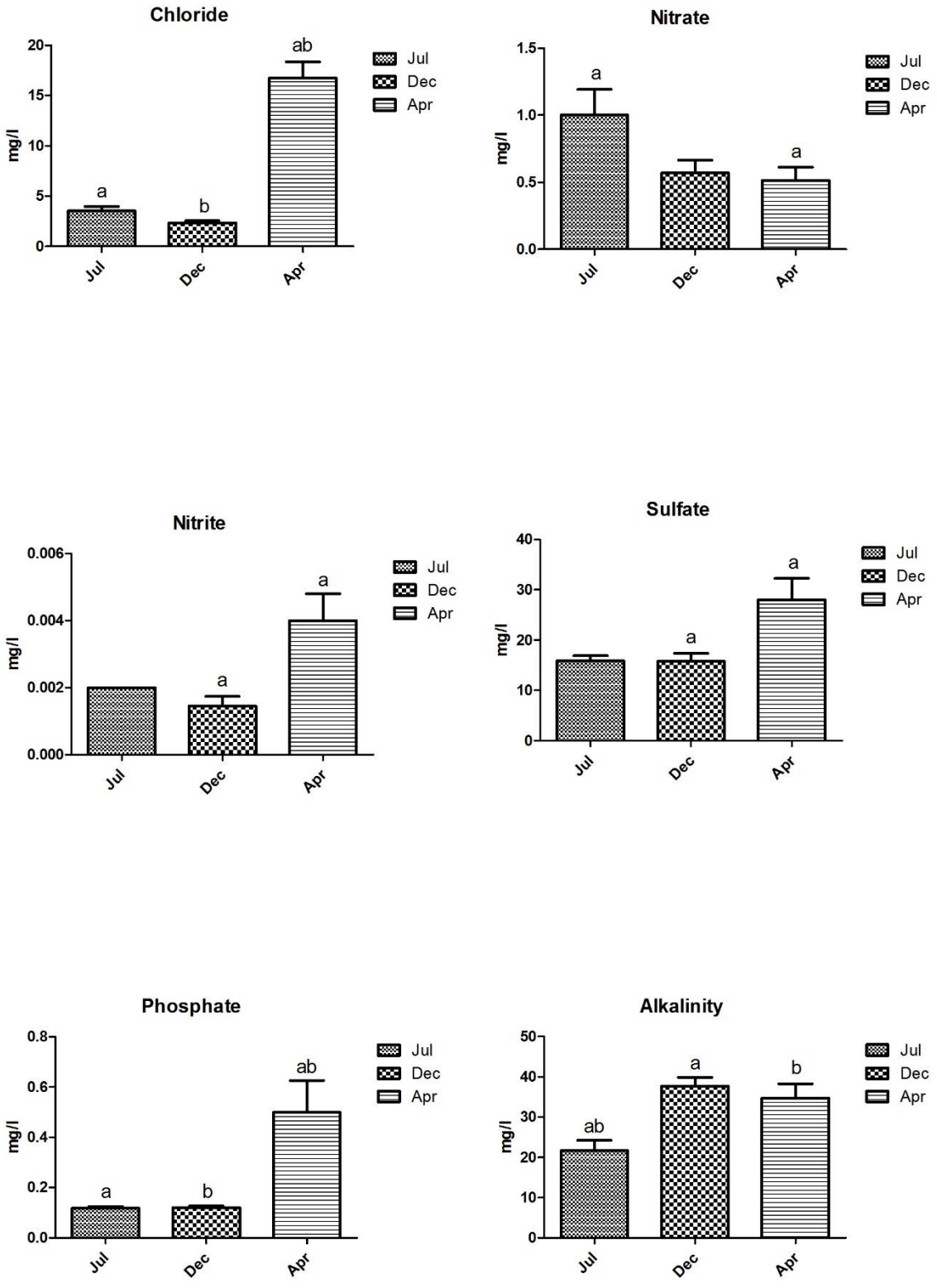

Figure 13: Temporal variation (July/winter, December/summer, and April/autumn) of nutrient concentrations in the water (mg/l). Bars indicate mean concentrations using different sites as replicates; error bars indicate standard error. Bars with common superscript differ significantly ($p < 0.05$).

The PCA bi-plot (Figure 14) explained 67.07% of the variation on the first and second axis. The first axis explained variation of 38.47% while the second axis explained 28.6 % of the variation. Groupings around seasonality emerged since sites sampled during the July (winter) survey (indicated in blue) were grouped together in the upper and lower right quadrants. December (summer) sites (indicated in green) were grouped together in the lower two quadrants, while the April (autumn) surveys (indicated in brown) were grouped together as well. The highest concentrations of water quality variables were located in the vicinity of the summer (December) and autumn (April) surveys; possibly due to the increase in runoff during the rainy seasons. The variables with the greatest influence were Mg and Ca, both having their highest values measured in December (summer) and April (autumn) (Appendix Table A1). These variables were closely followed by strontium (Sr), EC, and alkalinity (Figure 14). During the April (autumn) survey, chloride and antimony (Sb) had the greatest influence, followed by nitrite and phosphate (Figure 14). During the July/December (winter/summer) surveys, ammonium was found to have the greatest influence.

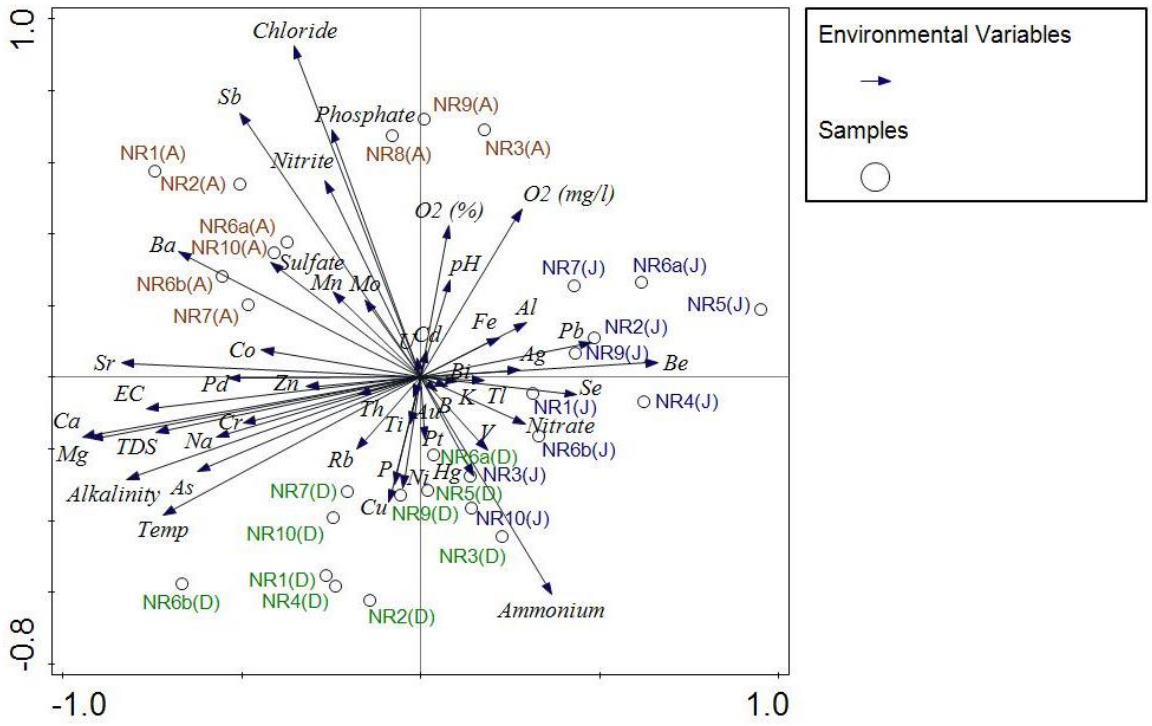

Figure 14: A principle component analysis (PCA) bi-plot showing relationships between sites (NR1 – NR10), seasons and water quality variables. The seasons are coloured to show groupings, with the July/winter (J) survey indicated in blue, the December/summer (D) survey indicated in green, and the April/autumn (A) survey indicated in brown. Water quality variables consist of metal concentrations and physico-chemical concentrations.

In order to ascertain the degree of variability explained by season, a RDA tri-plot was constructed. The RDA tri-plot (Figure 15) explained 51.95 % of the cumulative variation on the first and second axes. The first axis explained variation of 29.91 % while the second axis explained variation of 22.04 %. The RDA tri-plot is constructed so that all sites are grouped together with their respective seasons. The information displayed in Figure 15 concurred with the PCA bi-plot (Figure 14) in that the highest concentrations of water quality variables were located in the vicinity of the December (summer) and April (autumn) surveys. Also chloride, Sb, nitrite, and phosphate had the greatest influence during the April (autumn) survey, while ammonium had its greatest influence during the July (winter) and December (summer) surveys.

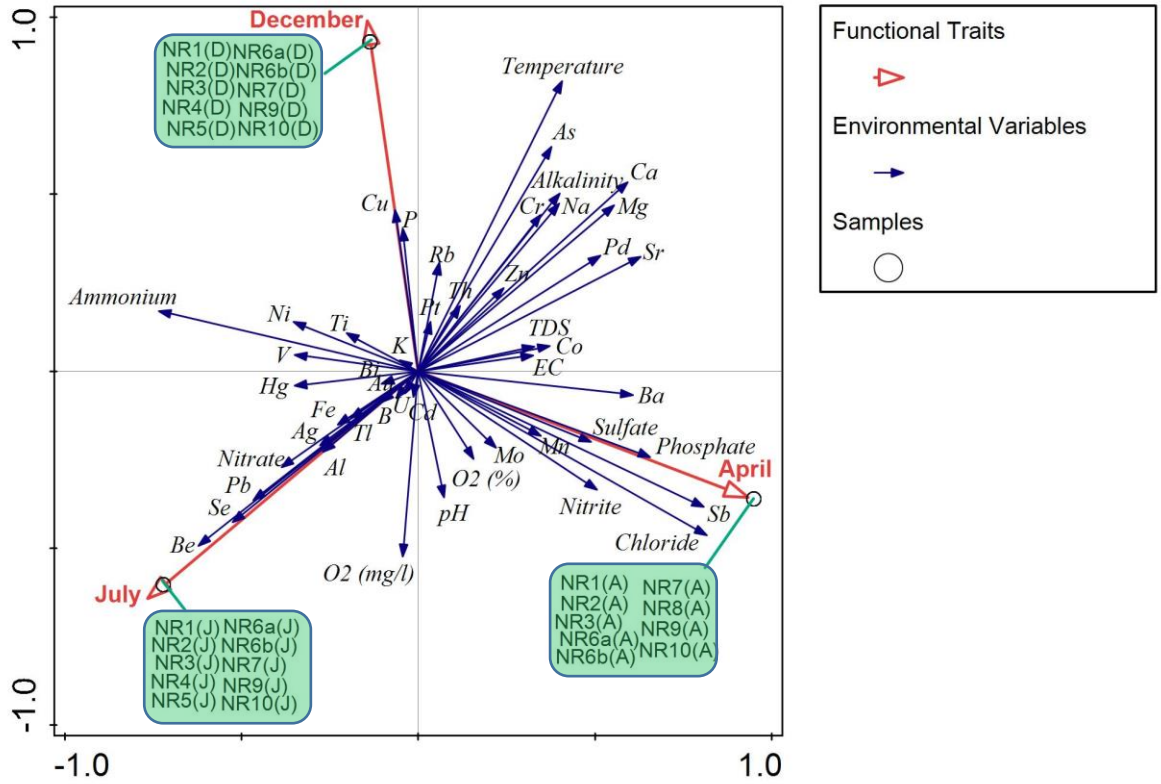

Figure 15: A redundancy analysis (RDA) tri-plot showing relationships between sites (NR1 – NR10), seasons, and water quality variables. Seasons consist of surveys conducted in July/winter (J), December/summer (D), and April/autumn (A). Water quality variables consist of *in situ* data, nutrient concentrations, and metal concentrations.

3.3.2 Sediment

The detailed sediment data are presented in Tables A3 – A4 in Appendix A. These data were used to construct the spatial and temporal graphs showing mean and standard error of metal concentrations within the wetland sediments, as well as to construct a RDA tri-plot representing the spatial variability of metal concentrations and grain sizes in the sediment of the Ntsikeni wetland complex.

The spatial variations of metal concentrations within the sediments of the wetland complex were calculated by using the different surveys (July, December, April) as replicates in order to determine the mean and standard error of each site (Figures 16 – 19). According to the statistical analyses, if $p < 0.05$ it is considered to be significant (de Klerk *et al.,* 2012). Only the metals Al, potassium (K), and zinc (Zn) indicated any significant differences. Aluminium (Al) indicated significant differences between sites NR3 and NR6a as well as between sites NR6a and NR10 (Figure 16). Both K and Zn indicated significant differences between sites NR1 and NR9 (Figures 16 and 18).

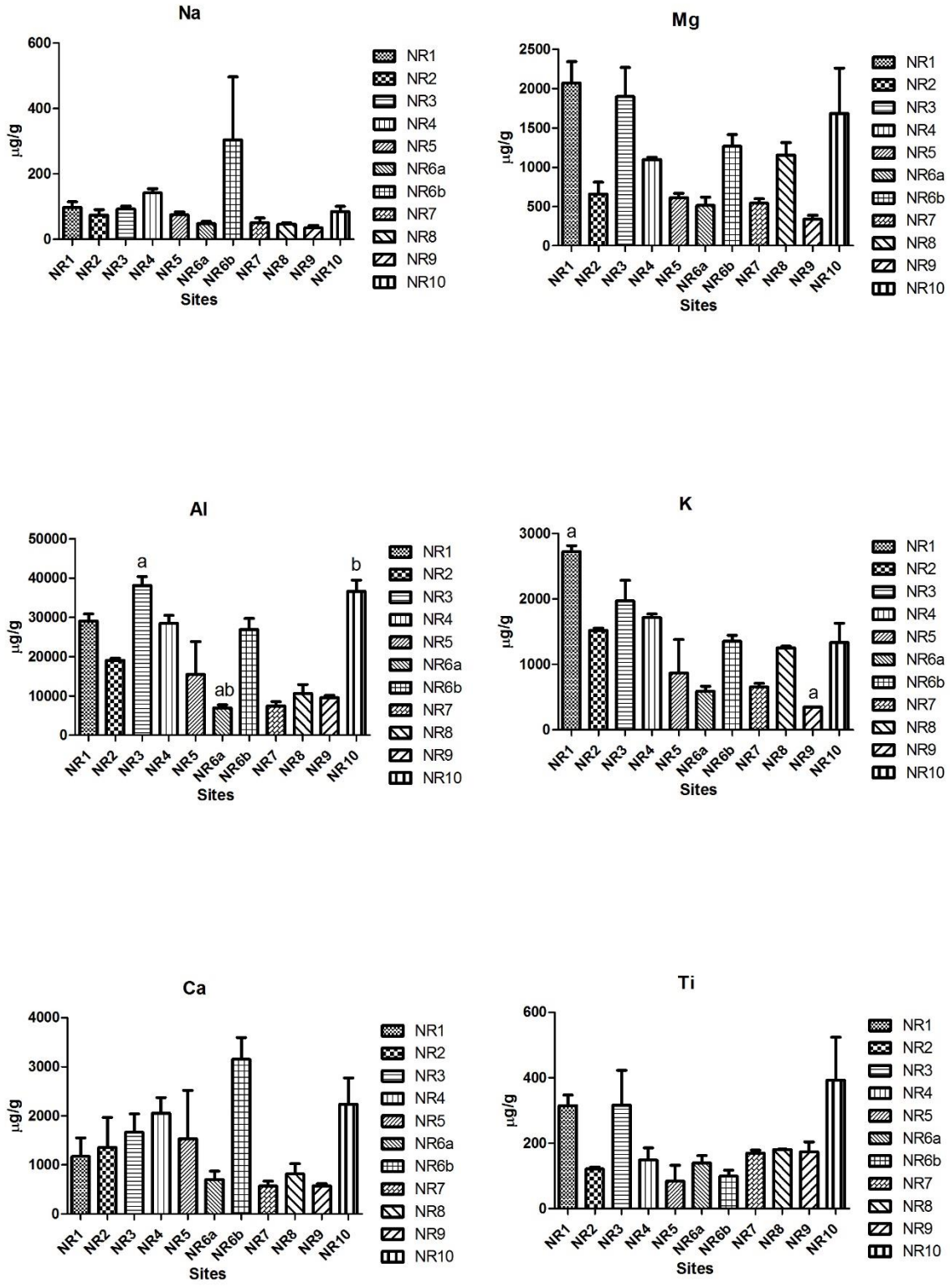

Figure 16: Spatial variation of sodium (Na), magnesium (Mg), aluminium (Al), potassium (K), calcium (Ca), and titanium (Ti) concentrations in the sediment (µg/g) across the Ntsikeni Nature Reserve's sampling sites (NR1 – NR10). Bars indicate mean concentrations using seasonal surveys as replicates; error bars indicate standard error. Bars with common superscript differ significantly ($p < 0.05$).

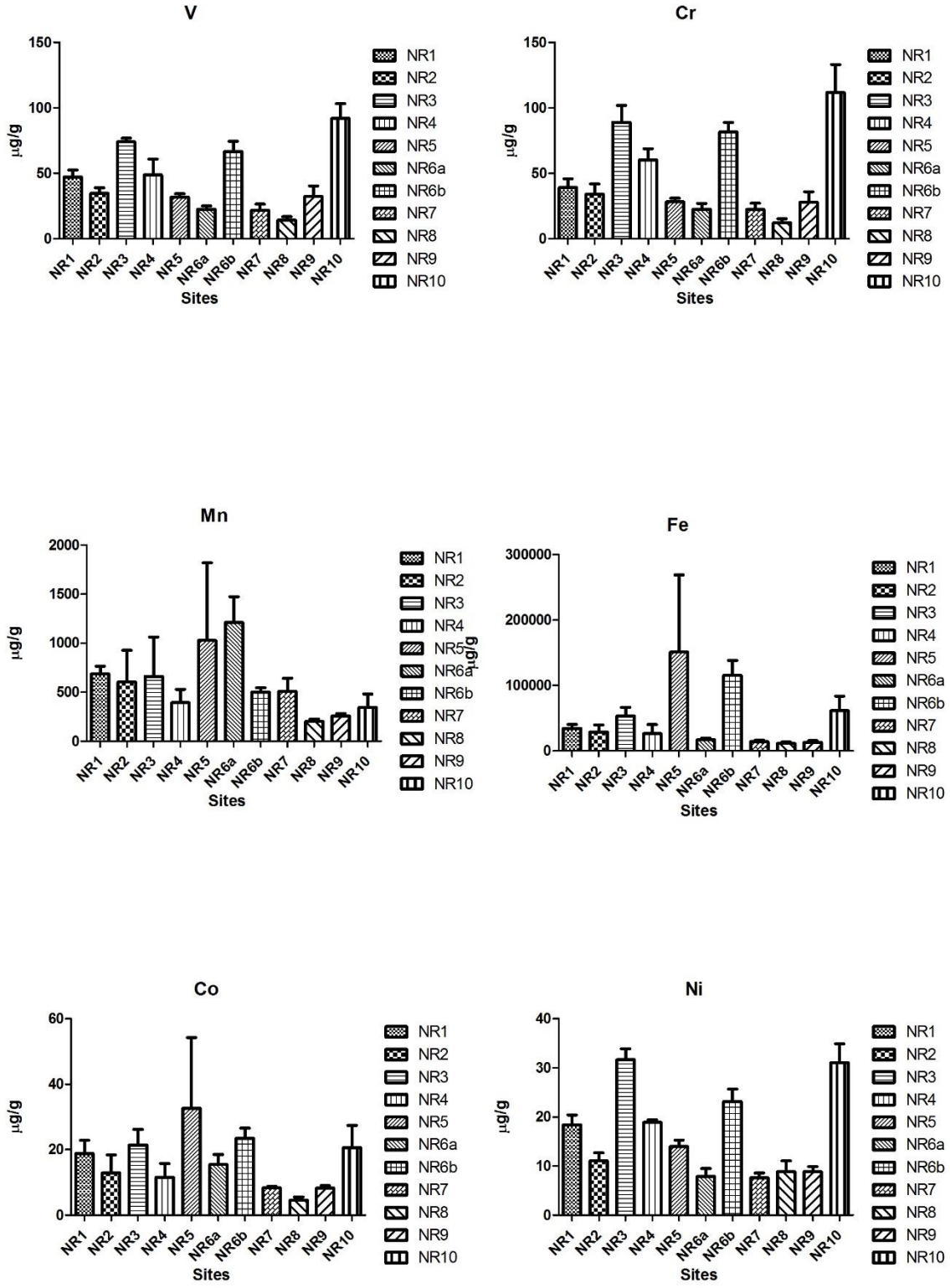

Figure 17: Spatial variation of vanadium (V), chromium (Cr), manganese (Mn), iron (Fe), cobalt (Co), and nickel (Ni) concentrations in the sediment (µg/g) across the Ntsikeni Nature Reserve's sampling sites (NR1 – NR10). Bars indicate mean concentrations using seasonal surveys as replicates; error bars indicate standard error. Bars with common superscript differ significantly ($p < 0.05$).

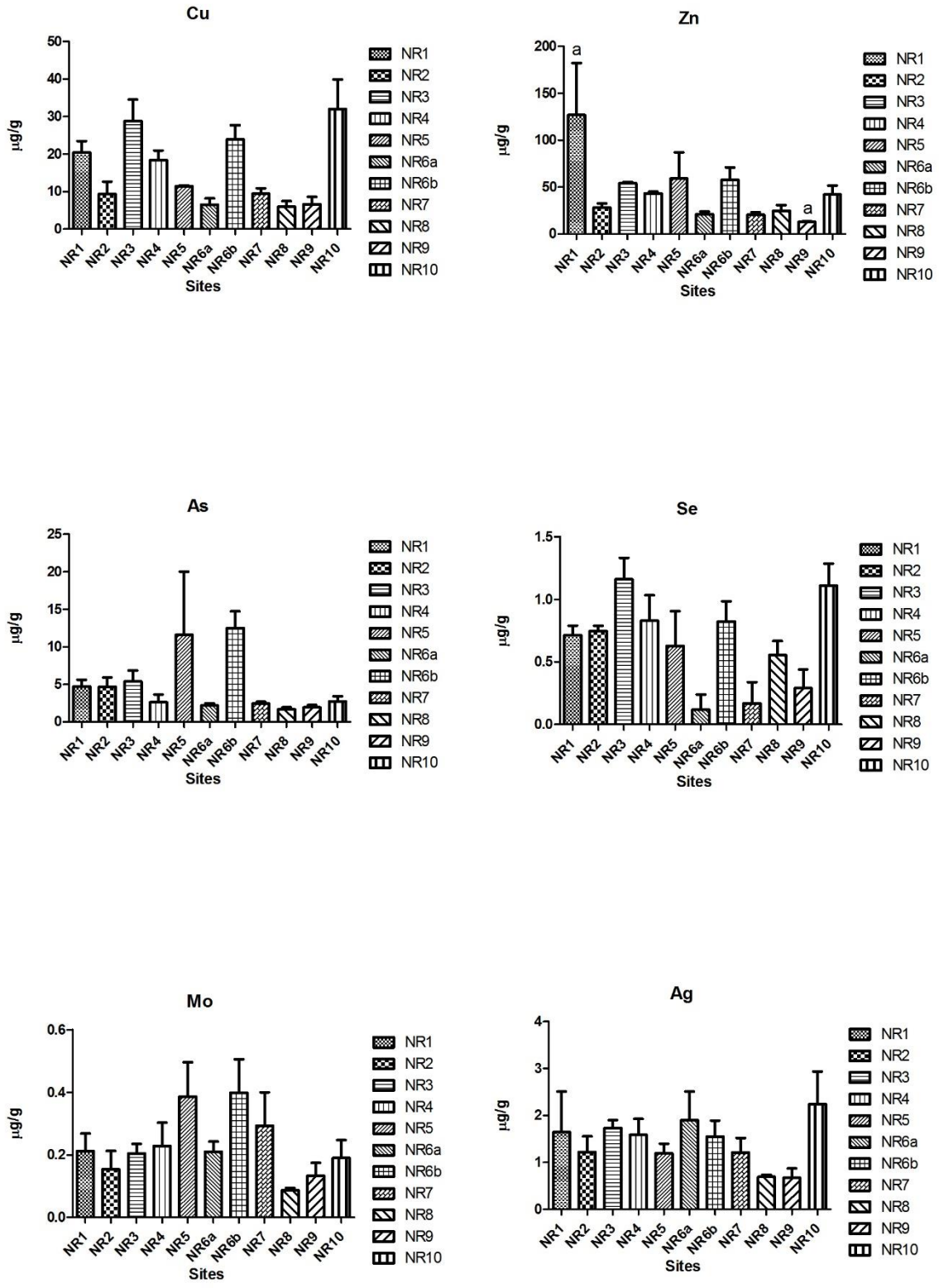

Figure 18: Spatial variation of copper (Cu), zinc (Zn), arsenic (As), selenium (Se), molybdenum (Mo), and silver (Ag) concentrations in the sediment (µg/g) across the Ntsikeni Nature Reserve's sampling sites (NR1 – NR10). Bars indicate mean concentrations using seasonal surveys as replicates; error bars indicate standard error. Bars with common superscript differ significantly ($p < 0.05$).

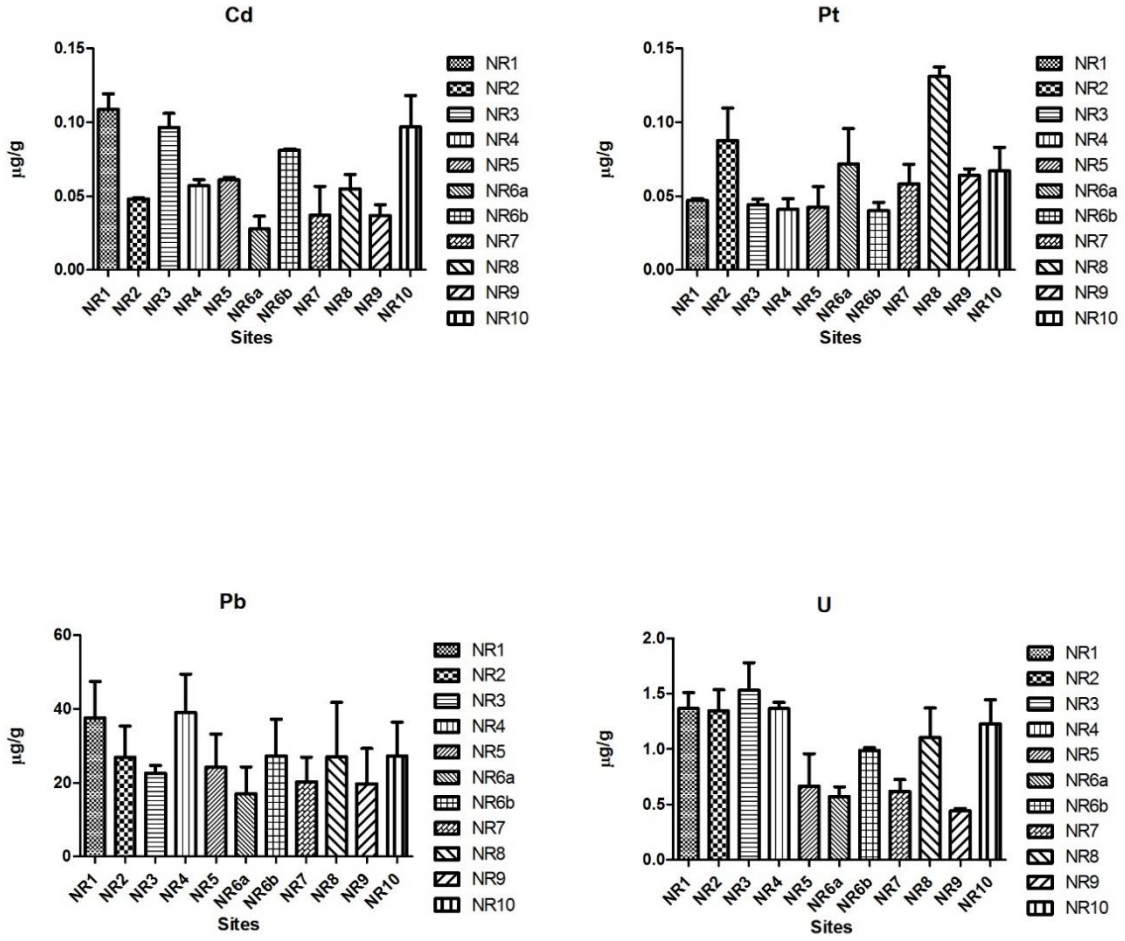

Figure 19: Spatial variation of cadmium (Cd), platinum (Pt), lead (Pb), and uranium (U) concentrations in the sediment (µg/g) across the Ntsikeni Nature Reserve's sampling sites (NR1 – NR10). Bars indicate mean concentrations using seasonal surveys as replicates; error bars indicate standard error. Bars with common superscript differ significantly ($p < 0.05$).

Temporal variations of metal concentrations in sediment throughout the wetland complex were calculated to show variation between different seasons using the different sites' data (NR1 – NR10) as replicates to determine the mean and standard error of each survey (Figures 20 – 23). Between the July (winter) and April (autumn) surveys Na, Co, molybdenum (Mo), silver (Ag), and Pb indicated significant differences (Figures 20 – 23). July (winter) is the dry season in KZN while rainfall was experienced during the April (autumn) survey. Metal compounds bind to sediment particles, which are mobalised by water flow (Greenfield *et al*., 2007). Thus, rainfall could explain the significant differences found. When comparing the July (winter) and December (summer) surveys, only the metals Mo and Pb indicated significant differences (Figures 22 – 23). No metal concentrations showed significant differences between the December (summer) and April (autumn) surveys.

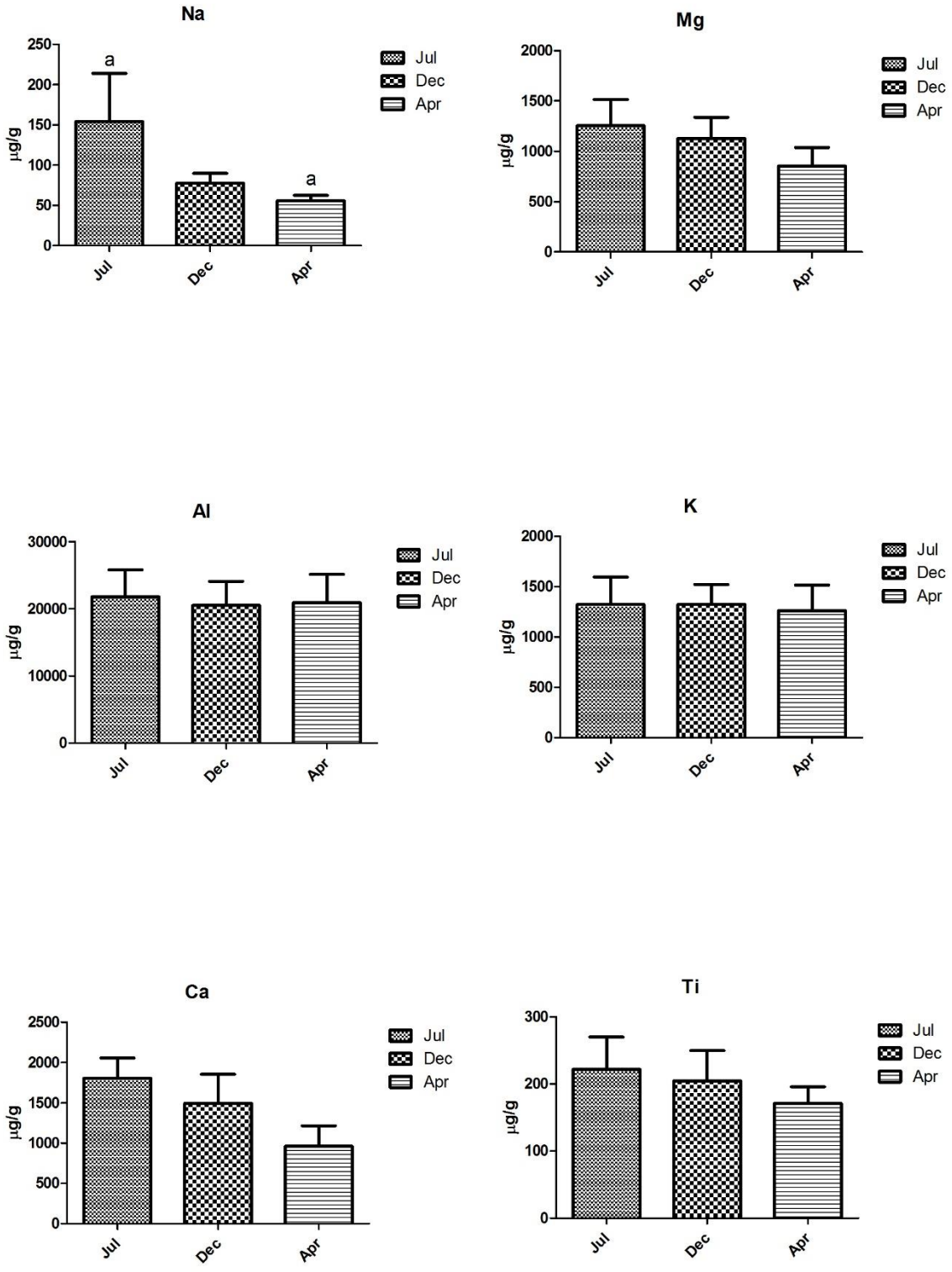

Figure 20: Temporal variation (July/winter, December/summer, April/autumn) of sodium (Na), magnesium (Mg), aluminium (Al), potassium (K), calcium (Ca), and titanium (Ti) concentrations in the sediment (µg/g). Bars indicate mean concentrations using different sites as replicates; error bars indicate standard error. Bars with common superscript differ significantly ($p < 0.05$).

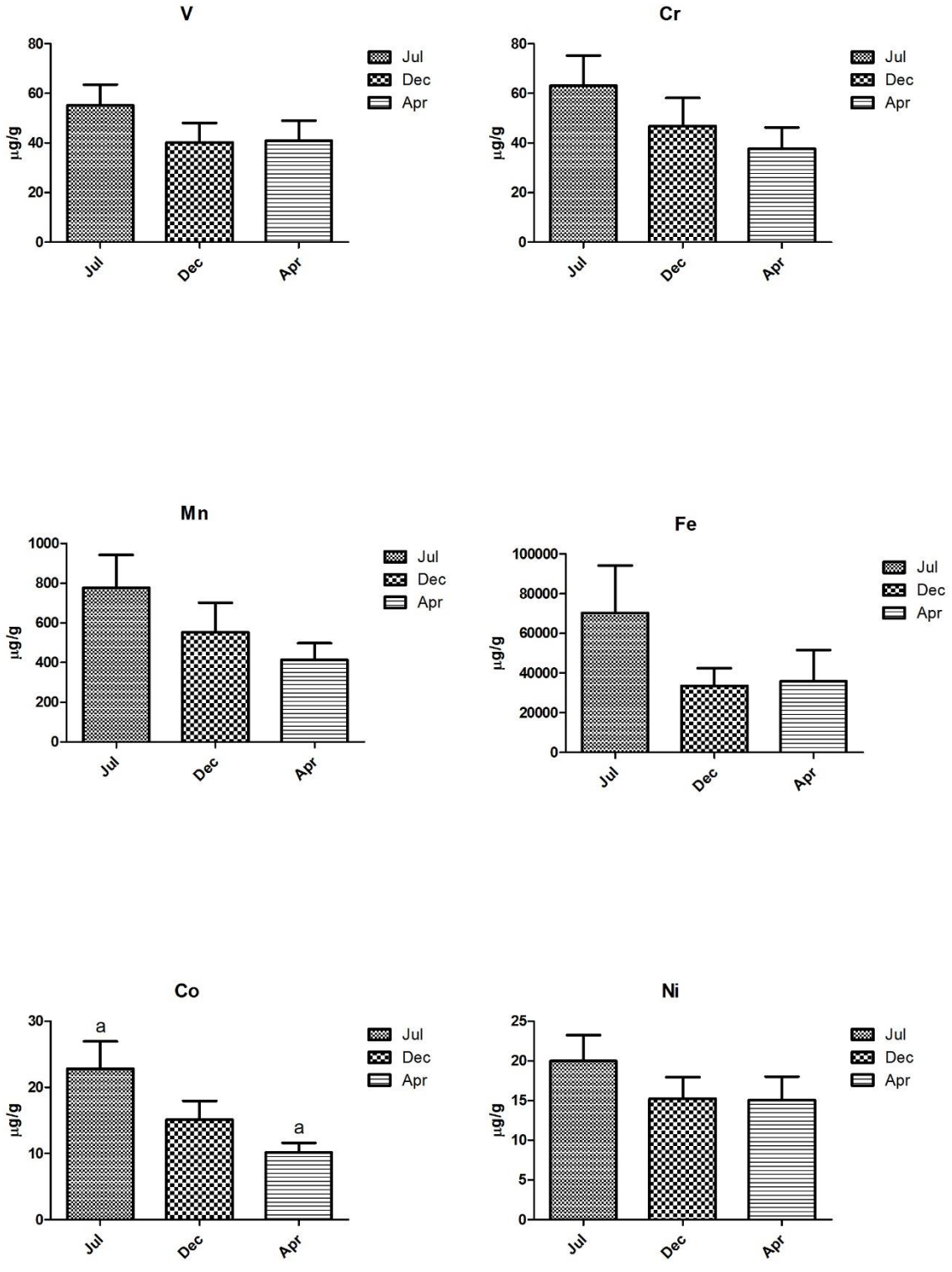

Figure 21: Temporal variation (July/winter, December/summer, April/autumn) of vanadium (V), chromium (Cr), manganese (Mn), iron (Fe), cobalt (Co), and nickel (Ni) concentrations in the sediment (µg/g). Bars indicate mean concentrations using different sites as replicates; error bars indicate standard error. Bars with common superscript differ significantly ($p < 0.05$).

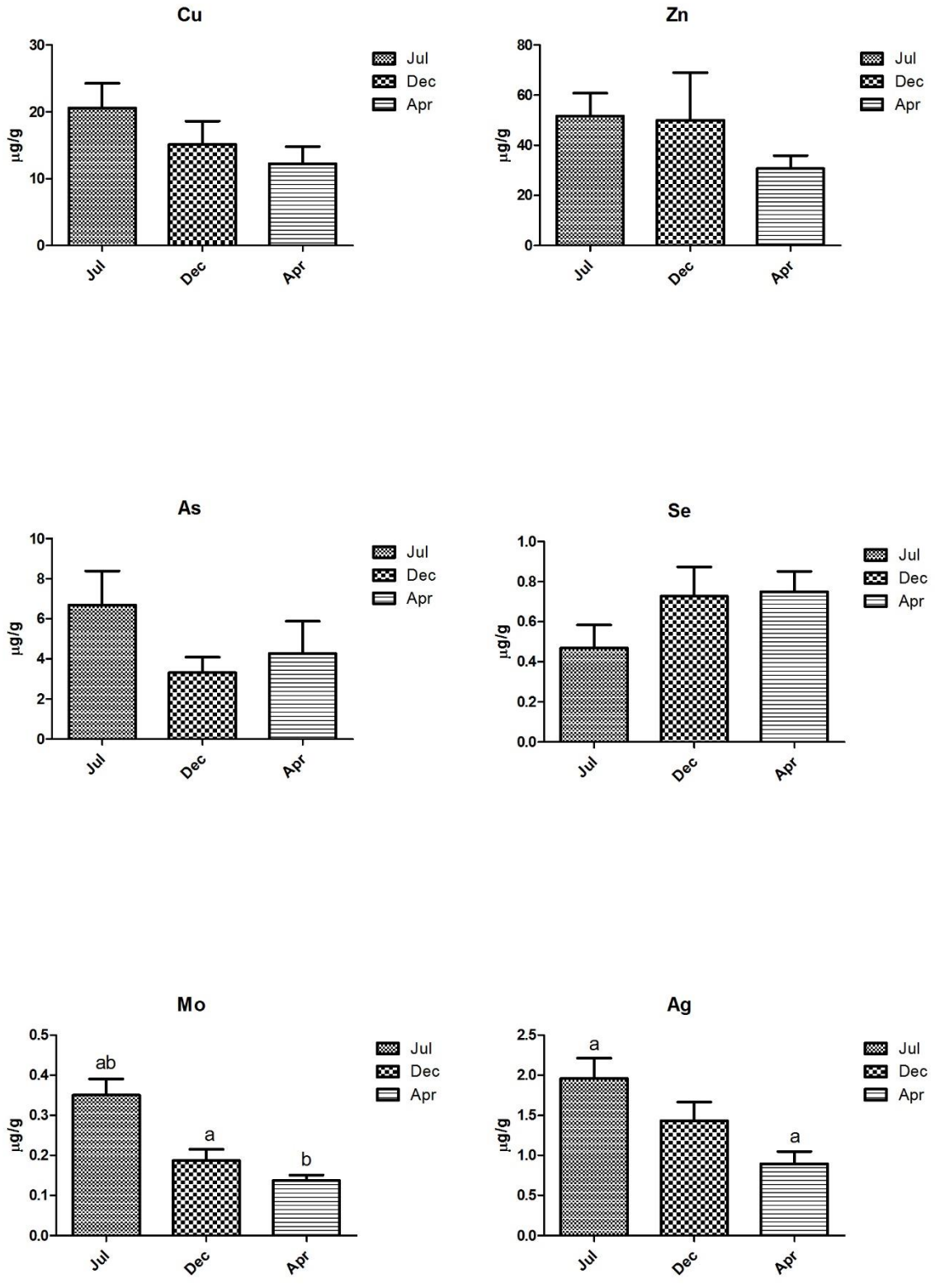

Figure 22: Temporal variation (July/winter, December/summer, April/autumn) of copper (Cu), zinc (Zn), arsenic (As), selenium (Se), molybdenum (Mo), and silver (Ag) concentrations in the sediment (µg/g). Bars indicate mean concentrations using different sites as replicates; error bars indicate standard error. Bars with common superscript differ significantly ($p < 0.05$).

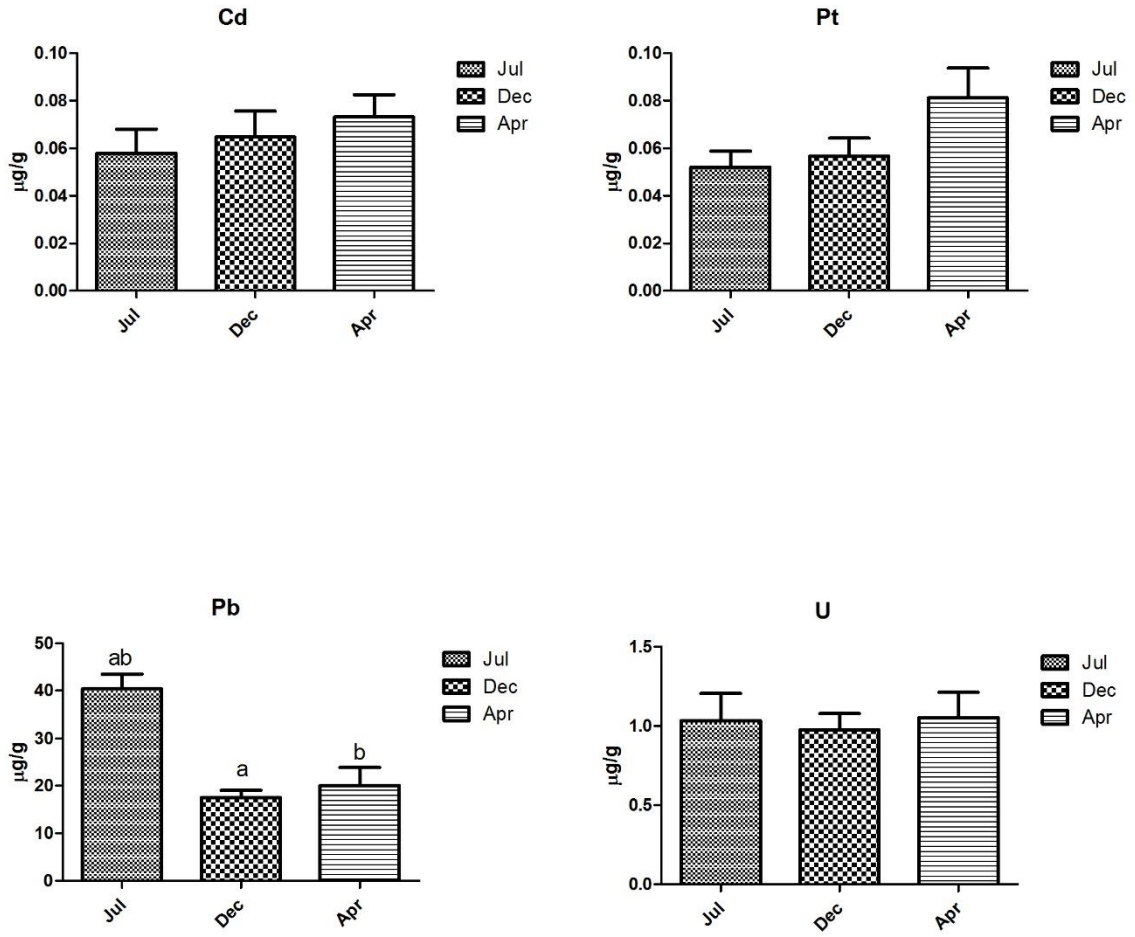

Figure 23: Temporal variation (July/winter, December/summer, April/autumn) of cadmium (Cd), platinum (Pt), lead (Pb), and uranium (U) concentrations in the sediment (µg/g). Bars indicate mean concentrations using different sites as replicates; error bars indicate standard error. Bars with common superscript differ significantly ($p < 0.05$).

The grain size analyses for July (winter) 2015, December (summer) 2015, and April (autumn) 2016 are presented graphically (Figure 24). Certain grain sizes predominated certain sites, but very coarse sand and silt/mud did not dominate any sites. Gravel dominated site NR8 during December. Coarse sand dominated sites NR1 (July and April), NR2 (July and April), NR3 (July and April), NR5 (December), NR6b (December and April), and NR10 (December and July). Medium sand dominated NR5 (July) and NR7 (July and December). Very fine sand dominated sites NR1 (December), NR2 (December), NR3 (December), NR4 (July and December), NR6a (July, December and April), NR6b (July), NR7 (April), NR8 (April), NR9 (July, December and April), and NR10 (April).

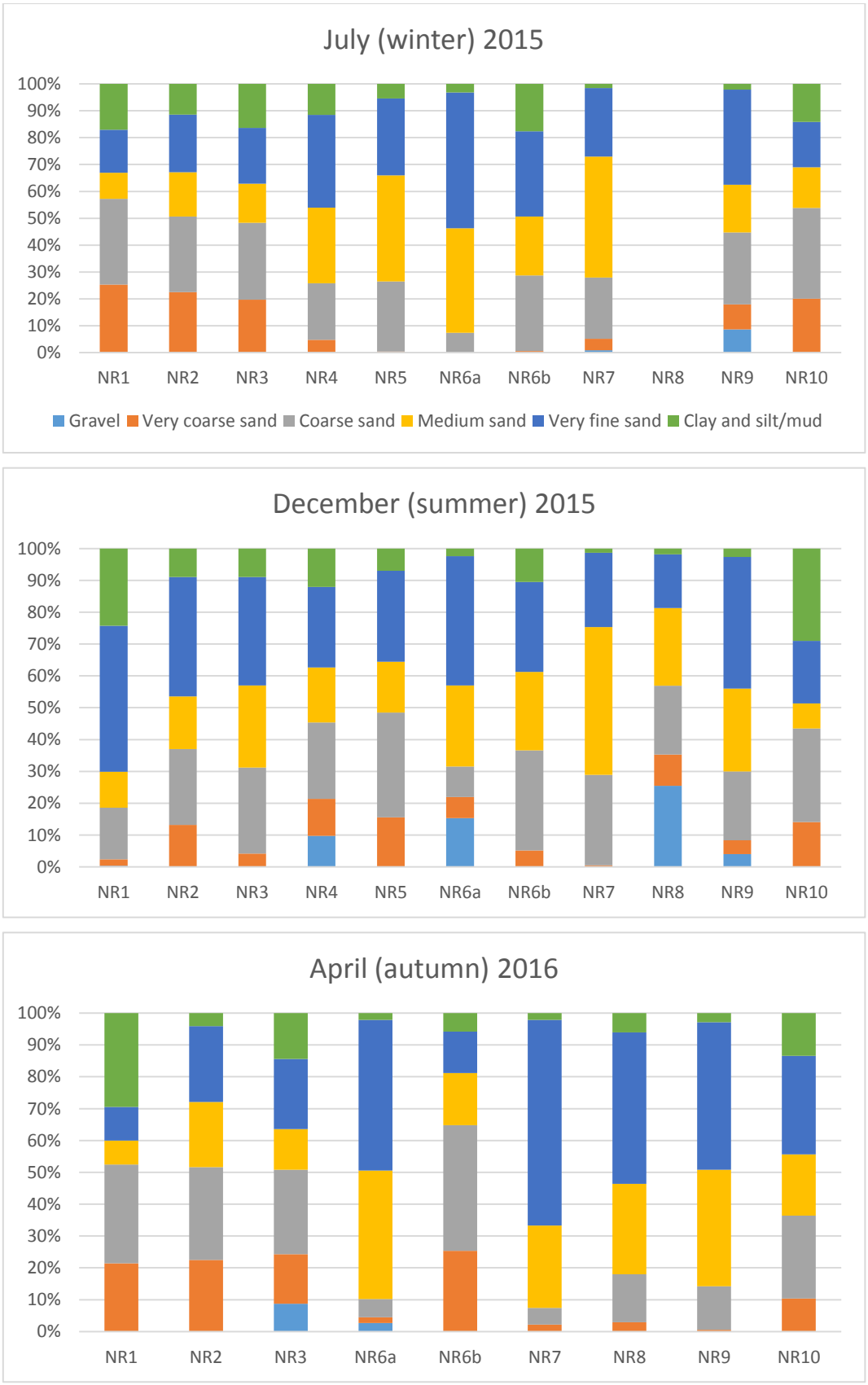

Figure 24: Sediment grain size distributions (percentages) across the Ntsikeni Nature Reserve's sampling sites (NR1 – NR10) during July/winter (J) 2015, December/summer (D) 2015, and April/autumn (A) 2016. Sites NR8 (J), NR4 (A), and NR5 (A) are not presented since no data was collected due to drying up of sites or lack of accessibility.

All sites that were sampled during all three surveys were combined with HGM types, metal concentrations, and grain size and used to construct a RDA tri-plot. All explanatory variables of the RDA tri-plot (Figure 25) accounted for 32.4 % of the total variation. The RDA tri-plot explained 30.29 % of the cumulative variation on the first and second axis. The first axis explained 26.96 % of the variation while the second axis explained only 3.33 % variation. A Monte Carlo permutation test on all axes revealed a p value of 0.004 thereby, indicating significant differences since $p < 0.05$. The sites were all concentrated on their respective HGM types (Figure 25). The sediment composition of sites (NR6a, NR7, and NR8) in close proximity to the river were dominated by gravel, medium, and very fine sand. In the area between unchannelled valley-bottoms and floodplains, the very coarse sand, coarse sand, and silt were dominant. Grain size can influence the ability of chemicals to accumulate in sediments (Malherbe *et al.*, 2015). The highest metal concentrations were measured between floodplain wetlands and unchannelled valley-bottom wetlands except for boron (B), thorium (Th), titanium (Ti), mercury (Hg), platinum (Pt), Mn, gold (Au), and Mo. The metals carrying the most influence were Cr, nickel (Ni), and vanadium (V) (Figure 25).

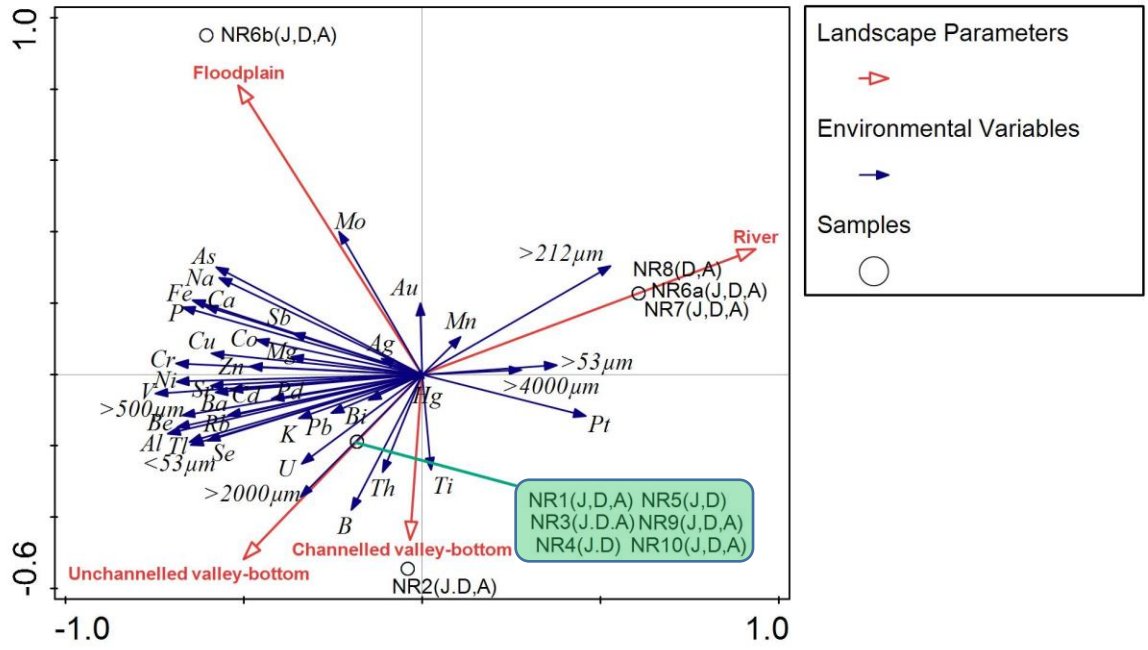

Figure 25: A redundancy analysis (RDA) tri-plot showing relationships between sites (NR1 – NR10), seasons, hydrogeomorphic (HGM) types, and sediment quality variables. Seasons consist of surveys conducted in July/winter (J), December/summer (D), and April/autumn (A). Sediment quality variables consist of metal concentrations and grain sizes.

3.4 Discussion

3.4.1 Water quality

The metals dissolved in the waters of the Ntsikeni Nature Reserve (Table A1 in Appendix A) were compared to the Target Water Quality Range (TWQR) as laid out by the South African Water Quality Guidelines (DWAF, 1996; Dallas and Day, 2004). Aluminium (Al) was always within the TWQR of ≤ 0.01 mg/l, except at two sites (NR6a and NR7; both during July). Arsenic (As), cadmium (Cd), Cr, Pb, Se, and Zn were always within the TWQR concentration for each metal. Copper (Cu) fell within the TWQR of ≤ 0.0003 mg/l on all occasions, with sites NR4, NR5, and NR9 (all during December) being the only exceptions. Manganese (Mn) fell within the TWQR of ≤ 0.18 mg/l on all occasions, with site NR5 (during April) being the only exception. A summary of the recorded ranges of dissolved metals in the Ntsikeni wetland complex in relation to the TWQR is given below (Table 2).

Aluminium (Al), Cu, and Mn exceeded the TWQR on occasions. However, according to the South African Water Quality Guidelines (DWAF, 1996), ninety percent of all dissolved Al, Cu, and Mn measurements for the area in question should fall within the TWQR. This holds true for the Ntsikeni wetland complex since no more than three sites at a time of the total 31 collected samples exceeded the TWQR.

Table 2: Comparison of the Target Water Quality Range (TWQR) as laid out by the South African Water Quality Guidelines (DWAF, 1996; Dallas and Day, 2004) and the Ntsikeni Nature Reserve's (NR) dissolved metal range recorded from all sites and surveys listed in Appendix Table A1. *Concentrations may vary depending on pH or water hardness (Dallas and Day, 2004).

Metal	Symbol	TWQR (mg/l)	NR value (mg/l)
Aluminium	Al	0.005 – 0.001*	7.97E-06 – 0.18
Arsenic	As	0.01	0.00028 – 0.00066
Cadmium	Cd	0.00015 – 0.004*	0.0001 – 0.00014
Chromium	Cr	0.007 – 0.012*	0.0002 – 0.00071
Copper	Cu	0.0003-0.0014*	0.00019 – 0.0035
Lead	Pb	0.0002-0.0012*	0.00017 – 0.00074
Manganese	Mn	0.18	1.82E-05 – 0.32
Selenium	Se	0.002	0.00072 – 0.0011
Zinc	Zn	0.002	8.35E-05 – 0.0097

The geology of an area has a major influence on the metal concentrations of the water (Vlok *et al.,* 2013). The Ntsikeni wetland complex contains several dolerite dykes and a major dolerite sill at the outlet of the wetland (Kotze, 2003). Dykes are discordant intrusions that appear as bands of igneous rock that slice across strata (Monroe *et al.*, 2007). According to Dallas and Day (2004), water affected by igneous rock is usually dominated by Ca and Mg cations, has a pH higher than 7, and has an electrical conductivity that often measures < 20 mS/m. It can, thus, be assumed that the waters of the Ntsikeni Nature Reserve were affected by igneous rock since Ca and Mg carried the greatest influence (Figure 14), while pH and EC (Figures 6 and 12) both fell within the ranges given by Dallas and Day (2004).

When assessing water quality (in terms of spatial distribution), no significant differences were found; however, when performing temporal analyses, significant differences between seasons were found amongst several water quality variables (Figures 8 – 13). Both Figures 14 and 15 showed seasonal groupings for each survey with water quality variables (such as Ca, Mg, Sr, EC, and alkalinity) carrying the greatest influence between the December (summer) 2015 and April (autumn) 2016 surveys. Flow rates, both into and out of a wetland, vary with rainfall, season, and evaporation and are often responsible for increased TDS in an area (DWAF, 1996; Dallas and Day, 2004). Seasonal climate changes, such as rainfall and solar heating, result in variations in the water balance (Chapman, 1996) which could lead to changes in water chemistry. Since December (summer) and April (autumn) are part of the rainy season in South Africa, an influx of metals and other particles are an explanation for the higher concentrations between summer and autumn, and the lack thereof during the winter survey.

When analysing the April (autumn) 2016 survey (Figure 14), it is evident that Sb and chloride carried the greatest influences during the autumn survey. The Sb concentrations measured in this study were, however, below 1 µg/l, indicating the source to be natural rock weathering and soil runoff (Filella *et al.*, 2002). In addition, due to the antagonistic effect of Ca, Mg, high levels of alkalinity, and high levels of TDS on trace metals such as Sb, Co, Cu, and Zn (Dallas and Day, 2004), the availability of these metals was expected to be low.

Chloride, having had the greatest concentrations during the April (autumn) survey as well as significant differences from the other two surveys (Figure 13), had the highest value of 24.3 mg/l at site NR1 (Appendix Table A2). Chloride ions are essential for living organisms since chloride is involved in the ionic, osmotic, and water balance of body fluids (Dallas and Day, 2004). In pristine freshwater chloride concentrations are usually < 10 mg/l (Chapman, 1996). Both the July (winter) and December (summer) surveys met this criteria with only the April (autumn) survey exceeding the < 10 mg/l range (Figure 13). Chloride enters surface waters via atmospheric deposition of oceanic aerosols, the weathering of some sedimentary rocks, and a little from igneous rock weathering (Chapman, 1996). Since rainfall was experienced during the April (autumn) survey it is possible that this provided an influx of chloride into the wetland complex.

When assessing all water quality variables, ammonium was found to be the only variable where both the July (winter) and December (summer) surveys differed significantly from the April (autumn) survey i.e. the April (autumn) survey had much lower concentrations than did the other two surveys (Figure 12). Ammonium is a reduced form of inorganic nitrogen that, in surface and ground water, is mostly derived from aerobic and anaerobic decomposition of organic material (DWAF, 1996; Dallas and Day, 2004). It contains little to no toxicity and forms part of the nitrogen cycle along with nitrite and nitrate (Dallas and Day, 2004). Nitrite, being the intermediate in the conversion of ammonia to nitrate (Dallas and Day, 2004), is usually produced via oxidisation under aerobic conditions (Chapman, 1996). In Figure 14, nitrite was grouped close to the high concentrations of dissolved oxygen while ammonium was located during the July/December (winter/summer) survey. Decomposition of organic matter must have been at its peak during the July/December (winter/summer) survey resulting in high ammonium concentrations, while nitrite concentrations were possibly affected due to the presence of oxygen.

When possible, water quality variables of the Ntsikeni wetland complex were also compared to those of the Blesbokspruit Wetland (Ambani and Annegarn, 2015), the Mkuze Wetland System (Barnes *et al.,* 2002; Ellery *et al.,* 2003), and the Nyl River floodplain (Greenfield *et al.,* 2012). All these water systems have been awarded Ramsar status, with the Mkuze Wetland System being protected along with Lake St Lucia (Barnes *et al.,* 2002). All these water systems have been impacted by human

activities in one way or another (Barnes *et al.,* 2002; Greenfield *et al.,* 2012; Ambani and Annegarn, 2015). Although the pH values were more or less similar, the water quality variables from the Ntsikeni wetland complex that were compared to the Blesbokspruit Wetland were much lower. When compared to the Mkuze Wetland System and the Nyl River floodplain, the majority of the Ntsikeni wetland's water quality variables were also found to be much lower than those recorded by Barnes *et al.,* (2002) and Greenfield *et al.,* (2012) respectively. These comparisons simply serve to support the fact that anthropogenic activities can cause a rise in water quality variables and that the Ntsikeni wetland complex is indeed an aquatic ecosystem with very low levels of water quality variables.

3.4.2 Sediment

The RDA tri-plot (Figure 25) indicated that the highest concentrations of metals, as well as percentages of grain sizes (very coarse sand, coarse sand, clay, and silt), were concentrated between the site NR6b floodplain and all the other unchannelled valley-bottom sites. The metal concentration data (Table A3 in Appendix A), revealed metal concentrations to be higher than those found in the water quality analyses. Sediments have a much higher storing capacity for contaminants than water does (Alhashemi *et al.,* 2011). In the hydrological cycle, less than 0.1 % of metals are dissolved in water with the majority contained in sediments and soils (Davies *et al.,* 2006; Pradit *et al.,* 2010; Alhashemi *et al.,* 2011). The higher concentrations of metals in sediments is most likely due to the presence of weathering underlying rocks belonging to the Karoo Supergroup (Retief *et al.,* 2006).

A comparison of the sediments of the Ntsikeni Nature Reserve with the Sediment Quality Guidelines (SQG) range (EPA, 1999) showed that the sediment of the Ntsikeni wetland complex had very low concentrations of metals overall (Table 3). All metals from the Ntsikeni Nature Reserve compared to the SQG fell within the given range, except for Cd which was lower than the given range.

Table 3: Comparison of Sediment Quality Guidelines (SQG) as laid out by the Environmental Protection Agency (EPA, 1999) and the Ntsikeni Nature Reserve's (NR) highest recorded values from all sites and surveys listed in Appendix Table A3.

Metal	Symbol	SQG (µg/g)	NR value (µg/g)
Arsenic	As	8.2 – 70	20.03
Cadmium	Cd	1.2 – 9.6	0.14
Chromium	Cr	81 – 370	137.47
Copper	Cu	34 – 270	42.59
Lead	Pb	46.7 – 218	56.61
Mercury	Hg	0.15 – 0.71	0.48
Nickel	Ni	20.9 – 51.6	36.26
Silver	Ag	1.0 – 3.7	3.38
Zinc	Zn	150 – 410	234.43

A floodplain wetland is characterised by the regular water and sediment contributions made by the associated river channel, while an unchannelled valley-bottom wetland is created by the accumulation of sediment and change in gradient which causes water to spread out over a wide area (Ollis *et al.,* 2013). This addition of sediment to an area that is spread out across a wide area, rather than being confined to a concentrated flow of water, is possibly responsible for the abundance of metals found between floodplains and unchannelled valley-bottoms as indicated by the RDA tri-plot (Figure 25).

Clay and silt were found to be of greater influence between floodplain and unchannelled valley-bottom wetlands than at other HGM types. It is known that contaminants are more prone to accumulate on smaller sediment grain sizes, such as clay, since their surface area is larger (Malherbe *et al.*, 2015). The presence of clay and silt could explain the accumulation of metals found between floodplains and unchannelled valley-bottom wetlands.

River type wetlands are associated with a concentrated flow of water (Ollis *et al.,* 2013). Sediment transportation can occur due to high flow volumes which results in smaller fractions of sediment being removed from certain sites (Malherbe *et al.*, 2015). This could explain the presence of certain grain sizes (gravel, medium sand, and very fine sand) and the absence of others (very coarse sand, coarse sand, clay, and silt) in the vicinity of river wetland areas (Figure 25).

According to a study by Greenfield *et al*. (2007) on the Nyl River in the Limpopo Province, all metal concentrations recorded in the Nyl River fell within the lower end of the SQG Range, with Zn being the only exception. Metal concentrations of the Ntsikeni Nature Reserve were compared to the study by Greenfield *et al*. (2007). Of all metals found in the Ntsikeni Nature Reserve sediments, iron (Fe) and Al had the highest concentrations (Appendix Table A3) with Cr and Ni carrying the greatest influence (Figure 25). All metal concentrations in the Ntsikeni Nature Reserve were lower than those from the Nyl River. The only exceptions were Al and Cr which were found to be slightly higher in the Ntsikeni Nature Reserve. The SQG indicated that Cr is well within the guideline range (EPA, 1999), while Al is not given by the SQG since it is one of the most common metals found in sediment samples (Malherbe *et al.*, 2015). Greenfield *et al*. (2007) stated that metal concentrations in the Nyl River had little or no potential effect on the organisms in the system. This comparison serves to verify that if an anthropogenic impacted wetland, such as the Nyl River floodplain, which has higher sediment concentration than those of the Ntsikeni Nature Reserve, then the metals in the Ntsikeni sediment are no threat to the organisms within the system.

3.5 Conclusion

Analyses of the spatial distribution of water quality indicated no significant difference. Temporal analyses of water quality showed significant differences with more than one variable. This indicated that water quality variables differed between seasons.

With regards to sediment quality, significant differences were found both spatially and temporally throughout the Ntsikeni wetland complex. The HGM types of the wetland complex had a significant influence on the concentration of the metals suspended in the sediments. The underlying geology also had an effect on which and what concentrations of metals entered the ecosystem.

When comparing the Ntsikeni wetland complex to the South African Water Quality Guidelines (DWAF, 1996) and the SQG range (EPA, 1999), it can be seen that metal concentrations, whether dissolved in water or stored in sediment, were rather low. Thus indicating little threat to the wetland’s organisms.

Chapter 4 – Zooplankton

4.1 Introduction

Zooplankton assemblages reflect the joint influences of prevailing biotic and abiotic environmental conditions (Hart, 2012). Zooplankton are planktonic organisms that include protozoans, rotifers, cladocerans, copepods, ostracods, water mites, and larvae of different insects. (van As *et al*., 2012). By grazing on phytoplankton and bacteria, zooplankton can aid in improving water quality, thus zooplankton are considered indicators of water quality (Dalu *et al.,* 2013). Zooplankton occupy different trophic levels and prefer different water clarities; they have been known to vary based on wetland quality and changing environmental conditions (Riato *et al.,* 2014). Since zooplankton cannot withstand strong currents they are largely absent from fast-flowing rivers but are more abundant in backwaters, pools, and floodplain wetlands (van As *et al*., 2012).

An important component of freshwater zooplankton is the crustacean taxa which includes Cladocera, Copepoda, and Ostracoda (van As *et al*., 2012). Cladocerans, considered to be secondary producers (Ali *et al.,* 2007), are filter feeders that continuously sift microscopic algae from the water as a means of obtaining food (van As *et al*., 2012). Cyclopoida, an order of the class Copepoda, are predators that feed on other members of the zooplankton community (van As *et al*., 2012). They are acid-sensitive and do not occur in water with a pH lower than 6, but they have been known to tolerate saline conditions (Riato *et al.,* 2014). Calanoida, another order of the class Copepoda, are filter feeders that feed on phytoplankton. Copepoda are abundant in freshwater and comprise a major component of most planktonic, benthic, and groundwater communities (Boxshall and Defaye, 2008). Ostracoda are been noted as typically phytophilous in that they are attached to solid plant surfaces (Ali *et al.,* 2007). They are small bi-valved crustaceans that can be found in a range of marine and non-marine water bodies and have even been found in moist semi-terrestrial habitats (Griffiths *et al.,* 2015).

According to studies by Hart (1990; 2012), Bird *et al.* (2014), Riato *et al.* (2014), and Dube *et al.* (2017), crustacean zooplankton are abundant in South African water bodies. Studies on wetlands in the Mpumalanga Highveld region (Riato *et al.,* 2014), alkaline wetlands in the Western Cape (Bird *et al.,* 2014), and floodplain wetlands of the Phongolo River (Dube *et al.,* 2017) have recorded species from a wide range of taxanomic orders which include Cladocera, Calanoida, Copepoda, Cyclopoida, Ostracoda, and Rotifera.

In general, little is known about zooplankton communities in wetlands in South Africa (Riato *et al.,* 2014). With regards to the Ntsikeni Nature Reserve, Blackmore (2010) listed only one species from the order Cladocera that was known to occur within the wetland complex. Other than this, no information is available on presence and biodiversity of zooplankton taxa within the Ntsikeni Nature Reserve. The aims of this chapter are to determine spatial and temporal distribution of zooplankton in the Ntsikeni Nature Reserve and to determine what physico-chemical factors drive their distribution.

4.2 Materials and Methods

4.2.1 Sampling procedure

Zooplankton sampling was undertaken during July (winter) 2015, December (summer) 2015, and April (autumn) 2016. Samples were collected at the same time as water and sediment samples were collected from each site (Section 3.2). However, not all sites were sampled for zooplankton during all three surveys due to a severe drought and the lack of water availability. Sites not sampled were NR3, NR4, NR5, NR6b, and NR8 during July (winter) as well as site NR4 during April (autumn). Sampling was done using methods similar to those of Riato *et al.* (2014) and de Necker *et al.* (2016) by having a certain volume of water pass through a plankton net and preserving the collected material on site.

Sites were sampled using a plankton net with a mesh size of 50 µm. The net was placed in flowing water for approximately five to ten minutes to allow zooplankton to accumulate. At sites that lacked flowing water 200 litres of water was filtered through the net. The samples were preserved on site using 5 – 10 % neutrally buffered formalin (NBF). In the laboratory, samples were rinsed under running water using a sieve with

a mesh size of 50 µm. Zooplankton were separated from all other collected matter with the aid of a Nikon, C-LEDS dissection microscope. Zooplankton were identified with the aid of a compound microscope (Nikon Eclipse 80i) to the lowest possible taxonomic level using relevant identification guides (Day *et al*., 1999; 2001a; Griffiths *et al.,* 2015). All zooplankton taxa were thereafter preserved in 70 % ethanol (Riato *et al.,* 2014; de Necker *et al.,* 2016).

4.2.2 Statistical analyses

Zooplankton data were used to perform both univariate and multivariate analyses. The univariate diversity indices consisted of Margalef's species richness (d), Shannon diversity index (H'), Pielou's evenness index (J'), and a distributional k-dominance plot. Margalef's species richness (d) accounts for sample size and effort when calculating species richness while the Shannon diversity index (H') is used to integrate both species richness and equitability components (de Necker *et al.,* 2016). Pielou's evenness index was calculated to determine overall evenness (de Necker *et al.,* 2016), with a value of 1 representing even distribution of abundance throughout the wetland complex and a value of 0 representing uneven distribution of abundance (Wolmarans *et al.,* 2014). The indices were drawn up to explain species abundance relationships, diversity, and evenness among zooplankton invertebrate communities across the different sites and surveys of the Ntsikeni wetland complex (de Necker *et al.,* 2016). These analyses were performed using Primer Version 7.

In order to verify the significance of the total taxa (S), total individuals (N), Margalef's species richness (d), Shannon diversity index (H'), and Pielou's evenness index (J'), both spatial and temporal differences were determined using ANOVA. The data were tested for normality using the Kolmogorov-Smirnov test with $p < 0.05$. If $p < 0.05$ for normality test, Tukey's *post hoc* statistical analysis test was used to determine significant difference; if $p > 0.05$, the Kruskal-Wallis *post hoc* test was performed (methods adapted from de Klerk *et al.,* 2012). These additional analyses were completed on Graphpad Prism version 6.

The multivariate analyses, used for temporal and spatial assessment, consisted of the Bray-Curtis similarity matrix, hierarchical clustering, NMDS, and RDA in order to explain the effect of environmental variables on community structure (van den Brink *et al*., 2003; de Necker *et al.,* 2016). These multivariate analyses were drawn up using Canoco Version 5 as well as Primer Version 7.

Both the hierarchical cluster analyses and the NMDS plots were constructed using the Bray-Curtis similarity matrix to identify patterns in the zooplankton assemblages (Malherbe *et al.,* 2010; de Necker *et al.,* 2016). An NMDS plot (a more informative summary than the corresponding cluster analysis) is a configuration of samples which attempts to satisfy all the conditions which are imposed by the rank (dis)similarity matrix (Clarke and Warwick, 2001). The NMDS plot is advantageous in that it is not restricted to the Euclidean distance measure but any (dis)similarity measure can be used; this (dis)similarity allows for better dealing with missing data since these can be calculated from the measured variables (van den Brink *et al*., 2003).

An analysis of similarities (ANOSIM) is an analogue of a 1-factor analysis of variance which is based on multispecies data (Chapman and Underwood, 1999). The ANOSIM is used to determine if *a priori* groupings found in the hierarchical clusters and NMDS plots had significant statistical differences that could be interpreted (Malherbe *et al.,* 2010). Performing an ANOSIM produces an R value which is scaled to lie between -1 and +1 with zero indicating a null hypothesis with no differences found among samples (Chapman and Underwood, 1999).

One-way similarity percentage (SIMPER) analyses were constructed using the S17 Bray-Curtis similarity resemblance with a 70 % contribution cut-off. The SIMPER analysis is used to calculate the average contribution of individual terminal fragments to the average dissimilarity that exists between samples (Rees *et al.,* 2005). The SIMPER procedure is a more explanatory analysis rather than a statistical testing framework (Clarke and Warwick, 2001).

4.3 Results

All collected zooplankton taxa from all surveys at the Ntsikeni Nature Reserve were identified and classified according to taxonomic level (Table 4). In total, 25 taxa were identified (Table B1 in Appendix B) consisting of three classes, five orders, eight families, and 22 genera. All zooplankton taxa identified belonged to the kingdom Animalia, phylum Arthropoda, and subphylum Crustacea. With regards to order, Cladocera had the most individuals (564) while Harpacticoida had the least number of individuals (15) (Appendix Table B1).

Table 4: Summary of zooplankton taxa identified from all sites sampled within the Ntsikeni wetland complex in July (winter) 2015, December (summer) 2015, and April (autumn) 2016.

Class	**Order**	**Family**	**Genus**
Branchiopoda	Cladocera	Chydoridae	*Acroperus* sp.
			Alona sp.
			Chydorus sp.
		Daphniidae	*Ceriodaphnia* sp.
			Simocephalus sp.
		Moinidae	*Moina* sp.
Maxillopoda	Calanoida	Diaptomidae	*Lovenula* sp.
			Metadiaptomus sp.
	Cyclopoida	Cyclopidae	*Ectocyclops* sp.
			Eucyclops sp.
			Macrocyclops sp.
			Microcyclops sp.
			Paracyclops sp.
	Harpacticoida		
Ostracoda	Podocopida	Cyprididae	*Cypricercus* sp.
			Kapcypridopsis sp.
			Parastenocypris sp.
			Potamocypris sp.
			Stenocypris sp.
			Zonocypris sp.
		Darwinulidae	*Darwinula* sp.
			Vestalenula sp.
		Ilyocyprididae	*Ilyocypris* sp.

4.3.1 Univariate diversity indices

From the five orders identified, Cladocera had 564 individuals; Calanoida had 68 individuals; Cyclopoida had 457 individuals; Harpacticoida had 15 individuals; and Podocopida (class: Ostracoda) had 479 individuals (Appendix Table B1). Site NR2 had the highest number of taxa (14) in April (autumn) and the lowest number of taxa (2) in July (winter) (Figure 26a). The remaining sites had taxa numbers between four and 12 taxa per site. Neither spatial nor temporal analyses of total taxa data indicated significant differences (Figures 28 and 29). During December (summer) the most individuals (344) were found at site NR5, while the least individuals (8) were found at site NR7 (Figure 26b). The remaining sites had 10 – 178 individuals per site. No significant differences were found either spatially or temporally (Figures 28 and 29).

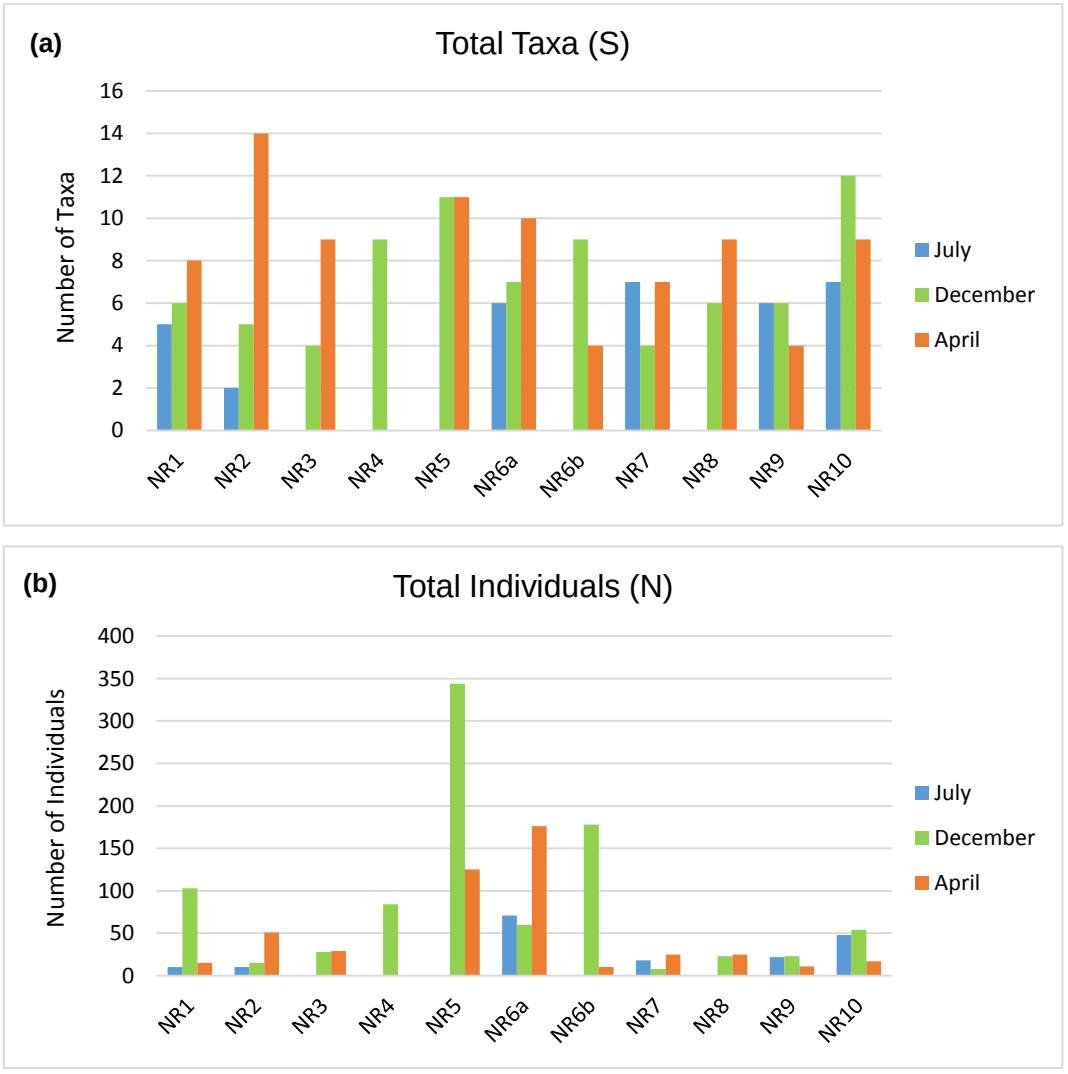

Figure 26: Total number of zooplankton found at each site during all surveys in July (winter) 2015, December (summer) 2015, and April (autumn) 2016 indicating (a) total taxa (S) and (b) total individuals (N). Sites NR3, NR4, NR5, NR6b, and NR8 were not sampled during all surveys.

Similar to Figure 26a, both the Margalef's Species Richness (d) (Figure 27a) and the Shannon Diversity Index (H') (Figure 27b) indicated that site NR2 contained both the highest and lowest values of the study. According to Margalef's Species Richness, site NR2 had the highest value (3.31) during the April (autumn) survey and the lowest value (0.43) during the July (winter) survey. All other values ranged between 0.90 and 2.82 (Figure 27a).

The Shannon Diversity Index (H') indicated that site NR2 had the highest value (2.41) during the April (autumn) survey and the lowest value (0.33) during the July (winter) survey; all other values ranged between 0.71 and 2.07 (Figure 27b). When assessing the spatial distribution data of both Margalef's Species Richness and Shannon Diversity Index, no significant differences were found (Figure 28). However, when assessing the temporal distribution data, the difference between the July (winter) and April (autumn) survey was found to be significant in both indices (Figure 29).

In Pielou's Evenness Index (J') (Figure 27c), the April (autumn) survey had the highest value (0.96) at site NR1 while the July (winter) survey had the lowest value (0.40) recorded at site NR6a. All remaining values ranged between 0.40 and 0.91. The values of Pielou's Evenness Index ranged closer to 1 than it did to 0, thus, no dominant taxon in terms of abundance were found. The distribution of zooplankton abundance in the Ntsikeni Nature Reserve was considered to be even. Figures 28 and 29 concur with Figure 27c by showing that no significant differences exist, either spatially or temporally, throughout the wetland complex.

Figure 27: Univariate diversity indices indicating (a) Margalef's species richness index, (b) Shannon diversity index, and (c) Pielou's evenness index. Sites NR3, NR4, NR5, NR6b, and NR8 were not sampled during all surveys.

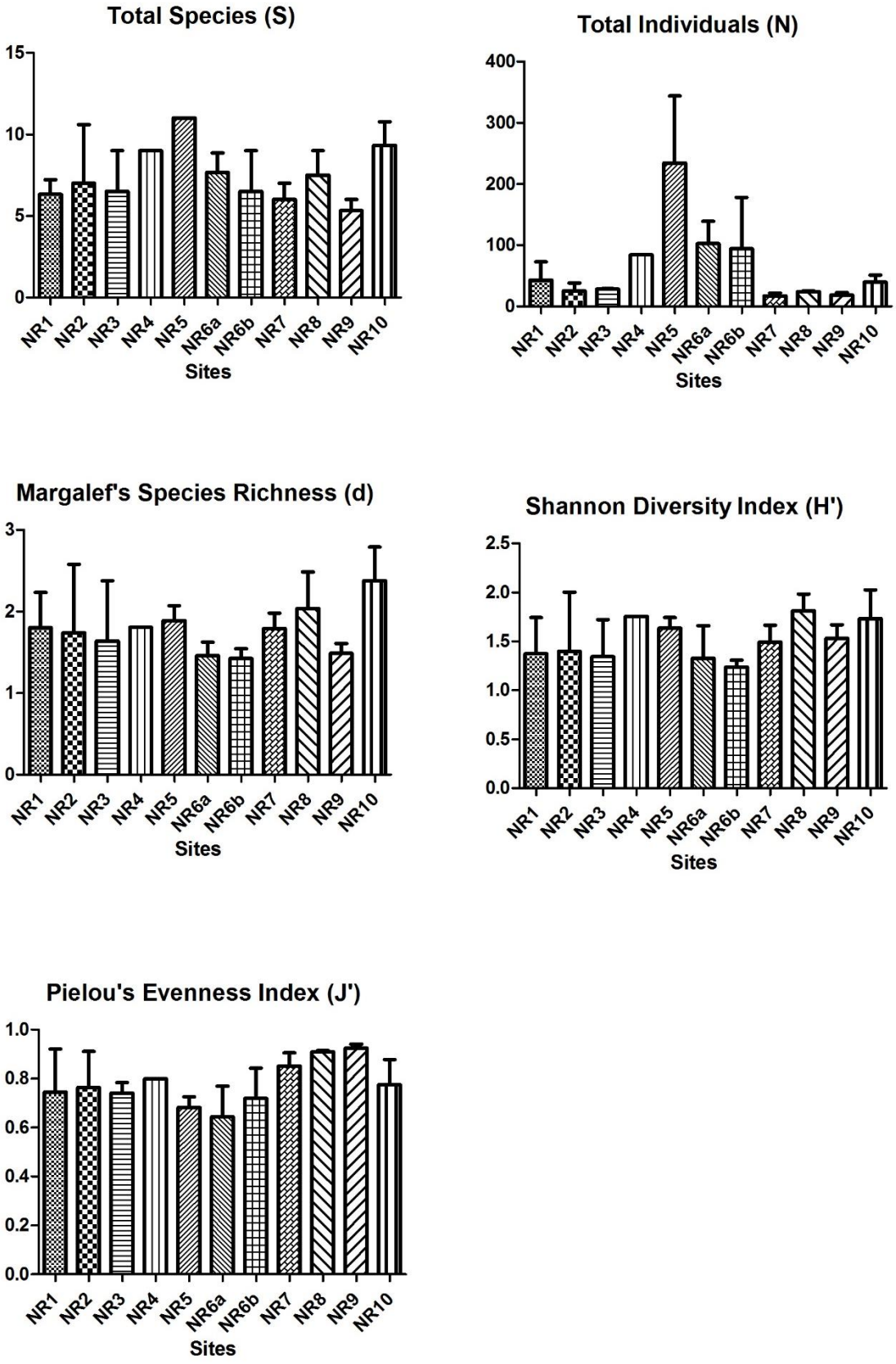

Figure 28: Spatial variation of zooplankton collected from the Ntsikeni Nature Reserve showing mean and standard error of diversity indices (Figures 26 and 27) using the three surveys as replicates.

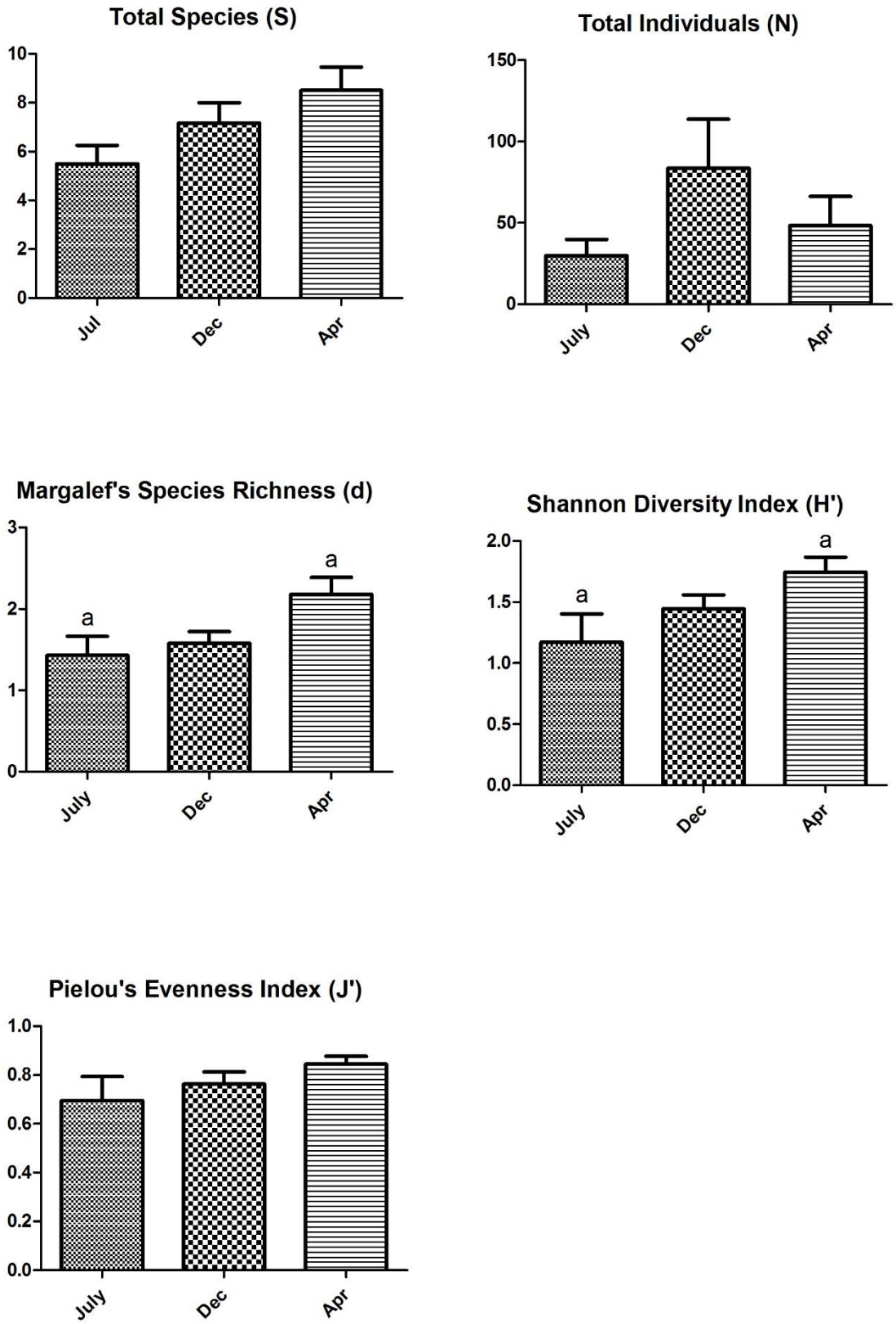

Figure 29: Temporal variation (July/winter, December/summer, April/autumn) of zooplankton collected from the Ntsikeni Nature Reserve showing mean and standard error of diversity indices (Figures 26 and 27) using different sites as replicates. Bars with common superscript (a) differ significantly ($p < 0.05$).

A k-dominance plot (Figure 30) was assembled by calculating the importance of taxa in terms of abundance and ranking these in a descending order (Malherbe *et al.,* 2010). The k-dominance plot illustrated that site NR2, during the July (winter) survey, had the highest degree of dominance with only two taxa and 10 individuals. Site NR6a, during the July (winter) survey, had the second highest degree of dominance with six taxa and 71 individuals. Site NR2, during the April (autumn) survey, had the lowest degree of dominance (14 taxa and 51 individuals).

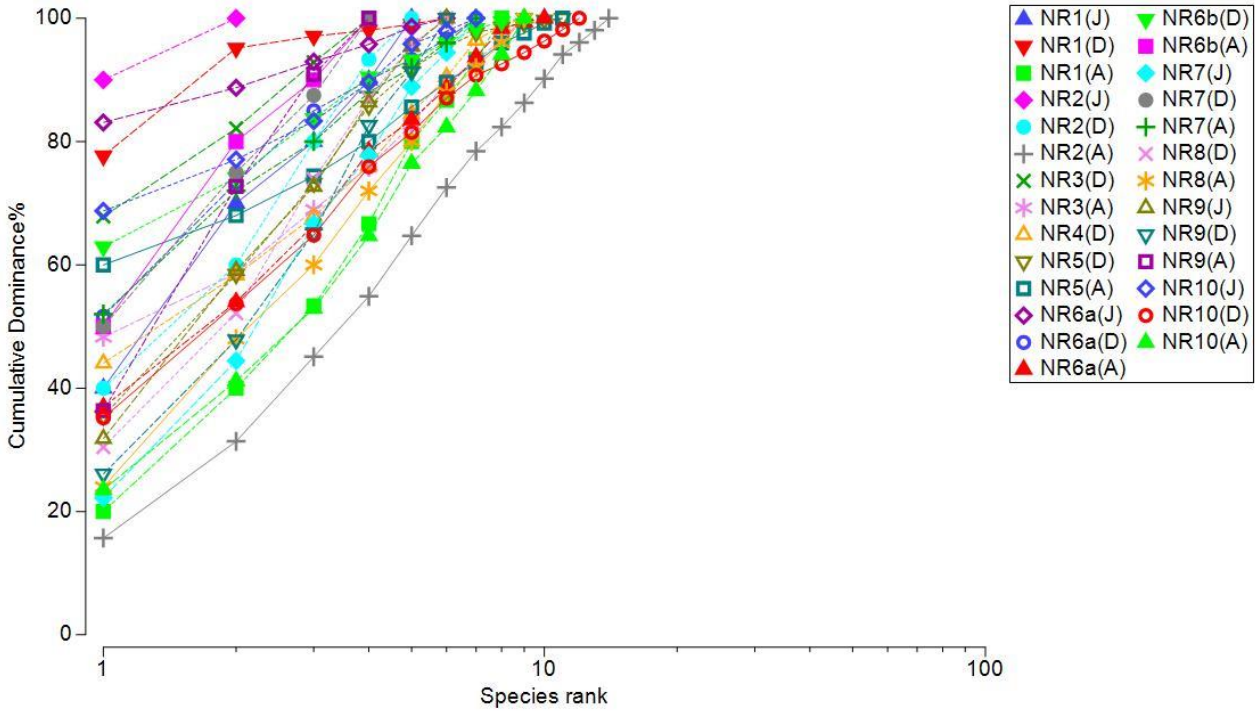

Figure 30: K-Dominance curves of the relative abundance of zooplankton taxa for each site sampled during July/winter (J), December/summer (D), and April/autumn (A).

4.3.2 Multivariate analyses

The first hierarchical cluster analysis (Figure 31a) was constructed to show groupings/similarities between season. When analysing Figure 31a at a similarity cut-off of 32 %, two groupings were revealed. The upper grouping showed all December (summer) surveyed sites were clustered together, except for three sites (NR3, NR7, NR8). The lower grouping showed all July (winter) and April (autumn) surveyed sites were clustered together, except for three April (autumn) surveyed sites (NR2, NR5, NR6a). At a similarity cut-off of 35 %, all July (winter) surveyed sites were grouped together. The NMDS plot (Figure 31b) showed that although groupings between seasons can be seen there are some sites that did not group with season. When performing an ANOSIM, an R value of 0.242 was obtained i.e. seasonal groupings were not significant.

The second hierarchical cluster analysis (Figure 32a) was constructed to show groupings between different sites sampled. When analysing Figure 32a, a similarity cut-off of 35 % revealed that all surveys of site NR7 were located in the upper cluster. Site NR7 was the only site with three sampling trips found within one cluster since all other sites showed more dispersion. The NMDS plot (Figure 32b) confirmed that the majority of sites seemed to be scattered without noteworthy groupings based on the sampling sites. When performing an ANOSIM, an R value of 0.046 was obtained indicating that there were no significant differences among sites (Chapman and Underwood, 1999).

The third hierarchical cluster analysis (Figure 33a) was constructed to show groupings between the four different HGM types. At a similarity cut-off of 32 %, Figure 33a revealed that all river types were grouped together with the exception of site NR6a during both the December (summer) and April (autumn) surveys. The NMDS plot (Figure 33b) showed that there were no evident groupings and that the sites were scattered. When performing an ANOSIM, an R value of -0.021 was obtained. Since this negative R value is so close to 0 it supports the null hypothesis in that no differences were found among the sampling sites (Chapman and Underwood, 1999).

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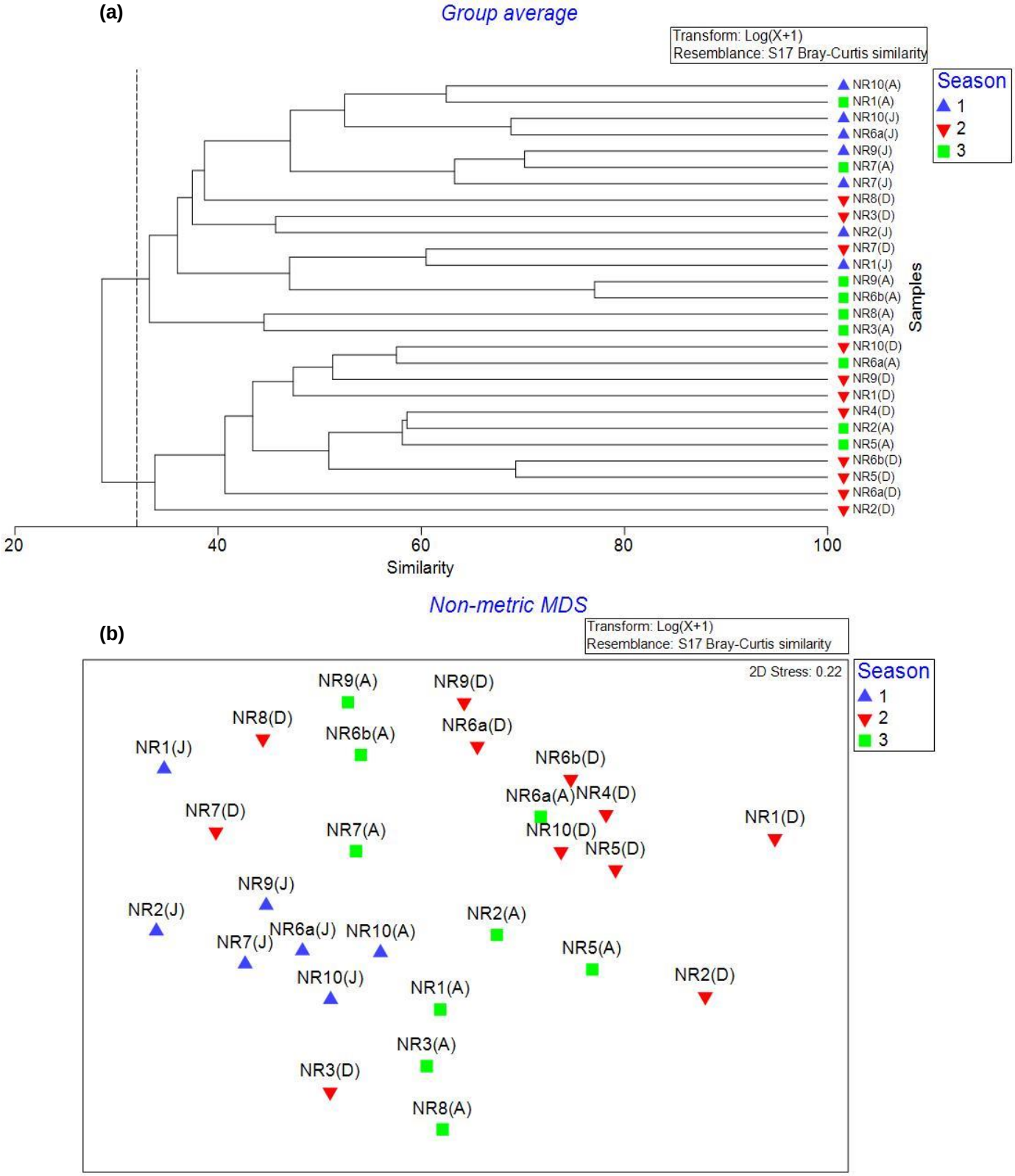

Figure 31: (a) Hierarchical cluster analysis indicating 32 % similarity and (b) non-metric multidimensional scaling (NMDS) plot based on Bray-Curtis similarity matrix showing groupings between seasons. Seasonal surveys to the Ntsikeni Nature Reserve (NR) occurred during the winter (1) month of July (J) 2015, the summer (2) month of December (D) 2015, and the autumn (3) month of April (A) 2016.

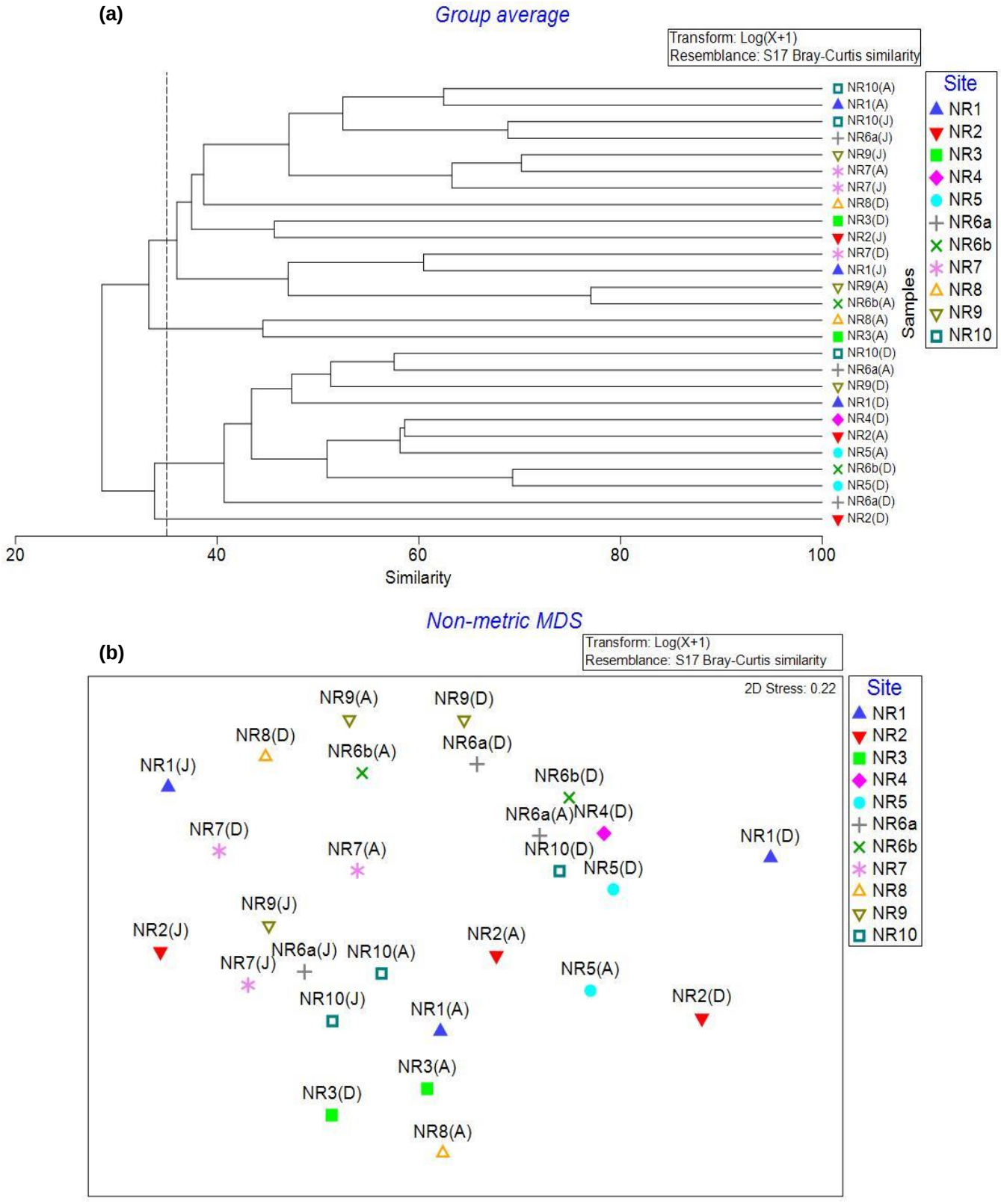

Figure 32: (a) Hierarchical cluster analysis indicating 35 % similarity and (b) non-metric multidimensional scaling (NMDS) plot based on Bray-Curtis similarity matrix showing groupings between sites sampled across the Ntsikeni wetland complex.

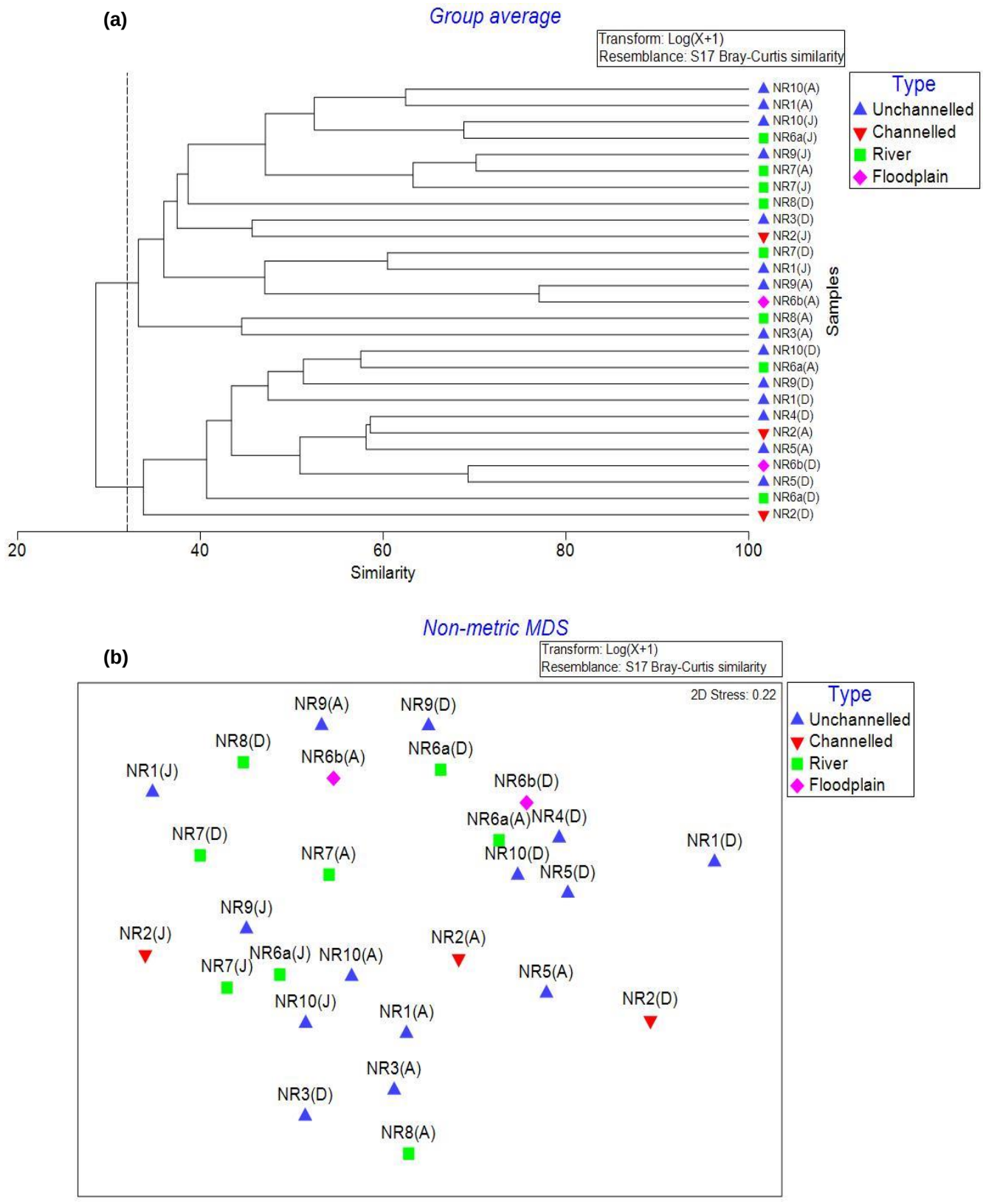

Figure 33: (a) Hierarchical cluster analysis indicating 32 % similarity and (b) non-metric multidimensional scaling (NMDS) plot based on Bray-Curtis similarity matrix showing groupings between hydrogeomorphic (HGM) types.

In order to help explain the influence of seasons, sites, and HGM types on zooplankton assemblages, SIMPER analyses were constructed for all aspects. However, SIMPER analyses of site NR4 (Table 6) were not calculated since only one sampling survey was possible due to the absence of water and lack of biotype availability. The July (winter) survey (Table 5) had an average similarity of 47.20 %, the December (summer) survey had an average similarity of 33.88 %, and the April (autumn) survey had an average similarity of 40.43 %. In all three surveys *Alona* sp. were found to have the highest contribution percentage with a percentage value of 45.62 % during July (winter), 21.47 % during December (summer), and 30.15 % during April (autumn). Cyclopoida had the second highest contribution percentage (17.89 %) during the July (winter) survey; *Ectocyclops* sp. had the second highest value (16.27 %) during the December (summer) survey; while *Zonocypris* sp. had the second highest value (17.85 %) during the April (autumn) survey.

Table 5: Similarity percentage analysis (SIMPER) with a 70 % contribution cut-off showing which zooplankton taxa are responsible for groupings in each season. Columns indicate average abundance (Av.Abund), average similarity (Av.Sim), similarity/standard deviation (Sim/SD), contribution percentage (Contrib%), and cumulative percentage (Cum.%).

July (winter)					
Average similarity: 47.20					
Taxa	**Av.Abund**	**Av.Sim**	**Sim/SD**	**Contrib%**	**Cum.%**
Alona sp.	2.28	21.53	2.62	45.62	45.62
Cyclopoida	1	8.44	1.53	17.89	63.51
Diaptomidae	0.91	7.19	1.45	15.24	78.75
December (summer)					
Average similarity: 33.88					
Taxa	**Av.Abund**	**Av.Sim**	**Sim/SD**	**Contrib%**	**Cum.%**
Alona sp.	1.48	7.27	1.06	21.47	21.47
Ectocyclops sp.	1.8	5.51	0.74	16.27	37.74
Zonocypris sp.	1.64	4.65	0.86	13.71	51.46
Cyclopoida	1.1	4.57	0.91	13.48	64.94
Chydorus sp.	1.14	3.95	0.77	11.67	76.61
April (autumn)					
Average similarity: 40.43					
Taxa	**Av.Abund**	**Av.Sim**	**Sim/SD**	**Contrib%**	**Cum.%**
Alona sp.	1.69	12.19	1.8	30.15	30.15
Zonocypris sp.	1.32	7.22	1.24	17.85	48
Ceriodaphnia sp.	0.92	4.18	1.01	10.34	58.33
Ectocyclops sp.	1.33	3.99	1.04	9.86	68.2
Paracyclops sp.	0.88	3.28	0.72	8.12	76.32

Alona sp. (Table 6) had the highest contribution percentage at sites NR3, NR6a, NR6b, NR7, NR8, and NR9, with a value of 24.37 % (NR6a) being the lowest. *Alona* sp. had the second highest contribution percentages at sites NR1, NR2, and NR10 with a value of 18.05 % being the lowest. Cyclopoida had the highest contribution percentage at sites NR1, NR2, and NR10, with a value of 20.27 % (NR1) being the lowest. Cyclopoida made significant contributions at sites NR5, NR6a, and NR7. *Ectocyclops* sp. (Table 6) had the highest contribution percentage at site NR5 (with a value of 30.74 %), and the third highest contribution percentage at site NR2 (with a value of 20.51 %). *Ceriodaphnia* sp. made significant contributions at sites NR1, NR6a, and NR10, although it never contributed the highest percentage value at any site.

Table 6: Similarity percentage analysis (SIMPER) with a 70 % contribution cut-off showing which zooplankton taxa are responsible for groupings found within each site sampled across the Ntsikeni wetland complex. Columns indicate average abundance (Av.Abund), average similarity (Av.Sim), similarity/standard deviation (Sim/SD), contribution percentage (Contrib%), and cumulative percentage (Cum.%).

NR1					
Average similarity: 19.36					
Taxa	**Av.Abund**	**Av.Sim**	**Sim/SD**	**Contrib%**	**Cum.%**
Cyclopoida	0.73	3.92	0.58	20.27	20.27
Alona sp.	0.69	3.49	0.58	18.05	38.32
Ceriodaphnia sp.	0.6	3.49	0.58	18.05	56.37
Diaptomidae	0.69	3.49	0.58	18.05	74.42
NR2					
Average similarity: 24.30					
Taxa	**Av.Abund**	**Av.Sim**	**Sim/SD**	**Contrib%**	**Cum.%**
Cyclopoida	1.39	10.46	2.48	43.06	43.06
Alona sp.	1.36	5.3	0.58	21.83	64.89
Ectocyclops sp.	1.38	4.98	0.58	20.51	85.39
NR3					
Average similarity: 45.63					
Taxa	**Av.Abund**	**Av.Sim**	**Sim/SD**	**Contrib%**	**Cum.%**
Alona sp.	2.16	17.94	SD=0!	39.31	39.31
Acroperus sp.	2.19	15.45	SD=0!	33.86	73.17
NR4					
Less than 2 samples in group					

Table 6 (continued):

NR5					
Average similarity: 56.30					
Taxa	**Av.Abund**	**Av.Sim**	**Sim/SD**	**Contrib%**	**Cum.%**
Ectocyclops sp.	4.57	17.31	SD=0!	30.74	30.74
Acroperus sp.	2.93	8.31	SD=0!	14.76	45.5
Cyclopoida	2.14	8.31	SD=0!	14.76	60.26
Zonocypris sp.	2.35	6.43	SD=0!	11.42	71.69
NR6a					
Average similarity: 39.28					
Taxa	**Av.Abund**	**Av.Sim**	**Sim/SD**	**Contrib%**	**Cum.%**
Alona sp.	2.5	9.57	2.58	24.37	24.37
Ceriodaphnia sp.	1.64	8.24	3.95	20.98	45.35
Cyclopoida	1.5	7.26	3.54	18.49	63.84
Chydorus sp.	2.3	6.09	0.58	15.49	79.34
NR6b					
Average similarity: 31.25					
Taxa	**Av.Abund**	**Av.Sim**	**Sim/SD**	**Contrib%**	**Cum.%**
Alona sp.	1.79	14.46	SD=0!	46.28	46.28
Zonocypris sp.	3.06	11.19	SD=0!	35.81	82.09
NR7					
Average similarity: 50.67					
Taxa	**Av.Abund**	**Av.Sim**	**Sim/SD**	**Contrib%**	**Cum.%**
Alona sp.	1.67	23.23	6.04	45.84	45.84
Chydorus sp.	1.48	11.58	10.61	22.86	68.7
Cyclopoida	0.73	4.28	0.58	8.44	77.14
NR8					
Average similarity: 25.12					
Taxa	**Av.Abund**	**Av.Sim**	**Sim/SD**	**Contrib%**	**Cum.%**
Alona sp.	1.39	14.02	SD=0!	55.79	55.79
Paracyclops sp.	1.24	11.11	SD=0!	44.21	100
NR9					
Average similarity: 34.68					
Taxa	**Av.Abund**	**Av.Sim**	**Sim/SD**	**Contrib%**	**Cum.%**
Alona sp.	1.83	22.78	26.24	65.68	65.68
Chydorus sp.	1.29	6.73	0.58	19.41	85.09

Table 6 (continued):

NR10					
Average similarity: 37.87					
Taxa	**Av.Abund**	**Av.Sim**	**Sim/SD**	**Contrib%**	**Cum.%**
Cyclopoida	1.36	10.21	3.07	26.97	26.97
Alona sp.	2	9.95	7.65	26.26	53.23
Ceriodaphnia sp.	1.23	8.17	1.61	21.58	74.81

Alona sp. (Table 7) had the highest contribution percentage at all HGM types, except for channelled valley-bottoms. Unchannelled valley-bottom types had an average similarity of 33.74 %. *Alona* sp. had a contribution percentage of 31.1 % and was followed by Cyclopoida which had a contribution percentage of 14.31 %. Channelled valley-bottom type had an average similarity of 24.30 %. Cyclopoida had the highest contribution percentage of 43.06 %, followed by *Alona* sp. which had a contribution percentage of 21.83 %.

The river type had an average similarity of 37.90 %. *Alona* sp. had a contribution percentage of 37.75 % and was followed by *Chydorus* sp. which had a contribution percentage of 19.02 %. The floodplain type had an average similarity of 31.25 %. *Alona* sp. and *Zonocypris* sp. were the only taxa that contributed to the percentage cut-off. *Alona* sp. had a contribution percentage of 46.28 % and was followed by *Zonocypris* sp. which had a value of 35.81 %. *Zonocypris* sp. also made a contribution at unchannelled valley-bottoms with a value of 9.24 %. *Ceriodaphnia* sp. made a contribution at unchannelled valley-bottoms as well with a value of 8.62 %.

Table 7: Similarity percentage analysis (SIMPER) with a 70 % contribution cut-off showing which zooplankton taxa are responsible for groupings found within unchannelled valley-bottoms, channelled valley-bottoms, rivers, and floodplains. Columns indicate average abundance (Av.Abund), average similarity (Av.Sim), similarity/standard deviation (Sim/SD), contribution percentage (Contrib%), and cumulative percentage (Cum.%).

Unchannelled valley-bottom					
Average similarity: 33.74					
Taxa	**Av.Abund**	**Av.Sim**	**Sim/SD**	**Contrib%**	**Cum.%**
Alona sp.	1.75	10.49	1.48	31.1	31.1
Cyclopoida	1.09	4.83	0.91	14.31	45.41
Ectocyclops sp.	1.51	3.31	0.59	9.82	55.23
Zonocypris sp.	1.04	3.12	0.71	9.24	64.48
Ceriodaphnia sp.	0.78	2.91	0.71	8.62	73.1
Channelled valley-bottom					
Average similarity: 24.30					
Taxa	**Av.Abund**	**Av.Sim**	**Sim/SD**	**Contrib%**	**Cum.%**
Cyclopoida	1.39	10.46	2.48	43.06	43.06
Alona sp.	1.36	5.3	0.58	21.83	64.89
Ectocyclops sp.	1.38	4.98	0.58	20.51	85.39
River					
Average similarity: 37.90					
Taxa	**Av.Abund**	**Av.Sim**	**Sim/SD**	**Contrib%**	**Cum.%**
Alona sp.	1.91	14.31	2.72	37.75	37.75
Chydorus sp.	1.68	7.21	0.9	19.02	56.77
Metadiaptomus sp.	0.89	3.8	0.67	10.03	66.8
Cyclopoida	0.84	3.29	0.69	8.67	75.47
Floodplain					
Average similarity: 31.25					
Taxa	**Av.Abund**	**Av.Sim**	**Sim/SD**	**Contrib%**	**Cum.%**
Alona sp.	1.79	14.46	SD=0!	46.28	46.28
Zonocypris sp.	3.06	11.19	SD=0!	35.81	82.09

A RDA plot was constructed to determine differences in composition at study sites (van den Brink *et al*., 2003; Malherbe *et al*., 2010). The RDA tri-plot (Figure 34) explained a cumulative variation of 33.74 %, with the first axis explaining 22.78 % and the second axis explaining 10.96 %. A Monte Carlo permutation test was conducted to determine which physico-chemical variables played a significant role in the zooplankton community structures. A test on all axes revealed a P value of 0.144, indicating no significant differences ($p > 0.05$).

Generally, the July (winter) surveys were grouped on the left side of the plot, while December (summer) and April (autumn) surveys were more spread out. The plot illustrated higher concentrations of oxygen and pH close to river sites in the upper left quadrant. Temperature, alkalinity, and TDS also illustrated higher concentrations close to the December (summer) survey in the upper right quadrant. Lower concentrations of nitrite, phosphate, chloride, and nitrate were found close to the July (winter) and April (autumn) surveys in the lower left quadrant. Electrical conductivity (EC), sulfate, and ammonium were found in lower concentrations close to the December (summer) and April (autumn) surveys near the centre point of the plot.

For the most part, the higher the system variable levels were the less zooplankton taxa were found. For instance, only *Ilyocypris* sp. and *Vestalenula* sp. were found in the area of high pH and oxygen concentrations. Only *Zonocypris* sp. was distributed in areas of the wetland complex with higher temperature ranges and high alkalinity concentrations. The distribution of the *Ectocyclops* sp., Cyclopoida, *Stenocypris* sp., *Ceriodaphnia* sp., *Macrocyclops* sp., and *Moina* sp. were not influenced by a single water quality variable, but a combination of all the variables.

Diaptomidae, *Kapcypridopis* sp., *Lovenula* sp., and *Alona* sp. were grouped close to phosphate and nitrite water quality variables. These taxa were also predominant during the July (winter) survey. All other taxa were more spread out between summer and autumn surveys. Most zooplankton taxa were shared between unchannelled and channelled valley-bottom wetland types. This is possibly due to the lack of flowing water, as compared to river types, and the fact that most sites sampled were classified as unchannelled valley-bottoms.

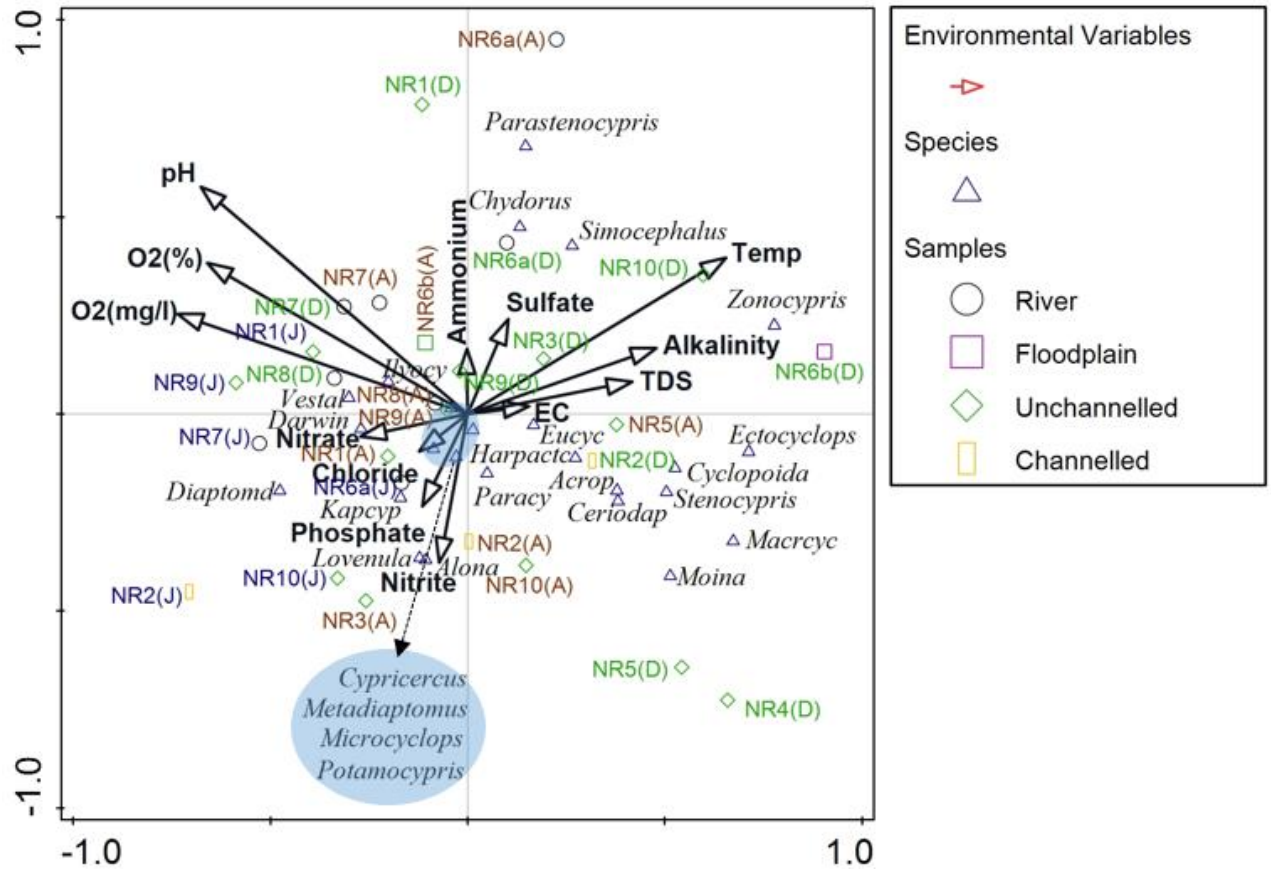

Figure 34: Redundancy analysis (RDA) tri-plot showing relationships between sites, seasons, zooplankton taxa, physico-chemical water variables, and hydrogeomorphic (HGM) types.

4.4 Discussion

4.4.1 Taxa characteristics

- Calanoida

The order Calanoida consists of very abundant organisms that occupy an intermediate position in the aquatic food chain (Griffiths *et al.,* 2015). Of this order all freshwater species in South Africa belong to the family Diaptomidae (Griffiths *et al.,* 2015). All African Diaptomidae are endemic to the continent and are often restricted in their distribution (Day *et al.,* 2001a; Griffiths *et al.,* 2015). The *Lovenula* sp., identified at the Ntsikeni Nature Reserve, is often found in temporary or brackish waters (Day *et al.,* 2001a) and is known to be carnivorous (Griffiths *et al.,* 2015). It has not been recorded from low-lying areas and appears to be confined to the high plateau of Africa (above 1000 m) and is well adapted to the temperature extremes of these regions (Day *et al.,* 2001a).

- Cladocera

Most species of Cladocera are found in waters with a pH ranging between 6.5 and 8.5; the majority are filter feeders but some forms are scrapers (Day *et al*., 1999). These microscopic crustaceans live mostly in lakes, wetlands, and temporary waters (Griffiths *et al.,* 2015). Taxa found at the Ntsikeni Nature Reserve (Table 4) included *Moina* sp., *Alona* sp., *Simocephalus* sp., and *Ceriodaphnia* sp.

Moina sp. is mostly restricted to small temporary pools or saline or alkaline water bodies (Day *et al*., 1999; Griffiths *et al.,* 2015), but some species are opportunistic and can be found in brackish water and have been commonly found in very turbid water soon after heavy summer rains (Day *et al*., 1999).

Alona sp. is a bottom dwelling taxa that is commonly eaten by larger crustaceans (Griffiths *et al.,* 2015). While *Simocephalus* sp. is littoral and *Ceriodaphnia* sp. is limnetic (Day *et al*., 1999).

- Cyclopoida

Cyclopoida is the most abundant and successful group of freshwater copepods that are distributed globally (Day *et al.,* 2001a; Griffiths *et al.,* 2015). They can be found in all types of water habitats such as lakes, ponds, wells, streams, rivers, and temporary pools (Day *et al.,* 2001a). Taxa found in the Ntsikeni Nature Reserve were *Ectocyclops* sp. and *Macrocyclops* sp.; representatives of both genera have a cosmopolitan distribution (Day *et al.,* 2001a).

- Ostracoda

The class Ostracoda can be found in a variety of marine and non-marine aquatic environments, ranging from humid to harsh environmental conditions, and fresh to highly saline waters (Day *et al.,* 2001a; Griffiths *et al.,* 2015). Aquatic habitats include marginal vegetation, streams, interstitial water, lake benthic zones, and temporary environments (Day *et al.,* 2001a), even occurring in some moist semi-terrestrial habitats (Griffiths *et al.,* 2015). In this class, only the order Podocopida were found in the Ntsikeni Nature Reserve; *Zonocypris* sp., *Stenocypris* sp., *Kapcypridopsis* sp., *Darwinula* sp., and *Vestalenula* sp. were notable according to the RDA tri-plot (Figure 34).

4.4.2 Community structure

Zooplankton taxa respond differently to their environments based on feeding methods, food preference and availability, trophic state, water clarity, as well as vulnerability to predators such as macroinvertebrates (Hart, 2012; Riato *et al.,* 2014). When both univariate and multivariate analyses were analysed it became evident that zooplankton taxa had both spatial and temporal distributional differences across the wetland complex.

Sites NR6a and NR7, which were both classified as river types, had low ranking values according to total individuals (Figure 26b) and Pielou's evenness index (J') (Figure 27c). Since rivers are lotic environments that contain flowing water, which is concentrated within a distinct channel (Ollis *et al.,* 2013), zooplankton are largely absent from such areas since they are known to be unable to withstand fast-flowing waters (van As *et al*., 2012). Site NR2, classified as a channelled valley-bottom, had the highest diversity and abundances during the April (autumn) survey and lowest during the July (winter) survey.

Many organisms are adapted to seasonal changes in temperature (Dallas and Day, 2004). Since water temperature changes are considered to be one of the more important factors governing seasonal variations in aquatic invertebrate community structures, changes in assemblages are to be expected (de Necker *et al.,* 2016). Rainfall in April (autumn), changes in temperature, and water volumes could be responsible for the variation in taxa numbers between autumn and winter. Finding more taxa and individuals during the autumn survey was a trend that de Necker *et al.* (2016) also observed in pans from three provinces in South Africa.

The multivariate analyses indicated that seasonality, sites, and HGM types did not have a significant effect on zooplankton community assemblages (Figures 31, 32, and 33), since all ANOSIM values showed < 0.5. The RDA tri-plot (Figure 34) indicated that water quality variables did not have a significant influence on the zooplankton community structure.

During this study Calanoida, Cladocera, Cyclopoida, and Podocopida were loosely associated with dissolved oxygen, nitrogen, and pH (Figure 34). These water quality variables have been found to be influential explanatory variables for determining zooplankton community assemblages in floodplain pans of the Phongolo River (Dube *et al.,* 2017). Nitrate is usually very abundant in the aquatic environment and its uptake is regulated by water temperature, oxygen availability, and pH (DWAF, 1996). Decreased water temperatures can result in an increase in dissolved oxygen and have been known to increase pH (Dallas and Day, 2004). This could explain the distribution of July (winter) sites close to the high dissolved oxygen and pH concentrations.

With regards to endemic cladoceran distribution in South Africa, van Damme *et al.* (2013) indicated that *Alona natalensis, Ovalona meridionalis, Pleuroxus carolinae, Dumontiellus africanus,* and *Ilyocryptus martensi* are restricted to mountain localities with an altitude greater than 1000 m in the eastern portion of South Africa. *Eurycercus freyi*, *Alona capensis, Alona striolata, Leydigia microps, Ilyocryptus africanus, Macrothrix sarsi,* and *Ceriodaphnia producta* are species known only from the Western Cape, while *Leydigia propinqua, Leydigia macrodonta,* and *Tretocephala colletti* are species that are widely distributed in South Africa in both mountain and lowland areas. When comparing genera found at the Ntsikeni Nature Reserve with those listed by van Damme *et al.* (2013), only *Alona* sp. and *Ceriodaphnia* sp. were found at Ntsikeni, indicating that there could possibly be more taxa which have not yet been found.

In order to establish overall biodiversity, the zooplankton community of the Ntsikeni Nature Reserve was compared to a study done on pans in the Highveld Region of the Mpumalanga Province by Riato *et al.* (2014). Although these two locations share similar altitude and geology, Ntsikeni receives more rainfall. Also, Ntsikeni's sites had lower pH values and much lower EC values than did the majority of the sites in Riato *et al.* (2014) (Figures 6 and 12). Riato *et al.* (2014) identified Rotifera as part of their study. However, when only comparing Arthropoda, Riato *et al.* (2014) identified 22 species belonging to 18 genera in six families. In the Ntsikeni Nature Reserve, 22 genera were identified belonging to eight families (Table 4).

The Ntsikeni wetland complex was also compared to a study on the freshwater floodplain wetlands of the Phongolo River completed by Dube *et al.* (2017). Although having similar pH values, the EC of the Phongolo wetlands were much higher and the dissolved oxygen values were slightly lower than that of the Ntsikeni wetland complex. In the Phongolo wetlands eight genera from the Cladocera were identified, with calanoids and cyclopoids also being found. In the Ntsikeni wetland complex, only six Cladocera genera were found (Table 4); five of these genera were also recorded by Dube *et al.* (2017). Calanoida and Cyclopoida were also found in the Ntsikeni wetland complex (Table 4).

Results from the Ntsikeni Nature Reserve was also compared to a study conducted by D'Ambrosio *et al.* (2016) on a wetland system located in the Llancanelo area, Argentina. The Llancanelo basin has a high altitude (> 1300 m) with very similar pH and dissolved oxygen values to that of the Ntsikeni wetland complex. Although the wetland receives less than 250 mm annual rainfall its water supply is supplemented by snowmelt. Thus, TDS values were found to be much higher in the Llancanelo basin (800 – 39700 mg/l) than that of the Ntsikeni wetland complex (Appendix Table A2). When comparing zooplankton biodiversity under the phylum Arthropoda, D'Ambrosio *et al.* (2016) identified 11 genera belonging to six families. As previously mentioned, 22 genera were identified belonging to eight families in the Ntsikeni Nature Reserve (Table 4). This indicates that the Ntsikeni Nature Reserve has a rich biodiversity of zooplankton spread throughout the wetland complex.

4.5 Conclusion

The univariate diversity indices and multivariate analyses indicated that there were differing zooplankton community structures in the Ntsikeni Nature Reserve. Originally it was thought that seasonality was responsible since variations within seasons were found. During July (winter), 13 taxa were found; during December (summer), 18 taxa were found; and during April (autumn), 20 taxa were found. The SIMPER analysis indicated that taxa, such as Cyclopoida, were noteworthy during the July (winter) and December (summer) surveys, but not during the April (autumn) survey. Taxa such as *Zonocypris* sp. and *Ectocyclops* sp. were important taxa during the December (summer) and April (autumn) surveys, yet not during the July (winter) survey. Although it appeared as if seasonality had an influence on zooplankton community structure, the ANOSIM indicated that seasonality was not significant to be considered a determining factor of zooplankton community structure.

Water quality variables such as dissolved oxygen, pH, temperature, salinity, and nitrogen are known to have effects on zooplankton community assemblages (de Necker *et al.,* 2016; Dube *et al.,* 2017). The RDA tri-plot indicated some taxa were found to be associated with dissolved oxygen concentrations and water temperatures. However, none of the water quality variables had a significant influence on zooplankton community structures.

When the zooplankton biodiversity of the Ntsikeni wetland complex was compared to other wetlands, both nationally and internationally, it showed that the Ntsikeni wetland complex had a relatively high diversity of zooplankton taxa. However, when comparing taxa found in the Ntsikeni wetland complex to those listed by van Damme *et al.* (2013) as being restricted to mountain localities in excess of 1000 m in the eastern portion of South Africa, then it can be assumed that the Ntsikeni wetland complex may have taxa that are still unidentified. Overall, it was found that the Ntsikeni Nature Reserve has quite a rich biodiversity of zooplankton taxa located throughout the wetland complex.

Chapter 5 – Macroinvertebrates

5.1 Introduction

Inland wetlands, which have a worldwide distribution, are unique due to their support of both terrestrial and aquatic biota (Muñoz, 2010). Wetlands are important habitats for birds, mammals, fish, reptiles, and amphibians of which macroinvertebrates occupy a central role in a wetland's food web in that they link primary producers to higher trophic level animals (Cooper *et al.,* 2009).

Macroinvertebrates have been known to retain and break down organic material, recycle minerals and nutrients, as well as contribute to the energy processes at different trophic levels (Malherbe *et al*., 2010). Some macroinvertebrates graze on living plant tissue, some on plant detritus, and some feed on algae; yet others are predators that prey on other macroinvertebrates and zooplankton (Cooper *et al.,* 2009). A "healthy" aquatic habitat with high habitat diversity and good water quality tends to support a greater diversity of macroinvertebrates (Hill *et al.,* 2005). Therefore, macroinvertebrate diversity and community structures can be used as an indicator of aquatic ecosystem health (Kemp *et al.,* 2014).

Macroinvertebrates have been used to analyse the ecological status of aquatic ecosystems since the 1990s (Azevêdo *et al.,* 2015) as they form an important link between primary productivity and higher trophic levels (Cooper *et al.,* 2009). Aquatic macroinvertebrates are regarded as one of the five groups of taxa referred to in wetland bio-assessment; the others being plants, waterbirds, amphibians, and fish (DWAF, 2004). Macroinvertebrates are among the most sensitive components of aquatic ecosystems; they are ubiquitous and represent diverse taxonomic groups and are easily influenced by physical and chemical changes (Dallas and Day, 2004; Azevêdo *et al.,* 2015). Each species has different sensitivities to stress and a large number of species within a community can offer a wide spectrum of responses to stresses that occur in the environment (Dallas and Day, 2004).

Although analyses of macroinvertebrates remain a useful bio-assessment tool, there are some disadvantages associated with their use as indicator organisms. Macroinvertebrate species diversity tends to be quite high with heterogeneous distribution (Dallas and Day, 2004; Cooper *et al.,* 2009), requiring a high level of expertise which can be expensive and time-consuming (Hill *et al.,* 2005). Another

reason is that each wetland has its own unique biotic and abiotic attributes which can lead to difficulty understanding macroinvertebrate community structures within specific wetlands (Cooper *et al.,* 2009). It should also be kept in mind that macroinvertebrate populations can be subject to rapid change usually due to extraneous factors such as hydrology, climate, and human land uses (Hill *et al.,* 2005; Cooper *et al.,* 2009). Despite these apprehensions the advantages far outweigh the disadvantages (Foster *et al.,* 2015). Macroinvertebrates, as biological indicators of environmental quality, still remain the mainstay of environmental monitoring as far as freshwater ecosystems are concerned (Malherbe *et al*., 2010).

Available information with regards to aquatic macroinvertebrates within the Ntsikeni Nature Reserve is limited. The most recently known information was compiled by Blackmore (2010) whilst declaring the Ntsikeni Nature Reserve a Ramsar site. Within this publication, Blackmore (2010) only lists 48 taxa, with most taxa only being identified to family level. According to EKZN (2016) invertebrates are important yet usually poorly understood. This is especially true for the Ntsikeni Nature Reserve.

The research aims of this chapter were to establish the community structure and distribution of aquatic macroinvertebrates of the Ntsikeni Nature Reserve. The information was also used to identify the links between macroinvertebrates and the surrounding abiotic factors such as water quality and sediment quality.

5.2 Materials and Methods

5.2.1 Procedure

Each of the ten selected sites was sampled using a sweep net measuring 30 cm x 30 cm, with a mesh size of 1 mm. At each site the marginal vegetation was sampled for 3 – 5 min across a representative area that included different vegetation and different flow velocities, if present. Wherever possible, as is the case with sites NR7, NR6a, and NR8, the biotopes consisting of stones and/or gravel, sand, and mud (GSM) areas were also sampled. All samples collected were preserved on site using 5 – 10 % NBF, with rose Bengal as a vital dye. The above methodology was adapted from Foster *et al.* (2015) and de Necker *et al.* (2016).

In the laboratory, all samples were rinsed with tap water using a sieve with a 250 µm mesh size. Macroinvertebrates were separated from the plant matter under a Nikon, Model C-LEDS microscope. Once separated from the plant matter, all macroinvertebrates were identified to the lowest taxonomic level using relevant identification guides (Day *et al.,* 1999; 2001a; 2001b; 2003; Day and de Moor, 2002; Gerber and Gabriel, 2002; de Moor *et al.,* 2003a; 2003b; Stals and de Moor, 2007; Griffiths *et al.,* 2015). All macroinvertebrates were thereafter preserved in 70 % ethanol.

5.2.2 Statistical analyses

The collected macroinvertebrate data were used to perform both univariate and multivariate analyses. The univariate diversity indices consisted of Margalef's species richness (d), Shannon diversity index (H'), Pielou's evenness index (J'), and a distributional k-dominance plot (Kemp *et al.,* 2014; Foster *et al.,* 2015). The diversity indices were drawn up to show the distribution of macroinvertebrate taxa in relation to their numbers between different sites and surveys (Kemp *et al.,* 2014). All these analyses were completed using Primer Version 7. To verify the significance of the total species (S), total individuals (N), Margalef's species richness (d), Shannon diversity index (H'), and Pielou's evenness index (J'), both spatial and temporal differences were determined using ANOVA. The data were tested for normality using Kolmogorov-Smirnov test with $p < 0.05$. If $p < 0.05$ Tukey's *post hoc* statistical analysis test was used to determine significant difference; if $p > 0.05$, the Kruskal-Wallis *post hoc* test was performed (methods adapted from de Klerk *et al.,* 2012). These additional analyses were completed on Graphpad Prism version 6.

The multivariate analyses were completed to determine both temporal and spatial variation within the macroinvertebrate community structure (Foster *et al.,* 2015). These analyses consisted of hierarchical clustering, NMDS, and RDA (Ferreira *et al.,* 2009; Malherbe *et al.,* 2010; Foster *et al.,* 2015). The multivariate analyses were performed using Canoco Version 5 as well as Primer Version 7.

The constructed multivariate analyses — hierarchical cluster, NMDS, ANOSIM, SIMPER — were based on the Bray-Curtis similarity matrix and were used to identify macroinvertebrate assemblages (Malherbe *et al.,* 2010; Foster *et al.,* 2015). An NMDS plot is a more informative summary than the corresponding cluster analysis (Clarke

and Warwick, 2001). The NMDS plot uses an iterative algorithm that converts multidimensional data of a similarity matrix into a minimal dimensional space (Rees *et al.,* 2005), in this case a two dimensional plot.

Following the hierarchical cluster analyses and NMDS plots, an ANOSIM was carried out for each plot based on a *priori* selected groups for season, wetland type, and site. The ANOSIM was used to examine statistical significance between samples (Rees *et al.,* 2005). Performing an ANOSIM produces an R value which is scaled to lie between -1 and +1 with zero indicating the null hypothesis to be true (Chapman and Underwood, 1999; Rees *et al.,* 2005). Values close to 1 (i.e. $p \geq 0.5$) indicate substantial differences while values closer to 0 (i.e. $p < 0.5$) show great similarity (de Necker *et al.,* 2016).

One-way similarity percentage (SIMPER) analyses were constructed with a 70 % contribution cut-off to be used as exploratory analyses rather than statistical testing frameworks (Clarke and Warwick, 2001). The SIMPER analysis was used to calculate the average contribution of individual terminal fragments to the average dissimilarity that exists between samples (Rees *et al.,* 2005). This procedure explains which macroinvertebrate taxa are responsible for groupings observed (Foster *et al.,* 2015). Finally, a RDA plot was constructed to indicate the differences and influences between sites, water quality variables, and the macroinvertebrate data (Malherbe *et al.,* 2010).

5.3 Results

All taxa collected and identified in the Ntsikeni Nature Reserve are listed in Table 8. A more detailed layout of the number of taxa found both spatially and temporally is given in Table C1 in Appendix C. In total, 129 macroinvertebrate taxa were identified with 84 taxa found in July (winter), 95 taxa found in December (summer), and 94 taxa found in April (autumn) (Appendix Table C1). According to Table 8, Coleoptera was the order with the most taxa identified, totalling 35 taxa. Ephemeroptera was the order in which the most individuals were counted, totalling 9757 individuals.

Table 8: Aquatic macroinvertebrate taxa sampled from all sites in the Ntsikeni Nature Reserve during all surveys (July/winter 2015, December/summer 2015, and April/autumn 2016).

Kingdom	Phylum	Subphylum	Class	Order	Family	Species
Animalia	Annelida		Clitellata	Arhynchobdellida	Salifidae	*Salifa africana*
				Rhynchobdellida	Glossiphoniidae	*Batracobdelloides tricarinata*
						Helobdella stagnalis
						Theromyzon cooperi
			Oligochaeta	Tubificida	Haplotaxidae	*Haplotaxis africanus*
					Naididae	
	Arthropoda	Chelicerata	Arachnida	Acarina	Pontarachnidae	
				Araneae	Lycosidae	
					Pisauridae	
					Tetragnathidae	
		Crustacea	Branchiopoda	Cladocera	Daphniidae	*Ceriodaphnia* sp.
						Scapholeberis sp.
			Malacostraca	Decapoda	Potamonautidae	*Potamonautes sidneyi*
				Amphipoda	Sternophysingidae	*Sternophysinx* sp.
			Ostracoda	Podocopida	Cyprididae	*Potamocypris* sp.
		Hexapoda	Insecta	Coleoptera	Chrysomelidae	*Donaciasta* sp.
					Curculionidae	*Stenopelmus rufinasus*
					Dytiscidae	*Canthyporus* sp.
						Cybister sp.
						Hydaticus sp.
						Hydroglyphus sp.
						Hyphydrus sp.
						Laccophilus sp.
						Methles sp.
						Philaccolus sp.
						Rhantus sp.
					Elmidae	*Pachyelmis* sp.
					Gyrinidae	*Aulonogyrus* sp.
						Dineutus sp.
						Gyrinus sp.
						Orectogyrus sp.
					Haliplidae	*Haliplus* sp.

Table 8 (continued):

Kingdom	Phylum	Subphylum	Class	Order	Family	Species
Animalia	Arthropoda	Hexapoda	Insecta	Coleoptera	Hydraenidae	*Coelometopon* sp.
						Hydraena sp.
						Parasthetops sp.
					Hydrophilidae	*Berosus* sp.
						Enochrus sp.
						Laccobius sp.
						Regimbartia sp.
					Noteridae	*Hydrocanthus* sp.
					Psephenidae	*Afrobrianax ferdyi*.
						Afropsephenoides sp.
					Ptilodactylidae	
					Spercheidae	*Spercheus* sp.
				Diptera	Athericidae	
					Ceratopogonidae	
					Chironomidae	*Paraphaenocladius* sp.
					Culicidae	*Anopheles* sp.
						Culex sp.
						Uranotaenia sp.
					Dixidae	*Dixa bicolor*
						Dixella harrisoni
					Empididae	*Hemerodromia* sp.
					Ephydridae	
					Muscidae	
					Sciomyzidae	
					Simuliidae	*Simulium (Nevermannia)* sp.
					Stratiomyidae	
					Tabanidae	
					Tipulidae	*Antocha* sp.
						Dolichopeza sp.
						Gonomyia sp.
						Limonia (Dicranomyia) capicola
						Tipula sp.
				Ephemeroptera	Baetidae	*Acanthiops* sp.
					Caenidae	*Afrocaenis* sp.
					Heptageniidae	*Afronurus* sp.
					Leptophlebiidae	*Adenophlebia* sp.
						Aprionyx sp.
						Castanophlebia sp.
						Hyalophlebia sp.
					Tricorythidae	*Tricorythus* sp.
				Hemiptera	Belostomatidae	*Appasus* sp.
					Corixidae	*Micronecta* sp.
						Sigara sp.
					Gerridae	*Aquarius distanti*
						Eurymetra natalensis

Table 8 (continued):

Kingdom	Phylum	Subphylum	Class	Order	Family	Species
Animalia	Arthropoda	Hexapoda	Insecta	Hemiptera	Naucoridae	*Laccocoris* sp.
					Nepidae	*Laccotrephes* sp.
						Ranatra sp.
					Notonectidae	*Anisops* sp.
						Enithares sp.
						Nychia limpida
					Paraphrynoveliidae	*Paraphrynovelia* sp.
					Pleidae	*Plea* sp.
					Veliidae	*Ocellovelia* sp.
						Rhagovelia sp.
						Xiphoveloidea major
				Lepidoptera	Crambidae	*Nymphula* sp.
				Odonata	Aeshnidae	*Aeshna* sp.
						Anax sp.
					Coenagrionidae	*Ceriagrion* sp.
						Pseudagrion sp.
					Corduliidae	*Phyllomacromia* sp.
					Gomphidae	*Onychogomphus* sp.
					Lestidae	*Lestes* sp.
					Libellulidae	*Notiothemis jonesi jonesi*
					Synlestidae	*Chlorolestes* sp.
				Plecoptera	Notonemouridae	*Aphanicercella* sp.
					Perlidae	*Neoperla* sp.
				Trichoptera	Ecnomidae	*Ecnomus* sp.
					Hydropsychidae	*Cheumatopsyche* sp.
					Hydroptilidae	*Hydroptila* sp.
						Oxyethira velocipes
					Leptoceridae	*Athripsodes* sp.
						Leptecho sp.
	Mollusca		Bivalvia	Veneroida	Sphaeriidae	*Pisidium* sp.
			Gastropoda	Hygrophila	Planorbidae	*Bulinus* sp.
						Ferrissia sp.
						Gyraulus sp.
					Lymnaeidae	*Lymnaea truncatula*
				Stylommatophora	Succineidae	*Oxyloma patentissima*
	Nematoda		Chromadorea	Monhysterida	Monhysteridae	*Monhystera* sp.
	Platyhelminthes		Rhabditophora	Tricladida		
						Sponge A

5.3.1 Univariate diversity indices

During the April (autumn) survey (Figure 35a), the highest number of taxa (48) were sampled at site NR6a. During the December (summer) survey (Figure 35a), the least number of taxa (8) were recorded at site NR4. The distribution of taxa, both spatially and temporally, indicated no statistical significance (Figures 37 and 38). The most individuals (2451) were recorded at site NR7 during July (winter) (Figure 35b). Site NR4, during the December (summer) survey, had the least individuals, totalling only 41. No significant differences were found when assessing the spatial analysis (Figure 37). However, the July (winter) survey, where the most individuals were collected, indicated significant difference from the December (summer) survey (Figure 38).

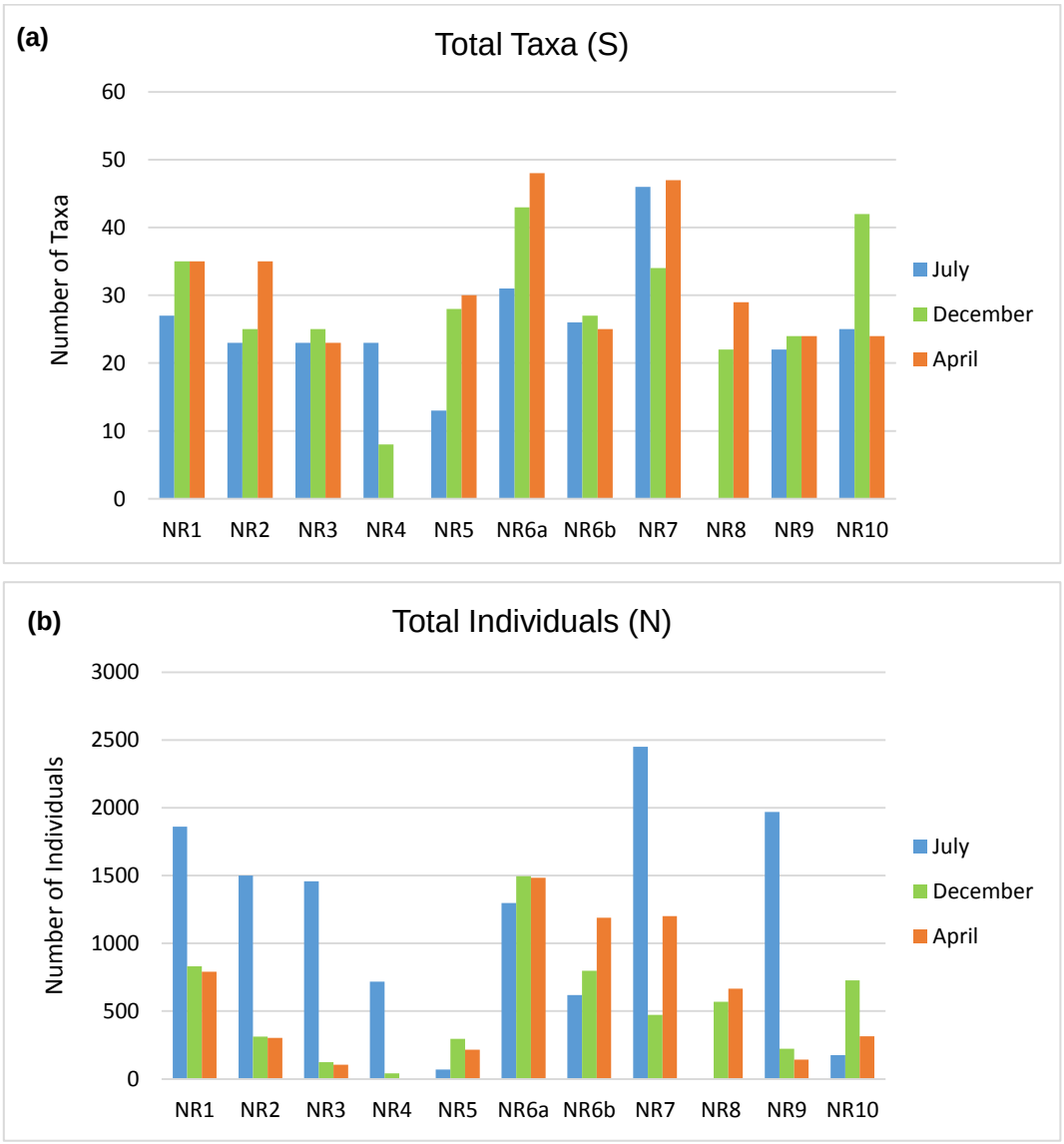

Figure 35: Total number of macroinvertebrates found at each site during all surveys at the Ntsikeni Nature Reserve in July/winter 2015, December/summer 2015, and April/autumn 2016 indicating (a) total taxa (S) and (b) total individuals (N).

Margalef's Species Richness (d) is calculated by incorporating the total number of individuals and the number of species present at a particular site (Clarke and Warwick, 2001). The Margalef's Species Richness results (Figure 36a) indicated that the April (autumn) survey had the highest value (6.49) at site NR7, while the December (summer) survey had the lowest value (1.88) at site NR4. All remaining values ranged between 2.76 and 6.44. No significant differences were found when assessing the spatial distribution data (Figure 37), yet the temporal analysis indicated significant difference between the July (winter) and April (autumn) surveys, with the April (autumn) survey containing the highest mean value (Figure 38).

The Shannon Diversity Index (H') is a proportional index that sums up species based on their relative abundance (Stirling and Wilsey, 2001). It indicated that, during the December (summer) survey, the highest value (2.92) was recorded at site NR3, while the July (winter) survey had the lowest value (1.20) at site NR2 (Figure 36b). All remaining values ranged between 1.41 and 2.87. No significant differences were found when assessing the data either spatially or temporally (Figures 37 and 38).

The Pielou's Evenness Index (J') values ranged between 0 and 1, with the value 0 representing an uneven distribution of abundance and the value 1 representing even distribution of abundance among different taxa (Stirling and Wilsey, 2001; Wolmarans *et al.*, 2014). The results (Figure 36c) indicated that the December (summer) survey had the highest value (0.90) at site NR3, while the July (winter) survey had the lowest value (0.38) at site NR2. The remaining values ranged between 0.42 and 0.85. Since the majority of the values were closer to 1 than to 0, the distribution of macroinvertebrate abundance relative to each toxon was considered to be even. If consistent patterns of distribution evenness are found within a certain community then that ecosystem is generally considered to be healthy (Wolmarans *et al.*, 2014). Figures 37 and 38 concur that there are no significant differences to be found either spatially or temporally throughout the wetland complex.

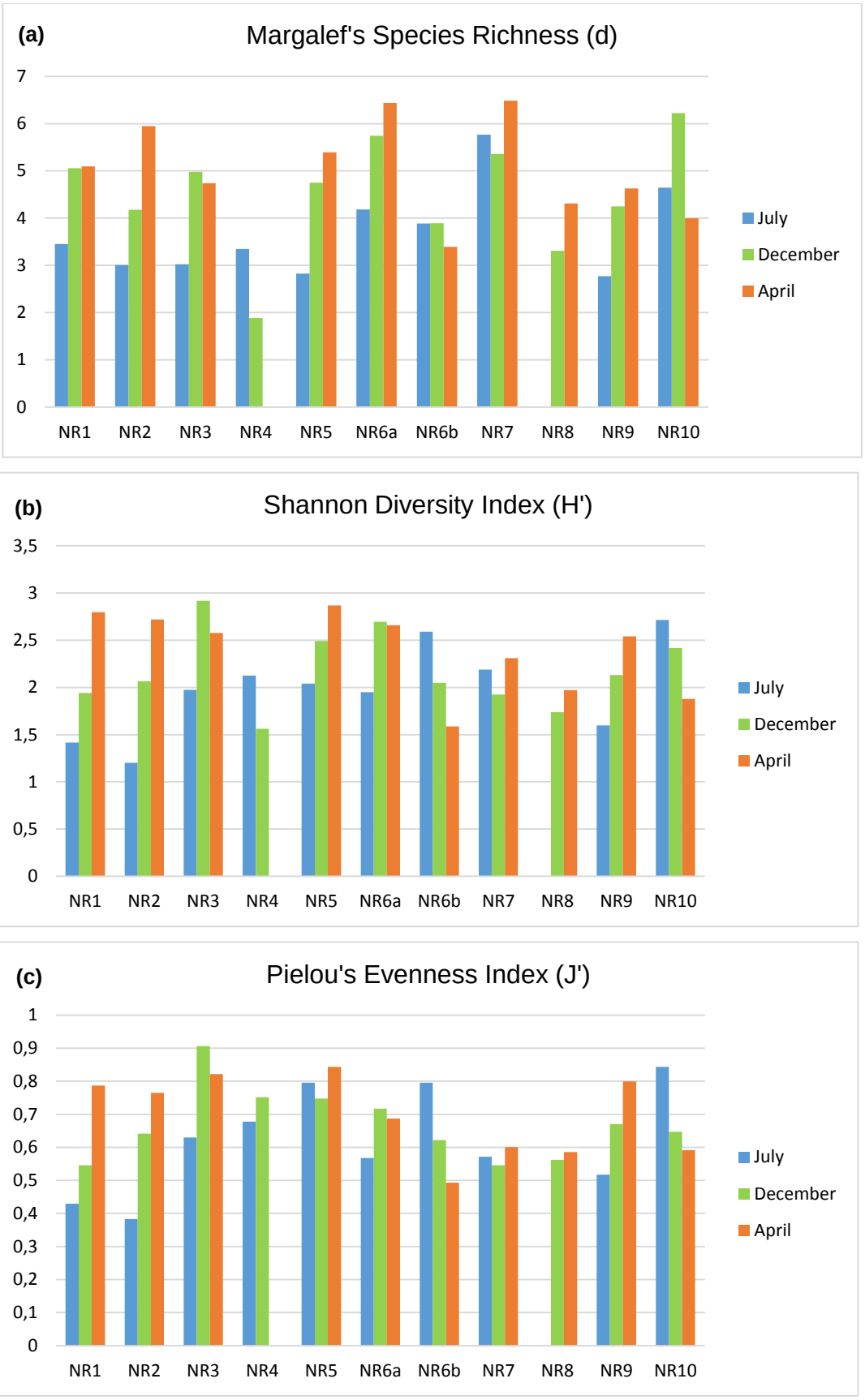

Figure 36: Univariate diversity indices indicating (a) Margalef's species richness index, (b) Shannon diversity index, and (c) Pielou's evenness index.

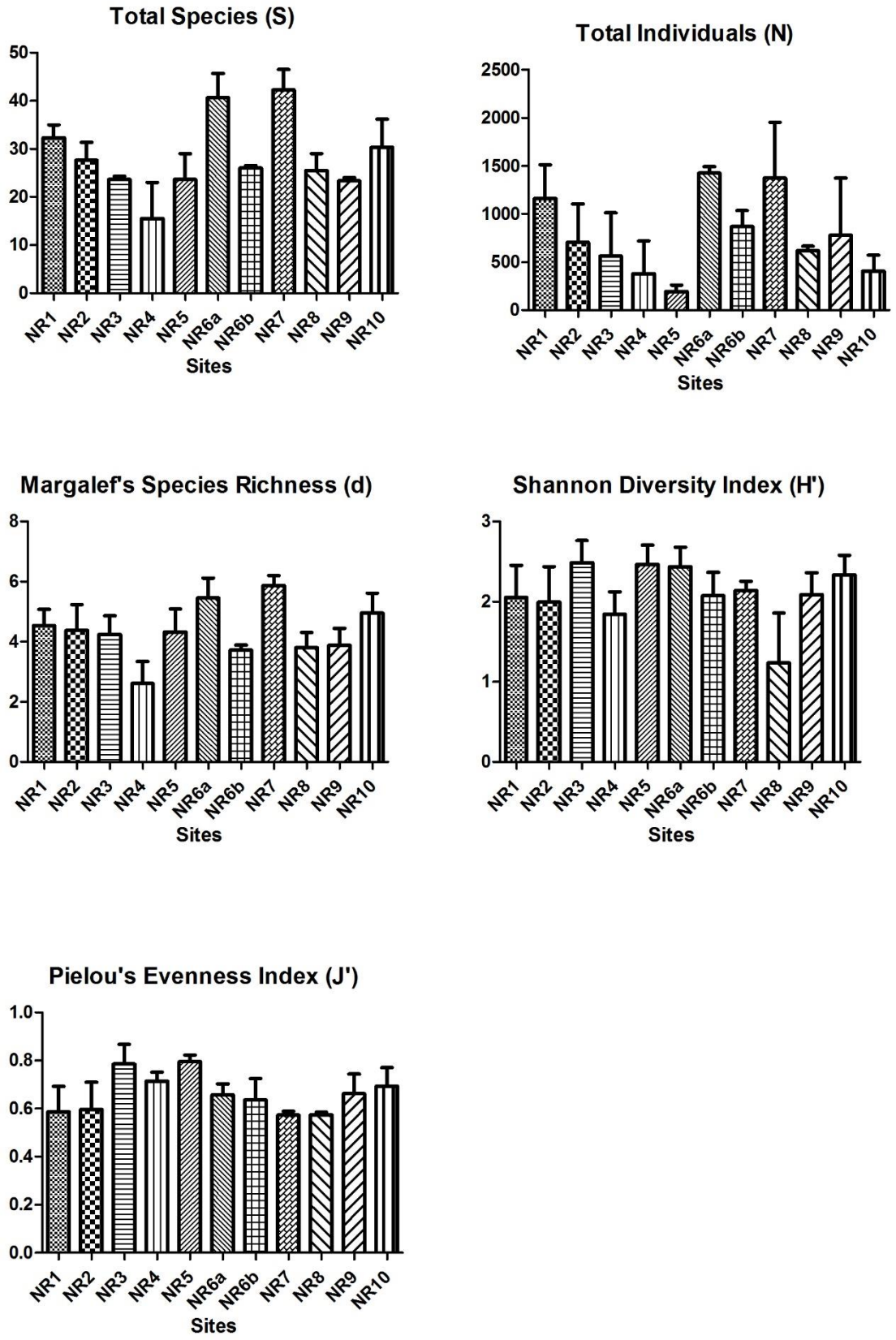

Figure 37: Spatial variation of macroinvertebrates collected from the Ntsikeni Nature Reserve showing mean and standard error of diversity indices (Figures 35 and 36).

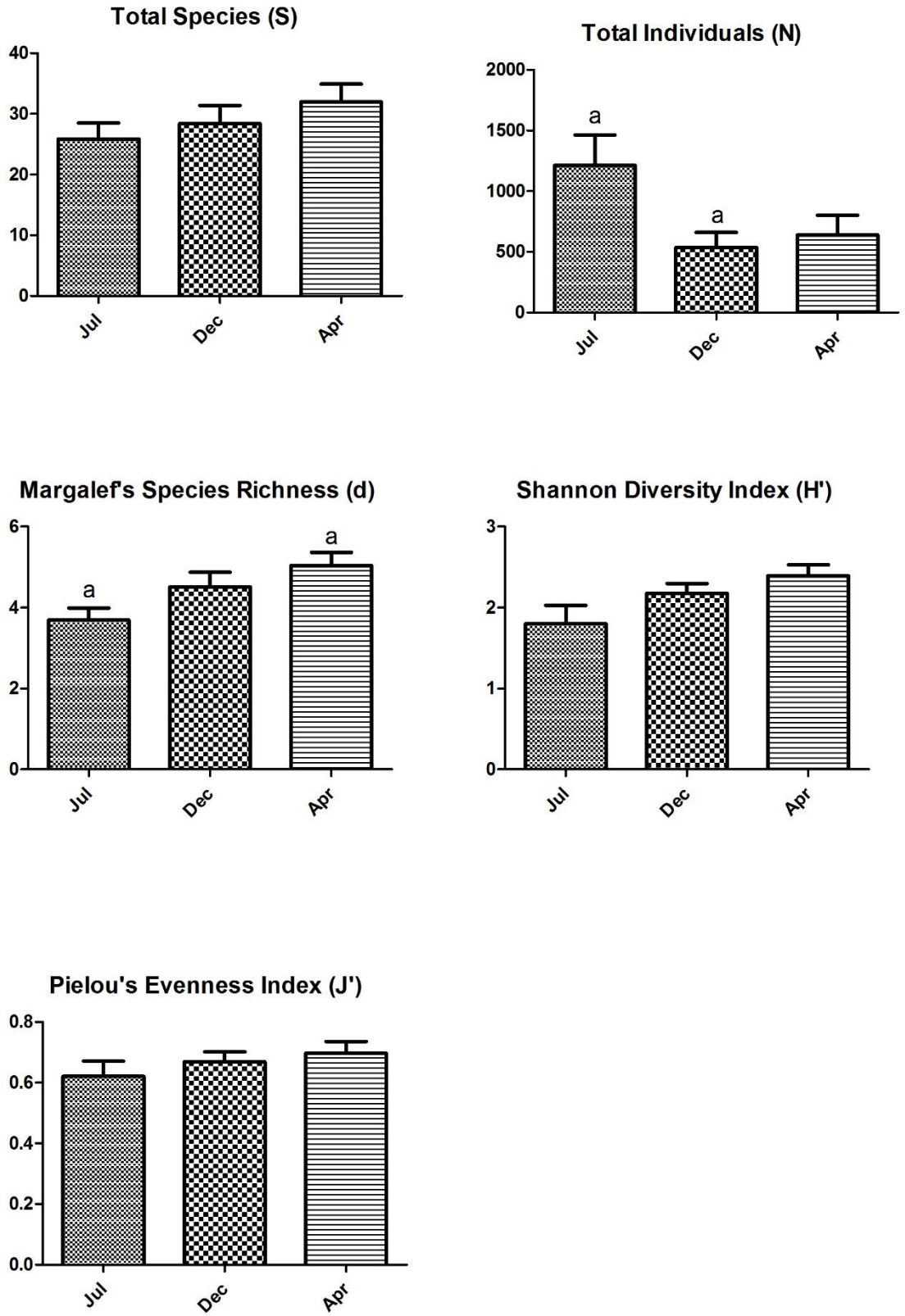

Figure 38: Temporal variation (July/winter, December/summer, April/autumn) of macroinvertebrates collected from the Ntsikeni Nature Reserve showing mean and standard error of diversity indices (Figures 35 and 36). Bars with common superscript (a) differ significantly ($p < 0.05$).

By using the macroinvertebrate, site, and survey data collected from the Ntsikeni Nature Reserve, a distributional k-dominance plot was assembled. This plot was constructed by determining the order of importance in terms of abundance and ranking the different taxa in a decreasing order to determine the dominant taxa (Malherbe *et al.,* 2010). The k-dominance plot (Figure 39) indicated that site NR2 during the July (winter) survey had the highest degree of dominance, with a total of 23 taxa and 1500 individuals. Site NR1, during the same July (winter) survey, was ranked with the second highest degree of dominance and contained 27 taxa and 1862 individuals. Site NR3, during the December (summer) survey, had the lowest degree of dominance having 25 taxa with only 124 individuals.

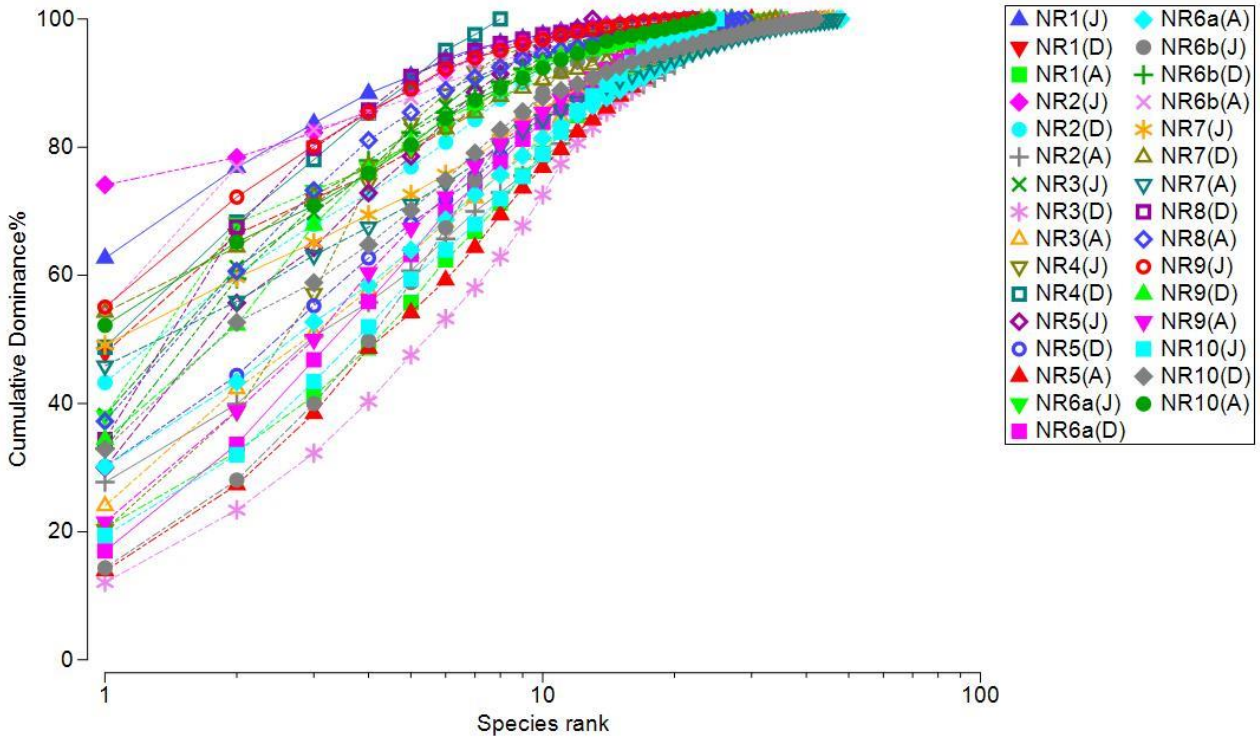

Figure 39: K-Dominance curves of the relative abundance of taxa for each site sampled during July/winter (J), December/summer (D), and April/autumn (A).

5.3.2 Multivariate analyses

The multivariate analyses were used to determine the effects of season, site, and HGM type on the macroinvertebrate community structure using the Bray-Curtis similarity matrix, hierarchical cluster, and NMDS analyses (Figures 40, 41, and 42). The first hierarchical cluster analysis (Figure 40a) was constructed to show groupings or similarities that occurred within different seasons. The December (summer) and April (autumn) surveys were mostly grouped in the upper two thirds of the hierarchical cluster analyses, while the July (winter) surveys were mostly grouped in the lower third. At a similarity cut-off of 44 %, all July (winter) data, with the exception of three sites, were grouped together (Figure 40a). The NMDS plot (Figure 40b) concurred in that it showed the July (winter) surveys were grouped together. When performing ANOSIM on the different seasons, an R value of 0.114 was obtained indicating no significant difference between seasonal groupings.

The second hierarchical cluster (Figure 41a) showed groupings between different sites sampled, irrespective of the sampling season. At a similarity cut-off of 41 %, sites NR8(D), NR8(A), NR9(D), and NR9(A) were grouped together, with site NR8 being a river type and site NR9 being an unchannelled valley-bottom (Figure 41a). The NMDS plot (Figure 41b) showed this in the upper left corner (encircled in grey). At a similarity cut-off of 41 %, sites NR1 (D and A), NR5 (D and A), NR6b (J, D, and A), and NR10 (J, D, and A) were grouped together. When calculating an ANOSIM of the different sites, an R value of 0.509 was obtained indicating that sites have great difference and the least similarity between each other when compared to other clusters.

The third, and final, hierarchical cluster analysis (Figure 42a) was constructed to show if macroinvertebrate communities grouped together by HGM types. At a similarity cut-off of 34 %, two main groups were found (Figure 42a). The group located at 36 % revealed that all river types were grouped together. The group located at 34 % revealed that all river types were absent and the cluster consists of only unchannelled valley-bottoms, channelled valley-bottoms, and floodplains. At a similarity cut-off of 57 %, all floodplain types were grouped together (Figure 42a). The NMDS plot (Figure 42b) indicated that river types were grouped relatively close together and floodplain types were grouped in one area. The ANOSIM of the different HGM types revealed an R value of 0.061. This indicated that no significant difference between different HGM types was present at the Ntsikeni Nature Reserve.

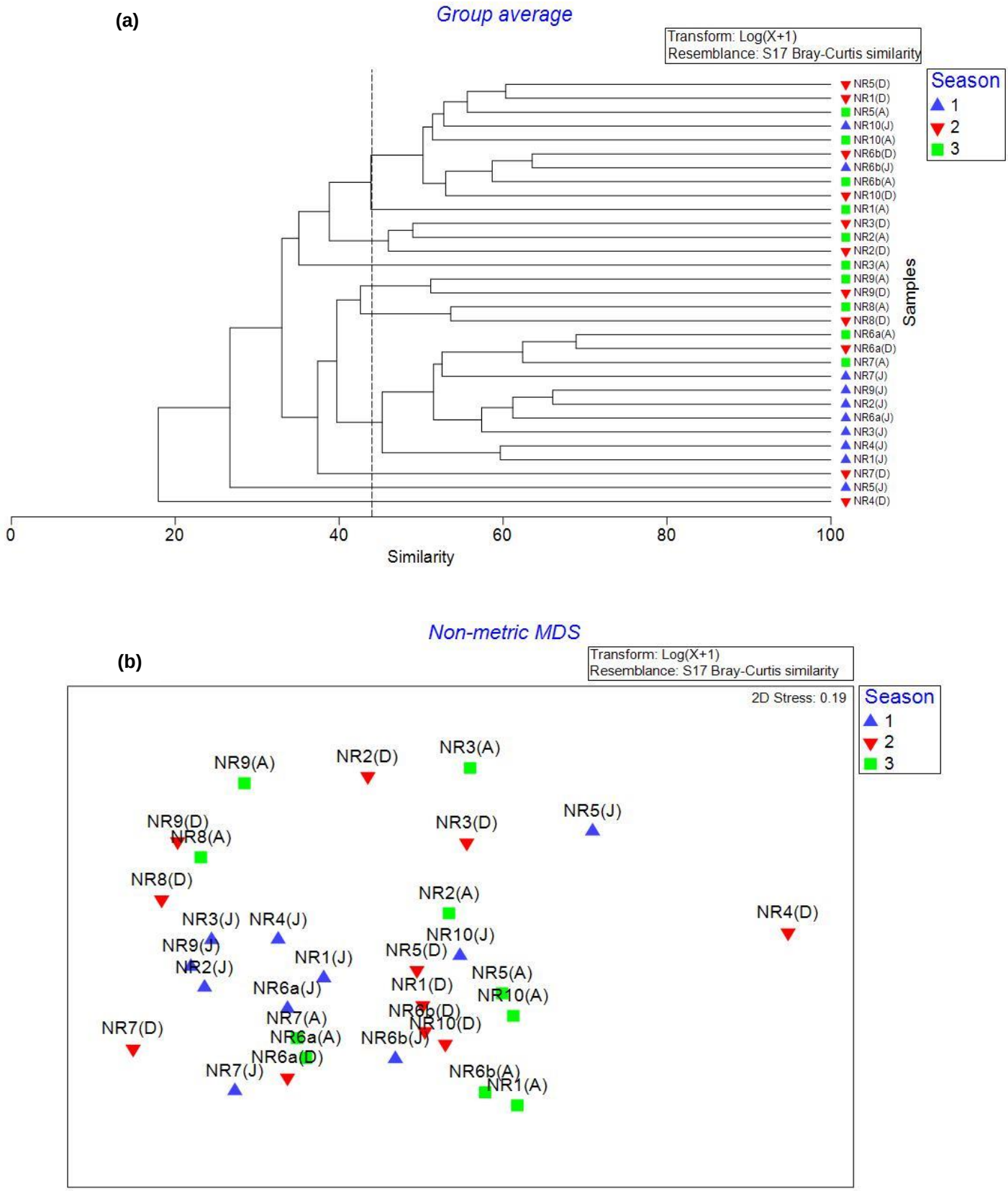

Figure 40: (a) Hierarchical cluster analysis indicating 44 % similarity and (b) non-metric multidimensional scaling (NMDS) plot based on Bray-Curtis similarity matrix showing groupings based on seasons. Seasonal surveys to the Ntsikeni Nature Reserve (NR) occurred during the winter (1) month of July (J) 2015, the summer (2) month of December (D) 2015 and the autumn (3) month of April (A) 2016.

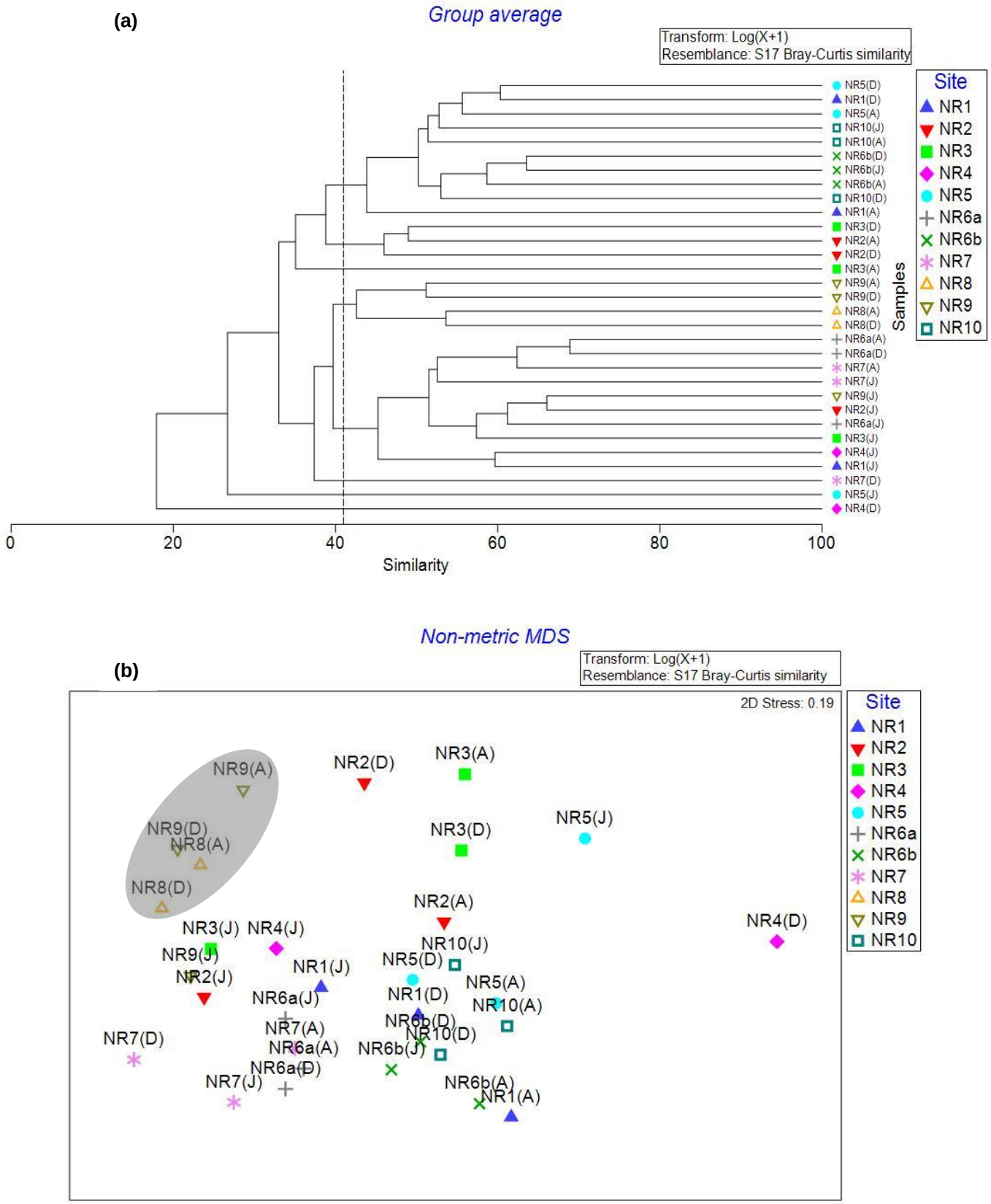

Figure 41: (a) Hierarchical cluster analysis indicating 41 % similarity and (b) non-metric multidimensional scaling (NMDS) plot based on Bray-Curtis similarity matrix showing groupings based on sites sampled across the Ntsikeni wetland complex.

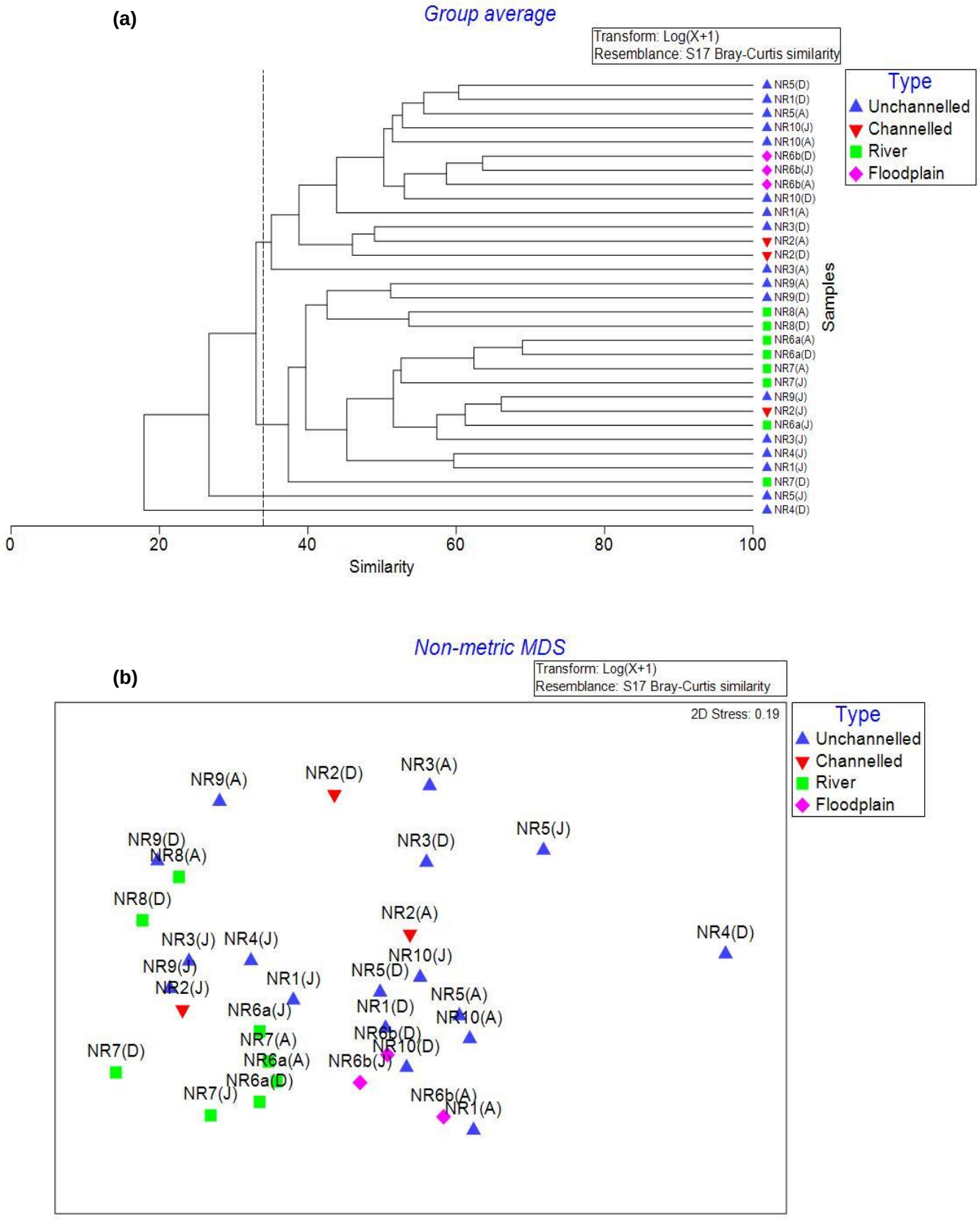

Figure 42: (a) Hierarchical cluster analysis indicating 34 % similarity and (b) non-metric multidimensional scaling (NMDS) plot based on Bray-Curtis similarity matrix showing groupings based on hydrogeomorphic (HGM) types.

To help explain the influence of seasons, sites, and HGM types on macroinvertebrate community structures, SIMPER analyses were constructed (Tables 9, 10, and 11). The July (winter) survey (Table 9) had an average similarity of 43.44 %. Baetidae had the largest percentage contribution of 15.48 %, followed closely by Chironomidae which had 10.92 % contribution. During this survey *Adenophlebia* sp. and *Ecnomus* sp. had contributions of 7.19 % and 3.85 % respectively, but did not feature again in either the December (summer) or April (autumn) surveys.

The December (summer) survey (Table 9) had an average similarity of 34.28 %. Baetidae had a contribution of 12.7 %, once more followed closely by Chironomidae which had a contribution of 12.11 %. *Oxyethira velocipes*, *Pseudagrion* sp., and Ceratopogonidae had contributions of 5.33 %, 5.01 %, and 3.05 % respectively, yet did not make significant contributions in either the July (winter) or April (autumn) surveys.

The April (autumn) survey (Table 9) had an average similarity of 37.45 %. Chironomidae had the largest percent contribution of 14.59 %, followed by *Anax* sp. with a contribution of 9.04 %. *Ceriagrion* sp., *Ocellovelia* sp., *Plea* sp., *Pontamonautes sidneyi,* Dytiscidae, and *Hyphydrus* sp. (listed in descending order) had contributions ranging from 3.46 % to 1.96 %. These taxa were only significant contributions during the April (autumn) survey and did not feature in either of the previous surveys.

Table 9: Similarity percentage analysis (SIMPER) with a 70 % contribution cut-off showing which macroinvertebrate taxa are responsible for groupings in each season. Columns indicate average abundance (Av.Abund), average similarity (Av.Sim), similarity/standard deviation (Sim/SD), contribution percentage (Contrib%), and cumulative percentage (Cum.%).

July (winter)					
Average similarity: 43.44					
Taxa	**Av.Abund**	**Av.Sim**	**Sim/SD**	**Contrib%**	**Cum.%**
Baetidae	5.11	6.72	3.44	15.48	15.48
Chironomidae	3.71	4.74	1.79	10.92	26.4
Afrocaenis sp.	3.45	3.88	1.58	8.93	35.33
Sigara sp.	2.86	3.45	1.26	7.94	43.27
Adenophlebia sp.	3.18	3.12	1.39	7.19	50.46
Micronecta sp.	2.85	2.95	1.61	6.8	57.26
Oligochaeta	2.92	2.59	1.06	5.95	63.21
Ecnomus sp.	1.8	1.67	1.11	3.85	67.06
Bulinus sp.	1.69	1.61	0.81	3.7	70.76

Table 9 (continued):

December (summer)					
Average similarity: 34.28					
Taxa	**Av.Abund**	**Av.Sim**	**Sim/SD**	**Contrib%**	**Cum.%**
Baetidae	3.44	4.35	1.36	12.7	12.7
Chironomidae	2.95	4.15	1.44	12.11	24.81
Micronecta sp.	2.36	2.43	1.09	7.08	31.89
Bulinus sp.	2.29	1.94	1.14	5.66	37.55
Oxyethira velocipes	1.5	1.83	1.32	5.33	42.88
Anisops sp.	2.17	1.75	0.79	5.11	47.99
Pseudagrion sp.	2.04	1.72	0.73	5.01	52.99
Afrocaenis sp.	1.92	1.42	0.67	4.14	57.13
Anax sp.	1.61	1.38	0.75	4.03	61.16
Oligochaeta	1.82	1.28	0.78	3.73	64.89
Ceratopogonidae	0.93	1.04	1.23	3.05	67.94
Sigara sp.	1.42	0.95	0.63	2.78	70.72
April (autumn)					
Average similarity: 37.45					
Taxa	**Av.Abund**	**Av.Sim**	**Sim/SD**	**Contrib%**	**Cum.%**
Chironomidae	3.96	5.46	3.61	14.59	14.59
Anax sp.	2.34	3.39	2.61	9.04	23.63
Baetidae	3.18	3.11	1.49	8.31	31.94
Oligochaeta	2.19	2.61	1.6	6.96	38.9
Bulinus sp.	2.25	1.73	0.85	4.61	43.51
Sigara sp.	2.17	1.59	0.79	4.26	47.77
Anisops sp.	1.48	1.48	1.22	3.96	51.73
Micronecta sp.	1.94	1.39	1.22	3.71	55.44
Ceriagrion sp.	1.86	1.3	0.62	3.46	58.9
Ocellovelia sp.	1.23	1.09	1.13	2.91	61.8
Plea sp.	1.59	1.09	0.84	2.9	64.7
Pontamonautes sidneyi	1.23	1.06	1.03	2.83	67.54
Dytiscidae	1.2	0.84	0.76	2.25	69.78
Hyphydrus sp.	0.98	0.73	0.66	1.96	71.74

Chironomidae (Table 10) made the largest contribution at sites NR1, NR3, NR4, NR6b, and NR10, with a contribution percentage of 13.04 % (NR10) being the lowest contribution. Baetidae had the largest contribution percentages at sites NR6a, NR7, NR8, and NR9. Baetidae also made contributions at sites NR1, NR2, NR3, NR5, and NR10. *Sigara* sp., listed amongst the top four most contributed taxa, was found in sites NR1, NR5, NR6b, and NR10.

Bulinus sp., listed among the top five most contributed taxa, was found at sites NR1, NR5, NR6a, NR6b, NR7, and NR10. *Pseudagrion* sp. was found to be the highest contribution at site NR2 with a contribution percentage of 16.24 %. This taxa was also found at sites NR5, NR6a, NR7, and NR9, having lower contribution percentages than site NR2. Overall, site NR6a had the highest similarity percentage of 64.35 %, while site NR4 had the lowest similarity percentage with only 15.31 % similarity.

Table 10: Similarity percentage analysis (SIMPER) with a 70 % contribution cut-off showing which macroinvertebrate taxa are responsible for groupings found within each site sampled across the Ntsikeni wetland complex. Columns indicate average abundance (Av.Abund), average similarity (Av.Sim), similarity/standard deviation (Sim/SD), contribution percentage (Contrib%), and cumulative percentage (Cum.%).

NR1					
Average similarity: 42.33					
Taxa	**Av.Abund**	**Av.Sim**	**Sim/SD**	**Contrib%**	**Cum.%**
Chironomidae	4.14	5.72	12.17	13.51	13.51
Sigara sp.	3.67	4.63	5.71	10.95	24.45
Bulinus sp.	3.12	4.08	8.4	9.64	34.1
Micronecta sp.	3.59	3.63	3.51	8.57	42.66
Oligochaeta	4.35	3.21	0.58	7.58	50.24
Anax sp.	2.22	2.27	6.26	5.37	55.61
Baetidae	2.83	2.06	1.16	4.85	60.47
Batracobdelloides tricarinata	1.56	1.6	10.94	3.78	64.24
Ceriagrion sp.	2.82	1.53	0.58	3.62	67.86
Notiothemis jonesi jonesi	1.25	1.19	4.34	2.82	70.68
NR2					
Average similarity: 35.39					
Taxa	**Av.Abund**	**Av.Sim**	**Sim/SD**	**Contrib%**	**Cum.%**
Pseudagrion sp.	3.48	5.75	16.64	16.24	16.24
Baetidae	4.14	4.92	3.39	13.89	30.13
Afrocaenis sp.	2.53	3.11	1.68	8.78	38.91
Coelometopon sp.	2.54	2.39	0.58	6.74	45.66
Micronecta sp.	1.87	2.35	1.28	6.65	52.31
Bulinus sp.	2.31	2.3	5.68	6.5	58.81
Anax sp.	1.71	1.63	0.58	4.61	63.42
Chironomidae	2.03	1.62	0.58	4.58	67.99
Oxyethira velocipes	1.96	1.61	2.9	4.54	72.54

Table 10 (continued):

NR3					
Average similarity: 37.44					
Taxa	**Av.Abund**	**Av.Sim**	**Sim/SD**	**Contrib%**	**Cum.%**
Chironomidae	3.42	6.33	5.15	16.91	16.91
Baetidae	3.63	5.52	6.24	14.73	31.65
Anax sp.	2.38	4.41	4.68	11.77	43.42
Dixa bicolor	1.55	2.81	5.95	7.52	50.94
Pisauridae	1.63	2.76	2.77	7.37	58.3
Oligochaeta	1.27	2.49	4.68	6.65	64.95
Anisops sp.	1.52	1.95	0.58	5.21	70.17
NR4					
Average similarity: 15.31					
Taxa	**Av.Abund**	**Av.Sim**	**Sim/SD**	**Contrib%**	**Cum.%**
Chironomidae	3.97	9.64	SD=0!	62.95	62.95
Oligochaeta	2.31	3.48	SD=0!	22.72	85.67
NR5					
Average similarity: 45.06					
Taxa	**Av.Abund**	**Av.Sim**	**Sim/SD**	**Contrib%**	**Cum.%**
Sigara sp.	3.12	7.51	4.47	16.66	16.66
Baetidae	2.35	4.96	2.48	11.01	27.68
Bulinus sp.	1.76	4.09	2.87	9.07	36.75
Anax sp.	1.93	3.54	5.06	7.86	44.61
Pseudagrion sp.	2.13	2.97	1.31	6.6	51.21
Micronecta sp.	1.9	2.82	5.06	6.25	57.46
Plea sp.	1.34	2.23	5.06	4.96	62.42
Oligochaeta	2.55	2.07	0.58	4.59	67.01
Enochrus sp.	1.4	2.04	18.15	4.54	71.54
NR6a					
Average similarity: 64.35					
Taxa	**Av.Abund**	**Av.Sim**	**Sim/SD**	**Contrib%**	**Cum.%**
Baetidae	5.94	6.07	7.51	9.43	9.43
Afrocaenis sp.	5.48	5.47	6.28	8.49	17.92
Pseudagrion sp.	4.38	4.2	42.54	6.53	24.45
Chironomidae	4.12	4.15	15.25	6.45	30.9
Bulinus sp.	4.19	3.65	21.77	5.68	36.58
Simulium (Nevermannia) sp.	3.55	3.57	6.26	5.55	42.13
Athripsodes sp.	3.92	3.53	3.08	5.48	47.61
Oligochaeta	3.26	3.27	5.23	5.08	52.69
Sigara sp.	2.79	2.15	1.74	3.35	56.04

Table 10 (continued):

NR6a (continued)					
Average similarity: 64.35					
Taxa	**Av.Abund**	**Av.Sim**	**Sim/SD**	**Contrib%**	**Cum.%**
Laccocoris sp.	2.59	1.98	6.6	3.07	59.11
Cheumatopsyche sp.	2.61	1.97	2.74	3.06	62.17
Oxyethira velocipes	1.94	1.95	16.22	3.03	65.2
Pontamonautes sidneyi	1.92	1.83	5.77	2.84	68.04
Anisops sp.	2.28	1.69	1.69	2.63	70.67
NR6b					
Average similarity: 60.30					
Taxa	**Av.Abund**	**Av.Sim**	**Sim/SD**	**Contrib%**	**Cum.%**
Chironomidae	5.52	8.15	6.7	13.51	13.51
Sigara sp.	4.65	6.92	31.52	11.47	24.98
Micronecta sp.	4.42	6.47	9.21	10.72	35.7
Bulinus sp.	3.26	5.12	17.48	8.49	44.19
Oligochaeta	3.06	4.01	4.54	6.65	50.85
Anisops sp.	2.9	3.88	2.45	6.43	57.28
Ceriagrion sp.	2.66	3.71	3.08	6.16	63.44
Anax sp.	2.03	3.26	49.53	5.4	68.84
Stratiomyidae	2.38	3.26	49.53	5.4	74.24
NR7					
Average similarity: 46.66					
Taxa	**Av.Abund**	**Av.Sim**	**Sim/SD**	**Contrib%**	**Cum.%**
Baetidae	6.32	6.49	8.91	13.92	13.92
Micronecta sp.	4.38	3.75	6	8.04	21.96
Pisidium sp.	3.57	3.63	7.71	7.78	29.73
Pseudagrion sp.	3.01	2.83	3.5	6.06	35.8
Bulinus sp.	2.6	2.64	3.92	5.66	41.45
Afrocaenis sp.	3.19	2.56	4.04	5.49	46.94
Athripsodes sp.	2.71	2.38	15.48	5.11	52.06
Anisops sp.	2.19	2.12	3.31	4.54	56.6
Chironomidae	3.12	2.06	1.67	4.42	61.02
Oligochaeta	3.02	1.79	1.1	3.85	64.86
Tricladida	1.46	1.56	5.78	3.35	68.22
Larainae	1.87	1.5	5.58	3.21	71.43

Table 10 (continued):

NR8					
Average similarity: 53.61					
Taxa	**Av.Abund**	**Av.Sim**	**Sim/SD**	**Contrib%**	**Cum.%**
Baetidae	5.38	11.02	SD=0!	20.56	20.56
Afrocaenis sp.	4.86	9.33	SD=0!	17.41	37.97
Adenophlebia sp.	4.31	7.47	SD=0!	13.93	51.9
Chironomidae	3.7	7.21	SD=0!	13.46	65.36
Chlorolestes sp.	3.37	5.22	SD=0!	9.74	75.09
NR9					
Average similarity: 51.70					
Taxa	**Av.Abund**	**Av.Sim**	**Sim/SD**	**Contrib%**	**Cum.%**
Baetidae	4.94	8.45	6.56	16.35	16.35
Adenophlebia sp.	3.37	6.23	5.1	12.05	28.4
Ptilodactylidae	3.87	5.81	3.55	11.24	39.64
Pseudagrion sp.	2.86	5.61	5.41	10.86	50.5
Chironomidae	3.08	4.77	4.42	9.22	59.72
Orectogyrus sp.	1.8	3.52	5.57	6.81	66.53
Pisidium sp.	1.89	3.41	6.43	6.6	73.13
NR10					
Average similarity: 48.81					
Taxa	**Av.Abund**	**Av.Sim**	**Sim/SD**	**Contrib%**	**Cum.%**
Chironomidae	3.98	6.36	5.64	13.04	13.04
Bulinus sp.	3.59	5.09	4.72	10.43	23.47
Ceriagrion sp.	3.22	4.95	3.45	10.15	33.62
Sigara sp.	2.72	4.71	5.86	9.64	43.26
Anax sp.	2.86	4.5	5.18	9.23	52.48
Baetidae	2.8	3.84	2.14	7.87	60.35
Oligochaeta	2.33	3.51	1.23	7.19	67.54
Ecnomus sp.	1.88	3.29	5.87	6.75	74.29

Chironomidae (Table 11) was found to be a major contributor at all HGM types; however, it had the highest contribution percentage at unchannelled valley-bottom and floodplain wetland with contribution percentages of 15.62 % and 13.51 % respectively. *Micronecta* sp. had a high contribution at all types, yet the highest contribution percentage was found at only 10.72 % (floodplain type).

Bulinus sp. also had a high contribution percentage at all HGM types, but the highest contribution percentage was found to be 8.49 % (floodplain type). Baetidae and *Pseudagrion* sp. (Table 11) had a high contribution percentage at all HGM types except for floodplain types. Baetidae had the highest contribution at river types, with a percentage contribution of 15.55 %. *Pseudagrion* sp. had the highest contribution value at channelled valley-bottom types, with a percentage contribution of 16.24 %.

Table 11: Similarity percentage analysis (SIMPER) with a 70 % contribution cut-off showing which macroinvertebrate taxa are responsible for groupings found within different hydrogeomorphic (HGM) types sampled. Columns indicate average abundance (Av.Abund), average similarity (Av.Sim), similarity/standard deviation (Sim/SD), contribution percentage (Contrib%), and cumulative percentage (Cum.%).

Unchannelled valley-bottom					
Average similarity: 35.46					
Taxa	**Av.Abund**	**Av.Sim**	**Sim/SD**	**Contrib%**	**Cum.%**
Chironomidae	3.38	5.54	2.06	15.62	15.62
Baetidae	3.05	4.24	1.58	11.97	27.58
Oligochaeta	2.36	2.53	1.09	7.12	34.7
Sigara sp.	2.09	2.28	0.88	6.43	41.13
Anax sp.	1.76	2.19	1.14	6.17	47.3
Micronecta sp.	1.9	1.8	1.06	5.07	52.38
Bulinus sp.	1.62	1.41	0.73	3.98	56.36
Oxyethira velocipes	1.07	1.21	0.77	3.42	59.77
Pseudagrion sp.	1.37	1.12	0.53	3.16	62.93
Adenophlebia sp.	1.63	1.05	0.51	2.97	65.91
Pontamonautes sidneyi	1	1.03	0.92	2.9	68.8
Ecnomus sp.	1.11	0.89	0.65	2.51	71.32
Channelled valley-bottom					
Average similarity: 35.39					
Taxa	**Av.Abund**	**Av.Sim**	**Sim/SD**	**Contrib%**	**Cum.%**
Pseudagrion sp.	3.48	5.75	16.64	16.24	16.24
Baetidae	4.14	4.92	3.39	13.89	30.13
Afrocaenis sp.	2.53	3.11	1.68	8.78	38.91
Coelometopon sp.	2.54	2.39	0.58	6.74	45.66
Micronecta sp.	1.87	2.35	1.28	6.65	52.31
Bulinus sp.	2.31	2.3	5.68	6.5	58.81
Anax sp.	1.71	1.63	0.58	4.61	63.42
Chironomidae	2.03	1.62	0.58	4.58	67.99
Oxyethira velocipes	1.96	1.61	2.9	4.54	72.54

Table 11 (continued):

River					
Average similarity: 46.28					
Taxa	**Av.Abund**	**Av.Sim**	**Sim/SD**	**Contrib%**	**Cum.%**
Baetidae	5.94	7.2	4.45	15.55	15.55
Afrocaenis sp.	4.47	4.72	2.41	10.19	25.74
Chironomidae	3.64	3.87	2.48	8.36	34.1
Athripsodes sp.	2.94	2.81	3.49	6.06	40.17
Micronecta sp.	2.84	2.09	1.32	4.52	44.68
Pseudagrion sp.	2.77	1.86	0.95	4.02	48.7
Oligochaeta	2.56	1.76	1.26	3.81	52.51
Larainae	1.65	1.69	6.14	3.66	56.17
Bulinus sp.	2.55	1.61	1	3.48	59.64
Adenophlebia sp.	2.18	1.35	0.65	2.91	62.55
Anisops sp.	1.76	1.28	1.19	2.78	65.32
Pontamonautes sidneyi	1.41	1.12	1.48	2.42	67.74
Simulium (Nevermannia) sp.	2	1.04	0.71	2.24	69.98
Elminae	1.26	0.99	1.5	2.13	72.1
Floodplain					
Average similarity: 60.30					
Taxa	**Av.Abund**	**Av.Sim**	**Sim/SD**	**Contrib%**	**Cum.%**
Chironomidae	5.52	8.15	6.7	13.51	13.51
Sigara sp.	4.65	6.92	31.52	11.47	24.98
Micronecta sp.	4.42	6.47	9.21	10.72	35.7
Bulinus sp.	3.26	5.12	17.48	8.49	44.19
Oligochaeta	3.06	4.01	4.54	6.65	50.85
Anisops sp.	2.9	3.88	2.45	6.43	57.28
Ceriagrion sp.	2.66	3.71	3.08	6.16	63.44
Anax sp.	2.03	3.26	49.53	5.4	68.84
Stratiomyidae	2.38	3.26	49.53	5.4	74.24

The RDA tri-plot (Figure 43) explained a cumulative variation of 25.71 %, with the first axis explaining 15.63 % of the variation while the second axis explained 10.08 % of the variation. A Monte Carlo permutation test on all axes revealed a p value of 0.006, thereby indicating significant differences. The July (winter) survey (Figure 43) was mostly grouped in the lower left quadrant, while the December (summer) and April (autumn) surveys were mixed together and mostly grouped in the upper right quadrant. Since the first axis explained more variation than the second axis, pH, nitrate, and dissolved oxygen carry more value than sulfate and EC.

Ammonium, which is dependent on temperature and pH concentrations (Chapman, 1996), had its greatest influence close to the July (winter) survey. High concentrations of dissolved oxygen were found close to the July (winter) survey; lower temperatures result in higher oxygen concentrations (Dallas and Day, 2004; Ferreira *et al.,* 2009). High concentrations of dissolved oxygen (O_2 mg/l and O_2 %) were located close to sites NR6a and NR7. Since these sites were both classified as rivers (Ollis *et al.,* 2013), higher levels of dissolved oxygen can be expected (Bowd *et al*., 2006b).

Eight macroinvertebrate taxa were grouped together close to pH and nitrate in the July (winter) survey area. These taxa were located nearest the first axis, thus they carry more significance than the other taxa do. Baetidae and *Afrocaenis* sp. were the two taxa lying in closest proximity to the first axis. Another 13 taxa were grouped together in the upper right quadrant close to high alkalinity, temperature, EC, TDS, and sulfate concentrations between the December (summer) and April (autumn) surveys.

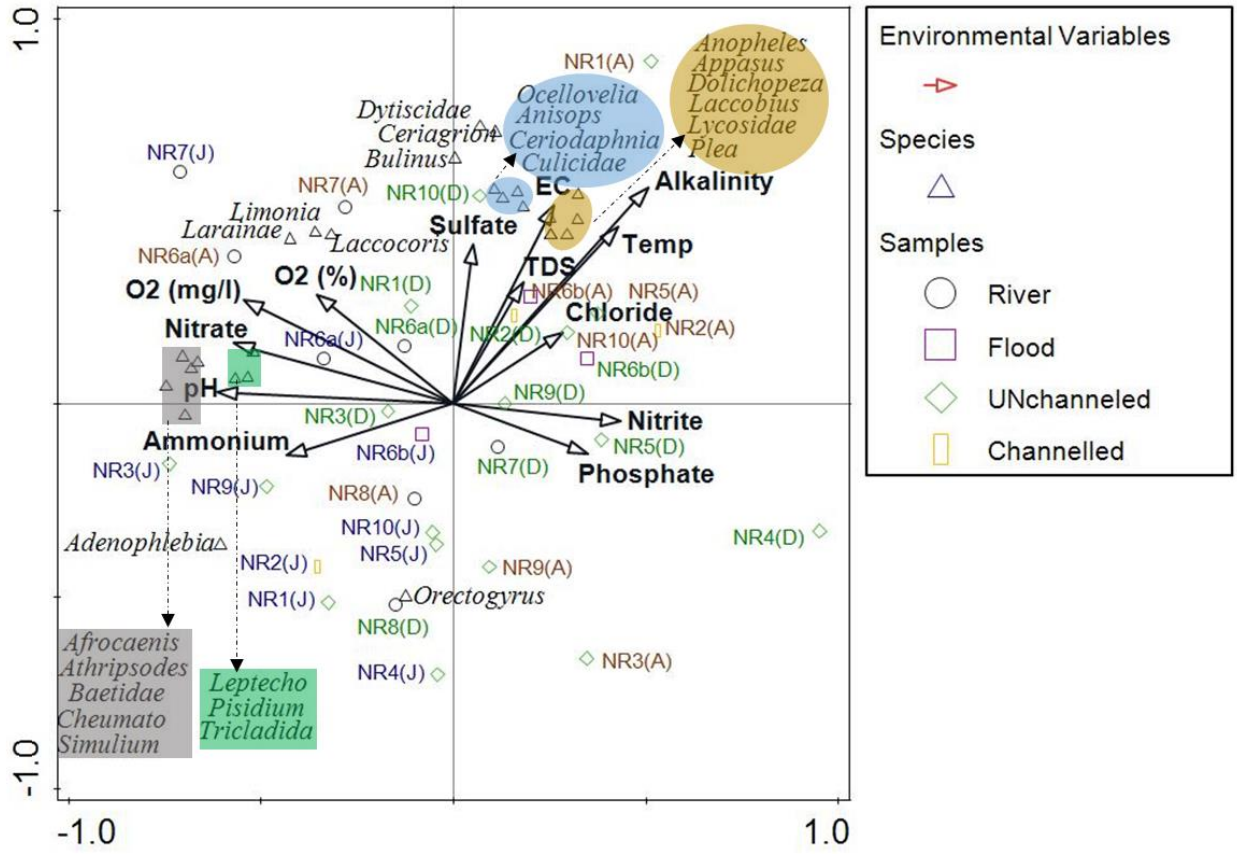

Figure 43: Redundancy analysis (RDA) tri-plot showing relationships between sites, seasons, aquatic macroinvertebrates, physico-chemical water variables, and hydrogeomorphic (HGM) types.

5.4 Discussion

5.4.1 Community structure

When assessing both univariate diversity indices and multivariate analyses, it becomes evident that the macroinvertebrate community structure across the wetland complex was variable. Communities were found to be site specific since sites NR6a (April/autumn) and NR7 (July/winter) had the most taxa and individuals collected, respectively (Figure 35). Site NR3 (December/summer) had the highest Shannon Diversity Index and the highest Pielou's Evenness Index (Figures 36b and 36c). Both sites NR6a and NR7 were river types where stone biotopes could be sampled; site NR3 was densely vegetated. This indicated that macroinvertebrates are indeed adapted to living in specific environmental conditions (Ferreira *et al.,* 2009).

With regards to HGM types, no clearly defined macroinvertebrate communities were found (Figure 42). However, there was a stronger grouping of riverine sites than any other HGM type thereby, possibly suggesting a river community type. Season in itself did not seem to define different community types (Figure 40) although there were some trends present with regards to number of taxa and individuals (Figure 35). Water quality and flow, however, were found to be linked to macroinvertebrate community structures. Water quality has an influence on macroinvertebrate community structure (Ferreira *et al.,* 2009; Malherbe *et al.,* 2010; Eady *et al.,* 2013; Foster *et al.,* 2015). The RDA tri-plot (Figure 43) showed that water quality variables had a significant effect on the macroinvertebrate community structures of the Ntsikeni Nature Reserve ($p < 0.05$). Also, certain families of macroinvertebrates are associated with stones biotopes (Malherbe *et al.,* 2010) which have been shown to significantly affect macroinvertebrate abundances (Bird *et al.,* 2014). Increased habitat complexity allowed for an increase in macroinvertebrate abundance (Bird *et al.,* 2014). All in all geological features, location, and climate all play a role in influencing macroinvertebrate community structure (Foster *et al.,* 2015).

5.4.2 Taxa characteristics

The eight macroinvertebrate taxa that were grouped together closest to the first axis (Figure 43) were *Afrocaenis* sp., *Athripsodes* sp. Baetidae, *Cheumatopsyche* sp., *Leptecho* sp., *Pisidium* sp., *Simulium* sp., and Tricladida. *Afrocaenis* sp. nymphs are associated with conditions with little or no flow; whereas in streams they are usually found in backwaters and among aquatic vegetation (de Moor *et al.,* 2003a). *Afrocaenis* sp. were found to be important constituents during the July (winter) and December (summer) survey, yet they were absent from the April (autumn) survey (Table 9). Baetidae are associated with a variety of water body types and can be found in anything ranging from rapidly flowing streams to still ponds (Griffiths *et al.,* 2015). The nymphs of Baetidae are generally found in flowing waters, but certain genera have been found in still and temporary water bodies (de Moor *et al.,* 2003a). According to the SIMPER analysis (Table 10), Baetidae had the highest contribution percentage at sites NR6a, NR7, NR8, and NR9. All these sites, except site NR9, are classified as rivers and have flowing water (Ollis *et al.,* 2013).

Cheumatopsyche sp., *Athripsodes* sp., and *Leptecho* sp. are all part of the order Trichoptera (Table 8). The larvae of Trichoptera are abundant in all types of freshwater ecosystems including mountain streams, rivers, springs, marshy wetlands, lakes, and temporary waters (de Moor *et al.,* 2003a). When found in lotic environments, Trichoptera larvae are usually the most diverse in terms of species and ecological specialisation since they have a great diversity of adaptations for freshwater life (de Moor *et al.,* 2003a; Griffiths *et al.,* 2015). Trichoptera larvae's use of cases has given them a large advantage in coping with the challenges posed by living in aquatic environments (de Moor *et al.*, 2003b).

Simulium sp. have been considered to be specialists of swift-flowing aquatic biotopes due to the dense colonies that can often be found on stones in rapids or areas of high current velocity (Day *et al*., 2003; Griffiths *et al.,* 2015). They are important primary consumers of planktonic matter and in turn act as prey for other aquatic organisms (Day *et al*., 2003). The SIMPER analysis (Table 10) indicated that the *Simulium* sp. was an important constituent at site NR6a which is classified as a river (Table 1) and also contains flowing water within its banks (Ollis *et al.,* 2013).

Tricladida are free-living, mobile, freshwater flatworms that are carnivorous and have been found in a range of habitats including the coast, high-altitude mountain streams, the sub-tropical areas of KwaZulu-Natal, and the semi-arid areas of the Karoo and Namibia (Day and de Moor, 2002; Griffiths *et al.,* 2015).

Pisidium sp. is found in both flowing and standing water bodies, usually within sandy mud, where they can occur in large numbers (de Moor and Day, 2002). The 13 taxa that were grouped together close to high alkalinity, temperature, EC, TDS, and sulfate concentrations were *Anisops* sp., *Anopheles* sp., *Appasus* sp., *Ceriodaphnia* sp., Culicidae, *Dolichopeza* sp., *Laccobius* sp., Lycosidae, *Ocellovelia* sp., and *Plea* sp.

Anisops sp. is often found in shady areas of clear standing or slow-flowing waters (de Moor *et al.*, 2003b; Griffiths *et al.,* 2015). They occur in permanent or seasonal pans, pools, dams, and vleis and have also been found in submerged vegetation along margins of stream and rivers (Griffiths *et al.,* 2015). They are predators in that they feed on fish, tadpoles, and other invertebrates (de Moor *et al.*, 2003b). The SIMPER analysis indicated *Anisops* sp. to be an important constituent at river and floodplain HGM types (Table 11). In addition to that, SIMPER analysis also indicated *Anisops* sp. to be an important constituents during the December (summer) and April (autumn) survey yet was absent from the July (winter) survey (Table 9). This is due to the fact that *Anisops* sp. has a life span of about five months from egg to adult (Griffiths *et al.,* 2015).

Culicidae larvae are filter feeders which live in a wide range of calm water habitats, including both natural and artificial areas such as temporary pools, water containers, swamps, reservoirs, and slow-moving streams (Day *et al*., 2003; Griffiths *et al.,* 2015). *Anopheles* sp. is a member of the Culicidae family and these larvae can survive in habitats ranging from high-salinity waters to freshwater streams and can even be found in mineral springs (Day *et al*., 2003). *Culex* sp. and *Uranotaenia* sp. are also members of the family Culicidae that were found in the Ntsikeni Nature Reserve (Table 8).

Appasus sp., the only member of the Belostomatidae found in the Ntsikeni Nature Reserve (Table 8), is found mainly in standing water as well as in marginal vegetation where it will remain submerged for most of its live (de Moor *et al.*, 2003b). All members of this family are predators — commonly ambush predators — that prey on fish, tadpoles, and other invertebrates (de Moor *et al.*, 2003b; Griffiths *et al.,* 2015).

The larvae of *Dolichopeza* sp., which is part of the Tipulidae, are usually found in waters that are well aerated and fast-flowing (Day *et al*., 2003) but have been associated with a remarkable variety of freshwater habitats (Griffiths *et al.,* 2015).

Laccobius sp. are better crawlers than swimmers and are thus mostly found in slow-moving water (Griffiths *et al.,* 2015). These beetles, however, can be encountered in almost any freshwater habitat, with some adults able to fly and can thus be encountered terrestrially too (Stals and de Moor, 2007).

Ocellovelia sp. are associated with floating vegetation since they glide or tread on the surfaces of water (de Moor *et al.*, 2003b). They were often found in seeps, slow rivulets, springs, and other small streams (Griffiths *et al.,* 2015).

Plea is the only genus known in the Pleidae. These bugs swim upside-down, often pulling themselves along via vegetation (de Moor *et al.*, 2003b). They are often found in areas with dense, tangled, submerged, and floating vegetation in stagnant water and feed on water mites, mosquito larvae, and planktonic crustaceans (de Moor *et al.*, 2003b; Griffiths *et al.,* 2015). According to SIMPER analyses, *Plea* sp. was found to be an important constituent during the April (autumn) survey (Table 9) as well as at site NR5 (Table 10).

Driving water quality variables that were responsible for groupings were oxygen and temperature (Ferreira *et al.,* 2009). The eight taxa that were located closest to the first axis (Figure 43) were located in close proximity to higher concentrations of dissolved oxygen (mg/l). The 13 taxa that were located in the upper right quadrant (Figure 43) were in close proximity to higher temperatures. Ferreira *et al.* (2009) noted changes in macroinvertebrate community structure due to increased chloride concentrations and the corresponding increase in EC. This is, to some extent, also true for Ntsikeni where groupings of macroinvertebrates were located around high EC concentrations.

5.4.3 Comparing biodiversity

The Ntsikeni Nature Reserve was compared to both national and international wetlands in order to ascertain aquatic macroinvertebrate biodiversity. In South Africa, Ntsikeni was compared to Melmoth Vlei which is located in the Karkloof Nature Reserve. According to Bowd *et al*. (2006b) the wetland is protected from degradation and modification, is located within the KwaZulu-Natal midlands, and has an altitude of 1595 m. Thereby, bearing similar characteristics as the Ntsikeni wetland complex. When comparing the Ntsikeni wetland complex to Melmoth Vlei it is evident that Ntsikeni contained a much greater diversity than Melmoth Vlei. Although Bowd *et al*. (2006b) only identified macroinvertebrates to family level they found 31 families belonging to 12 orders. In Ntsikeni 70 families were identified belonging to 22 orders (Table 8). In order to gain perspective of Ntsikeni's biodiversity, Coleoptera was assessed. Coleoptera, being the most diverse animal order, is considered to be a good indicator of biological diversity (Pérez-Bilbao *et al.,* 2014). In Melmoth Vlei only three Coleoptera were identified while in Ntsikeni 12 families were identified (Table 8).

Internationally, the Ntsikeni Nature Reserve was compared to freshwater alpine ponds in the Swiss National Park (Cirque of Macun) and to Yeal Lake in Gnangara Mound, western Australia. The Macun cirque ponds in Switzerland, according to Oertli *et al.* (2008), have an altitude of 2641 m, an average EC of 9.7 µS/cm, and experiences rainfall of 850 mm per year. In total, 47 taxa were identified from lentic environments with most being identified to species level. In the Ntsikeni Nature Reserve 129 taxa were identified, with most being identified only to genus level. Yeal Lake is a wetland which, according to Sommer *et al.* (2008), had the following water quality variables: temperature of 18.87 °C; dissolved oxygen of 75 %; EC of 1650 µS/cm; pH of 7.24; Mg of 30.6 mg/l; and Ca of 19.9 mg/l. In this wetland, only 50 taxa were identified with few organisms being identified beyond family level. In Ntsikeni, macroinvertebrates from 70 families were identified (Table 8).

In all three comparisons, Ntsikeni proved to contain a much higher biodiversity of aquatic macroinvertebrates than the other wetlands. Originally Blackmore (2010) submitted the information sheet on Ramsar wetlands only listing about 48 taxa that were known to occur in the Ntsikeni wetland complex. Therefore, this study made a significant contribution to increasing the knowledge on the composition of macroinvertebrate communities in the Ntsikeni Nature Reserve.

5.5 Conclusion

Season appeared to have an effect on changes in taxa due to varying numbers of taxa found during the winter in July (84), during the summer in December (95), and during the autumn in April (94). The SIMPER analysis highlighted the effect of seasonality. Some taxa, such as *Anisops* sp., were only found to be important constituents during the December (summer) and April (autumn) survey, possibly due to their life span (Griffiths *et al.,* 2015). While other taxa, such as *Plea* sp., were only found to be an important constituent during the April (autumn) survey. However, when performing an ANOSIM of the hierarchical cluster analysis constructed for season, the results were not significant even though seasonal groupings seemed evident.

Both water quality and sediment had an effect on macroinvertebrate community structure. Water quality variables, such as dissolved oxygen, EC, and temperature were found to play a role in macroinvertebrate composition. When comparing the results of this chapter with that of chapter 3, most sediment variables were found to be located between floodplain types and unchannelled valley-bottom types (Figure 25). This area was, however, most void of macroinvertebrates as can be seen on the RDA tri-plot (Figure 43).

Ntsikeni indicated significant differences between sites according to the ANOSIM analysis performed for the hierarchical cluster analysis constructed. Sites had varying habitats as well as varying biotopes. Heterogeneous habitats are known to support higher biological diversity than homogeneous ones do (Mlambo *et al.,* 2011). Overall, the Ntsikeni wetland complex is a water body that has a very rich biodiversity with regards to aquatic macroinvertebrates. This can be verified when comparing with Melmoth vlei (Bowd *et al*., 2006b) and the two international water bodies from Australia and Switzerland (Oertli *et al.,* 2008; Sommer *et al.,* 2008).

Chapter 6 – Conclusions and Recommendations

6.1 Conclusion

Wetlands are important ecosystems within the landscape since they provide valuable ecosystem and hydrological functions in relation to water quality and quantity (Dixon and Wood, 2003; Cowden *et al.,* 2014). Since wetlands are a connection between aquatic and terrestrial environments, organisms such as plants, fish, macroinvertebrates, reptiles, birds, and mammals have become dependent on them for their survival (Breedt and Dippenaar, 2013). Wetlands, being ecosystems that provide valuable services to people and are important biodiversity assets (SANBI and CSIR, 2016), are a subject of many socio-economic, environmental, and political issues (Dixon and Wood, 2003).

The need for international action with regards to wetland conservation is what led to the creation of the Ramsar Convention for Wetlands of International Importance (Matthews, 1993). The Ramsar Convention aims to conserve wetlands and waterfowl across international boundaries since they often transcend man-made boundaries (Breedt and Dippenaar, 2013). South Africa became the Convention's fifth signatory in 1975 (Breedt and Dippenaar, 2013) and added the Ntsikeni Nature Reserve to South Africa's list of Ramsar wetlands in 2010 (Ramsar, 2010). In total, South Africa has 23 wetlands designated as Ramsar Wetlands of International Importance (Ramsar, 2017).

The Ntsikeni wetland complex is particularly significant with regards to water storage and streamflow regulation since it is responsible for draining 2.7 % of the country's average annual runoff (Kotze, 2003). Since 35 – 60 % of South Africa's wetlands have been lost or severely degraded (SANBI and CSIR, 2016), the conservation of the Ntsikeni wetland complex remains within the best interests of the country. The Ntsikeni Nature Reserve has the potential to form an anchor for the development of further conservation initiatives in the KZN Province (EKZN, 2016). The research at the Ntsikeni Nature Reserve was conducted in response to a WRC workshop in 2013. As far as can be determined, available aquatic biodiversity information with regards to the Ntsikeni wetland complex was very limited. The current research at the Ntsikeni Nature Reserve was conducted in order to update and provide insight into aquatic biodiversity community composition of the Ntsikeni wetland complex.

In Chapter 3, the water quality results showed that seasonality played a role in the distribution and concentration of metals and other physico-chemical variables. The increased nutrients was attributed to rainfall, which is known to influence water quality (Chapman, 1996; Malan and Day, 2012). Significant spatial and temporal differences of metal concentrations in the sediments of the Ntsikeni Nature Reserve were found. The concentration and distribution of metals and grain sizes in sediments were influenced by the HGM types of the wetland complex. Climate, geomorphology, and geology of the area had an influence on water quality, distribution of sediment, and concentrations of metals (Barnes *et al.,* 2002; Dallas and Day, 2004). Both water quality variables and sediment metal concentrations of the Ntsikeni wetland complex were within national water quality and international sediment quality guidelines (DWAF, 1996; EPA, 1999; Dallas and Day, 2004).

The community structure of zooplankton appeared to differ both spatially and temporally. In total 25 taxa were identified, with 13 taxa found in July (winter), 18 taxa found in December (summer), and 20 taxa found in April (autumn). However, neither spatial diversity analyses nor ANOSIM analyses of sites and HGM types indicated any significant differences. With regards to temporal variation, significant differences were found between the July (winter) and April (autumn) surveys of Margalef's Species Richness (d) and Shannon Diversity Index (H'); however, the seasonal ANOSIM analysis indicated no significant differences. Variations found in zooplankton community structure can be due to environmental factors such as geography, habitat size, depth, food availability, and water chemistry (de Necker *et al.,* 2016). The RDA tri-plot indicated that some taxa were associated with dissolved oxygen concentrations and water temperatures. None of the water quality variables analysed had a significant influence on zooplankton community structures. The effects of sediment quality on zooplankton assemblages were not considered since zooplankton was not sampled from benthic areas. When comparing the Ntsikeni Nature Reserve to other wetlands, both nationally and internationally, it showed that the Ntsikeni wetland complex had a relatively high number of zooplankton taxa. The Ntsikeni wetland complex has proven to be an area rich in zooplankton biodiversity.

Water quality and sediment were found to have a significant effect on macroinvertebrate community structure. Water quality variables such as dissolved oxygen, EC, and temperature played a role in macroinvertebrate community composition. The physical characteristics of sediments (Chapter 3) were responsible for the low macroinvertebrate diversity in floodplain and unchannelled valley-bottom wetland types. However, the ANOSIM analysis for HGM types once again indicated no significant differences. Based on the ANOSIM results, significant differences in the spatial distribution of macroinvertebrate communities of the Ntsikeni wetland complex were seen. These differences are attributed to the varying habitats and biotypes of the different sites. Heterogeneous habitats are known to support higher biological diversity than homogeneous habitats (Mlambo *et al.*, 2011).

Based on the ANOSIM seasonal clusters, no significant differences were found. In total 129 macroinvertebrate taxa were identified, with 84 taxa found in July (winter), 95 taxa found in December (summer), and 94 taxa found in April (autumn). Thereby, indicating little change from one season to another. When comparing macroinvertebrate communities to those found within similar water bodies, both nationally and internationally (Bowd *et al*., 2006b; Oertli *et al.,* 2008; Sommer *et al.,* 2008), it can be confirmed that the Ntsikeni wetland complex was indeed very rich in biodiversity.

The first hypothesis, which states that water and sediment quality has an influence on the aquatic macroinvertebrate community structure of the Ntsikeni Nature Reserve's wetland complex, is accepted. Both water quality and sediment quality have an effect on macroinvertebrate community assemblages. The concentrations of these variables determined what taxa as well as how many macroinvertebrates were found in certain areas.

The second hypothesis, which states that the zooplankton and aquatic macroinvertebrate community structure of the Ntsikeni Nature Reserve will be influenced by season, is rejected. The ANOSIM analyses on both zooplankton community and macroinvertebrate community structures did not show significant variation.

In conclusion, the Ntsikeni Nature Reserve is a freshwater ecosystem that contains low water quality variables, low sediment quality variables, high diversity of zooplankton, and high diversity of macroinvertebrates. The results obtained from this study indicate that the Ntsikeni Nature Reserve supports a biologically diverse wetland complex that is subjected to minimal anthropogenic impacts.

6.2 Recommendations

This study at the Ntsikeni Nature Reserve was limited to the assessment of water quality, sediment quality, zooplankton community, and macroinvertebrate community structures over a period of one year consisting of three surveys. In future, it is recommended that:

- Surveys at the Ntsikeni Nature Reserve should be continued with more sampling trips being conducted over a longer period of time.
- Surveys should include all seasons of the year.
- Diatoms and algae samples should be collected and analysed, in addition to the samples already being collected.
- Amphibians should also be added to future studies so as to establish the full extent of aquatic biodiversity of the Ntsikeni Nature Reserve.
- Although no fish were observed at Ntsikeni, it may be useful to determine which fish species, if any, are present at the Ntsikeni Nature Reserve.

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Appendix A

Table A1: Water quality data showing metal concentrations dissolved in water samples (mg/l) collected from all sites at the Ntsikeni Nature Reserve (NR1 – NR10) during the July/winter (J) 2015, December/summer (D) 2015, and April/autumn (A) 2016 surveys.

	Be	B	Na	Mg	Al	P	K	Ca	Ti	V	Cr	Mn	Fe	Co	Ni	Cu	Zn	As
NR1(J)	0.00013	0.0027	3.091	0.76	0.00023	0.0011	0.40	1.70	0.00042	0.00067	0.00071	0.0038	0.0014	8.73E-05	0.00046	1.88E-04	0.002	0.00055
NR1(D)	0.00011	0.0013	4.02	2.61	0.00023	0.047	0.22	4.89	0.00087	0.00049	0.00053	0.00015	0.0063	0.00021	0.00059	0.00019	0.00092	0.00064
NR1(A)	0.00011	0.0013	3.55	2.50	0.00042	0.0058	0.26	6.55	0.00085	0.00049	0.00052	0.00027	0.0094	0.00015	0.00041	0.00019	0.0032	0.00046
NR2(J)	0.00012	0.00066	3.47	0.63	0.00023	0.0011	0.39	1.54	0.00046	0.00059	0.00052	0.003	0.0007	6.08E-05	0.0003	0.00019	8.35E-05	0.00042
NR2(D)	0.00011	0.0013	5.62	1.99	0.00023	0.18	0.37	4.59	0.00083	0.00065	0.00056	9.81E-05	0.0095	9.58E-05	0.00035	0.00019	0.000084	0.0006
NR2(A)	0.00011	0.0013	5.75	1.73	7.97E-06	0.013	0.38	4.45	0.00043	0.00054	0.00056	0.0004	0.0077	0.00012	0.00043	0.00019	0.00039	0.00066
NR3(J)	0.00011	0.0013	2.28	0.99	0.00023	0.0011	0.21	2.13	0.00045	0.00061	0.0004	0.0011	0.00057	2.77E-05	0.00021	0.00019	0.000084	0.00037
NR3(D)	0.00011	0.0013	2.65	1.29	0.00023	0.034	0.52	2.75	0.00069	0.00058	0.00053	0.000023	0.0049	7.01E-05	0.0001	0.00019	0.000084	0.00044
NR3(A)	0.0001	0.0013	1.90	1.06	0.00023	0.00066	0.16	2.16	0.00024	0.00047	0.00046	0.034	0.0042	0.00019	0.0001	0.00019	0.0036	0.00039
NR4(J)	0.00011	0.0013	3.25	0.33	0.00053	0.0062	0.58	0.66	0.0002	0.0005	0.00054	0.0014	0.0016	5.28E-05	0.00038	0.00019	0.000084	0.00044
NR4(D)	0.00011	0.0013	5.13	1.34	0.00023	0.028	0.97	3.66	0.00042	0.00054	0.0007	0.00073	0.026	0.00012	0.00051	0.0035	0.0048	0.0006
NR5(J)	0.00018	0.0013	1.88	0.24	0.0014	0.0011	0.57	0.56	0.00016	0.00049	0.0002	0.0023	0.0041	2.60E-05	0.00031	0.00019	0.000084	0.00029
NR5(D)	0.00011	0.0013	5.25	1.077	0.00023	0.015	1.35	4.52	0.00023	0.00049	0.00055	0.00025	0.014	8.46E-05	0.00033	0.0025	0.000084	0.00053
NR5(A)	0.0001	0.0013	4.38	2.66	0.0029	0.0022	0.30	5.82	0.00025	0.00048	0.00065	0.32	0.015	0.00065	0.00058	0.00019	0.0097	0.00053
NR6a(J)	0.00018	0.0013	2.24	0.46	0.068	0.0011	1.13	1.046	0.0011	0.00061	0.0003	0.0045	0.068	8.32E-05	0.00044	0.00019	0.000316	0.00033
NR6a(D)	0.00011	0.0013	4.11	1.26	0.00023	0.0011	0.79	2.96	0.00064	0.00048	0.00044	0.00011	0.0021	0.00015	0.0005	0.00019	0.000084	0.00059
NR6a(A)	0.00011	0.0013	4.74	2.24	0.00023	0.0066	0.85	5.02	0.00049	0.00048	0.0005	0.029	0.0076	0.00015	0.00045	0.00019	0.000084	0.00059
NR6b(J)	0.00017	0.0013	1.95	0.99	0.002	0.0011	0.43	1.90	0.0006	0.00052	0.0002	0.0099	0.0071	7.17E-05	0.00045	0.00019	0.000084	0.00033
NR6b(D)	0.00011	0.0013	2.75	4.44	0.0012	0.004	0.35	7.17	0.00099	0.00052	0.00054	0.0002	0.012	0.00019	0.0005	0.00019	0.0017	0.00056
NR6b(A)	0.00011	0.0013	3.044	2.83	0.00023	0.0037	0.43	5.6	0.00026	0.00047	0.00049	0.17	0.0077	0.00089	0.0003	0.00019	0.0018	0.00045
NR7(J)	0.00018	0.0013	2.10	0.93	0.18	0.00009	0.86	1.92	0.0026	0.00077	0.00047	0.0049	0.17	0.00012	0.00074	0.00019	8.35E-05	0.00033
NR7(D)	0.00011	0.0013	3.34	2.56	0.00023	0.0011	0.59	4.89	0.00077	0.00054	0.0005	0.000023	0.0044	7.83E-05	0.00039	0.00019	8.35E-05	0.00056
NR7(A)	0.0001	0.0013	3.79	2.38	0.00023	0.006	0.59	5.04	0.00086	0.00054	0.00051	0.000023	0.0072	0.000078	0.0003	0.00019	8.35E-05	0.00053
NR8(D)	0.00011	0.0013	3.14	1.58	0.00023	0.08	0.44	3.8	0.00096	0.0008	0.00048	5.01E-05	0.0053	3.76E-05	0.00011	0.00019	8.35E-05	0.0006
NR8(A)	0.00011	0.0013	3.68	1.35	0.002	0.0011	0.57	3.43	0.00045	0.00067	0.00049	0.00024	0.0074	3.70E-05	7.48E-05	0.00019	8.35E-05	0.00051
NR9(J)	0.00017	0.0013	1.94	0.88	0.0013	0.0011	0.42	1.8	0.00056	0.0006	0.00026	0.0011	0.0051	0.000043	0.00031	0.00019	0.00015	0.00031
NR9(D)	0.00011	0.0013	3.49	1.79	0.00064	0.51	0.24	4.81	0.0015	0.00088	0.00065	0.0012	0.01	0.00012	0.00038	0.002411	0.0059	0.00061
NR9(A)	0.00011	0.0013	2.49	1.27	0.00023	0.0011	0.29	2.39	0.00026	0.00052	0.00057	4.99E-05	0.0062	5.55E-05	0.00014	0.00019	0.0013	0.0004
NR10(J)	0.00017	0.0013	2.26	1.51	0.0039	0.0011	0.61	3.15	0.00078	0.0007	0.00031	0.0037	0.0091	5.49E-05	0.00032	0.00019	0.0011	0.00028
NR10(D)	0.00011	0.0013	3.34	2.25	0.00023	0.0011	0.40	4.00	0.00067	0.00071	0.00054	1.82E-05	0.0061	0.00024	0.00028	0.00019	0.0016	0.00047
NR10(A)	0.00011	0.0013	4.22	2.59	0.00073	0.0011	1.34	4.43	0.00079	0.00053	0.00056	0.018	0.0078	0.00019	0.00023	0.00019	0.0024	0.00045

Table A1 (continued):

	Se	Rb	Sr	Mo	Pd	Ag	Cd	Sb	Ba	Pt	Au	Hg	Tl	Pb	Bi	Th	U
NR1(J)	0.0011	0.00062	0.014	0.00041	0.00061	0.00053	0.00012	0.00022	0.007	0.00019	0.0017	0.0011	0.00018	0.00021	8.54E-05	0.00066	0.00017
NR1(D)	0.00077	0.00053	0.043	0.00019	0.00055	1.10E-04	0.0001	0.00019	0.0075	0.00017	0.00081	2.68E-05	0.00015	0.00018	6.35E-05	0.00066	0.00016
NR1(A)	0.00082	0.00056	0.055	0.00019	0.00058	0.00011	0.00012	0.00028	0.02	0.00019	0.00082	3.55E-06	0.00015	0.00019	5.90E-05	0.00065	0.00021
NR2(J)	0.00084	0.00047	0.013	0.00024	0.00052	0.0012	0.00012	0.0002	0.0062	0.00017	0.00096	0.0012	0.00016	0.0002	6.82E-05	0.00066	0.00017
NR2(D)	0.00082	0.0006	0.039	0.00027	0.00055	0.00011	0.00011	0.00019	0.0091	0.00017	0.00082	7.16E-05	0.00015	0.00018	0.00006	0.00066	0.00017
NR2(A)	0.0008	0.00063	0.039	0.0004	0.00056	2.44E-05	0.00012	0.00028	0.013	0.00018	0.00081	2.59E-05	0.00015	0.00018	5.95E-05	0.00066	0.00017
NR3(J)	0.00075	0.00036	0.015	0.0002	0.00052	0.00011	0.00011	0.00018	0.0044	0.00022	0.00085	0.0015	0.00015	0.00017	6.07E-05	0.00065	0.00016
NR3(D)	0.00072	0.0011	0.02	0.0002	0.00052	0.00011	0.00011	0.00019	0.0047	0.0011	0.00081	6.24E-05	0.00015	0.00017	0.000059	0.00065	0.00016
NR3(A)	0.00073	0.00036	0.014	0.00022	0.00061	0.00011	0.00012	0.00025	0.0056	0.00023	0.0009	1.08E-05	0.00015	0.00017	0.000059	0.00065	0.00016
NR4(J)	0.00091	0.001	0.0058	0.00021	0.00067	0.00011	0.00011	0.0002	0.0022	0.00039	0.00091	0.00034	0.00015	0.00019	6.36E-05	0.00066	0.00017
NR4(D)	0.0008	0.0024	0.026	0.00022	0.00053	0.00011	0.00012	0.0002	0.0067	0.00016	0.00082	0.0018	0.00015	0.00021	6.32E-05	0.00066	0.00017
NR5(J)	0.0009	0.00086	0.0055	0.0002	0.00027	0.00011	0.00012	0.0002	0.003	0.00017	0.00051	1.92E-05	0.00016	0.00031	6.35E-05	0.00063	0.00017
NR5(D)	0.0008	0.0025	0.019	0.0002	0.00058	0.00011	0.00012	0.00019	0.0091	0.00017	0.00084	7.18E-05	0.00015	0.00018	6.11E-05	0.00066	0.00017
NR5(A)	0.00084	0.00076	0.053	0.00021	0.00063	0.00011	0.00012	0.00025	0.018	0.00018	0.00086	0.0001	0.00015	0.00018	6.09E-05	0.00065	0.00016
NR6a(J)	0.00088	0.0016	0.0089	0.0002	0.00022	0.00011	0.00013	0.00021	0.0044	0.00017	0.0011	2.27E-05	0.00016	0.00045	6.54E-05	0.00064	0.00018
NR6a(D)	0.00079	0.0015	0.024	0.0002	0.00055	0.00011	0.00012	0.0002	0.0034	0.00017	0.00084	0.00012	0.00015	0.00018	6.31E-05	0.00066	0.00017
NR6a(A)	0.00081	0.0015	0.044	0.00022	0.00056	0.00011	0.00012	0.00026	0.013	0.00017	0.00082	6.78E-05	0.00015	0.00017	6.04E-05	0.00065	0.00016
NR6b(J)	0.00091	0.00076	0.009	0.0002	0.00023	0.00011	0.00012	0.0002	0.0039	0.00026	0.0007	5.29E-05	0.00016	0.00034	6.32E-05	0.00063	0.00017
NR6b(D)	0.00077	0.0014	0.03	0.0002	0.00054	0.00011	0.00013	0.00019	0.0055	0.00017	0.00082	0.00011	0.00015	0.00019	6.30E-05	0.00066	0.00017
NR6b(A)	0.00078	0.0013	0.025	0.00021	0.00052	0.00011	0.00011	0.00024	0.012	0.00016	0.00081	1.17E-05	0.00015	0.00018	0.000059	0.00065	0.00016
NR7(J)	0.00091	0.0014	0.014	0.00019	0.00033	0.00011	0.00013	0.00021	0.0077	0.00017	0.00054	5.85E-05	0.00017	0.00074	7.39E-05	0.00065	0.00019
NR7(D)	0.00078	0.0012	0.03	0.00021	0.00056	0.00011	0.00012	0.0002	0.0062	0.00052	0.00087	0.00018	0.00015	0.00018	6.39E-05	0.00066	0.00017
NR7(A)	0.00081	0.0011	0.036	0.00032	0.00067	0.00011	0.00011	0.00029	0.0087	0.00079	0.00095	2.56E-05	0.00015	0.00017	6.00E-05	0.00065	0.00016
NR8(D)	0.00076	0.00047	0.037	0.00022	0.00054	0.00014	0.00011	0.00019	0.0063	0.00018	0.00082	0.000079	0.00015	0.00017	0.000059	0.00065	0.00016
NR8(A)	0.00072	0.00051	0.036	0.00024	0.00055	0.00011	0.00014	0.00028	0.0067	0.00017	0.0008	1.39E-05	0.00015	0.00018	5.88E-05	0.00065	0.00017
NR9(J)	0.0009	0.00051	0.014	0.0002	0.00023	0.00011	0.00011	0.00021	0.0068	0.0002	0.00055	1.92E-05	0.00016	0.00031	6.32E-05	0.00063	0.00017
NR9(D)	0.00077	0.00055	0.023	0.00019	0.00053	0.00011	0.00011	0.0002	0.0083	0.00017	0.00081	3.01E-05	0.00015	0.00019	6.35E-05	0.00065	0.00017
NR9(A)	0.00072	0.0005	0.015	0.00024	0.00053	0.00011	0.00011	0.00023	0.007	0.00017	0.00081	9.18E-06	0.00015	0.00017	5.94E-05	0.00065	0.00016
NR10(J)	0.0009	0.00078	0.02	0.00022	0.00035	0.00011	0.00012	0.0002	0.0076	0.00034	0.00097	1.92E-05	0.00016	0.00033	6.30E-05	0.00063	0.00017
NR10(D)	0.00083	0.001	0.021	0.00022	0.00053	0.00011	0.00012	0.00023	0.0058	0.00017	0.00082	5.65E-05	0.00015	0.00018	6.23E-05	0.00066	0.00017
NR10(A)	0.00086	0.0024	0.027	0.00022	0.00054	0.00011	0.00012	0.0003	0.0085	0.00023	0.00081	1.30E-05	0.00015	0.00018	6.10E-05	0.00065	0.00016

Table A2: Water quality data showing physico-chemical and nutrient concentrations dissolved in water samples collected from all sites at the Ntsikeni Nature Reserve (NR1 – NR10) during the July/winter (J) 2015, December/summer (D) 2015, and April/autumn (A) 2016 surveys. EC – electrical conductivity; TDS – total dissolved solids; O_2 – dissolved oxygen; Temp – temperature.

	pH	EC (µS/cm)	TDS (mg/l)	O_2 (%)	O_2 (mg/l)	Temp (°C)	Ammonium (mg/l)	Chloride (mg/l)	Nitrate (mg/l)	Nitrite (mg/l)	Sulfate (mg/l)	Phosphate (mg/l)	Alkalinity (mg/lHCO^-_3)
NR1(J)	7.9	45.1	31.4	80.6	7.88	8.3	0.35	3.4	1	0.002	20	0.15	21.36
NR1(D)	8.55	58.7	41.9	118.2	9.72	27.7	0.27	1.5	0.25	0.002	12.5	0.14	42.71
NR1(A)	7.55	173	48.5	105.5	10.24	16.6	0.07	24.3	0.25	0.003	17	0.59	42.71
NR2(J)	8.4	42.6	27.5	87.3	8.32	6.3	0.17	4.3	1	0.002	14	0.1	12.2
NR2(D)	7.49	48.2	33.5	80.7	6.07	25.5	0.2	1.4	1	0.001	12.5	0.12	36.61
NR2(A)	8.1	70.2	49.3	77.6	7.23	16.5	0.07	21.7	0.25	0.01	18	0.98	36.61
NR3(J)	8	103.8	72.3	70.4	8.64	5.9	0.45	2.1	1.2	0.002	13	0.1	24.41
NR3(D)	7.85	29.5	20.3	62.2	5.15	21.4	0.26	1.9	0.9	0.001	13	0.09	24.41
NR3(A)	7.54	29.3	19.8	104.5	7.75	12.1	0.09	18.8	0.25	0.004	12.5	1.03	18.31
NR4(J)	8.04	29.9	18.9	34.4	4.39	5.3	0.13	3.5	1.1	0.002	15	0.16	24.41
NR4(D)	6.69	66.7	43.5	33.3	2.79	20.2	0.26	2.2	0.25	0.004	16	0.14	42.71
NR5(J)	7.8	23	17.1	75.8	9.5	3.1	0.12	3.5	0.5	0.002	12.5	0.11	12.20
NR5(D)	6.81	35.6	23.1	52.5	4.67	18.4	0.19	3.3	0.9	0.001	13	0.15	30.51
NR5(A)	7.42	64.1	44	77.4	7.53	15.2	0.1	12.7	0.5	0.003	51	0.15	42.71
NR6a(J)	7.8	30.9	18.9	108.1	12.42	7.9	0.26	5.1	0.5	0.002	17	0.11	12.20
NR6a(D)	8.05	38.1	25.6	104.9	9.1	23.3	0.26	3.2	0.3	0.001	14	0.1	30.51
NR6a(A)	8.36	52.9	35.7	85.3	8.33	16.8	0.16	14	0.7	0.001	31	0.16	30.51
NR6b(J)	7.64	37.4	26.2	124.4	12.54	12.8	0.34	2.2	0.3	0.002	17	0.1	24.41
NR6b(D)	7.23	107.2	80.5	35.7	3.08	22.7	0.16	3.1	0.5	0.001	13	0.12	48.82
NR6b(A)	8.24	70.4	47.5	80.1	8.13	15.4	0.1	12.3	0.8	0.005	39	0.17	54.92
NR7(J)	7.8	31	17.2	111.4	13.42	5.8	0.23	6	2.5	0.002	16	0.13	36.61
NR7(D)	8.3	54.9	25.9	95.3	8.03	21.7	0.2	2.9	0.3	0.001	15	0.1	42.71
NR7(A)	8.5	91.2	57.8	88.4	8.24	15.6	0.02	9	1	0.001	43	0.21	30.51
NR8(D)	-	-	-	-	-	-	0.3	1.5	0.7	0.002	12.5	0.11	42.71
NR8(A)	8.29	36.7	25.5	99.7	8.14	11.7	0.07	21.4	0.25	0.004	18	0.37	24.41
NR9(J)	8.3	40.3	24.9	87.3	9.79	6.4	0.27	3.6	1	0.002	22	0.12	18.31
NR9(D)	8.01	34.4	23.6	92.7	8.45	20.9	0.17	2.3	0.25	0.001	23	0.12	36.61
NR9(A)	7.67	34.7	33.4	95.8	8.94	9.5	0.07	20	0.25	0.004	17	1.13	24.41
NR10(J)	7.89	70.3	48	63.7	7.73	6.1	0.18	1.7	0.9	0.002	12.5	0.1	30.51
NR10(D)	7.38	53.4	36.7	74.2	6.4	26.8	0.25	2.5	0.9	0.001	29	0.14	36.61
NR10(A)	7.62	64	44.7	68.3	7.42	11.2	0.09	13.3	0.9	0.005	34	0.2	42.71

Table A3: Sediment quality data showing metal concentrations within sediment samples (µg/g) collected from all sites at the Ntsikeni Nature Reserve (NR1 – NR10) during the July/winter (J) 2015, December/summer (D) 2015, and April/autumn (A) 2016 surveys.

	Be	B	Na	Mg	Al	P	K	Ca	Ti	V	Cr	Mn	Fe	Co	Ni	Cu	Zn	As
NR1(J)	1.06	0.0013	123.26	2578.09	30466.12	653.53	2900.05	1903.89	359.92	55.48	51.35	838.06	46612.21	26.93	22.08	25.44	95.92	6.30
NR1(D)	0.69	1.94	103.77	1982.51	25640.39	262.32	2692.12	972.66	332.51	37.14	28.42	589.16	26650.25	15.36	15.01	14.77	234.43	3.33
NR1(A)	0.67	0.54	66.93	1653.71	31237.62	224.06	2569.31	637.87	252.23	48.96	37.72	633.17	29975.25	14.20	18.04	20.93	50.25	4.59
NR2(J)	1.02	0.0013	104.84	940.11	20162.00	697.59	1470.79	2565.05	111.95	43.13	49.48	1236.38	49680.90	23.65	14.27	15.88	36.72	7.17
NR2(D)	0.71	0.79	71.27	616.18	18723.04	203.33	1495.10	942.40	118.48	31.79	28.58	395.83	19490.20	9.29	9.80	6.80	26.35	3.26
NR2(A)	0.54	0.64	46.18	420.19	18408.65	176.59	1587.98	554.57	132.74	29.13	23.76	179.90	17632.21	5.90	9.10	5.32	21.08	3.56
NR3(J)	1.08	0.0013	108.33	2635.92	39781.55	782.52	2346.12	2184.22	496.84	75.15	114.83	282.28	47184.47	18.27	36.02	40.22	56.60	8.22
NR3(D)	0.93	0.78	92.46	1457.88	33596.06	854.68	1341.63	1867.00	128.89	78.23	77.88	1461.08	78916.26	30.89	28.60	22.18	51.85	4.69
NR3(A)	0.90	1.02	78.30	1594.90	40970.87	450.97	2215.29	946.36	323.30	68.96	74.47	245.87	34296.12	15.12	30.32	23.98	53.86	3.27
NR4(J)	0.99	0.0013	129.43	1125.78	26491.65	873.27	1666.35	1734.61	185.80	60.91	68.83	529.59	40143.20	15.72	19.37	20.92	45.13	3.66
NR4(D)	0.84	1.44	155.12	1072.30	30539.22	572.06	1767.65	2373.77	110.34	36.57	51.52	259.07	13002.45	7.30	18.47	15.76	41.23	1.59
NR5(J)	0.44	0.0013	83.65	550.14	7229.99	1783.99	356.56	2521.70	35.63	29.19	25.31	1818.23	268804.24	54.29	15.28	11.13	87.08	20.03
NR5(D)	0.87	2.24	66.86	668.13	23833.58	510.00	1380.67	543.19	132.63	34.31	30.94	236.24	32698.88	11.05	12.64	11.68	31.48	3.16
NR6a(J)	0.29	0.0013	59.06	589.84	6779.79	197.14	502.44	758.79	113.67	23.66	25.24	1359.86	17980.96	16.60	8.42	7.75	23.13	2.31
NR6a(D)	0.25	0.0013	49.59	646.60	8381.07	219.88	746.12	971.12	184.66	26.24	28.52	1575.00	20364.08	20.14	10.32	8.61	24.71	2.58
NR6a(A)	0.20	0.0013	35.74	302.94	5762.25	123.16	527.70	376.23	121.32	17.81	14.10	699.02	13904.41	9.53	4.95	3.25	15.83	1.70
NR6b(J)	1.08	0.0013	688.34	1214.01	29798.46	1153.31	1526.39	2586.37	92.99	76.01	83.25	468.57	93186.18	24.47	27.62	28.24	83.76	10.06
NR6b(D)	0.83	0.0013	134.73	1547.37	21305.84	1314.68	1221.16	4027.98	133.73	50.96	93.15	442.56	93127.15	28.28	18.91	27.10	41.58	10.55
NR6b(A)	0.70	0.07	87.97	1039.61	29685.99	1175.60	1314.25	2838.16	70.29	72.73	68.60	589.86	160845.41	17.65	22.95	16.50	47.68	16.94
NR7(J)	0.38	0.0013	79.48	656.78	8711.72	201.65	701.26	758.87	187.09	31.14	32.06	475.69	17926.59	8.89	9.38	8.79	24.40	2.70
NR7(D)	0.19	0.0013	31.75	495.91	5372.60	124.28	546.39	453.61	156.85	16.12	17.19	294.23	10783.65	7.31	6.04	7.25	14.92	1.94
NR7(A)	0.26	0.53	42.72	470.05	8396.04	143.04	721.78	505.45	165.10	17.87	17.80	757.43	13601.49	8.73	7.44	12.23	22.02	2.68
NR8(D)	0.33	0.55	42.24	995.67	8454.33	102.64	1227.40	606.97	181.47	11.67	9.40	176.97	9151.44	3.63	6.70	4.45	18.99	1.48
NR8(A)	0.46	0.02	50.22	1313.94	12956.73	173.03	1275.24	1024.04	179.83	16.88	15.33	226.83	13350.96	5.54	11.00	7.45	30.67	1.92
NR9(J)	0.35	0.0013	49.00	428.26	10724.53	179.60	349.83	660.45	232.86	48.33	43.78	212.75	17623.15	9.85	10.90	10.49	14.72	2.49
NR9(D)	0.30	1.39	32.97	325.12	8858.85	110.77	334.45	566.27	156.39	23.38	21.19	251.67	9956.94	7.58	7.69	5.24	12.14	1.46
NR9(A)	0.25	0.0013	24.98	265.29	9182.04	98.35	358.74	500.49	131.82	25.32	18.60	303.64	11827.67	7.21	7.97	4.19	11.89	1.93
NR10(J)	1.07	0.0013	116.22	1841.38	37493.96	591.74	1400.77	2367.46	402.95	108.84	137.47	544.18	102655.72	29.41	36.26	36.75	49.44	4.03
NR10(D)	1.09	1.52	73.15	2591.04	41067.91	335.38	1808.07	3093.01	615.65	96.56	128.69	404.28	55536.42	25.15	33.42	42.59	53.72	2.40
NR10(A)	0.59	1.13	68.11	619.90	31432.04	328.16	789.81	1258.74	157.96	70.44	68.93	87.21	26359.22	7.36	23.61	16.54	23.16	1.74

Table A3 (continued):

	Se	Rb	Sr	Mo	Pd	Ag	Cd	Sb	Ba	Pt	Au	Hg	Tl	Pb	Bi	Th	U
NR1(J)	0.57	53.44	25.85	0.33	0.81	3.38	0.11	0.11	256.85	0.04	0.68	0.06	0.39	56.61	0.38	10.45	1.41
NR1(D)	0.73	32.02	14.20	0.15	0.31	0.79	0.09	0.10	146.63	0.05	0.14	0.06	0.29	23.26	0.22	8.76	1.10
NR1(A)	0.84	32.33	10.65	0.15	0.37	0.76	0.12	0.08	155.59	0.05	0.13	0.01	0.33	32.87	0.27	10.64	1.59
NR2(J)	0.71	37.75	31.69	0.27	1.07	1.88	0.05	0.06	251.10	0.07	0.78	0.11	0.31	43.89	0.28	8.63	1.72
NR2(D)	0.83	24.56	14.11	0.10	0.42	0.99	0.05	0.08	150.54	0.06	0.17	0.04	0.25	17.99	0.16	7.80	1.21
NR2(A)	0.70	23.32	9.61	0.09	0.46	0.78	0.05	0.06	121.51	0.13	0.24	0.18	0.24	18.91	0.15	7.94	1.10
NR3(J)	0.84	48.71	28.88	0.26	0.94	1.76	0.08	0.05	244.42	0.04	0.69	0.04	0.39	24.85	0.36	8.71	1.71
NR3(D)	1.43	27.19	20.74	0.20	0.52	1.42	0.11	0.06	192.91	0.05	0.13	0.06	0.30	18.52	0.18	5.92	1.04
NR3(A)	1.21	35.51	16.28	0.15	0.66	2.01	0.11	0.08	200.12	0.05	0.14	0.09	0.39	24.61	0.29	8.89	1.85
NR4(J)	0.63	41.15	25.16	0.30	0.82	1.93	0.05	0.05	195.66	0.03	0.67	0.06	0.34	49.45	0.29	7.20	1.42
NR4(D)	1.03	31.67	33.09	0.15	0.50	1.25	0.06	0.07	212.94	0.05	0.14	0.07	0.34	28.63	0.22	6.19	1.31
NR5(J)	0.35	8.42	27.46	0.50	0.49	1.40	0.06	0.05	201.88	0.03	0.72	0.15	0.10	33.29	0.15	2.13	0.37
NR5(D)	0.91	22.50	7.79	0.28	0.39	1.00	0.06	0.12	136.80	0.06	0.29	0.34	0.29	15.27	0.23	6.47	0.96
NR6a(J)	0.00013	10.04	8.01	0.23	0.45	2.18	0.02	0.05	95.80	0.05	0.70	0.15	0.10	31.27	0.15	3.54	0.48
NR6a(D)	0.00013	13.73	11.45	0.25	0.44	2.79	0.02	0.04	131.77	0.04	0.68	0.02	0.12	12.86	0.16	6.37	0.75
NR6a(A)	0.36	6.47	4.61	0.15	0.15	0.72	0.04	0.07	68.46	0.12	0.14	0.01	0.09	7.12	0.06	3.65	0.49
NR6b(J)	0.63	26.27	16.62	0.58	0.74	2.13	0.08	0.09	197.94	0.04	0.72	0.15	0.32	47.14	0.24	4.95	1.04
NR6b(D)	0.70	35.00	28.42	0.40	0.74	1.59	0.08	0.09	232.28	0.03	0.71	0.05	0.29	16.54	0.22	4.88	0.95
NR6b(A)	1.14	28.02	21.86	0.22	0.46	0.93	0.08	0.07	213.24	0.05	0.24	0.11	0.27	18.07	0.15	5.13	0.97
NR7(J)	0.00013	9.42	5.81	0.51	0.48	0.85	0.02	0.08	64.95	0.06	0.78	0.48	0.11	33.74	0.45	3.90	0.53
NR7(D)	0.00013	9.61	4.68	0.21	0.41	1.83	0.02	0.04	57.62	0.04	0.70	0.02	0.09	13.42	0.16	4.45	0.50
NR7(A)	0.51	9.84	5.72	0.17	0.26	0.94	0.08	0.06	93.47	0.08	0.16	0.05	0.14	13.75	0.18	7.28	0.83
NR8(D)	0.44	11.73	8.36	0.08	0.22	0.65	0.05	0.05	62.19	0.12	0.14	0.05	0.13	12.35	0.13	4.69	0.84
NR8(A)	0.67	15.81	15.19	0.09	0.36	0.74	0.06	0.05	94.69	0.14	0.20	0.03	0.17	41.78	0.18	7.94	1.37
NR9(J)	0.00013	8.11	6.56	0.22	0.41	1.03	0.02	0.04	59.04	0.06	0.68	0.04	0.12	38.71	0.14	3.06	0.46
NR9(D)	0.47	6.35	5.90	0.09	0.19	0.65	0.04	0.07	55.19	0.07	0.37	0.15	0.12	11.80	0.08	3.24	0.47
NR9(A)	0.41	6.20	6.00	0.09	0.16	0.33	0.04	0.05	54.03	0.07	0.12	0.01	0.12	8.71	0.07	2.30	0.40
NR10(J)	0.96	29.84	19.28	0.30	0.93	3.06	0.08	0.05	188.94	0.10	0.71	0.11	0.33	45.24	0.27	7.25	1.21
NR10(D)	1.46	26.33	14.61	0.14	0.74	2.81	0.14	0.06	190.13	0.06	0.15	0.37	0.33	21.46	0.24	8.66	1.61
NR10(A)	0.91	15.25	15.10	0.12	0.40	0.85	0.07	0.06	98.52	0.05	0.12	0.04	0.23	15.24	0.13	4.67	0.87

Table A4: Sediment quality data showing grain size (µm) percentages within sediment samples collected from all sites at the Ntsikeni Nature Reserve (NR1 – NR10) during the July/winter (J) 2015, December/summer (D) 2015, and April/autumn (A) 2016 surveys.

	>4000 µm (%)	>2000 µm (%)	>500 µm (%)	>212 µm (%)	>53 µm (%)	<53 µm (%)
NR1(J)	0	24.47	30.94	9.31	15.49	16.53
NR1(D)	0.34	2.00	15.81	11.01	44.65	23.65
NR1(A)	0	20.85	30.20	7.33	10.26	28.72
NR2(J)	0	22.06	27.63	16.10	21.05	11.23
NR2(D)	0	12.99	23.45	16.28	36.92	8.78
NR2(A)	0	22.07	28.62	20.04	23.37	4.05
NR3(J)	0	19.29	28.01	14.19	20.29	16.01
NR3(D)	0	4.05	26.03	24.89	32.87	8.63
NR3(A)	8.56	15.23	25.97	12.47	21.54	14.13
NR4(J)	0	4.57	20.18	27.04	33.14	11.04
NR4(D)	9.30	11.18	22.91	16.52	24.20	11.51
NR5(J)	0	0.42	25.23	38.30	27.66	5.29
NR5(D)	0	15.31	32.23	15.70	27.99	6.89
NR6a(J)	0	0.09	7.22	38.58	50.07	3.13
NR6a(D)	15.20	6.59	9.49	25.30	40.32	2.38
NR6a(A)	2.64	1.77	5.72	39.93	46.76	2.10
NR6b(J)	0	0.56	27.33	21.20	30.82	17.10
NR6b(D)	0	5.01	30.56	23.96	27.41	10.24
NR6b(A)	0	24.72	38.51	16.05	12.69	5.65
NR7(J)	0.88	4.27	22.70	44.83	25.47	1.54
NR7(D)	0	0.48	28.24	46.11	23.19	1.35
NR7(A)	0	2.15	5.18	25.64	63.95	2.07
NR8(D)	25.22	9.68	21.41	24.10	16.71	1.79
NR8(A)	0	2.83	14.77	27.73	46.52	5.91
NR9(J)	8.55	9.27	26.54	17.55	35.07	2.15
NR9(D)	4.00	4.26	21.37	25.78	40.90	2.63
NR9(A)	0	0.56	13.42	36.05	45.67	2.79
NR10(J)	0	19.58	32.76	14.70	16.38	13.77
NR10(D)	0	13.78	28.73	7.66	19.13	28.35
NR10(A)	0	10.03	25.06	18.56	29.75	12.96

Appendix B

Table B1: Zooplankton taxa sampled from all sites in the Ntsikeni Nature Reserve (NR1 – NR10) during the July/winter (J) 2015, December/summer (D) 2015, and April/autumn (A) 2016 surveys. Total individuals and taxa collected in each season provided below.

Taxa	NR1 (J)	NR1 (D)	NR1 (A)	NR2 (J)	NR2 (D)	NR2 (A)	NR3 (D)	NR3 (A)	NR4 (D)	NR5 (D)	NR5 (A)	NR6a (J)	NR6a (D)	NR6a (A)	NR6b (D)	NR6b (A)	NR7 (J)	NR7 (D)	NR7 (A)	NR8 (D)	NR8 (A)	NR9 (J)	NR9 (D)	NR9 (A)	NR10 (J)	NR10 (D)	NR10 (A)	Total
CLADOCERA																												**564**
Acroperus sp.		1				1	19	3		43	7									1					1	6		82
Alona sp.	3		1	9		5	4	14	2	79	2	59	2	9	5	5	4	4	5	3	3	6	6	4	33	3	2	272
Ceriodaphnia sp.	1		2		3	5			12	2	2	4	2	8		1			1	5				1	3	1	4	57
Chydorus sp.	1								1	3			31	30	5		2	1	13	7		7	5			1		107
Moina sp.					2				7	2	5																	16
Simocephalus sp.											8			21												1		30
CYCLOPOIDA																												**457**
Cyclopoida		2	2	1	3	7	3		8	8	7	2	2	9	4		2		2			2			4	2	3	73
Ectocyclops sp.		18	1		6	8		2	37	122	75			9	17	1					1		4			6		307
Eucyclops sp.			2			2		1							1						6					3		15
Macrocyclops sp.						2			5	12					12								2			1		34
Microcyclops sp.						1															1							2
Paracyclops sp.			3			1	2	3			10								1	2	3						1	26
OSTRACODA																												**479**
Cypricercus sp.																					2							2
Darwinula sp.																	4											4
Ilyocypris sp.	4																											4
Kapcypridopsis sp.																									3			3
Parastenocypris sp.		80												65									4			10		159
Potamocypris sp.												1								*5*								6
Stenocypris sp.					1			2		51			7		20												1	82
Vestalenula sp.																						1						1
Zonocypris sp.		1	1			4			7	21	4		13	22	112	3		1	1		2		2	4		19	1	218
CALANOIDA																												**68**
Diaptomidae	1		3			2		2				2		1			1	2				3			3		2	22
Lovenula sp.						3		1			1						1				1				1		1	9
Metadiaptomus sp.						8				1	4	3		2	2		4		2		6	3					2	37
HARPACTICOIDA																												**15**
Harpacticoida		1				2		1	5				3											2		1		15

July total individuals	179	**July total taxa**	13	**Total (July. December. and April) individuals**	1583
December total individuals	920	**December total taxa**	18	**Total (July. December. and April) taxa**	25
April total individuals	484	**April total taxa**	20		

Appendix C

Table C1: Macroinvertebrate taxa sampled from all sites in the Ntsikeni Nature Reserve (NR1 – NR10) during the July/winter (J) 2015, December/summer (D) 2015, and April/autumn (A) 2016 surveys.

Taxa	NR 1 (J)	NR 1 (D)	NR 1 (A)	NR 2 (J)	NR 2 (D)	NR 2 (A)	NR 3 (J)	NR 3 (D)	NR 3 (A)	NR 4 (J)	NR 4 (D)	NR 5 (J)	NR 5 (D)	NR 5 (A)	NR 6a (J)	NR 6a (D)	NR 6a (A)	NR 6b (J)	NR 6b (D)	NR 6b (A)	NR 7 (J)	NR 7 (D)	NR 7 (A)	NR 8 (D)	NR 8 (A)	NR 9 (J)	NR 9 (D)	NR 9 (A)	NR 10 (J)	NR 10 (D)	NR 10 (A)	Total
ACARINA																																**3**
Pontarachnidae																													1	2		3
AMPHIPODA																																**1**
Sternophysinx sp.																											1					1
ARANEAE																																**83**
Lycosidae			8																	9									2		19
Pisauridae		4	6		1	1	2	10	3				2	2									4					6	6		47
Tetragnathidae		2	4			1			6													2	1	1							17
BIVALVIA																																**240**
Pisidium sp.	50	1	1	1		6	15	2		18					1	1	4				62	22	30			11	5	3		7		240
CLADOCERA																																**159**
Ceriodaphnia sp.			94			4								4			19															121
Scapholeberis sp.																					38											38
COLEOPTERA																																**1242**
Afrobrianax ferdyi																					2											2
Afropsephenoides sp.																					2											2
Aulonogyrus sp.				11											38		12									7						68
Berosus sp.																					9											9
Canthyporus sp.					3																											3
Coelometopon sp.					53	37		6														2								1		99
Cybister sp.									1																							1
Dineutus sp.																													1			1
Donaciasta sp.										1													1							2	1	5
Dytiscidae		5	35	6	1								6	6	2	1	2		15	5	28		2		1		1			26	5	147
Elminae																5	4				2	2	14	2	1							30
Enochrus sp.			8			1						1	10	2															1			23
Gyrinus sp.															2	1				1			3							1		8

Table C1 (continued):

Taxa	NR 1 (J)	NR 1 (D)	NR 1 (A)	NR 2 (J)	NR 2 (D)	NR 2 (A)	NR 3 (J)	NR 3 (D)	NR 3 (A)	NR 4 (J)	NR 4 (D)	NR 5 (J)	NR 5 (D)	NR 5 (A)	NR 6a (J)	NR 6a (D)	NR 6a (A)	NR 6b (J)	NR 6b (D)	NR 6b (A)	NR 7 (J)	NR 7 (D)	NR 7 (A)	NR 8 (D)	NR 8 (A)	NR 9 (J)	NR 9 (D)	NR 9 (A)	NR 10 (J)	NR 10 (D)	NR 10 (A)	Total
COLEOPTERA (continued)																																
Haliplus sp.						1																										1
Hydaticus sp.								2	1				1						1			2										7
Hydraena sp.		1	3						1	1		1								2									1		2	12
Hydraenidae	1																	4				1										6
Hydrocanthus sp.																															1	1
Hydroglyphus sp.	3	1														2							6			1						13
Hydrophilidae																						1								1		2
Hyphydrus sp.	3		3		13	3		2		3					1	8	5				6		6				1	3		3	6	66
Laccobius sp.			5																													5
Laccophilus sp.									2				3	7			11		2			1					1			3		30
Larainae														1	3	5	6			2	12	2	6	2	3							42
Methles sp.											1										1									4		6
Orectogyrus sp.	2			1		2	4		1	133					1	4	4					1		1	2	12	4	12				184
Pachyelmis sp.															2	5					4											11
Parasthetops sp.	1	1							1							2																5
Philaccolus sp.			1			3		6	4					3								38			2						3	60
Ptilodactylidae									1						1											338	40	7				387
Regimbartia sp.																														2		2
Rhantus sp.																														2		2
Spercheus sp.													1																			1
Stenopelmus rufinasus					1																											1
DECAPODA																																**96**
Pontamonautes sidneyi		1	1		1	15	12	1		4			6	9	8	4	6				7	1	3		3	4	5	2	1	1	1	96
DIPTERA																																**3065**
Anopheles sp.			56			6																	1									63
Antocha sp.																						2			2							4
Athericidae																	1															1

Table C1 (continued):

Taxa	NR 1 (J)	NR 1 (D)	NR 1 (A)	NR 2 (J)	NR 2 (D)	NR 2 (A)	NR 3 (J)	NR 3 (D)	NR 3 (A)	NR 4 (J)	NR 4 (D)	NR 5 (J)	NR 5 (D)	NR 5 (A)	NR 6a (J)	NR 6a (D)	NR 6a (A)	NR 6b (J)	NR 6b (D)	NR 6b (A)	NR 7 (J)	NR 7 (D)	NR 7 (A)	NR 8 (D)	NR 8 (A)	NR 9 (J)	NR 9 (D)	NR 9 (A)	NR 10 (J)	NR 10 (D)	NR 10 (A)	Total
DIPTERA (continued)																																
Ceratopogonidae	9	1		1	1		1	2		1			2			8	1	3	2				1	4			1			2		40
Chironomidae	87	46	59	27		15	94	14	19	132	20		22	11	45	61	82	89	263	649	43	2	86	30	52	101	5	16	22	39	165	2296
Culex sp.																	1												3			4
Culicidae			27			1											4						1				1				1	35
Dixa bicolor	1	1		2			4	6	2	3							1		1					1			1					23
Dixella harrisoni	2	1							3				2	1			1		4				1		2			4	1	16	1	39
Dolichopeza sp.			53																													53
Ephydridae																						1										1
Forcipomyiinae																						1										1
Gonomyia sp.	5	1					10	7		3						4				27								1		1	3	62
Hemerodromia sp.																					1			1								2
Limonia (Dicranomyia) capicola																					8		2							1		11
Muscidae															1		2						3	6								12
Paraphaenocladius sp.				2																												2
Sciomyzidae																														1		1
Simuliidae																1	1															2
Simulium (Nevermannia) sp.				2			123			7					41	23	41				12		15			15						279
Stratiomyidae			35			1				3						9	3	6	25	6			3			1				4		96
Tabanidae						2							1																			3
Tipula sp.																					9											9
Tipulidae								1						1			1			2											1	6
Uranotaenia sp.		1			10			9																								20
EPHEMEROPTERA																																**9757**
Acanthiops sp.																					2											2
Adenophlebia sp.	16	1		50	1		557		3	146					10			3			77		7	34	156	62	35	10	3			1171
Afrocaenis sp.	8	3		49	12	2	53			67					390	254	139	61	4		67	5	34	196	84	73	19	1	4			1525
Afronurus sp.																					21	3										24

Table C1 (continued):

Taxa	NR 1 (J)	NR 1 (D)	NR 1 (A)	NR 2 (J)	NR 2 (D)	NR 2 (A)	NR 3 (J)	NR 3 (D)	NR 3 (A)	NR 4 (J)	NR 4 (D)	NR 5 (J)	NR 5 (D)	NR 5 (A)	NR 6a (J)	NR 6a (D)	NR 6a (A)	NR 6b (J)	NR 6b (D)	NR 6b (A)	NR 7 (J)	NR 7 (D)	NR 7 (A)	NR 8 (D)	NR 8 (A)	NR 9 (J)	NR 9 (D)	NR 9 (A)	NR 10 (J)	NR 10 (D)	NR 10 (A)	Total
EPHEMEROPTERA (continued)																																
Aprionyx sp.																					8											8
Baetidae	128	1	18	1112	24	8	337	15	9	8		18	11	4	494	249	448	85	91		1204	256	551	189	248	1084	77	31	34	31	3	6768
Castanophlebia sp.																1					106	3			23							133
Hyalophlebia sp.																54									1							55
Tricorythus sp.																					67	3			1							71
GASTROPODA																																**954**
Bulinus sp.	12	31	27	2	3	84		3				6	6	3	23	197	59	26	18	33	8	12	20				1		13	239	13	839
Ferrissia sp.				6		1						4		4	1	34	8				7		5							8		78
Gyraulus sp.																					2	1						1				4
Lymnaea truncatula													5				1						1									7
Oxyloma patentissima			1									1		24																		26
HEMIPTERA																																**2938**
Anisops sp.		3	2		135	4		11	7				1	12	11	25	2	5	35	27	10	12	4		1					144	1	452
Appasus sp.			34																												5	39
Aquarius distanti																									1				2			3
Enithares sp.						5								2		1			2													10
Eurymetra natalensis		2	1		4	3																1	1		3					1		16
Laccocoris sp.		1			1	1									4	39	11		1		8	3	1							3		73
Laccotrephes sp.					2			1																				1				4
Micronecta sp.	264	19	8	14	8	1	9			25		2	32	2		6	45	38	211	69	258	15	121	14	2	19	1	1	3	43	1	1231
Nychia limpida																2			2													4
Ocellovelia sp.		6	10			3	1	6	2				1	1		2	3			10			5	1	2		1			7		61
Paraphrynovelia sp.																														2		2
Plea sp.			20		1	9		2			8	1	3	6		8	44	1	3	5			9						6	5	1	132
Ranatra sp.																														2		2
Rhagovelia sp.																							1		6			1				8
Sigara sp.	50	16	69	5		8			1	124	1	21	16	30	41	3	25	74	56	266	31			1		1			15	21	9	884
Xiphoveloidea major																						12	5									17

Table C1 (continued):

Taxa	NR 1 (J)	NR 1 (D)	NR 1 (A)	NR 2 (J)	NR 2 (D)	NR 2 (A)	NR 3 (J)	NR 3 (D)	NR 3 (A)	NR 4 (J)	NR 4 (D)	NR 5 (J)	NR 5 (D)	NR 5 (A)	NR 6a (J)	NR 6a (D)	NR 6a (A)	NR 6b (J)	NR 6b (D)	NR 6b (A)	NR 7 (J)	NR 7 (D)	NR 7 (A)	NR 8 (D)	NR 8 (A)	NR 9 (J)	NR 9 (D)	NR 9 (A)	NR 10 (J)	NR 10 (D)	NR 10 (A)	Total
HIRUDINEA																																**112**
Batracobdelloides tricarinata	2	2	11								3							46	20													84
Helobdella stagnalis	1	3									2							4	1	1												12
Salifa africana																		2														2
Theromyzon cooperi																		10	1	3												14
LEPIDOPTERA																																**102**
Crambidae				11		3									10	55	1			1				1	1					1		84
Nymphula sp.		2	10						2					1			3															18
MONOHYSTERIDA																																**5**
Monhystera sp.			1															1				2		1								5
ODONATA																																**1660**
Aeshna sp.	2			1	7								2						2										3			17
Anax sp.	3	27	6		11	13	6	6	25	1		2	8	11	2	6	13	6	8	6			8		8			2	7	34	18	237
Ceriagrion sp.		28	163			16		4						11	8			21	5	21	134		3						7	45	41	507
Chlorolestes sp.																	1				3	6		70	11			15				106
Lestes sp.					1											1					3						10					15
Notiothemis jonesi jonesi	1	2	6				3			2			1	2						3	1		43	1				1		3	14	83
Onychogomphus sp.																							11									11
Phyllomacromia sp.						7									1	7	13				1		39						1			69
Pseudagrion sp.		31		64	15	32	30	3				6	42	1	42	136	85				9	48	16			22	8	25				615
OLIGOCHAETA																																**2089**
Haplotaxis africanus																		2											8			10
Naididae												4						2														6
Oligochaeta	1167	398				3	2	2	4	33	2		89	22	30	31	17	56	7	20	78	1	53		4	13		3	20	2	16	2073
OSTRACODA																																**548**
Potamocypris sp.		154						1		1			2	29		99	196	53		12									1			548
PLECOPTERA																																**42**
Aphanicercella sp.									5								3								29			2				39

Table C1 (continued): Total individuals and taxa collected in each season provided below.

Taxa	NR 1 (J)	NR 1 (D)	NR 1 (A)	NR 2 (J)	NR 2 (D)	NR 2 (A)	NR 3 (J)	NR 3 (D)	NR 3 (A)	NR 4 (J)	NR 4 (D)	NR 5 (J)	NR 5 (D)	NR 5 (A)	NR 6a (J)	NR 6a (D)	NR 6a (A)	NR 6b (J)	NR 6b (D)	NR 6b (A)	NR 7 (J)	NR 7 (D)	NR 7 (A)	NR 8 (D)	NR 8 (A)	NR 9 (J)	NR 9 (D)	NR 9 (A)	NR 10 (J)	NR 10 (D)	NR 10 (A)	Total
PLECOPTERA (continued)																															
Neoperla sp.																					2										2
Notonemouridae																									1						1
TRICHOPTERA																															**1214**
Athripsodes sp.	1		1	26			96			1			1		66	110	16				12	6	36	9	3	34	4				422
Cheumatopsyche sp.				47			14								3	19	30				53		1			158	1				326
Ecnomus sp.	30	7		2	1					2			9	3	10	2	14	20	1	9	13	1	7			9	1		6	7	4	158
Hydroptila sp.																	70						18								88
Leptecho sp.							28											1			5		2								36
Oxyethira velocipes		27	4	58	2	1	3	2	1		4	3	10		5	7	6	1	16	1	12		9	1		3	1		6	1		184
TRICLADIDA																															**103**
Tricladida	13						55							1		3	20				3	3	4					1			103
Sponge A																									13						13

July total individuals	12122	**July total taxa**	84	**Total (July. December. and April) individuals**	24426
December total individuals	5888	**December total taxa**	95	**Total (July. December. and April) taxa**	129
April total individuals	6416	**April total taxa**	94		