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HIGH-ALTITUDE TEMPORARY SYSTEMS AND MACROINVERTEBRATE COMPOSITION IN LA NINA CYCLE, MALOTI-DRAKENSBERG

by

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A thesis submitted in fulfilment of the requirements for the degree of Master of Science in the Department of Zoology and Entomology, Rhodes University

NOVEMBER 2023

Abstract

The Maloti-Drakensberg Mountain is an important water catchment area for South Africa and Lesotho. It is rich in biodiversity in terms of endemic flora and fauna and has a high density of temporary wetlands in the form of Afromontane tarns and rock pools. There is, however, limited information on the distribution of macroinvertebrates in these ecosystems and environmental factors that drives their composition. To that end, this study investigated macroinvertebrate diversity and the effect of environmental factors on community assemblage in the Maloti-Drakensberg Mountain temporary wetlands, specifically tarns and rock pools. The main aims were to describe the abundance, diversity, and distribution of macroinvertebrates in temporary wetlands in the Maloti-Drakensberg Mountain. Furthermore, it aimed to investigate the effect of environmental variables on habitat structuring, spatial variations, and macroinvertebrate community composition in tarns and rockpools in the Maloti-Drakensberg Mountain. Seventy temporary wetlands (29 tarns and 41 rock pools) were surveyed over a once-off sampling (3 March – 7 April 2022). The wetlands were from 3 provinces including Eastern Cape, Free State, and KwaZulu-Natal.

15278 individual macroinvertebrates from 13 orders, 35 families, and 46 genera were collected and identified. Species accumulation curves incorporating both tarns and rock pools showed that observed results were significantly lower than Chao 1 and Chao 2 estimates meaning that the sampled sites were not enough to provide a comprehensive picture of the biodiversity in the region. This was also observed for rock pools when the temporary wetlands were analysed separately, however, the Chao 1 and Chao 2 values for tarns were almost equal to the observed values implying that sampled sites were enough to give a detailed picture of macroinvertebrate composition in tarns. Tarns and rock pools were significantly different in terms of species richness and Shannon-Wiener diversity index but showed no significant differences for relative abundance and Pielou's evenness. Of the 35 families identified, 19 were considered core taxa with Dytiscidae and Corixidae being the most dominant. Additionally, there was moderate Nestedness metric based on Overlap and Decreasing Fill (NODF) value of 33.05369, with 32 out of 35 families being idiosyncratic. Predators were the dominant feeding group with omnivores being the least common. Non-Metric multi-Dimensional Scaling (NMDS) and PERMANOVA results showed that tarns and rock pools supported distinctly different macroinvertebrate communities per wetland type and location respectively.

Physicochemically, tarns and rock pools were significantly different in terms of water temperature, depth, area, and chlorophyll-a concentration with tarns exhibiting higher values. Temporary wetlands in the sampled localities differed in terms of pH, EC, DO, water temperature, altitude, and phosphate. Principal Component Analysis showed that tarns were positively associated with area and depth whereas rock pools with phosphate. Akaike's Information Criterion (AICc) selection showed that area, pH, and water temperature were significantly influencing community assemblage in temporary wetlands. Furthermore, area significantly influenced all four measured biodiversity indices: relative abundance, species richness, Pielou's evenness and Shannon-Wiener Index, with water temperature also influencing relative abundance and altitude, species richness.

Overall, the species composition showed that the sites were sampled later in the hydroperiod as evidenced by the dominance of active dispersers. The extended distribution of *Prosthetops gladiator* in the Eastern Cape highlights the need for extensive surveys to provide valuable insight into the distribution patterns of macroinvertebrates in the region. Furthermore, the interplay between physicochemical variables and biodiversity indices emphasized area, water temperature and pH as significant factors. However, their limited explanatory power suggests that obscure factors also influence the diversity cementing the need for further exploration of these enigmatic systems. Taxonomic challenges, especially regarding large branchiopods, hindered precise species distribution and conservation status determination. This study is the first to provide a comprehensive list of macroinvertebrates from temporary wetlands in the Maloti-Drakensberg Mountain. These results establish a valuable baseline for future biological monitoring studies, particularly in assessing species composition and responses to anthropogenic activities. It is a timeous study considering the sensitivity of these systems to climate change as they act as sentinel sites hence the need for foundational data on which to make future projections.

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Acknowledgements

I would like to extend my sincere appreciation to the South African Weather Service (SAWS) for providing the valuable climate data for the Maloti-Drakensberg Mountain region, South Africa. I am deeply grateful to the Henderson Postgraduate Scholarship from Rhodes University for their generous funding which made my studies possible. I am also grateful to Rhodes University for the opportunity to pursue my postgraduate studies. I would like to thank the National Research Foundation (Dr. S.N. Motitsoe Thuthuka Grant: TTK210216586796) for providing financial support for this study. I am indebted to Albany Museum (Department of Freshwater Invertebrates) for the liquid gold (ethanol), sampling equipment and the ISUZU (hope it recovers!!!!!).

Forever grateful to my supervisors, Dr SN Motitsoe and Mr MC Mlambo. Sam, among other things, for patiently waiting an extra year when I had my visa issues, going above and beyond to ensure that I was comfortable and had all I needed to make my study a success and most importantly, for the open-door policy, which I constantly abused with silly questions and frequent pointless visits. Musa, for hosting me when I first arrived in Grahamstown, tough love and most memorably, the unplanned sleep-over at Prentjiesberg (you'll never hear the end of this one!). Skhu, for always ensuring I had a comfortable stay in KZN, pulling through when all plans failed, the frequent trips to Intercape and most importantly, the invaluable life talks. To my unofficial co-supervisor, THE WILDRTRAK, the best travel companion in rugged terrains, you made my trips comfortable. We took some pretty cool pictures together; you'll always have a special place in my heart!

Sive Kolisi, my Assistant Researcher, designated photographer, and fellow Mission Impossible actor, we should definitely feature on "I shouldn't be alive" for our experience in the Berg. I am forever grateful for the company and the long talks while doing nutrient analysis. If I were to ever re-do the experience, I wouldn't hesitate to take you with me. Ndiyabulela!

I want to thank my colleagues at Rhodes University, Kirk, Getrude Tshithuke, Nonkazimulo D. Mdidimba, Amanda Mgwadi, Lulutho Mancunga and Sonwabise Maneli; at Albany Museum, Abigail P. Kirklady and at University of Fort Hare Ms Grace and Mbeko Mqhokweni, for their assistance in various capacities.

Friends who became family, Tapiwa G. Mushore, my friend of 10 years and my daughter's godfather, although I am not the easiest person to deal with, you've consistently come through for me regardless, and I will forever be grateful. Tressia Chikodza, my human diary, and daughter's godmother, I appreciate our long-winding conversations, and I pray that someday, soon, we'll stop being so indecisive! Chipo P. Mungenge, my daughter's best friend and my only friend who understands temporary ponds, thank you for being raw, brutal, and unfiltered and for having 2MB of storage! Lenin D. Chari, many thanks for being a big brother, patiently listening to my rants, offering much-needed advice and for your assistance with data analysis.

Special thanks to my siblings Simangaliso S Moyo and Siyabonga M Moyo for the constant encouragement and long calls that cheered me up. My mother, my prayer warrior, MaQoma, MaZembe, Mtshali for the blessings and prayers that have covered me from day one. My father, for always reminding me to keep my eye on the ball and constantly validating me. My most tremendous appreciation goes to my sister, Nosimilo Gugulethu Moyo, for your selflessness in raising my daughter while I pursued my dreams. You never once complained and always encouraged me to reach for the stars. A special thank you to my brothers-in-law, Craig Change and Tesi Tshuma for always coming through when I needed a hand encouraging me to reach for the stars and not get blinded by the lights.

Kudzai Mashiri, my lucky charm, and ever-on-call-shrink; thank you for being so supportive and listening to my never-ending complaints. Thank you for the medical and emotional support when I was pregnant with our daughter. Words will never be enough to express how much I appreciate you!!!

To my guardian angel, my lily, my sleeping beauty, Sukoluhle Change, you were, are and will always be my number one cheer leader. Wish you were here for this one!!!

To my special, adorable, well-baked cupcake, Kudzwai Aurora Uriella Mashiri, thank you for being a trooper and the warm smile that kept me going!!!

Dedication

This thesis is dedicated to my late sister, my guardian angel, Sukoluhle Change.

Chapter 1

General Introduction

“I can speak to my soul only when the two of us are off exploring deserts or cities or mountains or roads”- P. Coelho (Photo credit - S. Kolisi)

1.1 An Overview of Wetlands

The world's arid regions contain numerous topographic lows, many of which are closed basins known as wetlands (Goudie & Wells, 1995; Ellery et al., 2016). Wetlands are estimated to cover 570 million hectares (5.7 million km^2), accounting for approximately 6% of the Earth's surface (Erwin, 2009; RAMSAR, 2016; Xu et al., 2019). They vary from endorheic water bodies ranging from small ponds to large chotts or sebkhas, as well as temporarily flooded margins of permanent bodies such as streams and lakes (Parra et al., 2021). Due to the dynamic nature of these systems, there is no universally accepted definition of wetlands, although several definitions have been proposed (e.g., RAMSAR, 1971; Cowardin et al., 1979; Dini et al., 1998; Keely & Zedler, 1998; NWA, 1998; Turner et al., 2003; Simeniuk & Simeniuk, 2004; Merot et al., 2006; Ollis et al., 2013; Dehnhardt et al., 2019; Reis et al., 2019). According to the RAMSAR Convention on Wetlands (2016), wetlands are 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 meters.

Wetlands are considered among the most productive of ecosystems and play a crucial role in many landscapes (Mitsch & Gosselink, 2000; Reddy & DeLaune, 2008; Wasserman & Dalu, 2022). Their high productivity enables them to support diverse flora (Cook, 2004; Ellery, 2004; Tiner, 2016) and fauna (Crisman, 2001; Bird et al., 2019; Acosta et al., 2020). Wetlands are prominent features in arid and semi-arid climates and serve as crucial water sources by regulating water quantity through groundwater recharge (Grizzetti et al., 2016, 2019; Davidson et al., 2019; Dube et al., 2020). In many developing countries, local communities face socio-economic challenges and therefore rely on wetlands to support their livelihoods through farming, drainage for irrigation, and as pastures for livestock (Marambanyika & Beckedahl, 2016; Calhoun et al., 2017; Phethi & Gumbo, 2019).

The physical, chemical, and biological components of wetlands enable them to provide essential ecosystem services (RAMSAR, 2016). They help mitigate the adverse effects of climate change by supplying the atmosphere with potential evapotranspiration and sequestering carbon dioxide (Russi et al., 2013; Xi et al., 2021). The ability of wetlands to retain water is crucial for flood and storm control (Williams, 1993; Reddy & DeLaune, 2008; Tang et al., 2020; Taylor & Druckenmiller, 2022). Water purification is another critical service provided by wetlands, a function that humans have exploited using both natural and artificial wetlands worldwide to treat industrial, agricultural, and domestic wastewater (Rai, 2008; Nilsson et al., 2010; Cronk & Fennessy, 2016; Cantonati et al., 2020; Dalu et al., 2020).

Permanent and temporary wetlands represent two distinct types of aquatic ecosystems that differ primarily in their hydrological characteristics and associated ecological dynamics (Calhoun et al., 2011; Jackson et al., 2014). Permanent wetlands exhibit relatively stable water levels and will generally have water except during extreme drought periods and provide a continuous habitat for various plant and animal species (Junk et al., 2014). They are often connected to permanent water sources, such as rivers, lakes, or groundwater, which sustain their water supply (Sakane et al., 2011). The stability of water availability in permanent wetlands allows for the establishment of diverse and specialized communities, including water-dependent invertebrates, plants, amphibians, fish, and waterfowl (Kingsford et al., 2016; Bird et al., 2019).

In contrast, temporary wetlands experience fluctuations in water levels and undergo periods of complete drying or significantly reduced water presence (Brendonck et al., 2017). They typically rely on rainfall, seasonal runoff, or periodic flooding events for their water supply. Their hydroperiod can range from a few weeks to several months. As water availability is intermittent, temporary wetlands are subject to dynamic ecological processes. During wet periods, temporary wetlands support a burst of biological activities, including germinating specialized plant species adapted to the short-lived aquatic conditions (Day et al., 2010; Boix et al., 2016; Mabidi et al., 2017). Despite the harsh conditions during dry phases, temporary wetlands exhibit remarkable resilience, with particular plant and animal species such as branchiopods (Bishop, 1967; Brendonck, 1996; Milne et al., 2021; Marrone et al., 2006), copepods (Wasserman et al. 2018) and chironomids (Cornette & Kikawada 2011; Cornette et al., 2022) possessing adaptations that allow them to survive and persist through extended periods of drought.

The contrasting hydrological characteristics between permanent and temporary wetlands influence the types of ecological communities they support and the ecosystem services they provide. While permanent wetlands offer stable habitats and support a wide array of biodiversity, temporary wetlands display dynamic patterns of species composition and play a vital role in the life cycles of numerous organisms adapted to their cyclic nature (Vanschoenwinkel et al., 2007). Understanding the distinctions between these wetland types is essential for effective conservation and management efforts, as their unique characteristics contribute to the overall ecological diversity and functioning of wetland landscapes (Ruhi & Batzer, 2014).

1.2 Temporary Wetlands Biology

Temporary wetlands are shallow, small aquatic water bodies occurring in various landscape settings. They are characterized by their annual or unpredictable drying to the point of lacking surface water (Brendonck et al., 2017). These wetlands are known by different names, including temporary pools, dayas, vernal pools, rock pools, temporary habitats, pans, or temporary ponds (Goudie & Wells, 1995; Brendonck et al., 2002; Zedler, 2003; Schwartz & Jenkins, 2007; Jocque et al., 2010; Ellery et al., 2016; Fritz & Whiles, 2021). They can occur naturally in depressional wetlands or be artificial, particularly in agricultural areas (Neff et al., 2019; Hammer, 2020). The lack of consensus on the specific characterization of these systems hinders assessment studies and efforts to quantify their environmental requirements (Zacharias et al., 2007; Mathilde et al., 2022; Wasserman & Dalu, 2022).

The regional climate plays a significant role in determining the formation of temporary wetlands by influencing the local precipitation/evaporation balance (Williams, 2006). They are most abundant in Mediterranean, arid, and semi-arid regions with distinct dry and wet seasons (Brendonck & Williams, 2000; Dube et al., 2020; Milne et al., 2020). Factors such as lack of fluvial integration, surface deflation, limited sand accumulation and infilling, weathering of the surface, suitable stratum, and geological structure drive the formation of temporary wetlands (Marshall & Harmse, 1992; Melly et al., 2017). These factors predominantly influence the physicochemistry of these systems (Hamer & Martens, 1998; Brendonck et al., 2000; Anusa et al., 2012; Mdidimba et al., 2021). Despite their relatively small area within watersheds, temporary wetlands play a disproportionate role in hydrological and biogeochemical functions and contribute to global cycles (Downing, 2010; Biggs et al., 2017; Cantonati et al., 2020).

The geographic isolation, local and regional distribution of temporary wetlands provide unique and strategic support for wildlife and migratory birds (Robinson, 1995; Tiner, 2003; Reynolds et al., 2017; Dube et al., 2020; Abbas, 2022). While temporary wetlands host various taxonomic groups of aquatic organisms, they are typically dominated by a high proportion of specialized, rare, and endemic aquatic fauna and flora with unique traits that aid in their survival (Zacharias et al., 2007; Bagella et al., 2016; Djamai et al., 2019; Mlambo et al., 2011; Mabidi et al., 2016; Schwentner et al., 2020; Rogers & Milne, 2022). High-altitude temporary wetlands are unique ecosystems occurring in mountainous regions around the world (Mdidimba et al., 2021; Dunnik et al., 2016; Hussien et al., 2018). They are common in the Andes Mountains in South America, the Himalayan Mountains in Asia, Ethiopian Highlands, the Matobo World Heritage Site in Zimbabwe and Maloti-Drakensberg Mountain in South Africa and Lesotho (Hamer and Martens, 1998; Gandarillas et al., 2016; Hussien et al., 2018). High-altitude temporary wetlands are considered sentinel systems due to their sensitivity to changes in the surrounding landscape and atmosphere (Dunnik et al., 2016; Curtis et al., 2023). Climate change is expected to impact different regions and influence aquatic systems (Poff et al., 2002; Allan et al., 2021). Understanding the responses of freshwater biodiversity to hydrological variation is crucial for predicting the consequences of changing hydrology due to human water use and climate change. High-altitude temporary wetlands, often in minimally disturbed areas, can be early warning systems for climate change and environmental disturbances.

1.2.1 Macroinvertebrates in temporary wetlands

The biological diversity of temporary wetlands supports ecosystem processes, functions, and services at various spatial scales (Calhoun et al., 2017). They are also convenient systems for ecological research, with simple food webs ideal for studying community interactions, evolutionary biology, and conservation (Blaustein & Schwartz, 2001; DeMeester et al., 2005; den Broeck et al., 2019). Macroinvertebrates comprise the bulk of the macrobiotic diversity in temporary wetlands. They are the primary trophic link between plant productivity (macrophytes and algae) and higher wetland vertebrates (fish and birds) in temporary wetlands (Batzer, 2013; Wissinger et al., 2016; Biggs et al., 2017). Temporary wetland communities tend to be simple, with limited carrying capacity, and few biotopes or micro-habitats due to their small size (Jocque et al., 2006; Brendonck et al., 2016; Smolak & Walsh, 2022).

Macroinvertebrates in temporary wetlands can be classified according to their strategy for tolerating or avoiding drought and their period of recruitment to the community, and mobility.

Wiggins et al. (1980) categorized macroinvertebrates into four groups. Group 1 are year-round residents incapable of active dispersal, which avoid desiccation either as resistant stages or burrowing into pool sediments, for example large branchiopods and ostracods. Group 2 are spring recruits which must oviposit on water but subsequently aestivate and overwinter in dry basin in various life histories, such as mayflies and dytiscid beetles. Group 3 are summer recruits ovipositing in the dry basin and overwintering as eggs and larvae, for example caddisflies and dragonflies. Group 4 are non-wintering migrants that leave the pool before the dry phase and spend the rest of the seasons in permanent water, returning in spring to breed. This group includes water boatmen and backswimmers. The group concept introduced by Wiggins et al. (1980) for distinguishing ecological strategies in temporary pond communities offers a sound base for community analysis and prediction.

Jocqué et al. (2010) refined the grouping system by categorising temporary wetland taxa into two major mobility groups: active dispersers and passive dispersers. Active dispersers are less restricted by hydroperiod because they fly or crawl overland from pond to pond to escape drying with the ability to colonise temporary wetlands almost immediately upon inundation. They include common insect taxa, such as hemipterans, coleopterans, and dipterans. Many of these insect taxa are predators that must consume high-protein prey as part of their diet to reach adulthood and become aerial dispersers before ponds dry (Washko & Bogan, 2019). On the contrary, passive dispersers employ strategies to survive drying in situ. This group is primarily dominated by crustaceans, such as branchiopods, and ostracods (Brendonck, 1996; Strachan et al., 2015; Brendonck et al., 2017; Bird et al., 2019; Vendramin et al., 2023).

The community succession and hydroperiod is illustrated in Figure 1.1. Upon inundation, dormant eggs hatch, resulting in a community of primary consumers (copepods, branchiopods, ostracods, and annelids) that feed on primary producers such as phytoplankton (Jocque et al., 2010; Dalu et al., 2017; Bird et al., 2019). With the progression of the hydroperiod, generalist taxa, such as insects like Notonectidae and Dytiscidae, invade as semi-aquatic adults, while others lay eggs that quickly hatch into predatory early life stages, like Odonata (O'Neill & Thorpe, 2014). Towards the end of the hydroperiod, generalist taxa must emigrate to survive, and specialist taxa prepare for the dry season by laying dormant eggs or going through a process called anhydrobiosis. Organisms that permanently inhabit temporary ponds rely on the production of resting or dormant stages to bridge dry season/desiccation and limited ecosystem productivity (Brendonck et al., 2002). Although the pattern of dry and wet phases is predictable, the exact start and length of the seasonal pools' hydroperiod vary spatially due to differences in pool and soil characteristics and temporally due to inter-annual climate variation. Hence, compared to their permanent counterparts, seasonal pools are stable but display intra- and inter-annual variation in hydroperiod and inundation frequency (Brendonck et al., 2017).

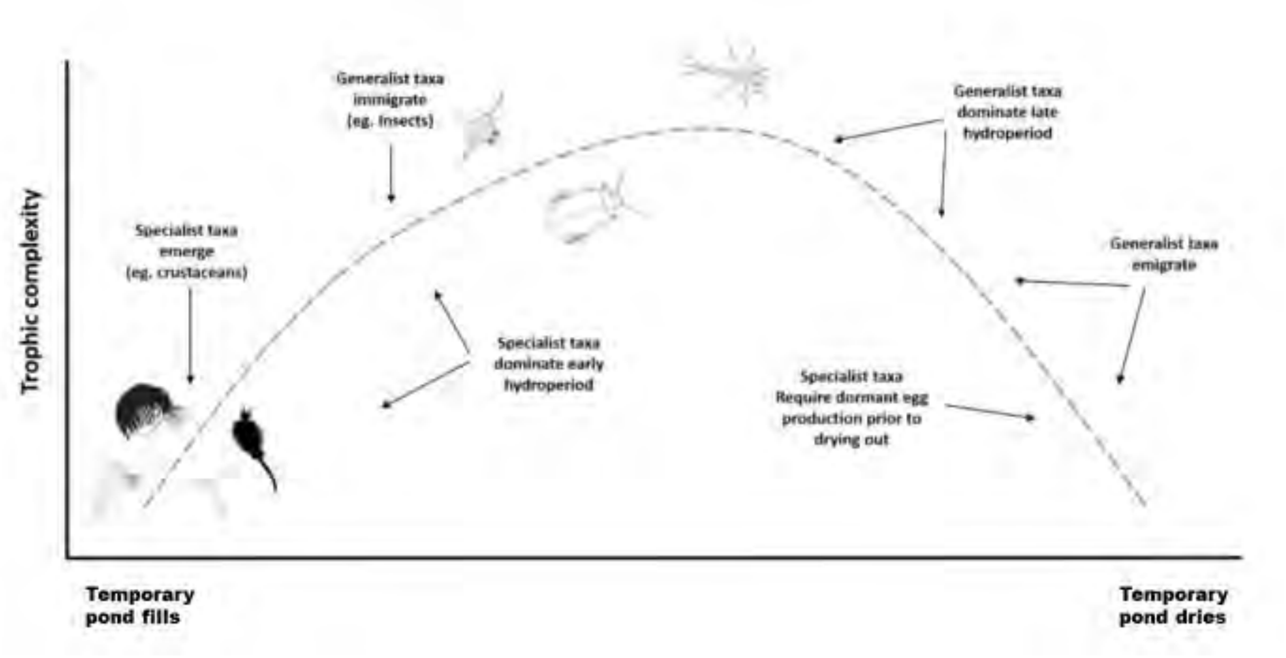

Figure 1.1 Theoretical illustration of temporary wetland community composition over the course of a hydroperiod (extracted from Tladi, 2021a).

Information on the community composition and diversity of macroinvertebrates can be used to assess temporary wetlands' status and ecological integrity (Dube et al., 2020) and as such sampling protocol have been developed to assess the ecosystem health of streams and rivers in southern Africa (Chutter, 1998; Lowe et al., 2013; Dalu et al., 2016; Mangadze et al., 2019). Macroinvertebrates, as suggested by Dickens and Graham (2002), along with fish (Kleynhans et al., 2005) and diatoms (de la Rey et al. 2004), have been utilized as bioindicators – organisms or communities providing environmental quality information (Ollis et al., 2006). The sensitivity of macroinvertebrates to pollution varies; some macroinvertebrates are tolerant of water pollution, while others are sensitive. For example, Caenidae (mayflies) are sensitive to water pollution, whereas Culicidae (mosquito’s larvae), Diptera (true flies), and Oligochaeta (worms) are tolerant to pollution (Dalu and Chauke, 2019). Ostracods have been noted for their sensitivity to environmental factors, salinity in particular, and can thus offer important insights into past environmental conditions (Ruiz et al., 2013; Spershott, 2017). In temporary wetlands, however, macroinvertebrate community assemblages are unreliable indicators (Cooper et al., 2007; Bird et al., 2013; Gleason & Rooney, 2017) because many inhabitant invertebrates are well adapted to cope with a range of conditions, and hence are inherently robust to environmental variation (Batzer, 2013). It is valuable to global efforts to manage hydrological regimes for biodiversity outcomes to counter the ever-growing threats to these ecosystems (Rolls et al., 2018). Hence prompting the need for research on potential bioindicators for temporary wetlands although this is beyond the scope of this study.

1.2.2 Threats to temporary wetlands biology

Human reliance on temporary wetland services makes them vulnerable to destruction through over-exploitation. At least 50% of the world's wetlands (permanent and temporary) have been lost since 1900 (Fluet-Chouinard et al., 2023). However, the provenance of this figure needs to be clarified since it was based on United States of America (USA) data only from the mid-20th century (Davidson, 2014). This estimation not only neglects the rest of the world, but it also does not account for the variable climatic conditions. Additionally, the USA is a first-world country with fewer socio-economic challenges than southern African countries, where studies have shown that anthropogenic activities contribute significantly to the threat of these systems (Mabidi et al., 2017; Dube et al., 2020; Mungenge et al., 2023). The lack of legislation protecting temporary wetlands has hindered conservation efforts, thereby exacerbating temporary wetland loss (Acuna et al., 2017; Calhoun et al., 2017; Balvanera et al., 2022; Chan & Satterfield, 2020). Destruction of temporary wetlands reduces their ability to perform essential services and causes excessive groundwater abstraction for agriculture, thus lowering the water table (Li et al., 2021).

When wetlands are destroyed, they lose their regulatory abilities rendering storms and floods catastrophic (Hinkel et al., 2014; Bradshaw et al., 2021). A decrease in carbon sequestration due to temporary wetland loss results in an immediate increase in carbon dioxide in the atmosphere resulting in a drastic increase in temperatures, consequently, global warming (Guldberg et al., 2018; Diaz et al., 2019). The rise in temperature and change in rainfall patterns will be detrimental to macroinvertebrates communities in warm/dry regions (Epele et al., 2022). Moreover, converting natural wetlands to artificial ones for rice production decreases biodiversity as homogenous environments are less diverse (Davidson et al., 2018). Deepening of temporary wetlands to increase their hydroperiod has adverse effects, as observed at Thomas Baines, South Africa, where the result was the extinction of a unique monotypic genus of fairy shrimps (Anostraca), *Rhinobranchipus martens* Brendonck 1995 (Martens & de Moor, 1995).

The 'disappearing' nature, coupled with competitive funding opportunities and conservation priorities, facilitates temporary wetlands typically “taking a back seat” in freshwater conservation, thus compromising the conservation agenda (Lopez et al., 2011; Calhoun et al., 2017). The lack of a charismatic species, as observed in vertebrates and limited public awareness, impedes efforts to include the community in the sustainable use of wetlands (Cohen et al., 2016; Biggs et al., 2017; Albert et al., 2021).There is ongoing conflict between human needs and ecosystem health (Aronson et al., 2020; Scheidel et al., 2020; Vogel et al., 2021). The increasing human population constantly needs more land for housing, industrial development to cater to the growing economies and agriculture for food security. There is an urgent need for sustainable use of these aquatic systems for human beings to benefit without degrading the systems.

1.3 Study Rationale

Traditionally, temporary wetlands have been considered 'wasted' land areas, potentially convertible to agriculture and human settlement once drained. In contrast, they are natural features of global landscapes representing unique habitats for unique and endemic fauna and flora (Williams, 2006; Zacharias et al., 2007; Rogers, 2020; Lopes et al., 2021). The ecological importance of macroinvertebrates in South Africa is often underappreciated because they are generally small and inconspicuous (Dube et al., 2020). Lower altitude temporary wetland systems have been extensively studied in southern Africa (e.g., Brendonck and Riddoch, 1999, 2001; Brendonck et al., 2000,2002; Balmford, 2003; de Roeck et al., 2007; Nhiwatiwa et al., 2009; Jocque et al., 2010; Mlambo et al., 2011; Anusa et al., 2012; Mabidi et al., 2016, 2017; Dube et al., 2020; Tladi et al., 2021; Mahed et al., 2022; Milne et al., 2020, 2022). The effects of anthropogenic activities on ecosystem functions are well-documented in these systems (Riato et al., 2014; Blanckenberg et al., 2019; Buxton et al., 2022; Mungenge et al., 2023). However, high-altitude temporary wetlands have yet to be addressed, with fewer studies on temporary wetland biology and their dynamics (Hamer and Martens, 1998; Dunnik et al., 2016; Mdidimba et al., 2021). One study solely focused on one taxonomic group, large branchiopods is that of Hamer and Martens (1998). Whilst the only other known study was about trophic dynamics of macroinvertebrates across hydroperiods (Mdidimba et al., 2021). Both studies left a knowledge vacuum regarding a holistic representation of the macroinvertebrate assemblages from temporary pools in the Maloti-Drakensberg mountains. High-altitude temporary wetlands are sentinel sites due to their relatively undisturbed nature, as they are often found in areas far from human settlement and are mostly largely natural. The Maloti-Drakensberg Mountain is considered a strategic water source in the southern Africa region especially in South Africa and Lesotho and it has a high density of high-altitude temporary wetlands in the form of Afromontane tarns and rockpools. Consequently, understanding their essential system functions is of paramount importance. These water bodies may function as reference systems for monitoring the effects of global climate change. Protecting water bodies for conservation requires an understanding of regional as well as local diversity.

1.3.1 Aim and Objectives

This thesis aimed to investigate the macroinvertebrate diversity and community assemblage in relation to environmental drivers, in temporary wetlands of the Maloti-Drakensberg Mountain, South Africa.

1.3.2 Objectives

The objectives of this thesis are to:

1. Describe the abundance, diversity, and distribution of macroinvertebrates in temporary wetlands in the Maloti-Drakensberg Mountain.
2. Investigate the effect of environmental variables on habitat structuring, spatial variations, and macroinvertebrate community composition in tarns and rockpools in the Maloti-Drakensberg Mountain.

In terms of biodiversity, it was hypothesised that: tarns will have higher diversity in the form of active dispersers than rock pools driven by the longer hydroperiod and presence of microhabitats that accommodate diverse taxa. Additionally, rockpools will have more specialist taxa in the form of passive dispersers than tarns as a result of the shorter hydroperiod and low nutrient levels. Secondly, it was hypothesized that due to their relative shallowness and small area, rock pools will have more variable environmental characteristics compared to tarns. Finally, tarns and rock pools will have different community assemblages and environmental drivers as they are distinct systems.

1.4 Thesis Structure

This thesis comprises of four chapters addressing the effect of environmental variables on macroinvertebrate diversity and community composition in temporary wetlands of the Maloti-Drakensberg Mountain, South Africa.

Chapter 1 introduces temporary wetland ecology and associated macroinvertebrates, highlighting the importance and related threats to these systems.

Chapter 2 describes macroinvertebrate diversity, distribution macroinvertebrate community assemblages in tarns and rock pools.

Chapter 3 describes environmental characteristics of tarns and rock pools and their influencing in shaping macroinvertebrates diversity and community assemblage.

Chapter 4 synthesises the results and provides a general discussion, conclusion with recommendations for future studies.

References Combined reference list for all chapters at the end to avoid repetition.

Chapter 2

From Scarcity to Surprises: Macroinvertebrate Diversity in Afromontane Tarns and Rockpools.

“I am dying by inches, from not having anybody to talk to about insects”- C. Darwin (Photo credit – S. Kolisi)

2.1 Introduction

Temporary wetlands are unique freshwater habitats that harbor a rich diversity of species with a high contribution to regional biodiversity. Despite their relatively small distribution over the world's surface (less than 1%), their services have far-reaching impacts (Wasserman & Dalu, 2022). They support local and regional aquatic biodiversity by hosting many rare, endemic, and specialist macroinvertebrate species (King et al., 1996; Nhiwatiwa et al., 2014). This section will provide an introduction of the concepts that will be covered in this chapter specifically macroinvertebrate diversity, community assemblage, core taxa and nestedness.

2.1.1 Macroinvertebrate diversity in temporary wetlands

Macroinvertebrate diversity in temporary wetlands offers insight into the functioning of these unique ecosystems. To better understand and assess the various aspects of biodiversity; relative abundance, species richness, Pielou's evenness, and the Shannon-Wiener Index, univariate analysis is used (Mendes et al., 2008; Daly et al., 2018). These concepts provide valuable insights into the composition, diversity, and structure of ecological communities, aiding researchers in understanding and managing ecosystems and their conservation (Morris et al., 2014).

Relative abundance refers to the total number of individuals present in an ecosystem. Assessing relative abundance helps ecologists understand which species are more or less dominant in an ecosystem, which can be used to determine ecological interactions, resource availability, and potential impacts on the ecosystem's functioning (Mueller et al., 2014). It provides information about the proportional representation of different species in a community, which can help identify dominant and rare species (Roswell et al., 2021). Additionally, it allows for easy comparison between different communities and can be useful for assessing changes in species composition over time given the current climate change scenarios and anthropogenic disturbances (Pavoine & Bonsall, 2011; Mouillot et al., 2013). However, it does not consider the overall diversity of the community and may not provide a comprehensive measure of diversity (Buckland et al., 2011; Blowes et al., 2022). It is very sensitive to changes in the abundance of dominant species, which can mask changes in the abundance of rare species (Pavoine & Bonsall, 2011). Relative abundance fails to account for the ecological importance or functional role of different species in the community (Cadotte et al., 2011; Roswell et al., 2021).

To counter the short fall of relative abundance, species richness quantifies the total number of different species present within a specific area or community (Chao and Chiu, 2016). A high species richness indicates a diverse ecosystem, while a low species richness suggests a less diverse system. Species richness is an essential metric for assessing the complexity of an ecosystem, as a higher species richness often correlates with increased ecosystem stability and resilience (Mendes et al., 2008). Species richness provides a simple and intuitive measure of diversity based on the number of different species present in a community. Since it is easy to calculate and understand, it can be a useful first step in assessing and comparing diversity among different communities. However, since it ignores the relative abundance of different species, it may not provide a complete picture of diversity (Roswell et al., 2021). Additionally, communities with the same species richness may have very different compositions in terms of dominant and rare species. Species richness does not account for the evenness of species abundances, which can be an important aspect of diversity.

Pielou's Evenness, also known as the Evenness Index, measures the distribution of individuals among different species in a community. It quantifies how evenly the species are distributed in terms of their abundance (Smith and Wilson, 1996). High evenness index indicates that species have similar population sizes, while low evenness suggests that one or a few species dominate the community (Camargo, 1995; Chao and Ricotta, 2019). Evenness is crucial for understanding the ecological balance within an ecosystem, as it can reveal whether certain species are monopolizing available resources or if there's a more equitable distribution (Bowles et al., 2022). It complements species richness and diversity by focusing on the distribution of abundances. However, it does not provide information about the actual number of species present in a community, which can be an important aspect of diversity. It also does not account for the identities of the species or their ecological roles and can be sensitive to changes in the evenness of dominant species, which may not reflect changes in overall community diversity (Roswell et al., 2021).

The Shannon-Wiener Index, also known as the Shannon Diversity Index, combines both species richness and evenness providing a quantitative measure of diversity (Bowles et al., 2022). It considers not only the number of species in a community but also the distribution of individuals among those species (Mendes et al., 2008). A higher Shannon-Wiener Index value represents greater biodiversity, considering both species variety and their relative abundances (Chase et al., 2018). This index is widely used to compare biodiversity across different ecosystems or study the impacts of environmental changes on community composition. It can be used to compare the diversity of different communities since it is sensitive to changes in the abundance of both dominant and rare species. Unfortunately, it can be affected by sample size, making it less useful for comparing communities with significantly different sample sizes. It does not provide information about the identity of individual species, which may be important in some ecological contexts (Chao and Ricotta, 2019). It is calculated using the formula:

$$H = -aP_i(\ln P_i)$$

Where H is the Shannon-Wiener Diversity Index and P_i is the proportion of each species in the sample.

In practice, these measures are often used in combination to gain a more comprehensive understanding of ecological diversity with the choice of which measure to use depending on the specific research question and the characteristics of the data being analysed (Chao and Ricotta, 2019). In this case, given the dynamic nature of temporary wetlands and their differences in terms of hydroperiod, they are bound to have different abundances and distributions which will affect the diversity indices. Hence a combination of relative abundance, species richness, Pielou's evenness and Shannon-Wiener Index will counter these differences and provide reliable biodiversity indices.

2.1.2 Macroinvertebrate community assemblage in temporary wetlands

Macroinvertebrate community assemblage in high-altitude freshwater temporary systems, specifically, are dominated by cold-adapted and drought-tolerant species that can survive harsh environmental conditions (Rani et al., 2020). However, the diversity and abundance of macroinvertebrates can vary significantly depending on factors like the size, depth, and location of the system, as well as the timing of precipitation events. The timing and duration of precipitation events are major drivers of macroinvertebrate community assemblage in these systems (Milner et al., 2023). Additionally, ecological interactions among organisms, such as competition, predation, and facilitation, also influence community assemblage in temporary wetlands (Wilbur et al., 1997; Mdidimba et al., 2021).

Functional feeding groups (FFGs) categorize organisms based on their feeding strategies, providing a framework to understand ecosystem dynamics and energy flow. Predators like dragonflies, regulate prey populations, exerting top-down control on food webs (Nhiwatiwa et al., 2009; Hershey et al., 2010; Vatandoost, 2021). They maintain species diversity by suppressing dominant competitors, thereby promoting coexistence (Bellingan, 2008). Herbivores such as snails and mayfly nymphs, consume primary producers and shape plant community composition and feeding behaviour influences plant growth, distribution, and diversity, ultimately impacting ecosystem structure and function (Duffy et al., 2007; Vilenica, and Ivković, 2020). Detritivores, including decomposers and scavengers, play a fundamental role in nutrient recycling by breaking down organic matter and facilitating decomposition processes thereby releasing essential nutrients back into the environment for primary producers' uptake (Chakraborty et al., 2021). Omnivores, catholic in their dietary habits, occupy multiple trophic levels, contributing to both top-down and bottom-up regulation within food webs (Benkendorf and Whiteman, 2021). Filter feeders like large branchiopods, characterized by their ability to strain suspended particles from the water column, are vital components of aquatic ecosystems (Riisgard et al., 2015; Merritt et al., 2017). They influence water clarity, nutrient cycling, and plankton dynamics, thereby shaping the productivity and biodiversity of aquatic habitats (Whalen and Stachowicz, 2017). Understanding FFGs provides valuable insights into ecosystem functioning, resilience, and stability. Their interactions drive energy flow, nutrient cycling, and trophic cascades, shaping the structure and dynamics of ecological communities. Incorporating FFGs into ecological research and management strategies enhances our ability to conserve biodiversity and maintain ecosystem services in the face of environmental change.

Conducting research in high-altitude temporary wetlands can be logistically challenging due to their remote locations and often inhospitable conditions (Rossini et al., 2017). High-altitude temporary wetlands ecosystems are particularly vulnerable to climate change, with rising temperatures and altered precipitation patterns affecting the timing and the availability of water

(Chatterjee et al., 2010; Dodds et al., 2019). These changes can have profound effects on macroinvertebrate communities. Given their often isolated and pristine nature, high-altitude temporary wetlands and their associated macroinvertebrate communities are of conservation concern (Jacobsen & Dangles, 2017).

Studying macroinvertebrate community assemblages in temporary wetlands can provide insights into the ecology and biodiversity of high-altitude ecosystems. This research is not only of scientific interest but also crucial for the conservation of these fragile and often isolated habitats. Consequently, this study contributes to our understanding of the importance of temporary wetlands and informs their management and conservation.

2.1.3 Core taxa in temporary wetlands

Core taxa are a subset of species or taxa that are consistently present or abundant and play a fundamental role in the structure and function an ecosystem (Batzer & Ruhi, 2013). In temporary wetlands, core taxa are the species or groups of species that are particularly important to the functioning and dynamics of these unique and often seasonally flooded habitats (Dube et al., 2021). The specific core taxa may vary depending on the geographical location, environmental conditions, and the type of temporary wetland but there are some common groups and species that are often central to these ecosystems (Batzer & Ruhi, 2013).

Core taxa possess morphological characteristics that enable them to thrive in temporary wetlands. They survive the dry phase by dispersing to nearby systems with longer hydroperiods such as permanent wetlands, lakes, and rivers (Strachan et al., 2015; Boix et al., 2016). These macroinvertebrates often exhibit traits such as desiccation resistance, rapid growth and reproduction, and efficient resource utilization (Williams, 2006). These traits allow them to persist during dry periods and rapidly colonize the wetland when water becomes available (Williams, 2006; Bird et al., 2019; Zarges et al., 2019). These are predominantly insect taxa such as hemipterans, coleopterans, dipterans and odonates (Mlambo et al., 2011; Dube et al., 2019).

Chironomids (dipterans) are often core taxa in temporary wetlands as their larvae are adapted to a variety of environmental conditions, and they are one of the most abundant and diverse groups of insects in aquatic habitats (Armitage et al., 2012; Siziba et al., 2013). They serve as an important food source for other organisms. Dytiscides (coleopterans), predaceous diving beetles, are often abundant and diverse in temporary wetlands and feed on other aquatic organisms such as chironomids. Similarly, to dytiscides, odonates, dragonfly and damselfly nymphs, are also predators feeding on a variety of prey, including other macroinvertebrates such as snails (Dube et al., 2019). Snails are also considered core taxa in some temporary wetlands, particularly those with longer hydroperiods and they can influence the composition of aquatic vegetation and participate in nutrient cycling (Mlambo et al., 2011).

Backswimmers and water boatmen (hemipterans), herbivores or predators, utilize the vegetation in temporary wetlands as food and are known to feed on other smaller macroinvertebrates (Burger et al., 2019). Crustaceans such as copepods and large branchiopods have short generation times, sometimes less than 30 days (Fahd et al., 2010). They thrive in temporary wetlands with short hydroperiods and oligotrophic as they are filter feeders and able to maximize environments with minimum nutrients (Wang and Chou, 2015). Coleopterans, odonates and hemipterans are known to be core taxa in temporary wetlands with longer hydroperiods and more microhabitats whereas crustaceans are often associated with those with shorter hydroperiods such as rock pools (Jocque et al., 2009).

The presence and abundance of these macroinvertebrate core taxa can provide insights into the health and ecological condition of temporary wetlands. Monitoring changes in the abundance and diversity of these core taxa can help assess the impacts of environmental changes and disturbances on temporary wetland ecosystems. It can also inform conservation efforts by highlighting the importance of preserving these key species and the habitats they depend on.

2.1.4 Nestedness in temporary wetlands

Temporary wetlands are characterized by variable conditions that create diverse habitats within the same wetland type. Nestedness is whereby the species composition in smaller, less diverse wetlands is a subset of the species found in larger, more diverse wetlands (Atmar & Patterson, 1993). This pattern suggests that certain species are found in all wetlands, while others are only present in a subset of wetlands, typically those with more stable or favourable conditions. Recolonization ensures that diverse species persist in temporary wetlands with varying hydroperiods, ensuring ecosystem stability (Smith et al., 2019). In a nested pattern, the species found in the more stable or favourable wetlands are also found in the less stable wetlands implying that wetlands with the greatest species diversity contain a superset of the species found in less diverse wetlands (Batzer & Ruhi, 2013).

Nestedness in temporary wetlands has important implications for biodiversity conservation and ecosystem functioning (De Meester et al., 2005). The isolation and fragmentation of these systems restrict the dispersal of macroinvertebrates between them, resulting in unique community assemblages in different systems (Ortelli et al., 2010b; Woodward et al., 2010). Nestedness can be influenced by a variety of factors, such as hydroperiod, physical characteristics of the wetlands, and the presence of certain species that can colonize a wide range of wetland types (Baber et al., 2004).

Species that are generalist (such as dipterans, odonates and coleopterans) and can adapt to different conditions may contribute to nestedness. Recognizing nestedness in temporary wetlands is important for conservation efforts since protecting and managing wetlands with higher species diversity becomes crucial, as they can act as sources of biodiversity and potentially help repopulate less diverse wetlands. Different wetlands may serve as specialized habitats for particular species, and the combination of these diverse habitats can support a variety of species (Leibowitz, 2003; Khudhair et al., 2019).

Within a population, individuals may exhibit idiosyncratic behaviours or life history strategies, such as variation in reproductive timing, migration patterns, or survival rates. These individual differences can impact population dynamics and have implications for population persistence and resilience (Batzer & Ruhi, 2013; Ruhi et al., 2013). Ecosystem processes and functions can be influenced by idiosyncratic factors, for instance, the presence or absence of key species or the occurrence of specific ecological interactions within an ecosystem can lead to unique patterns or outcomes that are not easily predicted based on general ecological principles (Kordas et al., 2011; Adler et al., 2013).

Overall, understanding the life history strategies, community assemblage, and taxonomy of these aquatic organisms provides insights into their ecological roles, conservation needs, and the functioning of freshwater ecosystems (Bird et al., 2019; Epele et al., 2022). Understanding the nestedness highlights the importance of maintaining diverse and stable wetlands, as they can support a wide range of species and contribute to regional and overall biodiversity. Similarly, recognising idiosyncratic characteristics highlights the need to consider the specific attributes and behaviours of individual species or populations when studying ecological processes, species interactions, and ecosystem dynamics (Wang & Bradburd, 2014; Brandl et al., 2023).

To contribute to the existing knowledge gap, this chapter aimed to assess the abundance, diversity, distribution, and community assemblage of macroinvertebrates in temporary wetlands within the Maloti-Drakensberg Mountain, South Africa. Macroinvertebrates were sampled from temporary wetlands characterised by varying depth and area, with a focus on contrasting sites with longer hydroperiods (tarns) and shorter hydroperiods (rock pools). The objectives of this chapter were to: i) Quantify and characterize the macroinvertebrate diversity and abundance in tarns and rock pools. ii) Investigate macroinvertebrate community assemblage in tarns and rock pools. iii) Compare the occurrence of core taxa in tarns and rock pools to identify key taxa that exhibit consistent presence. iv) Investigate nestedness in temporary wetlands.

This chapter hypothesised that, i) Tarns will have higher diversity than rock pools driven by the longer hydroperiod. ii) Rockpools will have more specialist taxa than tarns. iii) Tarns and rock pools will support different macroinvertebrate community assemblages.

2.2 Materials and Methods

2.2.1 Geology

The Maloti-Drakensberg Mountain originated as the eastern flank of the sub-continent when Gondwanaland was dismembered in the late Jurassic and is a product of erosion (King, 1944). The rocks which build up this mountainous area belong to the uppermost division of the Karoo formation and the Stormberg series (du Toit, 1905; Hamer & Martens, 1998). This series comprises Molteno, Clarens Formation Sandstones, and Drakensberg Basalts (Johnson et al., 2006). Maloti-Drakensberg soils vary with topography from weakly developed lithosols (Hoare & Bredenkamp, 2001) to deep sandy loams (Werger, 1980). They can be moderately deep and nutrient-rich (Clark et al., 2009), drained and very weathered (Hartmann, 1988), poorly developed and easily eroded (Clark et al., 2011). The geology of the Maloti-Drakensberg, coupled with abundant rainfall, has led to a diverse array of wetland habitats (Dely et al., 1999).

2.2.2 Rainfall and Temperature

The Maloti-Drakensberg Mountain has a temperate climate and mean annual rainfall varies from 1418 to 2017 mm between October and April, depending on altitude (Hamer & Martens, 1998). Rainfall and temperature data from January 2013 to April 2022 was obtained from the South African Weather Service (SAWS) for four weather stations in the Maloti-Drakensberg: Shaleburn (Station 0237618A9, -29.7860S 29.3520E, 1609 m.a.s.l), Ficksburg (Station 0296709AX, -28.8270S 27.9040E, 1628 m.a.s.l) Giant's Castle (Station 0268016AX, -29.2640S 29.5220E, 1759 m.a.s.l) and Royal Natal (Station 0298791 9, -28.6850S 28.9540E, 1392 m.a.s.l). To visualise the rainfall patterns, data was plotted per hydroperiod, that is, from September to April of the following year for example, from September 2013 to April 2014. This constituted one cycle—the typical cycle peaks in December or January. The mean temperature of the warmest month is below 22°C, and temperatures can go as low as –20°C in winter (Hamer & Martens, 1998). Rainfall data is illustrated in Appendix 1 and temperature in Appendix 2.

2.2.3 Study sites

The Maloti-Drakensberg region has different types of temporary wetlands in the form of rock pools and Afromontane tarns, hereafter referred to as tarns. Seventy sites comprising 29 tarns and 41 rock pools were sampled during this study at altitudes from 1100 to 3000 m.a.s.l in March 2022 (Figure 2.1). These temporary wetlands were identified using Google Earth, 1: 50 000 topographic maps, historical records (Hamer & Martens, 1998) and through communication with fellow researchers, farmer owners, reserve managers and environmental authorities in all three provinces of the Free State, KwaZulu-Natal, and Eastern Cape. Sites comprised of 18 rock pools and 9 tarns from the Eastern Free State, 17 rock pools and 17 tarns from KwaZulu-Natal and 6 rock pools and 3 tarns from Eastern Cape region of the Drakensberg (Figure 2.1).

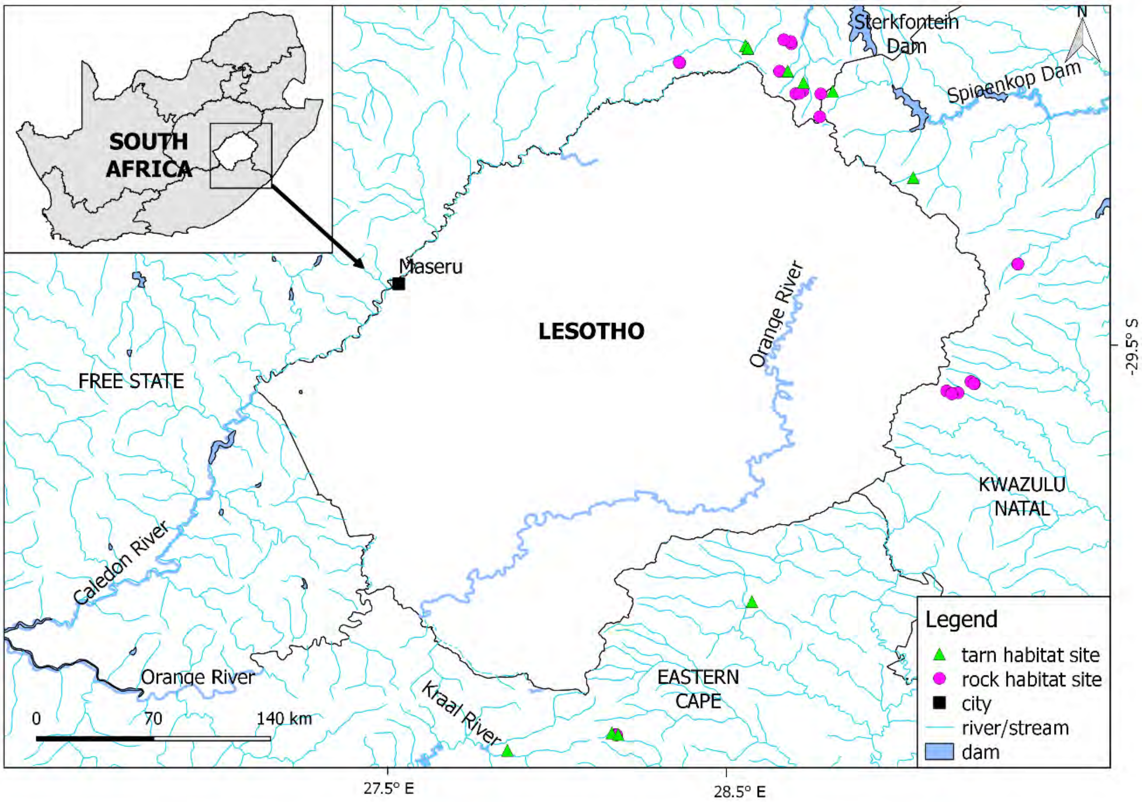

Figure 2.1: Map showing the distribution and location of sampled temporary wetlands in the Maloti-Drakensberg, South Africa.

2.2.4 Description of habitats

Hamer & Martens (1998) described the temporary wetlands of the Maloti-Drakensberg based on substratum, area, and depth, with the last two being critical factors in determining hydroperiod. Hydroperiod is the length of time a water body holds water. The authors classified the temporary wetlands into three groups: depressions in boulders and rocky outcrops, known as rock pools; tarns and pools in meanders of rivers and vlei systems. In this study, only rock pools and tarns were sampled. Rock pools directly result from bedrock weathering (Jocque et al., 2010) and are found in different types of bedrock but most often in granite, sandstone, and limestone (Brendonck, 2016). They are less than/equal to 1 metre in diameter (Hamer & Martens, 1998) and usually occur in exposed locations with highly variable hydroperiods influenced by local climate conditions (Jocque et al., 2010). Rock pools generally lack vegetation (Figure 2.2 a, c, d, f) although it may be present in some instances (Figure 2.2b, e) if there is enough sediment for support (Hamer & Martens, 1998; Brendonck et al., 2010).

Tarns are high-altitude lakelets with a diameter of more than 5 metres (Hamer & Martens, 1998; Dunnik et al., 2016; Mdidimba et al., 2021), a sandy or muddy substratum (Figure 2.3c, d) which can sometimes be situated on a rock base (Figure 2.3a, b) (Hamer & Martens, 1998). Tarns usually have some form of vegetation, be it emergent (Figure 2.3c) or submerged (Figure 2.3a, b, d). The vegetation varies from sub-aquatic to marsh plants such as *Eleocharis* sp. and *Eriocaulon sp.* (Hilliard & Burtt, 1987). They house endemic flora such as the Sehlabathebe lily *Aponogeton ranunculiflorus* (Guillarmod & Marais, 1972; Carbutt & Edwards, 2006).

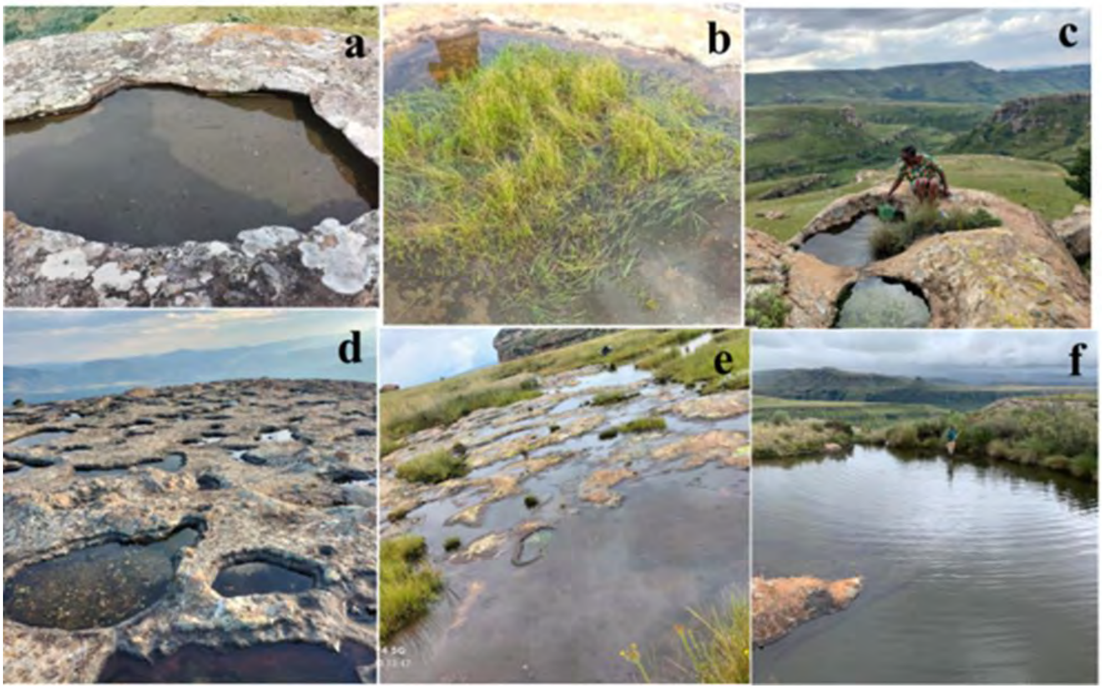

Figure 2.2: Examples of rock pools in the Maloti-Drakensberg: a. Isolated small depression in boulder (Prentjiesberg, Eastern Cape); b. Isolated small depression in boulder with vegetation (St Ford farm, Free State); c. Connected small depressions in boulder (Fika Patso, Free State); d. Series of rock pools on plateau (Prentjiesberg, Eastern Cape); e. Connected pools on plateau with vegetation (Qwaqwa, Free State); f. Series of large and small rock pools on plateau (Giant's Castle, KwaZulu-Natal).

Figure 2.3: Examples of Afromontane Tarns in the Maloti-Drakensberg: a and b A series of tarns (Cobham and Sani Pass, KwaZulu-Natal respectively) d. Tarn in proximity to a series of

rock pools with cattle in the background (Glen Avis Farm, Eastern Cape); c. An isolated tarn (Golden Gate Highlands National Park, Free State).

2.2.5 Data collection

Aquatic macroinvertebrates

Seventy temporary wetlands (29 tarns and 41 rock pools) were surveyed over a once-off sampling (3 March – 7 April 2022). Aquatic macroinvertebrates were collected from each site (rock pool and tarn) using a 1-metre SASS net, 30x30 cm square frame and 1 mm mesh size. Disturbing and sweeping available biotopes (i.e., open water, emergent and submerged vegetation biotopes), 27 aquatic net sweeps or nine sweeps per biotope were done per site (Mlambo et al., 2011). Aquatic macroinvertebrates per biotope were pooled and stored in 90% ethanol solution for later sorting, counting and identification in the laboratory. Rock pools on the other hand due to their small size were sampled using a small round shape aquatic net (15 cm diameter, 1 mm mesh size), and nine sweeps were considered sufficient to represent aquatic communities in rock pools as compared to large tarns (Mdidimba et al., 2021). Samples were also stored in 90% ethanol for further treatment in the laboratory at the Department of Zoology and Entomology, Rhodes University Makhanda (Grahamstown).

Aquatic macroinvertebrates samples were identified to the lowest possible taxonomic level i.e., genus and species using taxonomic keys for the southern African region (Day et al., 2001a, b; Day & de Moor, 200 b; de Moor et al., 2003a; Stals & de Moor, 2007; Griffiths, 2015; Fry, 2022) and the reference collection at Albany Museum, Makhanda, South Africa. Aquatic macroinvertebrates were assigned to Functional feeding Groups (FFGs) using trait data bases from Thorp and Covich (2010); Tachet et al. (2010) and Merritt et al. (2019).

2.2.6 Data analysis

Species accumulation curve was plotted using raw data to calculate Chao1 and Chao2 richness estimators in PRIMER 6 (ver. 6.1.16) and PERMANOVA + Version 1.0.6 add-on package (Anderson et al., 2008). These were plotted for tarns and rock pools combined and for the temporary wetlands individually. Chao 1 and Chao 2 are non-parametric estimators that estimate the total species in a given ecological community (Chao et al., 2009). They are commonly used in biodiversity studies when the observed species richness is likely underestimated due to incomplete sampling. Chao 1 and Chao 2 estimators consider both the number of rare or singleton species (i.e., species represented by only a single individual or occurrence) and the number of doubleton species (i.e., species represented by only two individuals or occurrences) to estimate the total number of species in the community. Chao 1 provides a conservative estimate of species richness by considering only the observed number of singleton species. It assumes that additional unobserved singleton species in the community are yet to be discovered (Chao et al., 2009). Chao 2 is a more refined estimator considering singleton and doubleton species. It assumes that unobserved singleton, doubleton, and higher-order rare species in the community have yet to be observed. Both Chao 1 and Chao 2 estimators provide a way to estimate the "true" species richness beyond what has been observed in a particular sampling effort. These estimators are helpful in situations where sampling efforts might be incomplete or biased, and they help researchers obtain a more accurate estimate of the total species richness in a given community (Chao et al., 2009).

Relative abundance, Species richness, Shannon-Weiner diversity index and Pielou’s evenness were computed on raw data using the Diverse function in PRIMER 6 (ver. 6.1.16) and PERMANOVA + Version 1.0.6 add-on package (Anderson et al., 2008) to investigate macroinvertebrate diversity in rock pools and tarns. Welch Two Sample t-test was used to test for significant differences in biodiversity indices between tarns and rock pools as it is more reliable when sample variances and sizes are unequal. Additionally, Dunn test was done to determine which groups were statistically significant.

To investigate nestedness and identify any association between taxa (family) and habitats (rock pools and tarns) data was organized into a table consisting of two habitats. Each taxon was assigned a value of 1 if it was present in the respective habitat or 0 if it was absent. ‘Absence’ of a taxon would more accurately be defined as ‘not detected’; as there was no way to be sure that a taxon that was not collected was not present or was under-sampled or just rare in some cases (Batzer and Ruhi, 2013). To determine the taxa driving nestedness, a nestedness rank analysis was performed. A presence/absence matrix was generated using observed data with sites as replicates. The analysis ranked each taxon based on its contribution to the observed nestedness pattern. Analysis was done using the "bipartite" package. The results provided a nestedness rank for each taxon, ranging from 0 to 1. Additionally, the nestedness rank for each habitat was also determined.

Core taxa in temporary wetlands are a group of species that play crucial ecological roles and exhibit consistent presence and abundance patterns.

To determine core taxa (≥10% occurrence across all habitats), the following formula was used:

$$\%\, occurence = \frac{O}{N} \times 100$$

Where O is number of occurrences of taxa in sites and N is Total number of observations.

Macroinvertebrate data was fourth root transformed to meet normality and recognise the abundance of rare taxa. A resemblance matrix based on the Bray-Curtis similarity coefficient was calculated. The 'Similarity Percentages' (SIMPER) procedure was carried out to quantify taxa contributing to the average Bray-Curtis similarity/dissimilarity among sites within each temporary pond type. Differences in assemblage composition were tested using non-parametric permutational MANOVA (PERMANOVA) using a two-way factorial design, which incorporated the factors 'type' (tarns and rock pools) and 'locality' (Eastern Cape (EC), Free State (FS) and KwaZulu-Natal (KZN)). Residuals were permuted under a reduced model (9999 permutations). The resemblance matrix was used to perform a non-metric multidimensional scaling (NMDS) ordination to illustrate differences in macroinvertebrate assemblage composition between temporary ponds. The 'Analysis of Similarities' (ANOSIM) procedure was done to test for differences between macroinvertebrate assemblages of tarns and rock pools. ANOSIM calculates a test statistic R, which ranges from −1 to +1. Values approaching |1| indicate good separation of the groups and values approaching 0 indicate weak separation. To further investigate the differences in macroinvertebrate assemblage between wetland types (tarns and rock pools) and locality (Eastern Cape, Free State and KwaZulu-Natal) a two-way factorial non-parametric permutational multivariate analysis of variance (PERMANOVA) was performed.

All statistical analyses were performed in R version 4.3.0 except where its mentioned. The a priori significance level for all statistical tests in this study was set at α = 0.05.

2.3 Results

Macroinvertebrates diversity patterns

A total of 15 278 individual macroinvertebrates were collected and identified during this study belong to 13 orders, 35 families and 46 genera. Hemiptera had the most families, nine including Corixidae, Notonectidae, Veliidae, Pleidae, Belostomatidae, Gerridae, Nepidae, Naucoridae and Hydrometridae (Table 2.1). Dytiscidae recorded the most genera with *Hyphydrus*, *Copelatus, Laccophilus*, and *Rhantus* being the most prevalent (Table 2.1). Lepidoptera, Odonata, and Podocopia were exclusively collected from tarns whereas Anostraca, Ephemeroptera, Spinicaudata and Trichoptera were exclusively collected from rock pools (Table 2.1). Although some species were collected in both tarns and rock pools; in Hemiptera, *Micronecta* sp., *Hydrometra* sp*, Naucoris* sp., and *Barborophilus* sp. were only found in tarns whereas *Sigara* sp. and *Nychia* sp. were only found in rock pools (Table 2.1). In Diptera, *Chaoborus* sp. and *Limnophora* sp. were only found in tarns whereas *Brachydeutera* sp. was only found in rock pools (Table 2.1). In Coleoptera, *Cybiste*r sp. and *Gyrinus* sp. were only found in tarns whereas *Prosthetops gladiator* and *Berosus* sp. were only found in rock pools (Table 2.1). In Mollusca, *Helisoma* sp. was only found in tarns (Table 2.1).

Table 2.1: Mean (±SD) of macroinvertebrate taxa relative abundances observed in temporary wetlands in the Maloti-Drakensberg.

Taxa	Tarn Mean±SD	Rock Mean±SD
Anomopoda		
Simocephalus sp.	13.56±48.06	1.69±13.55
Anostraca		
Branchipodopsis sp.	3.28±21.83	
Coleoptera		
Hydaticus sp.	0.6±1.4	0.51±3.4
Rhantus sp.	1.27±2.57	0.36±2.27
Hydroglyphus sp.	0.73±2.82	0.95±7.83
Laccophilus sp.	2.03±3.25	0.51±3.26
Copelatus sp.	3.27±8.61	0.36±2.4
Hyphydrus sp.	2.77±3.66	3.49±23.89
Philodytes sp.	0.57±1.52	0.08±0.58
Africophilus sp.	1.6±8.2	0.36±2.6
Cybister sp.	0.03±0.18	
Hydroporinae sp.	3.37±6.42	3.05±22.59
Colymbetinae sp..	0.47±1.04	0.46±3.3
Gyrinus sp.	0.13±0.73	
Haliplus sp.	0.37±1.65	0.05±0.38
Prosthetops gladiator		10.77±82
Helochares sp.	0.6±1.46	0.21±1.57
Enochrus sp.	0.03±1.15	0.1±0.72
Berosus sp.		0.21±1.76
Hydrocanthus sp.	0.53±.1.59	0.03±0.22
Decapoda		
Potamonautes sp.	0.1±0.31	
Diptera		
Chaoborus sp.	0.9±3.72	
Chironominae	6.93±23.14	66.38±408.85
Orthocladiinae	8.87±33.85	1.98±12.58
Tanypodinae	1.8±5.06	7.59±52.68
Culex sp.	51.9±77.76	22.26±142.04
Brachydeutera sp.	2.1±18.1	
Limnophora sp.	0.03±0.18	
Ephemeroptera		
Cloeon sp.	46±99.23	
Hemiptera		
Appasus sp.	4.7±9.42	0.15±1.02

Neomacrocoris sp.	0.1±0.31	0.05±0.38
Micronecta sp.	0.2±0.66	
Sigara sp.		23.92±147.65
Gerris sp.	3.13±4.31	1.46±9.54
Hydrometra sp.	0.07±0.37	
Naucoris sp.	0.03±0.22	
Ranatra sp.	0.83±1.49	0.07±0.53
Barborophilus sp.	0.03±0.18	
Enithares sp.	2.97±5.93	9.05±56.33
Anisops sp.	2.27±4.59	7±43.8
Nychia sp.		0.03±0.22
Plea sp.	6.23±15.53	0.05±0.44
Microvelia sp.	7.87±27.59	7.26±63.35
Lepidoptera		
Crambidae	4.53±7.44	
Mollusca		
Bulinus sp.	17.3±56.23	6.88±51.85
Helisoma sp.	4.4±24.1	
Odonata		
Anax sp.	1.5±2.73	
Aeshna sp.	0.57±1.33	
Psuedagrion sp.	38.33±36.62	
Tramea sp.	1.5±5.02	
Orthetrum sp.	11.83±14.34	
Podocopia		
Megalocypris sp.	5.93±29.12	
Spinicaudata		
Leptestheria sp.		0.44±3.53
Trichoptera		
Athripsodes sp.	0.02±0.22	0.1±0.72

The species accumulation curve analysis incorporating both tarns and rock pools revealed that the estimated species richness based on Chao 1 and Chao 2 estimators was higher than the observed values. Additionally, the curves did not reach a plateau, indicating that the sampled sites did not adequately represent the full diversity of the studied area (Figure 2.4a). A similar trend was observed when the species accumulation curve of rock pools only was plotted, implying that more sites needed to be sampled to gain an idea of the diversity of macroinvertebrates from these systems (Figure 2.4c). However, the species accumulation curve for tarns only showed no significant differences between observed values and Chao 1 and Chao

2 estimators meaning that the sampled sites represented the full diversity of the temporary wetland type (Figure 2.4b).

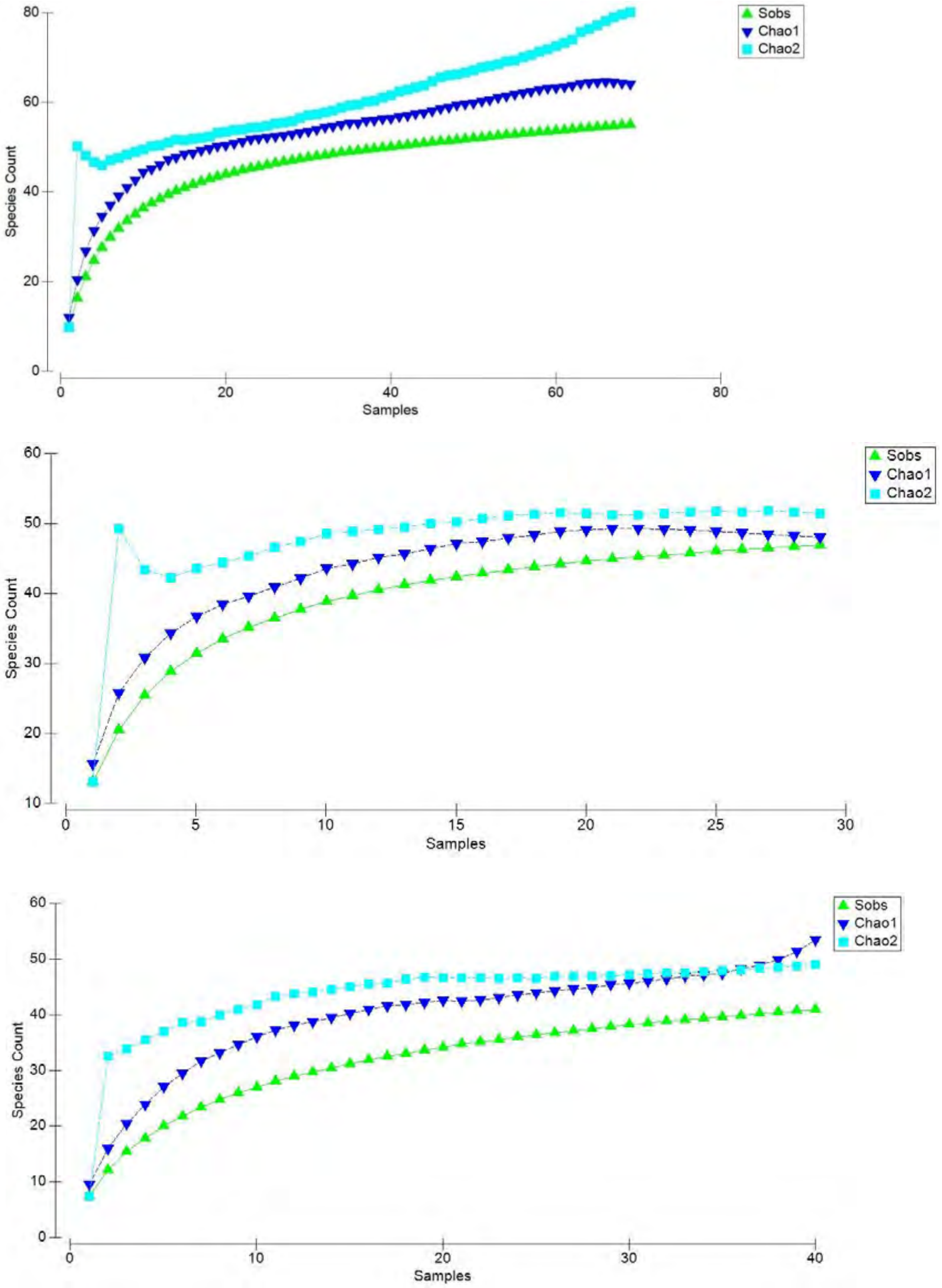

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Figure 2.4: Sample-based taxon accumulation curves of macroinvertebrate assemblages based on (a) whole data set for tarns and rock pools, (b) for tarns only, (c) for rock pools only.

There were no significant differences in relative abundance (Figure 2.5A) and Pielou's evenness (Figure 2.5C) between tarns and rock pools. However, tarns exhibited slightly higher values for both relative abundance (Figure 2.5A) and Pielou's evenness compared to rock pools (Figure 2.5C). Tarns and rock pools exhibited a significant difference in species richness, with tarns having nearly double the mean richness compared to rock pools (Figure 2.5B). Specifically, tarns had an average species richness of approximately 3, while rock pools had an average species richness of around 1.8 (Figure 2.5B). The Shannon-Wiener Index revealed a significant difference in diversity between tarns and rock pools, indicating that tarns exhibit higher diversity compared to rock pools (Figure 2.5D).

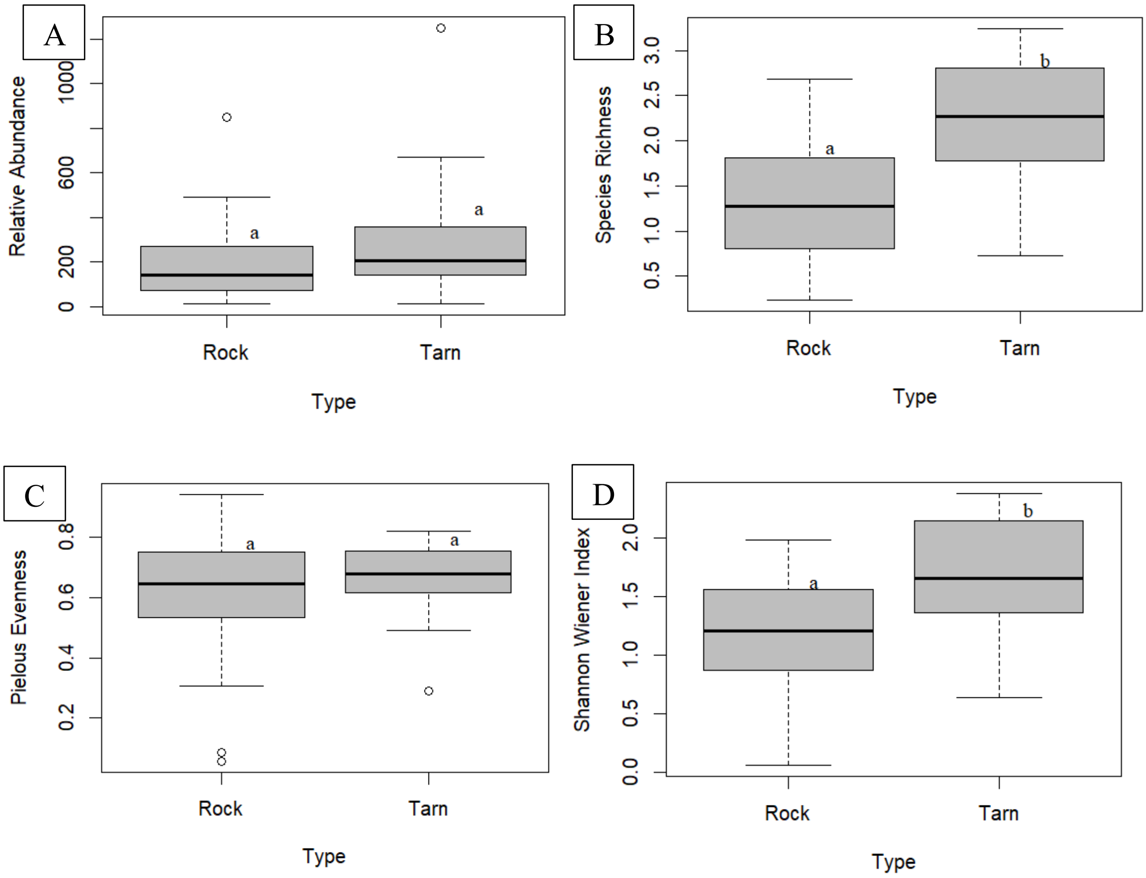


Figure 2.5: Aquatic macroinvertebrate biodiversity indices between rock pools and tarns; A. Relative abundance, B. Species richness, C. Pielou's evenness and, D. Shannon-Wiener Index. Middle line represents mean and lower-case letters represent significant differences ($p < 0.05$).

An NODF value of 33.05369 indicates that there is a moderate level of nestedness in the presence/absence matrix (Table 2.2).

Table 2.2: Rank results based on taxa driving nestedness.

Number of columns (Habitats)	100
Number of rows (taxa)	32.94118
NODF (Nestedness metric based on Overlap and Decreasing Fill)	33.05369
Matrix fill	0.9

Taxa with a percentage occurrence ≥10% are considered core taxa (Batzer &Ruhi, 2013). Of the 35 families recorded, 19 were core taxa with Dytiscidae and Corixidae being the dominant taxa (Table 2.3). Of the observed functional feeding groups, predators were dominant accounting for 57% with omnivores being the least common at 0.06% (Table 2.3). Nestedness analysis determined that 32 of the 35 taxa were idiosyncratic and 3 were nested, with all core taxa being idiosyncratic (Table 2.3).

Table 2.3: Percentage of occurrence, nestedness value and functional feeding groups of macroinvertebrate families collected from temporary ponds. Percentage of occurrence ≥10% (bold) considered core taxa. Nestedness value of <0.947 considered idiosyncratic and values ≥0.947 (bold) considered nested.

Family	% of occurrence	Nestedness value	Functional Feeding Group(s)
Leptoceridae	1	**1**	Omnivore
Hydrometridae	1	0.589	Predator
Ephydridae	1	**0.971**	Collector
Empididae	1	**0.941**	Predator
Muscidae	1	0.5	Collector/Predator
Planorbidae	1	0.206	Scrapper/Shredder
Gyrinidae	1	0	Predator
Chaoboridae	3	0.529	Predator
Sciomyzidae	3	0.823	Predator
Cyprididae	4	0.176	Collectors
Leptestheriidae	4	0.883	Collector
Hydraenidae	4	0.853	Scrapper/Shredder
Veliidae	7	0.765	Predator
Noteridae	7	0.029	Predator
Potamonautidae	9	0.323	Predator
Chydoridae	9	0.147	Collector/Herbivore
Haliplidae	**10**	0.059	Scrapper/ Shredder/Herbivore
Branchipodidae	**12**	0.912	Collector
Pleidae	**14**	0.706	Predator
Crambidae	**14**	0.265	Collector/Herbivore
Naucoridae	**17**	0.676	Predator
Nepidae	**17**	0.618	Predator

Hydrophilidae	**19**	0.882	Omnivore
Bulinidae	**22**	0.234	Scrapper/Shredder
Aeshnidae	**25**	0.412	Predator
Libellulidae	**29**	0.382	Predator
Baetidae	**30**	0.294	Collector
Belostomatidae	**33**	0.647	Predator
Coenagrionidae	**35**	0.353	Predator
Gerridae	**46**	0.588	Predator
Culicidae	**54**	0.471	Collector
Chironomidae	**61**	0.441	Collector/Predator
Notonectidae	**71**	0.794	Predator
Dytiscidae	**80**	0.118	Predator
Corixidae	**88**	0.735	Collector/Herbivore

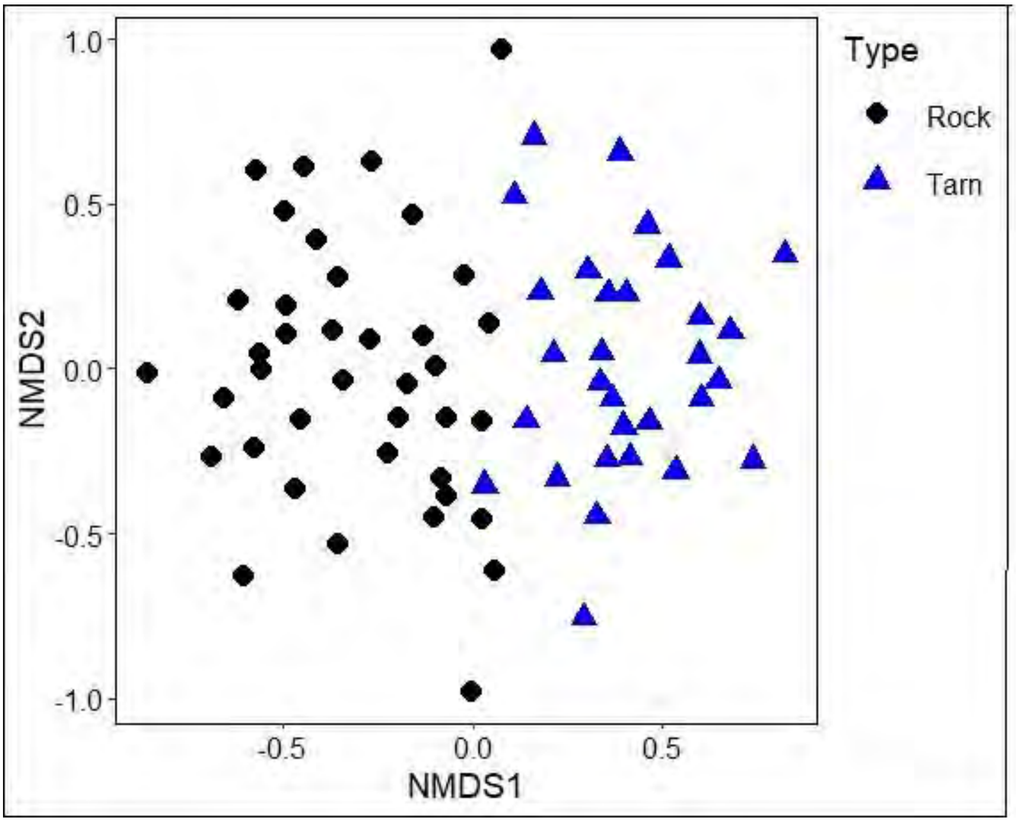
The average Bray-Curtis dissimilarity between tarns and rock pools was 77.35%, indicating substantial dissimilarity in macroinvertebrates assemblage and composition (Table 2.4). Major contributors to the Bray-Curtis dissimilarity in tarns were *Pseudagrion* sp., *Cloeon* sp. and *Sigara* sp.; and Chironominae, *Sigara* sp. and *Enithares* sp. in rock pools (Table 2.3).

Table 2.4: SIMPER listing the macroinvertebrate taxa that contributed to the average Bray-Curtis dissimilarity between rock pools and tarns. Taxa are listed in terms of their percentage contribution to the average Bray-Curtis dissimilarity and given taxa with a cumulative contribution of ~90% of the total similarity/dissimilarity. The average dissimilarity is 77.35.

Taxa	Group Rock Av. Abund.	Group Tarn Av. Abund.	Contrib. %	Cum.%
Pseudagrion sp	0	2.02	7.69	7.69
Chironominae	2.05	0.45	7.58	15.28
Culex sp	1.01	1.7	6.58	21.85
Cloeon sp	0	1.77	6.1	27.95
Sigara sp	1.63	1.75	4.23	32.18
Enithares sp	1.07	0.72	3.85	36.03
Orthetrum sp	0	1.08	3.75	39.78
Anisops sp	0.94	0.6	3.59	43.37
Gerris sp	0.46	0.84	3.06	46.44
Hyphydrus sp	0.48	0.74	3.04	49.48
Appasus sp	0.13	0.86	2.84	52.32
Hydroporinae larvae	0.43	0.66	2.73	55.04
Tanypodinae	0.58	0.34	2.71	57.75
Laccophilus sp	0.28	0.61	2.69	60.44
Bulinus sp	0.35	0.61	2.67	63.1
Orthocladiinae	0.37	0.39	2.32	65.42
Copelatus sp	0.2	0.56	2.27	67.68
Crambidae	0	0.63	2.13	69.81
Anax sp	0	0.58	2.07	71.89
Rhantus sp	0.27	0.41	2	73.88
Plea sp	0.03	0.55	1.88	75.76
Hydaticus sp	0.22	0.34	1.73	77.49
Ranatra sp	0	0.46	1.65	79.14
Pleuroxus sp	0.12	0.38	1.52	80.66
Branchipodopsis sp	0.37	0	1.38	82.04
Microvelia sp	0.13	0.29	1.38	83.42
Helochares sp	0.09	0.25	1.2	84.62
Africophilus sp	0.15	0.16	1.2	85.81
Colymbetinae larvae	0.12	0.3	1.18	86.99
Tramea sp	0	0.32	1.13	88.13
Aeshna sp	0	0.28	1.04	89.16
Hydroglyphus sp	0.12	0.18	1.02	90.18

Macroinvertebrates community assemblage

The non-metric multidimensional scaling (NMDS) analysis revealed two distinct clusters each representing tarns and rock pools, thus indicating differences in macroinvertebrates communities at each wetland types (Figure 2.8). The R-value was calculated as 0.4382, indicating a moderate level of dissimilarity between the communities (Figure 2.8). The statistical significance of the differentiation was confirmed by a p-value of 1e-04, suggesting a highly significant difference between the tarns and rock pools (Figure 2.8).



ANOSIM

R= 0.4382

p=1e-04

Figure 2.8: NMDS ordination plot of macroinvertebrate assemblage composition based on Bray-Curtis similarity between rock pools and tarns.

Further examination through PERMANOVA revealed significant distinctions in assemblages between rock pools and tarns ($p < 0.05$) (Table 2.4). However, there were no significant differences among rock pools within the sampled provinces ($p > 0.05$) (Table 2.4). Tarns exhibited notable variations in macroinvertebrate assemblages across provinces (Table 2.4), with distinct compositions observed in the three sampled provinces ($p < 0.05$) (Table 2.4). Furthermore, both rock pools and tarns within different provinces harbored dissimilar assemblages ($p < 0.05$) (Table 2.4).

Table 2.4: Non-parametric permutational MANOVA results for the two-way factorial model examining the effect of type and locality on macroinvertebrate assemblage in rock pools and tarns. Bold values indicate significant P values at α = 0.05.

Factors	df	SS	MS	Pseudo-F	P
Rock pools and tarns	1	21680	21680	11.336	**<0.05**
Rock pools	2	4684	2342	1.169	>0.05
Tarns	2	7971	3986	2.1609	**<0.05**
Locality	2	9665.9	4832.9	2.5271	**<0.05**
Type x Locality	2	12171	6085.5	3.182	**<0.05**

2.4 Discussion

This study presents an account of macroinvertebrate diversity and community assemblages in temporary wetlands of Maloti-Drakensberg Mountain, South Africa. Coleoptera, Diptera, Hemiptera, and Odonata were the most dominant taxa in terms of abundance with the latter exclusively found in tarns. Although tarns and rock pools showed significant differences in terms of relative abundance and Pielou's evenness, species richness and Shannon-Wiener Index were significantly different. Temporary wetlands supported significantly different community assemblages when grouped by type, locality and with type and locality combined. *Prosthetops gladiator,* which was initially reported from Prentjiesberg, Eastern Cape, showed an extended distribution in the province as it was reported from Glen Avis (EC). These results agree with our hypotheses and in line with what was previously reported by Mdidimba et al. (2021) in the region.

When analysed together, species accumulation curves showed significant differences between estimations and observations in tarns and rock pools. However, separately, tarns were almost equal to estimates whereas rock pools were not. These observations can be attributed to their differences in hydroperiod and physical environment. Tarns have a longer hydroperiod and support a range of vegetation types (Dunnik et al., 2016; Chatanga et al., 2019) and are nutrient-rich hence they offer more microhabitat options thus catering for a more diverse range of macroinvertebrate. Additionally, the fact that taxa use different components of the ecosystem makes even a once-off sampling event enough to provide a detailed snapshot of taxa composition thus estimated values were almost equal to the observed one. Rock pools on the other hand lack these microhabitats found in tarns and the species they support have more rapid life cycles. Timing is of the essence when surveying rock pools hence the need for either perfect timing or seasonal sampling in order to fully understand the composition. This is probably why the species accumulation curve results showed that more sites were needed before these indices do not account for the biological diversity of these ecosystems.

Tarns and rock pools showed no significant differences in relative abundance and evenness. The lack of differences in abundance can be attributed to taxa such as Chironomidae that occur in high numbers regardless of the nutrient-availability in an ecosystem. They are a common feature in temporary wetlands and give the illusion that rock pools can support a lot of individuals. This is where species richness clears this illusion as it focuses on the number of species present in spite of the abundance, hence the observed significant difference between tarns and rock pools. Additionally, Shannon-Wiener diversity index also showed significant differences as it combines both richness and evenness. This underscores the need for various univariate techniques to show the trends in sampled data as a single one might fall short thereby producing misleading results.

Physical developmental constraints are known to dispel insect taxa from rock pools with a shorter hydroperiod (Jeffries, 1994). Insects with mixed life cycles, that is, aquatic larvae and terrestrial-flying adults, connect ecosystems across ecotones and can occupy different ecological niches during their life span. This is the case for some Hydrophilidae diving beetles, with predatory larvae and herbivorous-scavenger adults (Labandeira, 1997). Species-specific requirements may also account for community structure (Jocque et al., 2010). Rock pools are known to be nutrient-poor hence the assemblages occupying them must be adapted to such conditions. Large branchiopods are flagship species of rock pools (Brendonck et al., 2008) and the behaviour, for example, of *Branchipodopsis* sp., of scraping the substratum with their phyllopods thus stirring up food particles allows them to survive in nutrient-poor habitats (Hamer and Martens, 1998). They are virtually absent from wetlands with a lengthy hydroperiod such as tarns due to their sensitivity to predation (Wiggins et al., 1980; Brendonck et al., 2000a) and larger insects such as Odonata are usually not found in smaller wetlands such as rock pools due to the limited time and resources available to grow and emerge as an adult (Williams, 2006). However, there has been instances of Odonata being found in rock pools, for example, Suhling et al. (2004) reported them from the Namib Desert, but the rock pools had an unusually long hydroperiod for a temporary habitat. Biotic interactions, strongly influence macroinvertebrate community composition however the impacts are structured by the integration of hydrology and organism life histories (Batzer, 2013).

In temporary wetlands with a short hydroperiod such as rock pools, community development is truncated by a shorter inundation period that limits the time available for a community to fully develop and mature (Jocque et al., 2007). Tarns, with a longer hydroperiod and vegetation, provide enough time for insect taxa to complete their life cycles and have food resources to support diverse organisms. Similar to our findings, Anusa et al. (2012) reported higher species composition in intermediate pools, followed by large ones and then small ones. Their findings were in line with the Intermediate Disturbance Hypothesis (IDH) which states that diversity will be highest at sites that have intermediate frequency of disturbance that prevents competitive exclusion and will be lower at sites that have experiences very high or very low disturbance frequencies (Connell, 1978). Similar results were also reported elsewhere, and the observations were attributed to the stability of a habitat with a longer hydroperiod (Serrano and Fahd, 2005; Jocque et al., 2007; Vanschoewinkel et al., 2010) which allows communities to build up towards some dominant species resulting in lower species numbers (Anusa et al., 2012).

The species composition dominated by 19 insect taxa which are known to be core taxa is similar to what was reported by Jocque et al. (2006), Vanschoewinkel et al. (2007) and Anusa et al. (2012). These taxa are subsets of those found in permanent wetlands thus showing nestedness (Rosset et al., 2017). Their ability to migrate between the different ecosystems enables them to thrive and utilise the services efficiently (Escriva et al., 2017). Diptera dominate temporary wetlands (McLachlan and Ladle, 2001; Oerteli et al., 2010; Suren et al., 2008; Anusa et al., 2012). The dipteran family, Chironomidae, contributes almost the same biomass as other insect taxa in both Neotropical and Afrotropical wetlands. Dytiscids are known to thrive in aquatic systems with long hydroperiods to avoid frequent dispersal when the habitat dries up. Macroinvertebrate their diversity and abundance are positively correlated with habitats with diverse vegetation, such as tarns (Mlambo et al., 2011; Plenzler, 2012; Plenzler et al., 2015; Mabidi et al., 2017). Predator-prey interactions in temporary wetlands can explain the distribution of specific species and assemblages (Jara, 2016, 2019; Jara & Pueta, 2023).

Predation is a major community structuring force in permanent waterbodies (Kerfoot and Sih, 1987) and temporary ponds also accommodate predatory taxa (Mlambo et al. 2011). Studies have shown that predator-prey interactions in rock pools can explain the distribution of specific species and assemblages (Jones, 1974; Dodson, 1987). Corixidae, ‘water boatmen’, were the most abundant hemipterans collected in both rock pools and tarns and they are known to be seen collecting mosquito larvae when they are present (Ingram et al. 1997). Competitive interactions, both inter- and intra- specific are common in rock pool habitats although their impact on local community structure and distributional patterns within a metacommunity are often not as distinct as those of predation (Jocque et al., 2010). Biotic interactions, especially predation, strongly influence macroinvertebrate community composition however the impacts are structured by the integration of hydrology and organism life histories (Batzer, 2013). Habitat size and hydroregime explain both shared and unique variation in community structure and diversity indicating that their individual effects on biota can be different (Vanschoewinkel et al., 2009). Species-specific requirements may also account for community structure (Jocque et al., 2010).

The taxonomic challenges associated with large branchiopods restricted the present study from further identifying to species level. Several *Branchipodopsis* sp. and *Leptestheria* sp. have been reported from the region (Hamer & Martens, 1998). While the fairy shrimps are taxonomically by far the most diverse group (Rogers, 2013), this could at least partly result from poor systematic knowledge of clam shrimps and tadpole shrimps which may hold many cryptic species (Brendonck et al., 2021). Limited systematic knowledge on clam shrimps and tadpole shrimps is mainly due to lack of apparent species-specific morphological features (except for egg morphology in Limnadiidae) and high intraspecific morphological variation (Hegna and Rogers, 2020). However, of late there has been progress in clam shrimp research including an updated catalogue (Rogers, 2020) and worldwide review (Schwentner et al., 2020) illustrating their biology, phylogeny, and systematics. There is still a lot of work to be done in resolution of this group's taxonomy and systematics.

2.5 Conclusion

This chapter offered insight into the biodiversity patterns of macroinvertebrates in temporary wetlands in the Maloti-Drakensberg Mountain. The dominance of active dispersers is indicative of the fact that sites were sampled later in the progression of the hydroperiod. The reported extended distribution of *Prosthetops gladiator* in the Eastern Cape fuels the need for rapid sampling and monitoring exercises to update distribution records in the region.

Chapter 3

Unravelling the Enigma: The Relationship between Physicochemical Variables and Macroinvertebrate Communities

"Only those who risk going too far can possibly find out how far they can go"- T.S. Eliot (Photo credit – S. Kolisi)

3.1 Introduction

The environmental conditions of temporary wetlands play a significant role in shaping the diversity and distribution of macroinvertebrates. This chapter will explore the physicochemical attributes of these aquatic ecosystems and their impact on macroinvertebrate diversity and community composition. This exploration, coupled with the biological insights from Chapter 2, is crucial for advancing our understanding of temporary wetland ecology.

3.1.1 Physical variables shaping macroinvertebrate diversity in temporary wetlands.

Physical variables are pivotal in shaping the community structure and distribution of macroinvertebrates in temporary wetlands (de Necker et al., 2016; Brendonck et al., 2020; Dalu & Chauke, 2020). Of particular significance are variables like depth and area, which serve as proxies for the wetland's hydroperiod (Mabidi et al., 2017; Mdidimba et al., 2021). These factors within temporary wetlands create microhabitats that, in turn, support diverse and distinct macroinvertebrate assemblages (Gleason & Rooney, 2018; Dube et al., 2020). To comprehend how these physical variables influence macroinvertebrate diversity, abundance, and composition, continuous monitoring of their fluctuations in temporary wetlands is imperative (Mabidi et al., 2016; Dube et al., 2017).

Temporary wetlands exhibit a range of depths to which macroinvertebrate species are uniquely adapted, depending on their life cycle or hydroperiod stages (Porst et al., 2012; Batzer & Boix, 2016). These adaptations encompass morphological and phenological features, often a combination of both (Dube et al., 2017). For instance, dytiscid beetles and backswimmers possess well-adapted swimming appendages, while odonates boast streamlined bodies (Smith et al., 2011; Porst et al., 2012; Pires et al., 2019). Conversely, water striders feature long legs, ideal for skating on the water's surface, as they primarily dwell at the interface between water and air (Lytle, 2015). These adaptive traits are instrumental in shaping the macroinvertebrate community composition within a temporary wetland at any given stage of the hydroperiod (Dube et al., 2018).

During the course of a hydroperiod, the dry phase is considered an ecological disturbance for its flora and fauna resulting in short hydroperiod reducing biotic interactions (Waterkeyn et al., 2008; Perez et al., 2015). As such, temporary wetlands with a longer hydroperiod such as tarns provide a comparatively low disturbance frequency thus providing a stable habitat resulting in increased diversity and colonisation of macroinvertebrates (Jocque et al., 2007; Bonada et al., 2020; Dube et al., 2020).

Rock pools, with shorter hydroperiods, will favour macroinvertebrates that can rapidly reproduce and have an obligatory dry phase during their life cycle (Brendonck et al., 2017; Tladi, 2020). With the progression of the hydroperiod, the chemical components of the ecosystem also change thereby affecting the diversity and distribution of macroinvertebrates in these systems. Consequently, the continuous observation of physical variables enables researchers and conservationists to comprehend better the intricate relationship between environmental conditions and macroinvertebrate communities in temporary wetlands. Such insights are vital for the preservation and sustainable management of these unique and ecologically significant habitats.

3.1.2 Chemical parameters influencing macroinvertebrate assemblages in temporary wetlands.

Temporary wetlands represent dynamic ecosystems where chemical factors interplay with the hydroperiod and environmental variables to shape macroinvertebrate community structure. Salinity levels in temporary wetlands undergo significant fluctuations as the hydroperiod unfolds, driven by evaporation and the accumulation of dissolved salts (Waterkeyn et al., 2008; Tuytens et al., 2014; Botwe et al., 2018). Salinity, in turn, exerts a profound influence on the osmoregulatory abilities of macroinvertebrates. Consequently, the varying osmoregulatory capacities of macroinvertebrates dictate their occupancy of the wetland at different stages of the hydroperiod (Chessman, 2015; Kefford et al., 2020; Zhao et al., 2021).

Water temperature within temporary wetlands is subject to seasonal and hydroperiod-related variations. Temperature profoundly affects the physiological processes of macroinvertebrates, including reproductive activities, growth, and metabolic rates (Sgro et al., 2016). Different species exhibit distinct thermal preferences, which, in turn, influence the composition of the macroinvertebrate community within the wetland (Tladi et al., 2021). Water temperature influences multiple factors since it affects the solubility of gases (e.g., oxygen) and pollutants, alters the toxicity of chemicals, influences pH, impacts water density, and plays a role in electrical conductivity (Salimi et al., 2021). Additionally, water temperature regulates nutrient cycling, organic matter decomposition, and primary production. (Bonacina et al., 2023).

Water temperature and water depth play a critical role in shaping dissolved oxygen levels. Increased temperature and decreased water depth can lead to decreased oxygen levels (Gale et al., 2006; Vilas et al., 2017). Macroinvertebrates exhibit varying requirements and tolerances for oxygen levels, influencing their distribution and abundance within the wetland at different hydroperiod stages and ecological niches (Porst, 2012; Waterkeyn et al., 2012).

Rainfall and groundwater attributes, as well as the composition of the wetland substrate, influence the pH of the water within temporary wetlands. As such, pH impacts the solubility of chemical compounds in the water, potentially altering their toxicity to macroinvertebrates (Sola et al., 2004). Additionally, pH levels have a profound effect on the availability of nutrients, subsequently influencing macroinvertebrate survival and growth. Nutrients are vital resources for primary producers such as algae and plants, serving as the foundation of the food web in temporary wetlands (Robinson et al., 1997; Nordstrom et al., 2014). An excess of nutrients can disrupt the balance, favouring opportunistic species over others and thereby modifying the physicochemical properties of the wetland (Boulton et al., 2014). This has cascading effects on macroinvertebrate assemblages.

In temporary wetlands, the intricate interactions and fluctuations of these physicochemical factors create a dynamic and diverse environment, directly influencing the variety of macroinvertebrates present (Williams, 1996; Gallardo et al., 2008; Bonada et al., 2020). The ability of these macroinvertebrates to adapt to these ever-changing conditions is a crucial determinant of their successful establishment and survival in these ecosystems. Comprehending these physicochemical variables and their impacts on macroinvertebrate communities is not only vital for effective conservation and management but also for gaining insights into the overall health and ecological stability of these aquatic habitats (Nicolet et al., 2004; Stenert et al., 2008; Mereta et al., 2012; Hill et al., 2021b). Careful monitoring and managing these variables is essential for safeguarding the biodiversity and ecological balance of temporary wetlands, thereby supporting the thriving macroinvertebrate populations that rely on them.

This chapter presents an investigation into the physicochemical characteristics of temporary wetlands within the Maloti-Drakensberg Mountain region and their potential influence on macroinvertebrates biodiversity indices and community structure. The objectives of this chapter were to: i) provide a comprehensive description of the physicochemical characteristics of tarns and rock pools in the Maloti-Drakensberg Mountain, ii) examine the relationship of physicochemical characteristics on diversity indices and macroinvertebrate community assemblages in both tarns and rock pools.

This chapter hypothesis that: i) tarns and rock pools would have distinct physicochemical parameters given the differences in hydroperiod, ii) physicochemical variables influencing biodiversity indices and community assemblages will be different for tarns and rock pools given their physical differences.

3.2 Materials and Methods

3.2.1 Data collection

Physicochemical parameters

Physicochemical parameters including pH, dissolved oxygen (DO: %), electrical conductivity (EC: µS), salinity (ppm) and water temperature (°C) were measured using a portable YSI multiparameter Pro Plus probe (YSI Integrated Systems and Services, USA). Water depth (cm) was measured using a custom-made water depth measuring stick. Additionally, an integrated 1000 ml water sample was collected with an opaque polyethene container for each site where; (i) 250 ml was used for chlorophyll-a analysis and 450 ml (150 ml, n=3) was used to determine water nutrients analysis per site. Water nutrients were analysed using a multiparameter benchtop photometer (Hanna Instruments model HI83306-02) and this include ammonium concentration (NH_4^+, mg/L) (range 0.00 to 10.00 mgL^{-1}, resolution 0.01 mgL^{-1}) and phosphate concentration (PO_4^{3-}, mg/L) (range 0.0 to 30.0 mgL^{-1}, resolution 0.1 mgL^{-1}). Physicochemical parameters, water depth and the water sample were collected at the centre and opposite ends of the temporary wetlands, whereas wetland area was calculated using Google Earth Pro.

Chlorophyll-a analysis

100 ml of the chlorophyll-a sub-sample was filtered through a 0.7 µm Whatman glass fibre filter (GFFs) (Diameter = 47 mm). GFFs were then placed in a marked zip-lock bags and kept on ice in the field. In the laboratory, GFFs were transferred into a reaction tube with screws, and 10 ml of 90% acetone was added and the tubes were stored in the dark for 48 hours at -20°C to allow chlorophyll-a extraction. Chlorophyll–a concentration was then determined fluorometrically using a Turner 10 AU fluorometer. Absorbances was taken for each sample before and after adding two drops of 1M Hydrochloric acid (HCl) (Holm-Hansen & Riemann, 1978; Gusha et al., 2021). Chlorophyll-a concentrations was later calculated following the Environmental Protection Agency (EPA) method 445.0 (Arar & Collins, 1997):

$$\mathbf{Chl - a}\left(\mathbf{mg}\boldsymbol{L^{-1}}\right) = \left(\frac{\mathbf{C_3H_6O}}{\mathrm{V}}\right) \times (\mathrm{F_0} - \mathrm{F_a}) \times \mathrm{C},$$

where Chl-*a* (mgL^{-1}) is the chl-*a* concentration in mg L^{-1}, C_3H_6O is the quantity of acetone used for extraction in ml, *V* is the volume of the water sample filtered in ml, F_0 is the chl-

a reading before acidification, *F*a is the chl-*a* reading after acidification with 1M HCl, and *C* is the constant value (0.325).

Macroinvertebrates

Macroinvertebrates collection, sorting, identification, and community assemblages determination followed a procedure described in Chapter 2, section 2.2.5, page 25.

3.2.2 Data analysis

Physicochemical variables were tested for normality using Shapiro-Wilks test and Levene's test for homogeneity of variances. Data was found to be not normally distributed (Shapiro-Wilks test, $p<0.05$) and variances not homogenous (Levene's test, $p>0.05$). Thus, a non-parametric test, Kruskal Wallis and Dunn post hoc tests were performed to investigate significant differences in physicochemical variables between tarns and rock pools. To analyse the differences in temporary wetland physicochemical variables across provinces (i.e., Eastern Cape, Free State and KwaZulu-Natal), data was transformed, and analysis of variance (ANOVA) test was performed. All auto-correlated physicochemical variables were removed from the analysis and each response matrix was constructed using the following variables: pH, EC, DO, water temperature, altitude, NH_4^+, PO_4^{3-}, and chlorophyll-a.

All physicochemical variables were log transformed ($\log x + 1$) to meet normality and the data was pre-treated and resembled into a Euclidean distance matrix (Mabidi et al., 2017b). Principal Component Analysis (PCA) was done to visualise physicochemical parameters patterns and similarities between temporary wetlands firstly grouped by type (tarns and rock pools) combined and individually. Additionally, they were analysed by locality (Eastern Cape, Free State and KwaZulu-Natal), firstly with tarns and rock pools combined and individually. PCA was conducted using PRIMER 6 Version 6.1.16 & PERMANOVA + Version 1.0.6 (Clarke and Gorley, 2006). Following PCA, differences in physicochemical variables between wetland types and locality were tested using a two-way factorial non-parametric permutational multivariate analysis of variance (PERMANOVA). To determine the relationship between macroinvertebrate abundance and physicochemical variables, RELATE function was used to compare the Bray-Curtis resemblance matrix (macroinvertebrates) against the Euclidean distance matrix (physicochemical variables) using a Spearman Rank correlation Coefficient

(Clarke and Gorley, 2006). Additionally, Distance-Based Linear Modelling (DistLM) was used to describe patterns in macroinvertebrate data using physicochemical variables.

A stepwise regression procedure with an Akaike's Information Criterion (AICc), as the selection criterion to deduce a subset of predictor variables associated with the macroinvertebrate assemblages was used to generate a dbRDA plot (Burnham and Anderson, 2002).

Then a multiple linear regression analysis was used to identify physicochemical variables that are significant predictors of biodiversity. Physicochemical variables (predictor variables); pH, EC, DO, water temperature, altitude, depth, area, NH_4^+, PO_4^{3-} and chlorophyll-a, and biodiversity indices (response variables); relative abundance, species richness, Pielou's evenness and Shannon-Wiener Index were log-transformed to meet the assumptions of linear regression. To explore the relationships between the physicochemical variables and biodiversity metrics, a full multiple linear regression model was used. To refine the model and identify the most influential predictors, a model selection process was employed. The StepAIC function from the 'MASS' package, which performed forward-backward selection of predictor variables was used. The selection criterion was the AIC, with a focus on minimizing the AIC score. Variables with p-values less than 0.05 were considered statistically significant and retained in the final model. The final model only included the significant predictor variables.

3.3 Results

In this study, tarns and rock pools showed significant distinctions between several physicochemical parameters. Tarns, which are characterized by their extended hydroperiods, exhibited noteworthy disparities when compared to rock pools. Tarns displayed slightly elevated water temperatures in comparison to rock pools, and this variation held statistical significance ($p<0.05$) (Table 3.1). The greater depth of tarns, as opposed to the relatively shallower rock pools, reinforced the notion of a prolonged hydroperiod in tarns. Tarns boasted a larger surface area than rock pools ($p<0.05$) (Table 3.1). This significant difference in surface area further validated the idea that tarns have longer hydroperiods. Tarns exhibited slightly higher levels of chlorophyll-a compared to rock pools ($p<0.05$) (Table 3.1). This difference, although not substantial, was statistically significant, indicating potential variations in primary productivity and nutrient availability between the two types of water bodies. Furthermore, while not reaching statistical significance, consistent trends were observed in other parameters, such as EC, NH_4^+, and PO_4^{3-} concentrations, where tarns consistently demonstrated higher concentrations compared to rock pools (Table 3.1). However, it is important to note that rock pools showed higher pH and DO values than tarns (Table 3.1). This study also revealed significant distinctions among temporary wetlands in sampled regions; Eastern Cape (EC), Free State (FS), and KwaZulu-Natal (KZN). The study observed significant differences in pH, EC, DO, water temperature, altitude and PO_4^{3-} (ANOVA $F_{2, 66}$, $p<0.05$) (Table 3.1). These findings underscore the ecological heterogeneity among these temporary wetlands and regions.

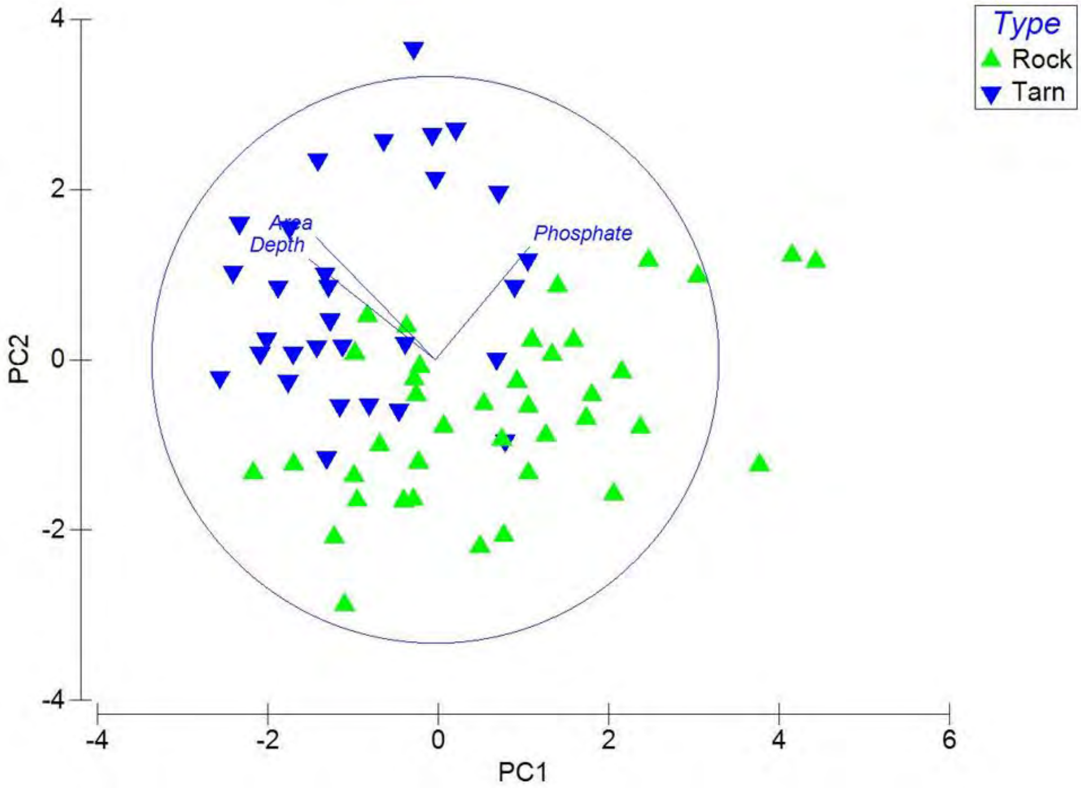
Table 3.1: Summary of physicochemical variables (mean ± standard deviation), Kruskal Wallis test, Dunn test and ANOVA from tarns and rock pools in the Maloti-Drakensberg Mountain. The bold values indicate significant differences ($p<0.05$).

Physicochemical variable	**Wetland Type**		**Locality**			**Kruskal-Wallis test**	**Dunn test**	**Anova df (2, 66)**
	Tarns	**Rock pools**	**Eastern Cape**	**Free State**	**KwaZulu-Natal**	**P-value**	**P-value**	
pH	6.23 (±0.69)	7.26 (±1.13)	8.22(±0.5)	6.54(±0.9)	6.72(±1.1)	>0.05	>0.05	**<0.05**
EC (ppm)	28.4 (±12.96)	19.94 (±27.82)	35.21(±52.4)	33.74(±15)	11.39(±11.2)	>0.05	>0.05	**<0.05**
DO%	71.88 (±19.84)	80.75 (±19.97)	7.05(±1.4)	6.31(±1.7)	7.31(±1.4)	>0.05	>0.05	**<0.05**
Water Temperature (°C)	22.28 (±4.3)	19.63 (±3.3)	22.59(±5.6)	22.28(±4.4)	18.98(±2.9)	**<0.05**	**<0.05**	**<0.05**
Altitude (m.a.s.l.)	1837.9 (±440.92)	1985.36 (±95.66)	1957.71(±54.73)	1772.73(±307.5)	2079.38(±284.1)	>0.05	>0.05	**<0.05**
Depth (cm)	23.4 (±19.95)	18.72 (±13.16)	16.71(±8.1)	23.57(±17.6)	28.06(±16.8)	**<0.05**	**<0.05**	>0.05
Area (m^2)	1402 (±2502.4)	562.34 (±568.3)	478.14(±648.9)	1646(±3078.6)	819.69(±757.44)	**<0.05**	**<0.05**	>0.05
NH_4^+ (mgL^{-1})	0.6 (±0.42)	0.24 (±0.29)	0.26(±0.3)	0.49(±0.5)	0.33(±0.3)	>0.05	>0.05	>0.05
PO_4^{3-} (mgL^{-1})	2.04 (±1.36)	1.22 (±0.6)	1.1(±0.7)	1.98(±1.3)	1.29(±0.7)	>0.05	>0.05	**<0.05**
Chl-a (mgm^{-3})	0.05 (±0.07)	0.04 (±0.09)	0.03(±0.03)	0.04(±0.1)	0.03(±0.03)	**<0.05**	**<0.05**	>0.05

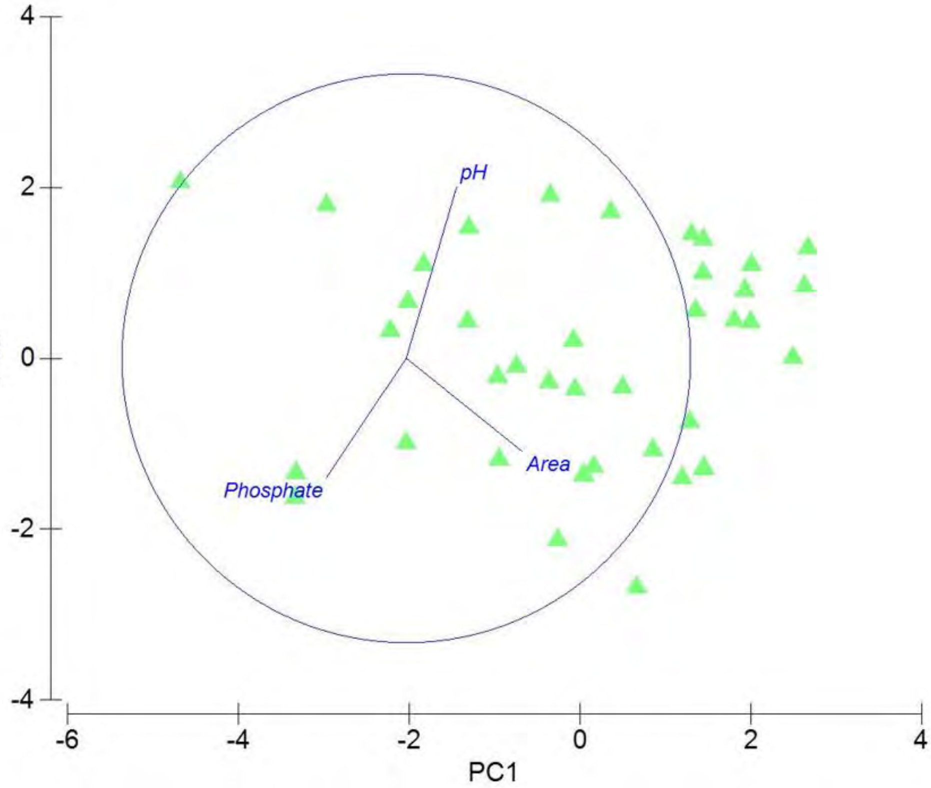
Principal Component Analysis (PCA) revealed two distinct clusters, each closely aligned with specific environmental attributes associated with either tarns or rock pool (Figure 3.1a). Tarns were positively associated with depth and area (Figure 3.1a). In contrast, rock pools exhibited a positive association with PO_4^{3-} concentrations, implying that these environments tend to have higher phosphate levels compared to tarns (Figure 3.1a). A deeper exploration within the cluster of rock pools revealed notable correlations with pH and PO_4^{3-} (Figure 3.1b). While less pronounced, area was positively correlated with some sites indicating that a larger area may be a characteristic of certain rock pools (Figure 3.1b). Among tarns, our analysis indicated positive associations with altitude, and a few tarns showed links with EC and DO (Figure 3.1c). These associations suggest that certain tarns may be situated at higher altitudes and may exhibit variability in EC and DO levels (Figure 3.1c). When examining the data based on locality, area and depth were positively correlated with wetlands from the Free State (FS) (Figure 3.2a). Intriguingly, none of the variables displayed significant correlations with wetlands from either KwaZulu-Natal (KZN) or the Eastern Cape (EC) (Figure 3.2a). Rock pools in the Free State were positively associated with pH, PO_4^{3-}, and area (Figure 3.2b). Most sites from KZN did not align with the explained ordination space (circle boundary) (Figure 3.2b). Similarly, most tarns from KZN did not conform to the explained ordination, and only a few, along with those from the Free State, showed positive associations with altitude (Figure 3.2c).

Overall, the vectors representing these associations in the PCA plots (Figure 3.1a, b, c) are relatively short. This indicates that the relationships between the physicochemical variables and the differentiation of habitat types (tarns vs. rock pools) and localities are relatively weak. The cumulative explained variance of the two principal component axes provides insight into the extent of association. For tarns, the variance explained was 23.2% and 15.6% (Figure 3.1c, 3.2c), for rock pools it was 32.6% and 15.2% (Figure 3.1b, 3.2b), and for both combined it was 22.3% and 18.5% (Figure 3.1a, 3.2a). These values suggest that the physicochemical variables have limited explanatory power when it comes to differentiating habitat types.

a


Type
Rock
Tarn
Area
Depth
Phosphate
PC2
PC1
4
2
0
-2
-4
-4
-2
0
2
4
6

b


pH
Area
Phosphate
PC2
PC1
4
2
0
-2
-4
-6
-4
-2
0
2
4

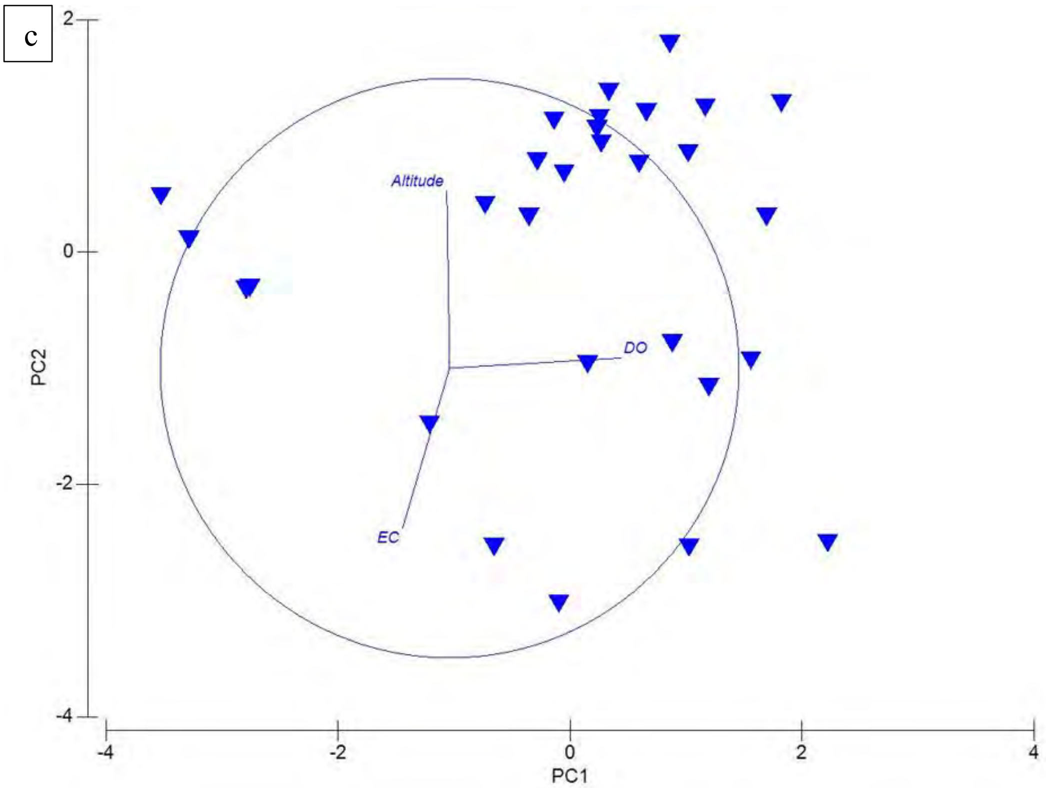
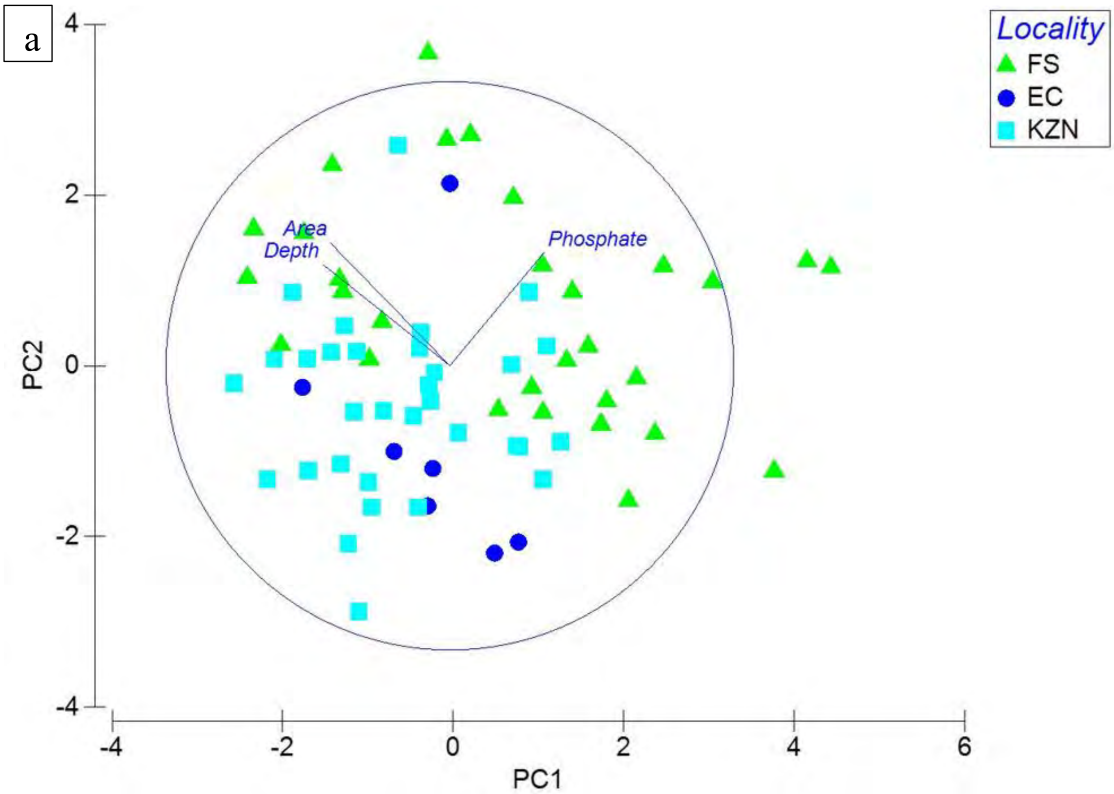
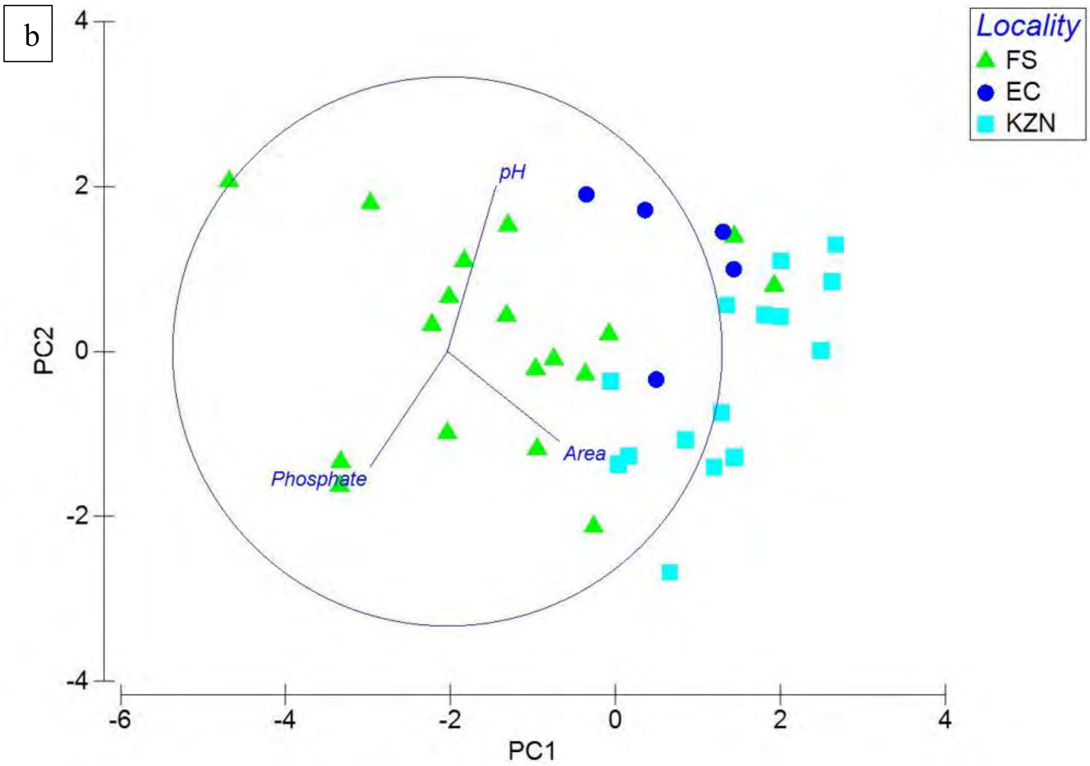


Figure 3.1: Principal Component Analysis (PCA) of physicochemical characteristics in temporary wetlands grouped by type (a) rock pools and tarns combined with PC1 and PC2 explaining 23.2% and 15.6%, respectively. (b) rock pools only with PC1 and PC2 explaining 32.6% and 15.2%, respectively. (c) tarns only with PC1 and PC2 explaining 22.3% and 18.5%, respectively. Only variables with a moderate correlation (>0.5) are shown.

a


Locality
FS
EC
KZN
Area
Depth
Phosphate
PC2
PC1
4
2
0
-2
-4
-4
-2
0
2
4
6

b


Locality
FS
EC
KZN
pH
Phosphate
Area
PC2
PC1
4
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-2
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-6
-4
-2
0
2
4

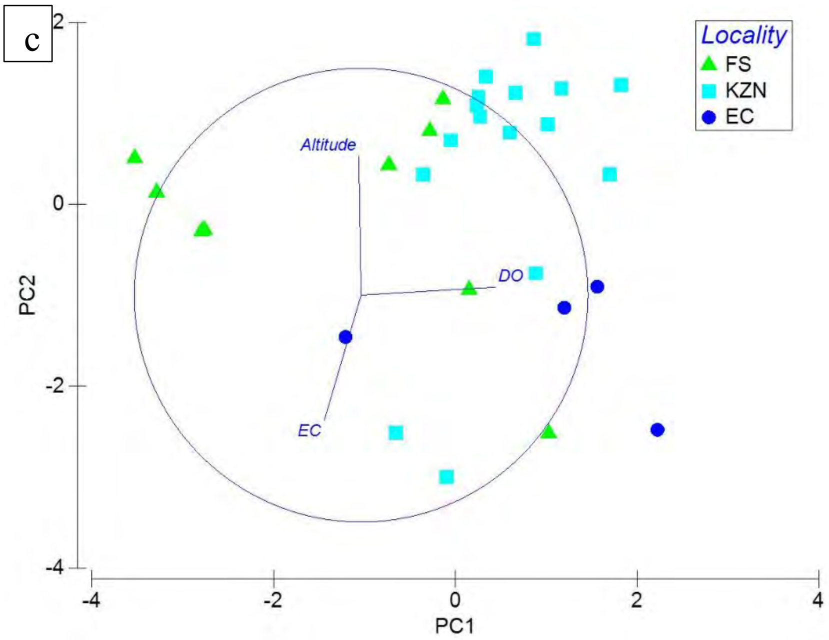

Figure 3.2: Principal Component Analysis (PCA) of physicochemical characteristics in temporary wetlands grouped by locality (a) rock pools and tarns combined with PC1 and PC2 explaining 23.2% and 15.6%, respectively (b) rock pools with PC1 and PC2 explaining 32.6% and 15.2%, respectively. (c) tarns with PC1 and PC2 explaining 22.3% and 18.5%, respectively. Only variables with a moderate correlation (>0.5) are shown.

The outcomes of the PERMANOVA analysis have provided compelling evidence of significant differences in the physicochemical variables among temporary wetlands, and these distinctions have attributed to both the type of wetland and its geographical location (Table 3.2). Moreover, the interaction of wetland type and locality as factors also emerged as a significant contributor to the observed variations (Table 3.2). This finding underscores the importance of considering both the nature of the wetland (tarn or rock pool) and the specific geographic context when evaluating the influencing factors behind the physicochemical characteristics of these temporary wetlands.

Table 3.2: Non-parametric permutational MANOVA results for the 2-way factorial model examining the effect of temporary wetland type and locality on physicochemical variables from tarns and rock pools in the Maloti-Drakensberg Mountain. Bold values indicate significant values at α=0.05.

Factors	df	SS	MS	Pseudo-F	P-value
Type	1	54.812	54.812	6.9184	**<0.01**
Locality	2	78.402	39.201	4.948	**<0.01**
Type x Locality	2	71.913	35.957	4.5385	**<0.01**

The RELATE analysis revealed a weak yet significant association (R = 0.268, p < 0.01) between the Euclidean distance of physicochemical variables and the Bray-Curtis distances of macroinvertebrates matrices. This highlights the nuanced interplay between physicochemical variables and macroinvertebrate matrices, suggesting that while the association may not be strong, it is statistically meaningful.

Table 3.3: Non-parametric multivariate regression results (dbRDA, AICc selection criteria) for physicochemical variables that best explained variation in macroinvertebrate assemblages in the temporary wetlands.

Type	Variable	AICc	Pseudo-F	P	% Variation	Res. df
Rock pools and tarns	Area	526.29	12.82	**<0.01**	16.07	67
	Temperature	524.39	4.02	**<0.01**	4.82	66
	pH	523.36	3.17	**<0.01**	3.68	65

The results of the AICc selection have provided insights into the significant variables influencing the patterns of macroinvertebrate assemblages in temporary wetlands (Table 3.3). Among the physicochemical variables examined, area, water temperature, and pH were identified as significant factors that explain variations in macroinvertebrate assemblages in these temporary wetlands (Table 3.3). Notably, area emerged as the most influential factor, accounting for 16% of the observed variation in macroinvertebrate assemblages (Table 3.3). Temperature and pH also played significant roles, with temperature contributing 4.82% of the variation and pH accounting for 3.68% (Table 3.3). The direction of environmental vectors showed that area, temperature, and pH influences macroinvertebrate assemblage in tarns with no vector pointing towards rock pools (Figure 3.3). However, all the vectors showed a weak relationship with temporary wetland type (Figure 3.3).

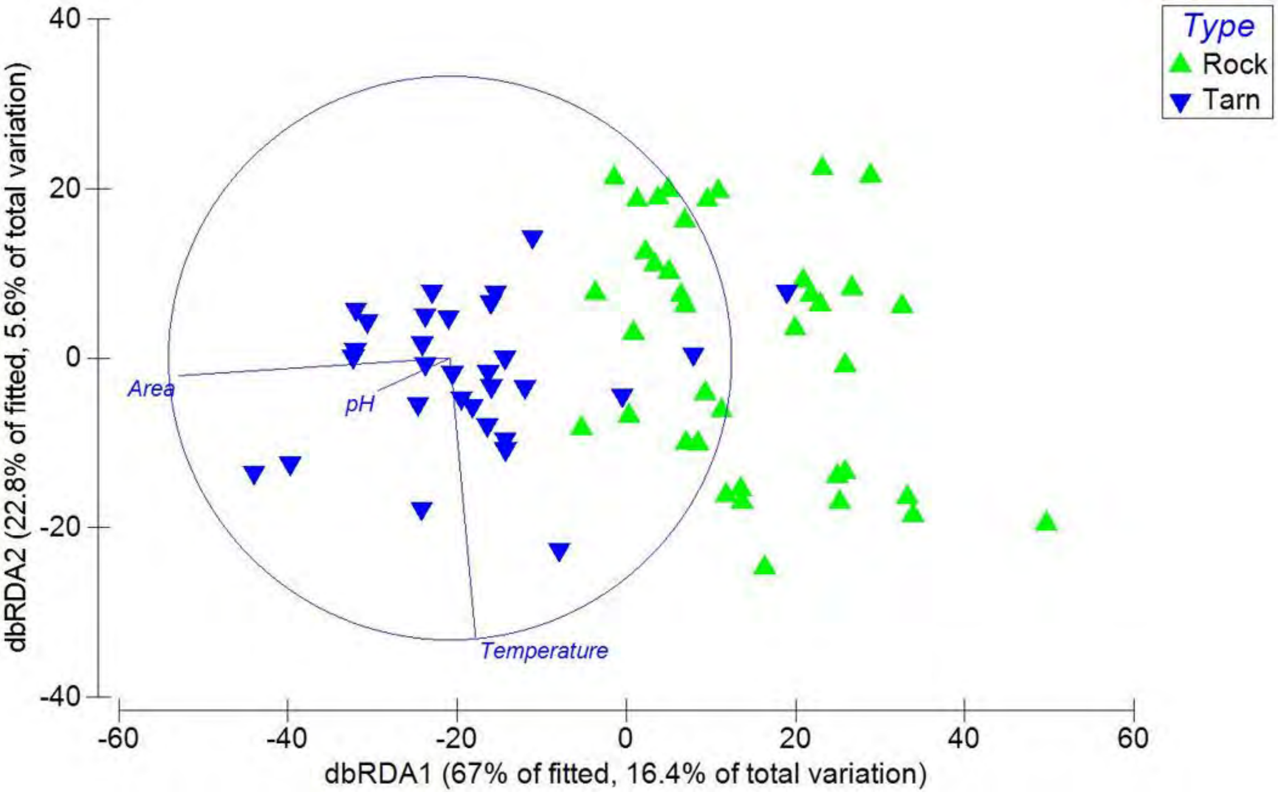

Figure 3.3: dbRDA ordination plot (AICc selection criterion) of macroinvertebrate assemblage composition among sites (Bray-Curtis similarity) constrained by the environmental variables. Explained variation in the fitted model and total variation are indicated for each axis.

Multiple linear regression analysis was performed to determine which physicochemical variables (pH, EC, DO, water temperature, area, depth, chlorophyll-a, NH_4^+, PO_4^{3-}) significantly influenced biodiversity indices (relative abundance, species richness, Pielou’s evenness and Shannon-Wiener Index). The model demonstrated a statistically significant relationship (F-statistic = 5.187, p-value = 0.008084), indicating that at least one of the predictor variables, temperature, and area, had a significant effect on the response variable like relative abundance (Table 3.4). The estimated coefficient is 1.51901 (p-value = 0.0143), suggests that a one-unit increase in temperature is associated with a significant increase in relative abundance (Table 3.4). The estimated coefficient is 0.16425 (p-value = 0.0162), indicates a statistically significant positive relationship between area and relative abundance (Table 3.4). The adjusted R-squared value was 0.1097, suggesting that approximately 10.97% of the variance in relative abundance was explained by the combination temperature and area (Table 3.4).

The linear regression model exhibited strong overall performance, as indicated by an F-statistic of 29.94 (p-value of 5.581e-10), suggesting that area and altitude explain a significant portion of the variance in species richness (Table 3.4). Both area (p-value = 3.58e-09) and altitude (p-value = 0.0321) were found to be statistically significant predictors of species richness (Table 3.4). Altitude showed a positive coefficient estimate of 0.36724, suggesting that an increase in altitude is associated with an increase in species richness (Table 3.4). Area showed a higher positive coefficient estimate of 0.12577, indicating a more substantial impact on species richness (Table 3.4). The model's adjusted R-squared value was 0.4598, signifying that approximately 45.98% of the variability in species richness was accounted for by altitude and area (Table 3.4).

Area demonstrated a strong statistical significance with a p-value of 6.1e-10 (Table 3.4) to Pielou’s evenness. Area exhibited a positive coefficient estimate of 0.13417, implying that an increase in area is associated with a corresponding increase in Pielou’s evenness (Table 3.4). The model's adjusted R-squared value was 0.4292, signifying that approximately 42.92% of the variance in Pielou’s evenness was explained by area (Table 3.4).

Area demonstrated a strong statistical significance with Shannon-Wiener Index (Table 3.4). Area had a positive coefficient estimate of 0.10097, implying that an increase in area is associated with a corresponding increase in Shannon-Wiener Index (Table 3.4). The adjusted R-squared value was 0.4423, indicating that approximately 44.23% of the variance in the Shannon-wiener index was explained by the area (Table 3.4). Area was the most common predictor variables influencing all 4 indices (Table 3.4). Additionally, relative abundance was also influenced by temperature and species richness by altitude (Table 3.4).

Table 3.4: Multiple linear regression analyses showing the relationship between physicochemical parameters and macroinvertebrate diversity indices. The bold values indicate significant differences ($p<0.05$).

Explanatory Variables	Predictor Variables	Estimates	SE	t value	P	Adj. R^2	df	F	P
Relative Abundance	Intercept	-0.54822	1.96794	-0.279	0.7814	0.1097	2, 66	5.187	**0.00804**
	Area	0.16425	0.06654	2.468	**0.0143**				
	Temperature	1.51901	0.60363	2.516	**0.0162**				
Species Richness	Intercept	-2.55978	1.24927	-2.049	**0.044**	0.4598	2, 66	29.94	**5.581e-10**
	Area	0.12577	0.01848	6.806	**3.58e-09**				
	Altitude	0.36724	0.16773	2.189	**0.0321**				
Pielou’s evenness	Intercept	0.16484	0.11299	1.459	0.149	0.4292	1,67	52.13	**6.104e-10**
	Area	0.13417	0.01858	7.220	**6.1e-10**				
Shannon Wiener Index	Intercept	0.25625	0.08283	3.094	**0.00288**	0.4423	1, 67	54.93	**2.766e-10**
	Area	0.10097	0.01362	7.411	**2.77e-10**				

3.4 Discussion

This chapter was conducted to characterize the physicochemical characteristics of temporary wetlands in the Maloti-Drakensberg Mountain region, focusing on tarns and rock pools. Additionally, it aimed to explore the influence of these variables on the diversity and community structure of macroinvertebrates. As anticipated by the initial hypothesis, distinct physicochemical profiles were observed between tarns and rock pools, with key differentiating factors being depth, area, temperature, and chlorophyll-a. Confirming our second hypothesis, that macroinvertebrate assemblage patterns in tarns were primarily influenced by area and pH, while temperature played a crucial role in rock pools. Interestingly, despite these observed associations, the collective impact of the studied physicochemical variables appeared insufficient in explaining the macroinvertebrate community dynamics within these temporary wetlands. The lack of conclusive patterns suggests the potential involvement of other unexamined factors in driving the observed macroinvertebrate patterns.

Results from this study confirm the distinct physicochemical characteristics of tarns and rock pools within the Maloti-Drakensberg Mountain region, contributing to their divergent ecological profiles. With their larger surface area and greater depth, tarns demonstrated a longer hydroperiod, aligning with prior research (Hamer and Martens, 1998; Sieben et al., 2010; Chatanga et al., 2019; Mdidimba et al., 2021). The temperature differential observed between rock pools and tarns is consistent with earlier studies (see Hamer and Martens, 1998; Mdidimba et al., 2021), where rock pools exhibited lower temperatures than tarns. Similarly, the lower chlorophyll-a levels in both tarns and rock pools are in line with findings by Hamer and Martens (1998), Dunnik et al. (2016), and Mdidimba et al. (2021) within the region. This suggests limited productivity, potentially influenced by limited external inputs and invertebrates' grazing and oligotrophy (Wasserman et al., 2015; Leach et al., 2018).

The variation in conductivity, ranging from 19 to 40 µS/cm in rock pools, corresponds to earlier observations (Hamer and Martens, 1998; Jocque et al., 2007a; Anusa et al., 2012; Mdidimba et al., 2021). As products of bedrock weathering (Jocque et al., 2010), rock pools exhibited low buffering capacity and quick physicochemical fluctuations (Scholnick, 1994). This brevity in hydroperiod is reinforced by the absence of vegetation and higher evaporation rates in rock pools (Brendonck et al., 2010). Contrastingly, tarns, owing to their larger size and depth

(Chatanga et al., 2019), support stable ecosystems with extended hydroperiods, allowing for intricate food chains and heightened biological diversity (Mdidimba et al., 2021). Increased diversity confirms the role of hydroregime in shaping the stability and adaptability of local biota in temporary ponds (Vanschoenwinkel et al., 2010; Mabidi et al., 2017; Dube et al., 2020). The interplay between hydroperiod, environmental conditions, and biotic interactions contributes to the diverse faunal assemblages observed across these aquatic habitats (DeMeester, 2005; Mlambo et al., 2011, Mabidi et al., 2017; Dube et al., 2020).

Our findings demonstrated that among the predictor variables, area, temperature, and pH emerged as crucial factors driving macroinvertebrate assemblage patterns within these temporary wetlands. Area exhibited substantial influence, explaining 16% of the variation observed in the macroinvertebrate communities. Since area is the one of the primary determinants of temporary wetland type, this asserts the fact that tarns and rock pools support different communities due to their variabilities in size. This is in line with what was previously reported in the region by Mdidimba et al. (2021) and elsewhere by Johnson et al. (2004), Stenert & Maltchik (2007) and Mabidi et al. (2017). Temperature and pH played secondary yet significant roles, accounting for 4.82% and 3.68% of the variance, respectively. The significance of temperature in these wetlands is subjective given they were all not sampled at the same time of the day and under different conditions. Despite these individual variables been significance, the physicochemical variables collective explanatory power over macroinvertebrate assemblages was limited and a similar trend was observed by Hancock and Timms (2002). This suggests that additional factors beyond those considered in this study likely contribute significantly to the complex dynamics of these aquatic ecosystems. A similar trend was observed by Dube et al., (2020) where the authors postulated that unmeasured factors such as hydroperiod were driving community composition. This suggestion was based on observations from previous studies (Waterkeyn et al., 2008; Nhiwatiwa et al., 2017).

The positive correlation observed between area and tarns signifies the potential role of habitat size in fostering unique ecological conditions. Similarly, the positive trend between pH and macroinvertebrate assemblages, particularly in the KwaZulu-Natal region, implies a potential influence of pH-driven ecological gradients on the distribution of aquatic species. The majority of sampled temporary wetlands in KZN are on a rock base therefore it is not surprising that pH was a major structuring factor owing to the poor buffering capacity of wetlands with a rock base (Brendonck et al., 2000, 2010; Jocque et al., 2010). Additionally, several studies have highlighted the importance of pH in structuring macroinvertebrate community assemblage (Feldman and Connor, 1992; Rosemond et al., 1992; Bird and Day, 2016; Mabidi et al., 2017). Notably, DO emerged as a significant factor in explaining relative abundance, reaffirming its importance in shaping the community composition of macroinvertebrates. Similarly, Dube et al., (2017) identified dissolved oxygen as one of the key factors in affecting macroinvertebrate and zooplankton distribution in lower altitude wetlands whose characteristics are similar to tarns.

Furthermore, area was a significant explanatory variable for relative abundance, species richness, Pielou's evenness and Shannon-Wiener index. Given that area is one of the major differentiating factors between tarns and rock pools, it is no surprise that it drives the diversity in these ecosystems. Furthermore, this supports the notion that the bigger the wetland, the steadier the environment and the more diverse the organisms it supports. Temperature was a key factor in determining relative abundance further confirming it's importance as a base factor influencing other physicochemical variables. The influence of altitude on species richness underscored the need for more robust sampling factoring in different altitudes in the sample design to get a better appreciation of high-altitude temporary wetlands. The fact that only three factors were shown to affect biodiversity indices indicates the potential impact of unmeasured ecological drivers and intrinsic characteristics on these indices.

3.5 Conclusion

This chapter presents a comprehensive analysis of the relationship between physicochemical attributes and macroinvertebrate assemblages in temporary wetlands. The results underscore the complex interplay between these variables, highlighting the significance of area, temperature, and pH in shaping these aquatic ecosystems. However, the limited overall explanatory power of the considered physicochemical variables suggests the presence of diverse, unexplored factors influencing the observed biodiversity patterns. These findings provide valuable insights into the intricate dynamics of temporary wetland ecosystems and set the stage for further investigation into the multifaceted drivers of aquatic biodiversity.

Chapter 4

General Discussion

“Scenery without history is like wine without age: a juvenile product lacking those subtleties acceptable to a cultured taste.”- L.C. King (Photo credit- P.S. Kubheka)

4.1 Synthesis

The observed changes in rainfall and temperature patterns are likely to alter the hydroperiod of temporary wetlands. This in turn might affect the physicochemical and macroinvertebrate assemblages supported by these systems. Temporary wetlands in high altitudes such as the Maloti-Drakensberg Mountain are pristine systems that can offer early warnings due to their sensitivity to the slightest changes (Dunnik et al., 2016). Hence there is an urgent need to document the diversity and assemblages of the flora and fauna in these systems to have baseline information as a foundation for long-term monitoring. In that regard, the aim of this thesis was to investigate macroinvertebrate diversity, community assemblage and the influence of environmental drivers on assemblage and diversity in temporary wetlands of the Maloti-Drakensberg Mountain, South Africa. This study offered crucial insights into high-altitude temporary wetland macroinvertebrate diversity (**Chapter 2**) thereby consolidating available information from Hamer & Martens (1998) and Mdidimba et al. (2021). Further exploration to determine the effect of environmental variables on diversity and community assemblage (**Chapter 3)** showed that the measured variables poorly explained the observed patterns in **Chapter 2**.

The study findings reveal distinct patterns in the abundance and diversity of macroinvertebrates between tarns and rock pools from the Maloti-Drakensberg Mountain (**Chapter 2**). The study assessed 70 temporary wetlands (29 tarns and 41 rock pools) which were surveyed over a once-off sampling (3 March – 7 April 2022). The sampled macroinvertebrates were dominated by active dispersers such as Hemiptera and Coleoptera which are known to colonise the late stages of the hydroperiod (Jocque et al., 2009). This was attributed to the timing of sampling which was towards the end of the hydroperiod. Tarns had higher diversity than rock pools due to the structure of these ecosystems. Tarns are characterised by longer hydroperiod and microhabitats which offer different niches such as open water, emergent and submerged vegetation thus the observed diversity. The observed results supported our first hypothesis that tarns will have higher diversity in the form of active dispersers than rock pools driven by the longer hydroperiod and presence of microhabitats that accommodate diverse taxa. In contrast to rock pools which are dominantly monotonous systems, as was reflected by their lower diversity. However, the uniqueness of rock pools allows specialist taxa such as large branchiopods to dominate them hence the observed presence of large branchiopods and hydraenid beetles exclusively in rock pools. This supported the second part of our first hypothesis that rock pools will have more specialist taxa in the form of passive dispersers than tarns as a result of the shorter hydroperiod and low nutrient levels. Tarns and rock pools were shown to have distinct macroinvertebrate community assemblages (**Chapter 2**). The biology of the macroinvertebrates sampled in these systems proved to be a major community structuring factor as observed by the dominance of predators.

To further elucidate the observed patterns and differences in **Chapter 2**, this study explored the physicochemical attributes of temporary wetlands in the Maloti-Drakensberg Mountain region, and their influence on macroinvertebrate diversity and community structure (**Chapter 3**). The expected dissimilarities between these habitat types were confirmed, with distinctive physicochemical profiles attributed to varying factors such as depth, area, temperature, and chlorophyll-a. Depth, area, temperature, and chlorophyll-a were the key factors differentiating tarns and rock pools. This confirmed the first part of our second hypothesis that rock pools and tarns will have distinct physicochemical characteristics. Correspondingly, macroinvertebrate assemblages were significantly influenced by area and pH in tarns, and by temperature in rock pools. However, the combined impact of these variables on the macroinvertebrate communities was limited, indicating the probable involvement of unexamined factors. This further confirmed the second part of our second hypothesis that different environmental parameters will drive community assemblage in tarns and rock pools. However, the poor explanatory power of environmental parameters on macroinvertebrate assemblage suggests that there are key structuring factors that were not measured in this study warranting further research in the Maloti-Drakensberg Mountain.

The study highlights the intertwined influence of habitat size and hydroregime on community structure and diversity. Unique variation patterns emphasize the differing impacts of these factors on macroinvertebrates. Notably, rock pools, with their nutrient-poor nature, harbour specialized assemblages, like large branchiopods and hydraenid beetles.

4.2 Conclusion

Afromontane tarns have higher biodiversity than rock pools driven by their longer hydroperiod as expected, which is influenced by size and area. The diversity in tarns was mainly dominated by active dispersers with passive dispersers being limited to rock pools where they take advantage of the short hydroperiod which deters predators which are predominately active dispersers. Although there was a clear distinction in community assemblage between tarns and rock pools, that was not the case in terms of environmental variables. In fact, the variables poorly explained the differences in macroinvertebrate assemblage suggesting that unexplored factors such as vegetation structure were probably responsible for the observed differences. This study provided a detailed account of temporary wetlands in the Maloti-Drakensberg mountains, South Africa, substantially contributing to knowledge on these understudied systems. By establishing that these systems support distinct macroinvertebrate assemblages, we further affirm the need to have them co-existing in the ecosystem. Additionally, this study underscores the need for further research into high-altitude temporary wetlands in Southern Africa, especially compared to their lower-altitude counterparts. As these habitats are susceptible to environmental changes and often underrepresented in scientific investigations, our study provides a foundation for future inquiries. By unravelling the intricate relationships between physicochemical attributes and macroinvertebrate communities, we contribute to the broader understanding of aquatic ecology and emphasize the importance of holistic research approaches.

4.3 Limitations of the study

Sampling was conducted only once due to the remote nature of the habitats and financial constraints. This limited approach provided only one seasonal aspect into the rich biodiversity of the temporary wetlands within the Maloti-Drakensberg Mountain region. The highly dynamic nature of these wetlands over their hydroperiod cycles underscores the necessity for seasonal sampling, allowing for a more comprehensive understanding of community composition and succession dynamics. Furthermore, the lack of updated taxonomy keys for certain taxa, including Coleoptera, Hemiptera, Diptera, and notably Anostraca and Spinicaudata, posed challenges in accurately identifying specimens to the species level. This taxonomic limitation constrained our ability to provide a finer resolution of species identification. Future studies could greatly benefit from improved taxonomy resources to enhance the precision of species-level identification.

4.4 Recommendations

Among the notable challenges encountered in sampling temporary wetlands within the Maloti-Drakensberg Mountain region, the issue of timing stands out prominently. In response, environmental DNA (eDNA) emerges as a compelling and innovative solution. eDNA encompasses the analysis of genetic material obtained from environmental samples like soil, water, or air (Taberlet et al., 2012). This approach has demonstrated considerable success in diverse contexts (Ficetola et al., 2008; Turner et al., 2014b; Goldberg et al., 2013; Treguier et al., 2014). The potential of eDNA for species detection holds immense promise for advancing our understanding of aquatic ecology and species conservation. By sidestepping the need to directly sample organisms, eDNA presents a superior alternative that can mitigate impacts on sensitive species and amplify the efficacy of field surveys targeting rare and elusive organisms (Goldberg et al., 2016). This transformative methodology not only addresses the challenges posed by sampling timing in temporary wetlands but also opens new avenues for ecological research and conservation strategies. Considering the costs associated with eDNA processing and training required, this technique cannot replace traditional sampling methods which ultimately provide a voucher specimen for future use. Additionally, eDNA is only effective if there is an existing reference library rendering ineffective in habitats where most species have not been barcoded.

The study of genetic relatedness in macroinvertebrates found in high and low altitude wetlands and other regions reveals patterns of adaptation, speciation, and biodiversity. (Bertin et al., 2022). Comparative analysis aids in understanding evolutionary responses to altitude-specific pressures and climate change. This interdisciplinary approach combines ecology, genetics, and evolutionary biology to better understand biodiversity dynamics and inform conservation efforts in sensitive wetland ecosystems. Additionally, exploring the thermal biology of high-altitude species compared to low-altitude counterparts due to the diverse climates they experience (Tladi, 2020). Investigating how organisms from various altitudes adapt to temperature fluctuations can provide insights into their physiological responses to environmental changes. (Huey & Bennett, 1990). As a result, combining these techniques and developing a comprehensive reference library addresses the knowledge gap in macroinvertebrate monitoring in such a biodiversity-rich area.

Appendices

Appendix 1

Maloti-Drakensberg average rainfall data from January 2013 to April 2022.

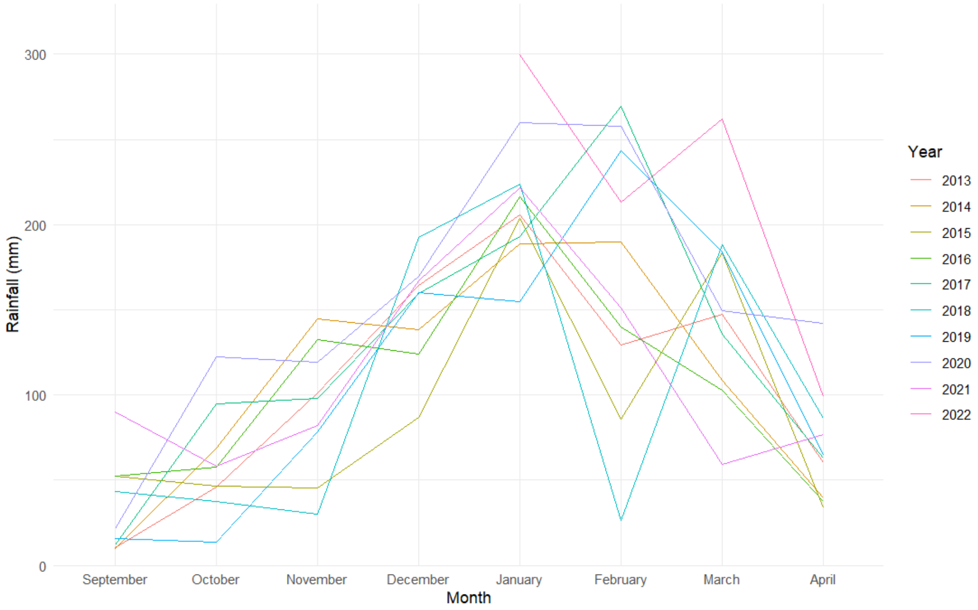

Figure 1a: Average monthly rainfall (September to April) recorded at Shaleburn weather station from 2013 to 2022.

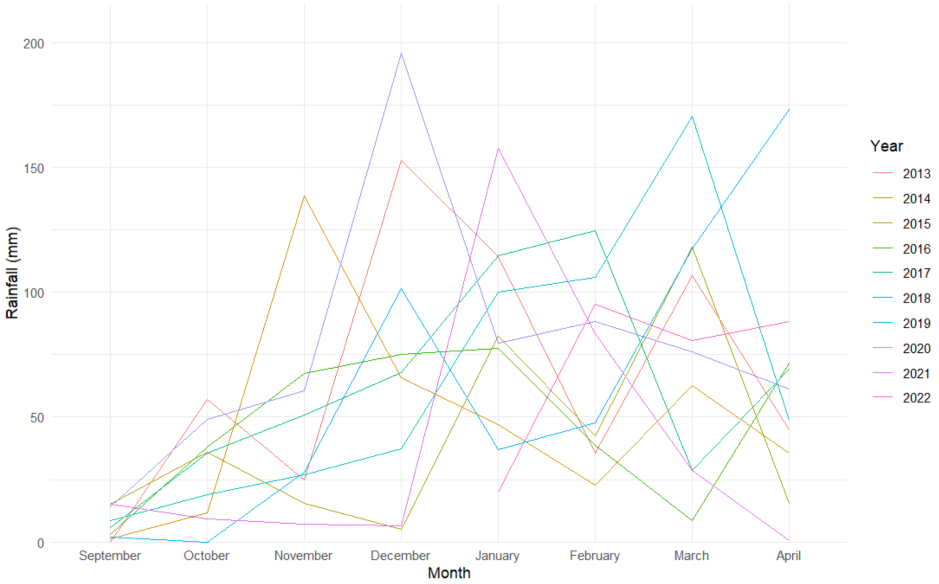

Figure 1b: Average monthly rainfall (September to April) recorded at Ficksburg weather station from 2013 to 2022.

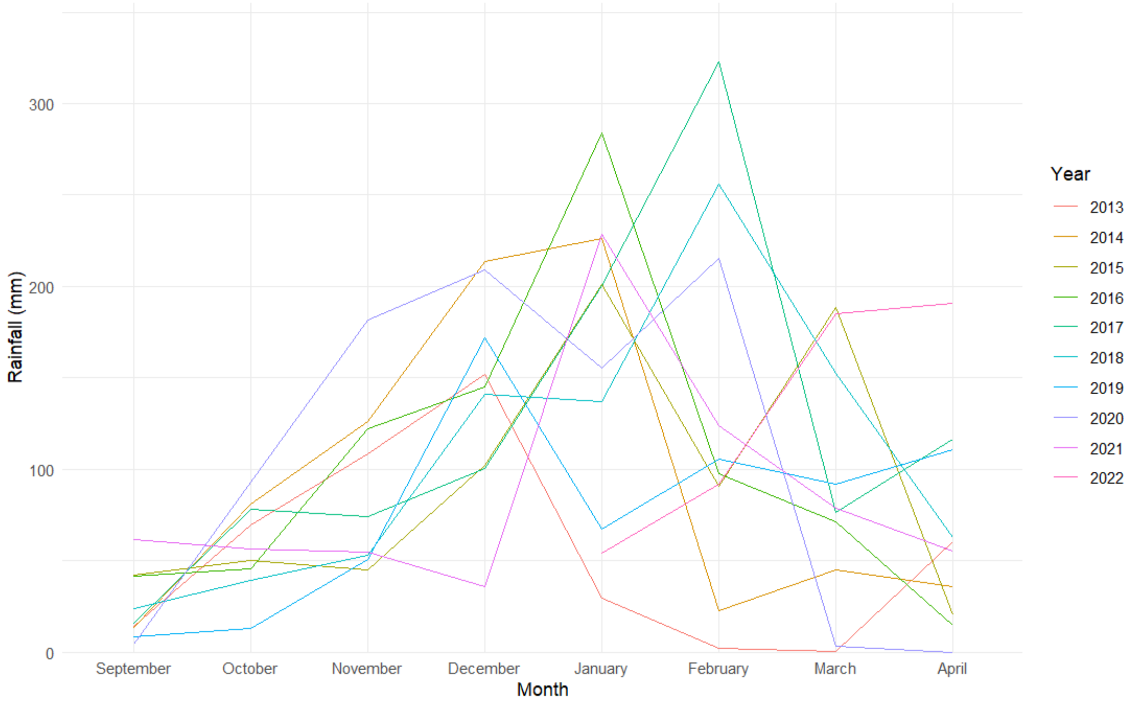

Figure 1c: Average monthly rainfall (September to April) recorded at Giant's Castle weather station from 2013 to 2022.

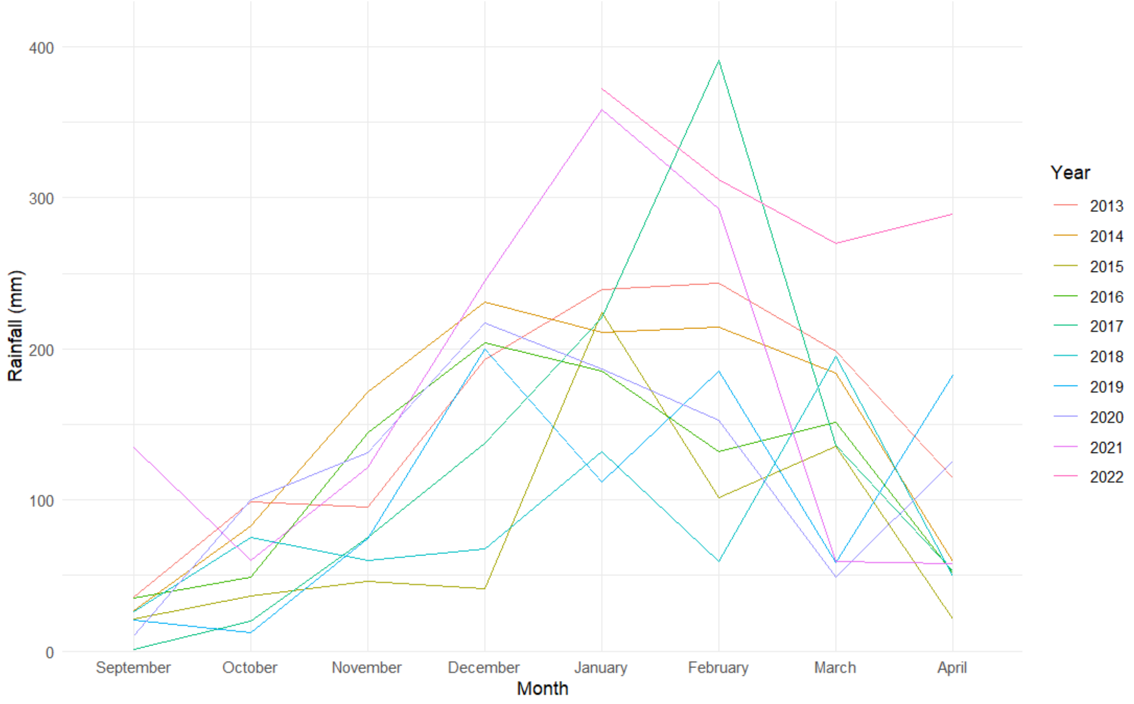

Figure 1d: Average monthly rainfall (September to April) recorded at Royal Natal weather station from 2013 to 2022.

Appendix 2

Maloti-Drakensberg average temperature data from January 2013 to April 2022. Solid lines indicate maximum temperature whereas dotted lines indicate minimum temperature.

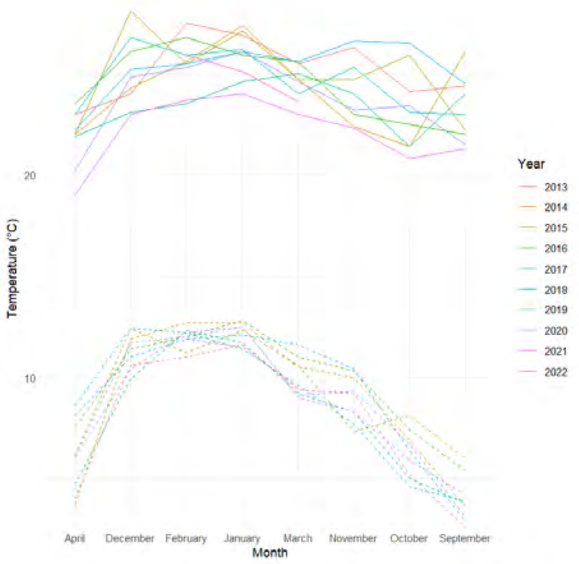

Figure 2a: Average monthly minimum and maximum temperatures (September to April) recorded at Shaleburn weather station from 2013 to 2022.

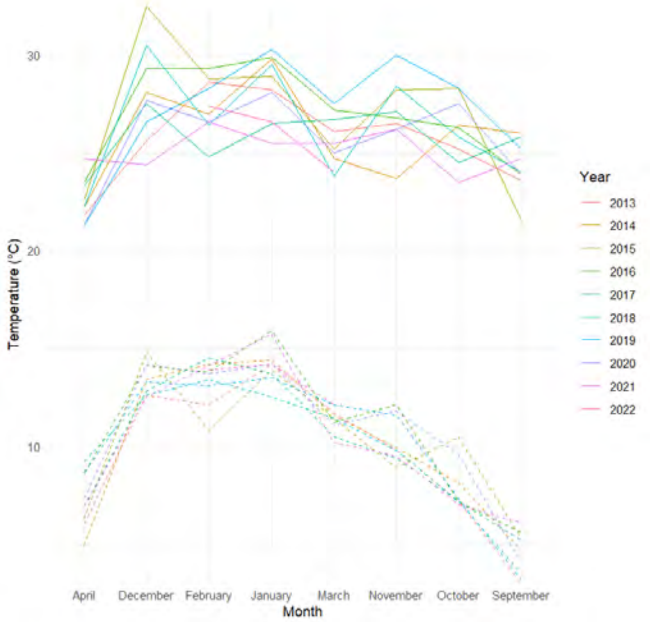

Figure 2b: Average monthly minimum and maximum temperatures (September to April) recorded at Ficksburg weather station from 2013 to 2022.

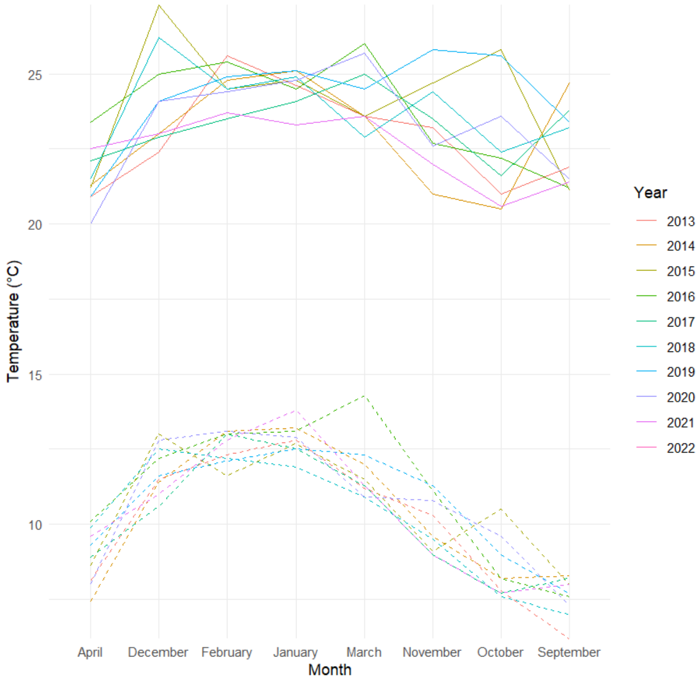

Figure 2c: Average monthly minimum and maximum temperatures (September to April) recorded at Giant’s Castle weather station from 2013 to 2022.

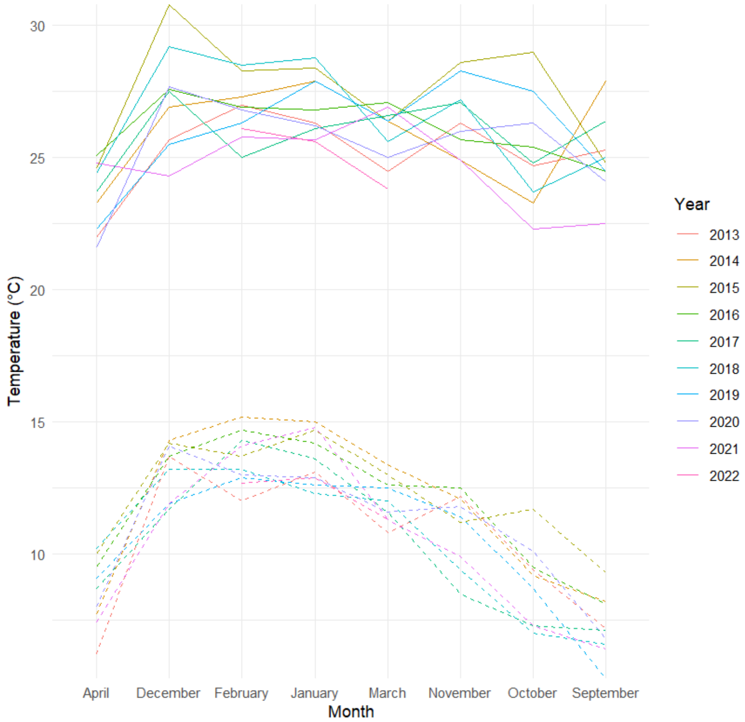

Figure 2d: Average monthly minimum and maximum temperatures (September to April) recorded at Royal Natal weather station from 2013 to 2022.

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