PLANKTON AND MACROINVERTEBRATE DYNAMICS IN THE KHAKHEA–BRAY TRANSBOUNDARY AQUIFER REGION

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GENERAL ABSTRACT

Temporary wetlands are prevalent in semi–arid and arid climates across the globe and harbour unique faunal assemblages that significantly contribute to regional aquatic biodiversity. This study aims to enhance our understanding of the ecological dynamics of temporary wetland ecosystems, focusing on plankton dynamics, large branchiopod diversity, trophic dynamics, water quality and the impacts of freshwater salinisation in temporary pans in the Khakhea Bray Transboundary aquifer region in the North west Province, South Africa. This body of work represents field, laboratory and writing components which span the period May 2021 to December 2023. The results from this study revealed seasonal patterns in plankton diversity with a winter peak in phytoplankton diversity dominated by Zygnematophyceae species, while Chlorophyceae were dominant in summer. Zooplankton diversity was high in summer as compared to winter, with both Rotifera and Copepoda dominant in both seasons. Six large branchiopod species were found in the region, and three of these were new distribution records for the North west Province, including the first record of *Phallocryptus spinosa* in the salt pan that was sampled in summer. Large branchiopod diversity was mainly influenced by water temperature and phosphorous in summer, while sediment sodium influenced the diversity in winter. The stable isotope analyses used to determine trophic dynamics in these temporary pans revealed that the food web had four trophic levels, with the top predators being the notonectids *Anisops* sp. The dominant consumers were predatory insects such as *Sigara* sp., *Anisops* sp., *Lestes* sp., *Rhantus* sp. and *Cybister* sp. adults and larvae, as well as detritivorous *Tomopterna* sp. tadpoles. High trophic niche overlaps were found between the notonectids and the dytiscids. These temporary pans are susceptible to anthropogenic impacts, and disturbed pans were found to have elevated pH, ammonium, phosphates and dissolved oxygen compared to the undisturbed pans. A strong positive relationship was observed between chl–*a* and temperature,

pH, dissolved oxygen, phosphates and ammonium. Chlorophyll–*a* concentration increased as surface area and the distance from kraals, buildings and latrines decreased. Freshwater salinisation was found to cause notable shifts in abiotic factors and benthic phytoplankton communities, favouring the proliferation of saline–tolerant diatom species at the cost of more sensitive taxa. The study also revealed that in interaction with salinisation, time also exerted a notable influence on shaping the benthic phytoplankton community. Salinity levels of 2.5 ppt and above led to significant decreases in emergent taxa richness and abundance, with Spinicaudata and Ostracoda being the most sensitive taxa to high salinities. There was a limited effect on community hatching phenology dynamics from salinity. This suggests that the main impact of salinisation in these systems will be reductions in hatching success and, hence, reduced recruitment. The study highlights the vulnerability of temporary pan ecosystems to anthropogenic influences and the complexities of interactions of organisms and the environmental conditions in these systems.

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PREFACE

This thesis is comprised of a general introduction (Chapter 1), a series of data chapters (Chapters 2, 3, 4, 5, 6, 7), and a general synthesis and conclusion (Chapter 8). The data chapters are presented as scientific papers, some of which have been published in peer–reviewed journals, with the remaining papers under review or in the final stages of preparation for submission (see below). The combined reference list at the end of the thesis was to ensure limited repetition.

- Mungenge, C. P., Wasserman, R. J., Dondofema, F., Keates, C., Masina, F. M., and Dalu, T. (**2023**). Assessing chlorophyll–*a* and water quality dynamics in arid–zone temporary pan systems along a disturbance gradient. *Science of The Total Environment*, 873, 162272. https://doi.org/10.1016/j.scitotenv.2023.162272
- Mungenge, C. P., Wasserman, R. J., Cuthbert, R. N., Dondofema, F., and Dalu, T. (**2023**). Salinisation of arid temporary pools alters crustacean hatching success but not phenology dynamics. Hydrobiologia, 1–13. https://doi.org/10.1007/s10750–023–05325–0
- Mungenge, C.P., Wasserman, R. J., Mofu, L., Keates, C., Dondofema, F. and Dalu,T. (**Under review**) Trophic dynamics in semi–arid temporary pan systems using stable isotope analyses. *Food Webs*.
- Mungenge, C.P., Wasserman, R.J., Dondofema, F. and Dalu, T. (**Awaiting submission to a journal**) Understanding seasonal dynamics of plankton communities in temporary pans of the Khakhea Bray Transboundary Aquifer region, South Africa.
- Mungenge, C.P., Wasserman, R.J., Mlambo, M., Keates, C. , Dondofema, F. and Dalu T. (**Awaiting submission to a journal**) Diversity of large Branchiopoda (Crustacea) in

semi–arid temporary pans, with a new record from the North west Province, South Africa.

- Mungenge, C. P., Wasserman, R. J., Cuthbert, R. N., Dondofema, F. and Dalu, T. (**Awaiting submission to a journal**) Increased salinisation effects on the benthic phytoplankton communities in semi–arid temporary pans.

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I want to acknowledge the financial support from the Southern African Development Community Groundwater Management Institute (SADC–GMI) and the National Research Foundation (NRF Grant no. 141656). I am also grateful to Rhodes University for funding conferences I attended.

To my brothers Mucha and Ngoni, your belief in me has been a source of inspiration, further fueling my determination. I am very grateful for the unwavering support and love from each of you. And to my friends, Taps (crazy stranger with some loose screws who became my brother from another mother), Taku (that one friend who would make me laugh any day when I felt low), Busi (my confidante, lunch partner, gossip partner), Frank (partner in crime, WEL labmate), Lenin (big brother to us all), Tress (mainini, always has a kind smile for me), Natasha (crazy a little bit but always believed in me and encouraged me that I could do this), Natanah (who always topped my prune jar and bought me sweets and food and introduced me to the world of coding in a pink R interface **highlight of my data analysis journey), Nawa (my unpaid therapist who I just vent to and annoy so much). You all have a special place in my heart; you get the worst of me sometimes. I vent my problems to you most of the time, but I also have so many fond memories of us all together, arguing over silly things, playing games, goofing around, and cooking together; the list is endless. Thank you also to my officemates Garyn and Erin; you guys are crazy, that's all I can say, but it was great sharing an office with you. I hope you stop your banter and start working next year.

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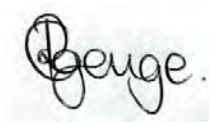
I thank the Lord God Almighty for this thesis, with whom I would never have reached this far. Thank you for giving me strength, holding my right hand, ordering my footsteps and being an ever–present help throughout this journey. In the silence, when the world was in deep sleep, and I was burning the midnight oil, you were there with me.

DEDICATION

I dedicate this thesis to my dearest best friend, my sweet mother, Eileen Musarurwa, whose unwavering support manifested through daily morning and evening texts from the day I left home until the triumphant conclusion of my journey. Your consistent presence, filled with love, provided a comforting warmth, and during moments of discouragement, your reassuring voice became my motivation to persevere, making every challenge worthwhile. In times when things did not go as planned and I cried, you stood as the sole witness to my struggles. Your ready availability for a phone call, your patient listening, and your subsequent prayers and words of encouragement during rough patches became the cornerstone of my strength. You believed in me in moments when I did not even believe in myself. You have always been my biggest cheerleader.

DECLARATION

I, Chipo Perseverance Mungenge (student number: g21m9745) hereby declare that the work described in this thesis is my work which was carried out in the Department of Zoology and Entomology, Rhodes University under the supervision of Prof. Ryan Wasserman and Dr Tatenda Dalu. The various components of this thesis comprise original work by the author and have not been submitted for any degree or assessment to any other university or tertiary institution.



Chipo Perseverance Mungenge

14 December 2023

CHAPTER 1: GENERAL INTRODUCTION

"We have short time to stay, as you,

We have as short a Spring,

As quick a growth to meet decay,

As you or anything."

– Robert Herrick

Plate 1. Aerial view of dry and inundated temporary pans in the Khakhea Bray Transboundary Aquifer region during winter, June 2021. Photo by Dr Adam Wyness

Freshwater biodiversity makes up a valuable natural resource, with freshwater ecosystems sustaining almost 9.5 % of all described species. However, the decline in biodiversity within freshwaters surpasses that observed in affected terrestrial ecosystems by a significant margin (Dudgeon et al., 2006; Dudgeon, 2019). The current threats to freshwater biodiversity include climate change, overexploitation, water pollution, destruction or degradation of habitats, freshwater salinisation, invasions and the cumulative impact of various stressors (Reid et al., 2019). These threats underscore the urgency of studying patterns in spatial and temporal variation in species endemism, abundance and richness, which are central to the various fundamental questions of conservation biology (Collen et al., 2014).

1.1 Temporary wetland ecology

Williams (2006) describes temporary wetlands as "*the Cinderellas of aquatic science*," akin to the neglected little sister of the large and more charismatic wetlands. The key defining feature of temporary wetlands is the recurrent dry phases of varying durations (Williams, 2006). Globally, temporary wetlands comprise considerable portions of inland surface waters in both arid and semi–arid regions of the world (Roshier et al.,2001; Calhoun et al., 2017; Nhiwatiwa et al., 2017; Mpakairi et al., 2022). These internally drained temporary systems (i.e., endorheic) are classified as wetlands but are often overlooked in monitoring and research (Williams, 2006; Bird et al., 2019). Temporary wetlands are often overlooked in research due to their small size and frequent drying, which pose challenges to their conservation (Williams, 2012; Calhoun et al., 2017).

The temporary wetland ecosystems are characterised by a complex interplay of various ecosystem features influenced by external processes (De Roeck, 2008; Figure 1.1). Among these features, the hydroperiod emerges as a crucial hydrological driving force shaping community dynamics and other ecological processes in temporary systems, often resulting in

a unique biotic community characterised by a number of specialist plant and animal species only found within these aquatic ecosystem types. (Calhoun et al., 2017; Kochi et al., 2020; Brendonck et al., 2022a). The key aspects of the hydroperiod that play a major role in shaping the community are hydroperiod length, predictability, inundation timing and frequency (Sim et al., 2013; Boix et al., 2020). Furthermore, the hydroperiod influences the water's physical and chemical attributes (Bird and Day, 2014). As the temporary wetlands dry out towards the end of the hydroperiod, when the rate of evapotranspiration exceeds the water input through precipitation and groundwater recharge, shifts in water chemistry occur resulting in increased nutrients, turbidity, ions and total suspended solids in the water column due to this evapo–concentration effect (Boone et al., 2006), with implications for dissolved oxygen, pH and other physical and chemical variables (Wasserman et al., 2018). Subsequently, during the rehydration phase, a dilution of the initial concentrations then occurs (Williams, 2012). Additionally, hydrological parameters such as surface area and depth significantly influence nutrients such as nitrogen and phosphorous dynamics (Anusa et al., 2012). The resulting physical and chemical conditions shape the aquatic biota in these wetlands, with variables such as conductivity, pH and dissolved oxygen influencing the taxa present (Nielsen et al., 2007; Mabidi et al., 2018). Aquatic biota can also reciprocally influence physicochemical variables as benthic organisms contribute to increased turbidity and nutrient release from sediment through bioturbation (Adámek and Maršálek, 2013).

The hydroperiod plays a pivotal role in shaping the aquatic biota within temporary wetlands. Species that dominate these communities exhibit distinct physiological or life cycle traits that enhance their resilience in these dynamic environments (Williams, 2012). The crustacean community in these habitats, such as the branchiopods and microcrustaceans, have dormant life stages and produce dormant propagules that remain viable through the dry phases, ensuring their persistence (Brendonck and De Meester, 2003; Brendonck, 1996). These dormant

propagules are transported over varying distances by various dispersal agents such as birds, wildlife, water, wind and/or humans (Vanschoenwinkel et al., 2011; Wasserman et al., 2023). These propagules remain dormant for extended periods until suitable environmental conditions are available to synchronise the life cycle for growth and reproduction in a favourable environment (Brendonck, 1996).

Biotic interactions in the aquatic biota occur throughout the hydroperiod. Extended hydroperiods provide an extended time frame for community development, facilitating the gradual occupation of available niches and successful colonisation and recruitment by a more diverse set of taxa (Boven and Brendonck, 2009). In the early stages of the hydroperiod, crustaceans like copepods, daphniids, spinicaudatans (clam shrimp) and anostracans (fairy shrimps) are among the first to establish in the temporary wetlands as they undergo their full life cycle in the habitat. The early hydroperiod stage is thus typically characterised by a short and simple food web dominated by crustaceans (Wasserman et al. 2016). Diversity and trophic complexity increase over time during wet phases, given the colonisation of the predator insects seeking to exploit foraging and breeding opportunities in these systems (Jocque et al., 2010; Dalu et al., 2017b). The later stages of these communities are, thus, increasingly comprised of predatory insects such as odonates, corixids, dyticids and notonectids at various life–stages (Brendonck et al., 2017). Consequently, hydroperiod dynamics are associated with variable diversity and trophic complexity (O'Neill and Thorp, 2014).

External processes also play a significant role in influencing the multiple features of the ecosystem. The geographical location assumes a crucial role in determining the climate patterns prevalent in regions that host temporary wetlands. Climate patterns form an intricate relationship with the hydrology in temporary wetlands. These temporary wetlands are particularly sensitive to shifts in climatic conditions such as temperature, precipitation or the

occurrence of extreme weather events like flooding and droughts, which alter the hydro regime (Boix et al., 2020). External processes such as anthropogenic activities within the catchment area, such as water abstraction and agriculture, also exert influence on the water chemistry within temporary wetlands. Overall, the interaction of both direct and indirect factors on the ecosystem is key in shaping the community assemblage within the temporary wetland ecosystems.

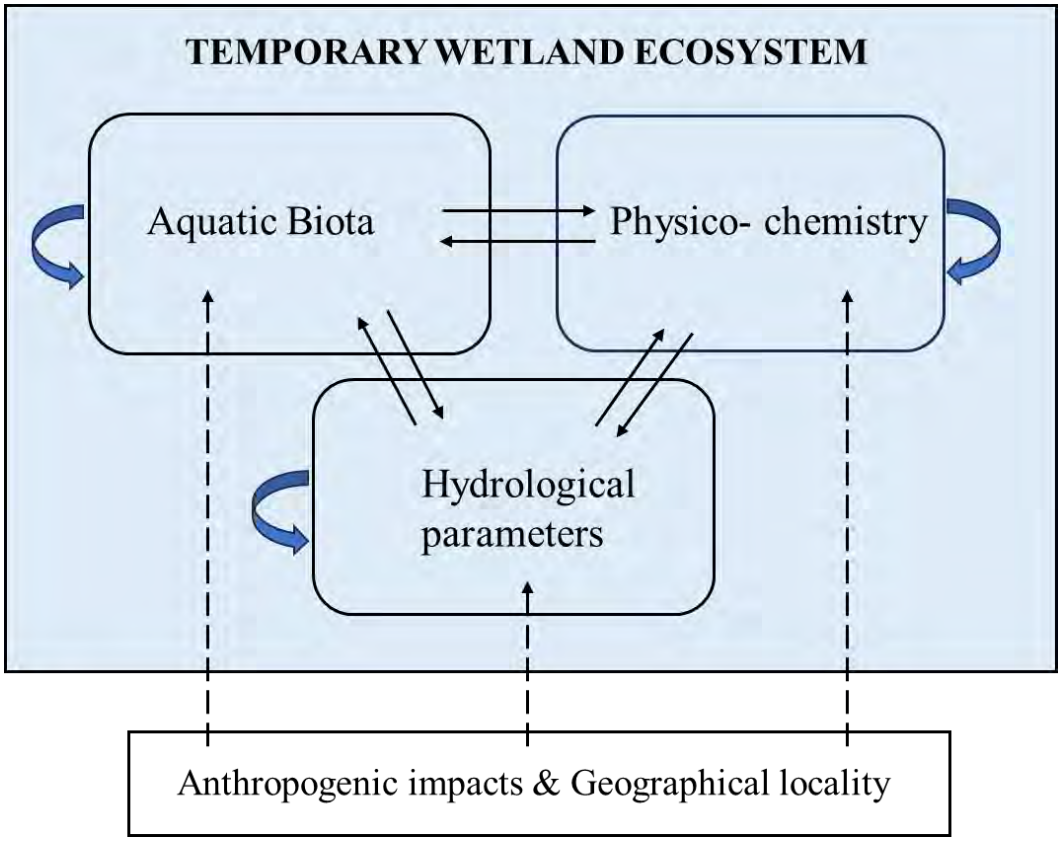


Figure 1.1. Simplified potential interactions between aquatic biota, physical, chemical and hydrological parameters in a temporary wetland ecosystem (solid black arrows). Blue curved arrows indicate interactions for an ecosystem feature. Dashed arrows represent external influences on the temporary wetland ecosystem. Modified from De Roeck (2008).

1.2 The value of temporary wetlands

The unique assemblage of temporary wetlands disproportionately contributes to the biodiversity of aquatic and semi–aquatic organisms (Boix et al., 2020). For example, faunal invertebrate diversity within temporary wetlands is high and is often higher than in permanent wetlands, establishing the former as biodiversity hotspots (Williams, 2000; Bird et al., 2019). These systems function as essential habitats for diverse invertebrate and vertebrate species. Additionally, the presence of shallow temporary waters makes them suitable foraging and breeding grounds (Smith et al., 2019). Temporary wetlands also provide various important ecosystem services in the major categories of provisioning, regulating, cultural and supporting (Table 1.1). These ecosystem services support a substantial population of the community around these temporary wetlands for their livelihood (Mitchell, 2013). The fluctuation in water availability in temporary wetlands not only fails to constrain wetland functions but, in fact, enhances them (Robinson, 1995).

Table 1.1.Ecosystem services derived from temporary wetlands (MA, 2005)

Ecosystem service	Definition	Examples
Provisioning	Products obtained from wetland ecosystems	• Food • Water • Fuel, wood and fiber • Medicines
Regulating	Benefits obtained from the regulation of wetland ecosystem processes	• Climate regulation • Water regulation • Water purification and waste treatment • Erosion regulation • Flood regulation
Cultural	Non–material benefits obtained from wetland ecosystems	• Spiritual • Recreational • Aesthetic • Educational
Supporting	Services necessary for the production of all other wetland ecosystems services	• Soil formation • Nutrient cycling • Primary production

1.3 Current threats to temporary wetlands

Despite the ecological importance of temporary wetlands, these systems are severely threatened worldwide, with degradation occurring at alarming rates. The increasing human population growth and resultant ecosystem loss and degradation due to changes in land use within the catchment pose significant threats to temporary wetlands, particularly in dryland areas (Calhoun et al., 2017; Parra et al., 2021). Changes in land use, pollution, water abstraction, urbanisation, agriculture, mining, drainage, habitat destruction and invasive species have been found to be major causes of temporary wetland degradation (De Roeck et al., 2007; Bird and Day, 2014; Brendonck et al., 2022a). Wetlands are currently facing heightened agricultural pressures compared to other aquatic ecosystems, and anthropogenic impacts may be more detrimental to these systems than to flowing systems (Wrubleski and Ross, 2011). Temporary wetlands subjected to agricultural pressures such as the increased input of pesticides, sedimentation, nutrient loading and exposure to invasive plant species will

result in shifts in the vegetation communities (Gordon et al., 2013). Over–exploitation poses a significant threat to the biodiversity and ecological balance of these temporary wetland ecosystems (Khudhair et al., 2019). This can further exacerbate the proliferation of nuisance species, such as mosquitoes, in these systems (Buxton et al., 2020). The escalating pollution and land drainage in temporary wetlands further compound these issues, affecting aquatic biota and leading to a decline in biodiversity, elevating the risk of extinction (Habib and Yosuf, 2015). Freshwater salinisation is an emerging threat to these systems as well, and coupled with climate change, this can have far–reaching impacts on their ecological integrity and the communities therein (Mabidi, 2018; Cunillera–Montcusí et al., 2022). Within Africa, temperatures have increased by 1–2 ºC in the past 30 years, and it is predicted that changes in rainfall events will occur, resulting in changes in the availability of surface water, reduced growing seasons and hydroperiods further negatively impacting the temporary freshwater ecosystems (Mitchell, 2013). Temporary wetland systems, because of their endorheic nature, are particularly susceptible and stand out as the most profoundly impacted systems in the face of climate change (Nicolet et al., 2004; Brendonck et al., 2022b).

1.4 Research in temporary wetlands: The South African context

In South Africa, geomorphic features and climatic conditions have given rise to the highest densities of temporary pans (Goudie and Wells, 1995; Bird et al., 2019). Despite this, their significance in the broader landscape and their contributions to biodiversity have only begun to garner significant recognition in the past decade. The comprehensive review by Bird et al. (2019) encompassed aquatic invertebrate research in southern African temporary wetlands. The review highlights that there has been a noticeable bias towards macroinvertebrates, particularly large branchiopods, which are often considered the more “charismatic” organisms in comparison to the smaller plankton taxa. Table 1.2 shows that while Mpumalanga and

Western Cape provinces have had substantial wetland research work done, North west Province, identified by Henri et al. (2014a) as also having high densities of pans, remains relatively underexplored with regards to plankton and macroinvertebrates (Table 1.2). With the current gaps in knowledge on the patterns, richness and endemism of species within temporary wetlands, Bird et al. (2019) recommended the need for updated information on community characterisation and distribution to facilitate effective conservation strategies for these ecosystems.

Table 1.2. Research articles on plankton and macroinvertebrates in temporary wetlands in the different provinces of South Africa

Province	Plankton	Macroinvertebrates	Reference
Kwa Zulu Natal		✓	Hamer and Appleton (1991)
		✓	Hamer and Martens (1998)
		✓	Dube et al. (2020)
Western Cape		✓	Bird et al. (2013)
		✓	Mlambo et al. (2011)
		✓	Bird and Day (2016)
		✓	De Roeck et al. (2007)
	✓		Suáreza–Morales and Rayner (2004)
	✓		Rayner (1998)
		✓	Martens (2007)
		✓	De Roeck et al. (2010)
		✓	De Moor and Day (2013)
		✓	Martens (2003)
Eastern Cape	✓	✓	Dalu et al. (2016)
	✓		Dalu et al. (2022a)
		✓	Brendonck (1995)
		✓	Mabidi et al. (2016)
	✓		Sua´rez–Morales et al.(2015)
	✓		Wasserman et al. (2016)
	✓	✓	Wasserman et al. (2018)
		✓	De Moor and Day (2013)
Mpumalanga	✓		Riato et al. (2014)
	✓	✓	De Necker et al. (2016)
		✓	De Klerk and Wepener (2013)
	✓		Ferreira et al. (2011; 2012)
		✓	Foster et al. (2015)
Limpopo		✓	Dalu and Chauke (2020)
Northern Cape		✓	Hamer and Rayner (1996)
		✓	Buschke et al., 2012
		✓	Meyer–Milne et al. (2020)

Gauteng		✓	Burger et al. (2019)
North west	✓		Szwarc et al.(2023)
	✓	✓	De Necker et al. (2016)
		✓	Henri et al. (2014b)
		✓	Foster et al. (2015)
Freestate		✓	Seaman et al. (1991b)
	✓	✓	Meintjes (1996)
		✓	Brendonck et al. (2000)
		✓	Mdidimba et al. (2021)
	✓	✓	Vanshoenwinkel et al. (2007)

1.5 The present study

The present study extends the existing research on the ecology of South African temporary wetlands, focusing on the understudied temporary pan systems in the Khakhea Bray transboundary aquifer (KBTA) region. With no prior published work on the region, the present study provides insights and data on these specific wetlands and contributes new information, addressing the existing knowledge gap. Additionally, this study holds broader ecological relevance, contextualising its findings within a larger ecological framework for temporary wetland ecology. The thesis specifically investigates the seasonal variation in phytoplankton community structure within these temporary wetlands, an aspect which has received little attention compared to larger taxa. Environmental drivers shaping plankton community structure throughout the seasons are also assessed. The study then explores large branchiopod diversity in the region, comparing findings with previous occurrences in the province to identify new distribution records. The thesis also investigates trophic interactions of the biota in these pans, using stable isotope analyses. With the area experiencing agricultural activities and pollution, the water quality and chlorophyll–*a* was assessed over a disturbance gradient. Finally, the study examines the impacts of freshwater salinisation on the hatching and phenology dynamics of the benthic phytoplankton communities and the crustaceans in these

temporary pans. This work contributes to the fundamental understanding of water quality, plankton dynamics and the environmental drivers within the context of anthropogenic pressures, contributing knowledge for effective conservation and management efforts of these systems. Various components of the study are also of global significance and address the growing relevance of studying temporary wetlands, given the projected shift of many freshwater systems towards more temporary states due to climate change.

1.6 Thesis outline

The thesis, as highlighted in the preface, comprises a general introduction (Chapter 1), followed by six data chapters (Chapters 2 to 7) and a synthesis and conclusions chapter, Chapter 8. Chapter 2 includes a description of the study site and focuses on the seasonal variation of plankton in temporary pans in the KBTA region, while Chapter 3 explores the diversity of large branchiopods in the province. Chapter 4 employs the use of stable isotope analyses to assess the trophic dynamics in these temporary pans systems. Chapter 5 examines changes in water quality along a disturbance gradient in these pans. Finally, Chapters 6 and 7 investigate the impact of freshwater salinisation on the benthic phytoplankton and crustaceans within these systems.

CHAPTER 2: PLANKTON DIVERSITY IN THE KHAKHEA BRAY TRANSBOUNDARY AQUIFER

"Science, for me, gives a partial explanation for life. In so far as it goes, it is based on fact, experience, and experiment."

– Rosalind Franklin

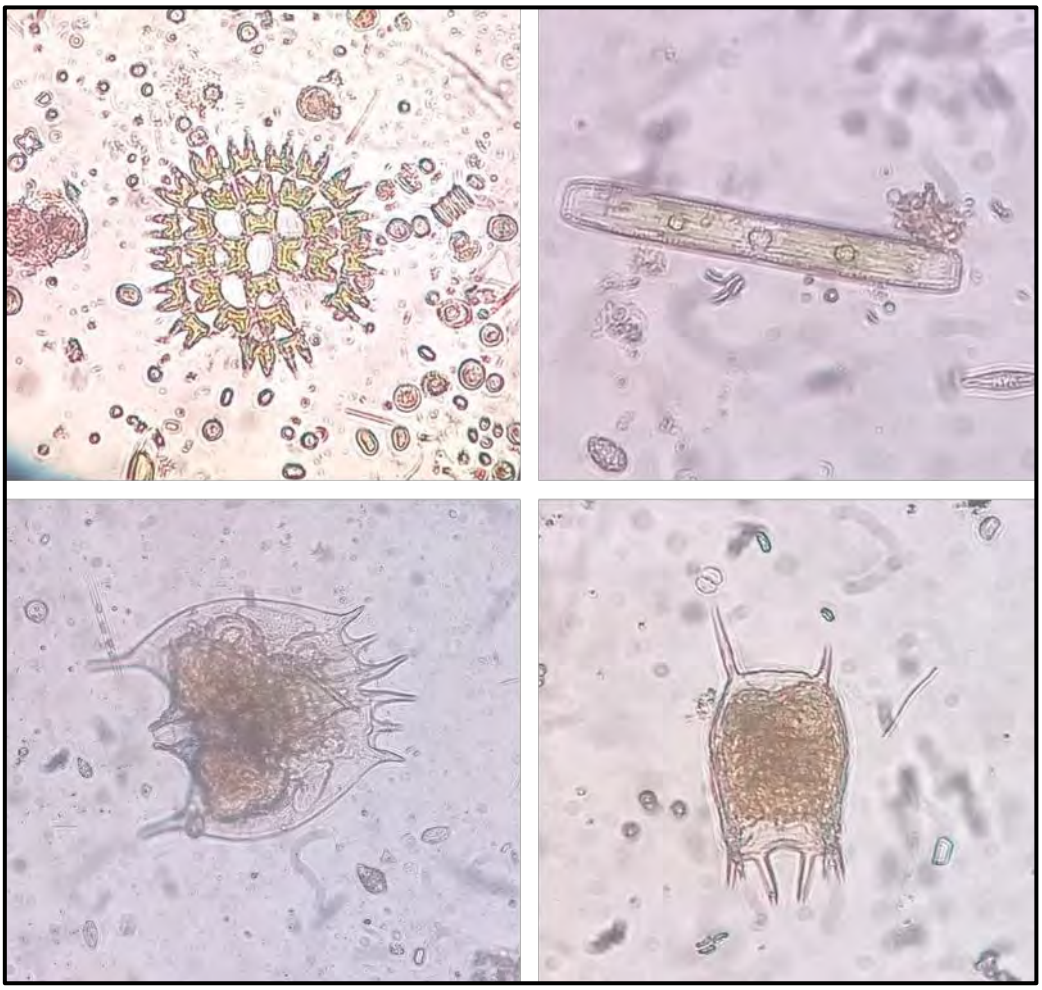


Plate 2. Selected phytoplankton (Top row: *Pediastrum*, *Scenedesmus*, *Pinnularia*, *Navicula*) and zooplankton (Bottom row: *Brachionus*, *Keratella*) species recorded in the Khakhea Bray Transboundary Aquifer region. Photo by Chipo P. Mungenge.

Abstract

Plankton dynamics play a crucial role in the ecological functioning of temporary wetland ecosystems, yet there is a notable gap in studies examining seasonal plankton dynamics within temporary wetland systems in Southern Africa. This study investigates the seasonal variations of plankton communities in temporary pans within the Khakhea Bray Transboundary Aquifer region, North west Province, South Africa and the environmental variables driving these communities. Phytoplankton and zooplankton samples were collected from ten pans during both summer and winter seasons and were identified to the lowest taxonomic level possible. The study revealed distinct seasonal variations in plankton communities, with a winter peak in phytoplankton diversity dominated by Zygnematophyceae species such as *Cosmarium caelatum*, *Straurastrum anatium*, *Straurastrum crenulatum*, *Straurastrum planctonica* and *Straurastrum vestitum*. Chlorophyceae were dominant in summer, with *Coelastrum cambricum*, *Oocystis borgei*, *Oedogonium cymatosporum* and *Pediastrum boryanum* being the dominant species. Zooplankton diversity was high in summer, and the dominant species were *Trichocerca elongata*, *Brachionus plicatilis*, *Cyclops* sp., copepod nauplii, *Diaphanosoma excisum* and Cypridídae, while in winter the dominant species were *Lovenula falcifera*, copepod nauplii, *Diaphanosoma excisum* and Cypridídae. Additionally, zooplankton species such as *Macrothrix spinosa*, Cypridídae and *Mytilina* sp., were also recorded in the temporary pans in this study.

Keywords: *temporary wetland, phytoplankton, zooplankton, South Africa*

2.1 Introduction

Seasonal dynamics of phytoplankton and zooplankton, along with their responses to environmental variables, have been comprehensively investigated, particularly in permanent systems (Mustapha, 2010; Ndebele–Murisa et al., 2010; Dalu et al., 2013; Mhlanga et al., 2017). Plankton plays a crucial role in freshwater ecosystems as an ecological tool for monitoring ecosystem health and providing insights into ecosystem functioning through their multifaceted roles in various ecosystem processes (Araujo et al., 2022). While plankton dynamics are key components in temporary wetland ecosystems, significantly influencing community succession across different inundation events and seasons (Dalu et al., 2022a), only a limited number of studies have investigated seasonal plankton dynamics in temporary wetland systems within Southern Africa (Anusa et al., 2012; Riato et al., 2014; Msiteli–Shumba et al., 2017; Nhiwatiwa et al., 2017; Dalu et al., 2022a).

Phytoplankton are essential photoautotrophic organisms, and they contribute to nearly 50 % of the global net production, serving as the primary energy source and functioning in nutrient–cycling processes within aquatic ecosystems, with temporary wetlands being no exception (Graham et al., 2009; Winder and Sommer, 2012; Dalu et al., 2016; Cai et al., 2019). Phytoplankton communities are diverse (15 major phyla) with contributing species characterised by distinct shapes, sizes and morphologies, with the most diverse groups being the Chlorophyta (chlorophytes) and Bacillariophyta (diatoms) (John et al., 2002; Reynolds, 2006). From the various phytoplankton groups, diatoms, cyanophytes and chlorophytes are more widely studied. Diatoms show remarkable adaptability, but their distinct habitat preferences make them valuable as bio–indicators in freshwater environment assessment (Soininen, 2007; Taylor et al., 2007). The chlorophytes are often dominant in fresh water, with a preference for warmer temperatures (Reynolds,1984). They are, however, more diverse, with

several kind of species with some being cosmopolitan and others being endemic and cannot be applied for biomonitoring like the diatoms. The cyanobacteria (blue–green algae), in addition to their role as primary producers, are a global problem due to their production of cyanotoxins and the ability to produce extensive blooms in nutrient–rich environments (Havens, 2008; Kimambo et al., 2019).

Zooplankton, as heterotrophic organisms, play a crucial role in the food web by linking primary producers and organisms in high trophic levels and also forming a significant proportion of the planktonic community in temporary wetlands (Sharma and Singh, 2012). Freshwater zooplankton consists of protozoa, rotifers, cladocerans, copepods and ostracods (Brendonck et al., 2022b). Among these groups, protozoans and rotifers represent the smaller taxa, with rotifers comprising benthic or periphytic forms which occur in a wide range of freshwater habitats, with genera such as *Brachionus*, *Lecane* and *Euchlanis* being cosmopolitan species that are predominantly littoral (Rajapaksa and Fernando, 1984; Fernando, 2002). Additionally, freshwater zooplankton includes small crustacean groups Copepoda and Cladodera, with copepods being the most diverse group featuring both free–living and symbiotic forms (Bird et al., 2019). Similar to phytoplankton, zooplankton also serve as highly responsive indicators of ecosystem health due to their acute sensitivity to environmental shifts (Guermazi et al., 2023).

The intricate community dynamics of organisms in temporary aquatic environments are influenced by the water regime encompassing factors such as timing, duration, frequency and predictability of the aquatic phase (Vanschoenwinkel et al., 2009). Hydroperiod and nutrient concentrations are important in structuring phytoplankton communities in temporary wetlands (Nhiwatiwa et al., 2017). The physical and chemical variables of temporary systems undergo

wide fluctuations throughout the hydroperiod, further exacerbated by their small sizes (Jocque et al., 2010). Notably, changes in water chemistry, particularly nutrient enrichment, substantially influence phytoplankton colonisation and composition. Following inundation, phytoplankton communities are initially dominated by short–residence green algae and then transition to blue–green algae in the later stages of the hydroperiod (Anusa et al., 2012), while in other cases, fast–growing phytoplankton species which are adapted to short hydroperiod phases colonise first in the temporary pans then large slow–growing species adapted to more stable conditions develop later (Dalu et al., 2022a). Environmental factors such as pH and salinity are pivotal in shaping the structure of phytoplankton communities within these systems (Chapter 6). Zooplankton taxon succession in temporary freshwater wetlands also follows the same trajectory as phytoplankton with initial colonisation by fast–maturing organisms such as copepods and ostracods with a shift to cladocerans later in the hydroperiod (Henri et al., 2014b; Mabidi et al., 2018; Mungenge et al., 2023). Geography, rainfall, temperature and hydroperiod are natural factors driving zooplankton communities (De Necker et al., 2016). Other local environmental factors include habitat size, water depth and chemistry (i.e., conductivity, salinity, turbidity, phosphates), food availability and predation pressure by other invertebrates and vertebrates (Nielsen et al., 2003a; Anusa et al., 2012; Nhiwatiwa et al., 2017). In these highly variable environments, phytoplankton and zooplankton also face additional threats from climate change and anthropogenic activities within the landscape (Roshier et al., 2001).

Zooplankton–phytoplankton interactions are central in the food webs of temporary wetland ecosystems. Within these systems, zooplankton not only consume phytoplankton at the base of the food web but are also preyed upon by other zooplankton, macroinvertebrates and vertebrates. Changes in phytoplankton and zooplankton community structures undergo seasonal succession due to nutrient availability, competition and predation (Sommer et al.,

1986; Wentzky et al., 2020). Hydroperiod primarily drives community succession and food web structure in temporary ecosystems (O'Neill and Thorp, 2014). Consequently, phytoplankton and zooplankton community composition shifts over the hydroperiod, creating temporal niches facilitating optimum feeding conditions (Vanschoenwinkel et al., 2010b). During the inundation period, increased depth and area from increased pool size result in additional spatial niches for feeding, which allows more species diversity to occur (Anusa et al., 2012).

Knowledge of the phytoplankton and zooplankton community structure and seasonal changes associated with environmental variables in temporary wetlands is scarce and fragmented, particularly for semi–arid regions of the global south. Therefore, understanding how plankton communities change over seasons can provide insights into the ecological drivers behind these shifts and the implications for ecosystem functioning. It may also shed light on plankton trophic interactions and, hence, the ecological resilience of these temporary systems. The present study, therefore, aims (a) to investigate seasonal phytoplankton and zooplankton species diversity in temporary pans of the Khakhea Bray Transboundary Aquifer (KBTA) region and (b) to explore seasonal fluctuations in environmental variables and examine how these influence plankton communities within temporary semi–arid wetland systems. Considering the seasonal environmental variability that characterises these temporary systems, we hypothesised that (i) phytoplankton community composition would be closely linked to seasonal changes in environmental variables in the temporary pans. Specifically, elevated temperatures and high water availability during summer are anticipated to result in a high proportion of chlorophytes, while low water levels and high pH will lead to diatoms dominating in winter. As such, (ii) high phytoplankton species diversity would be observed in summer, and ultimately (iii) in

summer, increased habitat availability and high resource abundance from the predominance of chlorophytes would subsequently lead to a peak in zooplankton species diversity.

2.2 Materials and methods

2.2.1 Study area

This study was done in temporary pans in the Khakhea Bray transboundary aquifer (KBTA) region, North west Province of South Africa. During this study, numerous temporary pans were sampled, varying across the data chapters. The entire study was carried out for the various components in three different periods: winter (June 2021), summer (January 2022), and winter (May 2022). Further details on sites, sampling procedures and study designs are outlined in subsequent data chapters. For this chapter, the study was conducted in June 2021 (low water period, winter) and January 2022 (high water period, summer) in ten pans located in the (KBTA) region (Table 2.1; Figure 2.1 and Figure 2.2).

The KBTA is supported by the low–yielding Khakhea–Bray dolomitic aquifer, with the main source of recharge being rainfall. Geological lineaments, shallow dolomite outcrops and sub–outcrops, banded ironstone formation and alluvial channels along the Molopo River serve as recharge areas for the aquifer (Godfrey and Van Dyk, 2002). The climate in the region is characteristic of a semi–arid conditions with low annual rainfall (average 376 mm) that comes in the summer months (October–March) (Godfrey and Van Dyk, 2002; Mpakairi et al., 2022). High temperatures are experienced in the region, with the mean temperature ranging from 22 °C (April) to 34 °C (October). The KBTA has low infiltration rates because of the thick Kalahari sands (>15 m) and has high evaporation rates (2 050–2 250 mm per annum) (Turton et al., 2006). Because of the low rainfall in the area, vegetation is characterised by bushveld savannah–type vegetation. The vegetation around the pans comprised bushveld (i.e., *Senegalia nigrescens* and *Vachellia grandicornuta*). The shrubland was intermixed with *Scorzonera*

humilis, *Eragrostis* sp., *Ziziphus mucronata*, *Leucas martinicensis* and *Lipia avani.* The region is also characterised by a large number of temporary pan ecosystems (Plate 1). Littoral zones of these pans house *Potamogeton* sp., *Marsilea* sp., and *Lagarosiphon* sp. The pan systems constitute most of the standing surface water in the region. Given that much of this region is rural and largely under–developed, these pans serve as a significant potable water source for domestic and agricultural requirements.

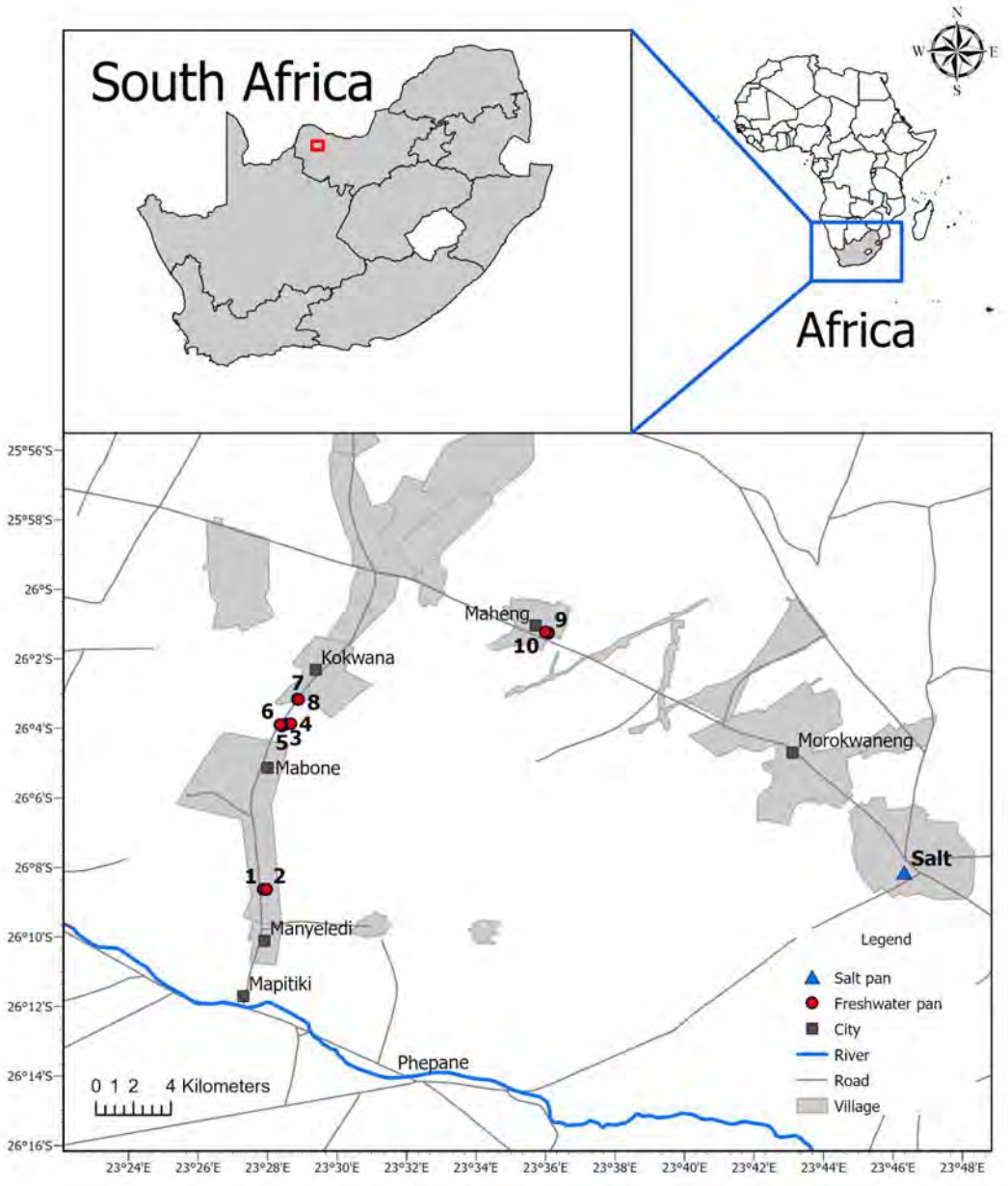


Figure 2.1. Map of the study area showing sampled pans in the Khakhea Bray Transboundary Aquifer region in January 2022 and May 2022

Table 2.1. Co–ordinates and surface area of all the sampled pans in the Khakhea Bray Transboundary Aquifer region

Pan	Latitude	Longitude	Surface area m^{-2}
1	23.4645	–26.1439	940.7
2	23.4661	–26.1438	450721.9
3	23.4754	–26.0646	2708.9
4	23.4777	–26.0645	15701.7
5	23.4735	–26.0656	630.2
6	23.4726	–26.0648	10563.8
7	23.4811	–26.0525	5465.7
8	23.4815	–26.0528	1474.0
9	23.6013	–26.0209	2985.4
10	23.5998	–26.0205	7622.4
Salt pan	23.7721	–26.1357	474851.9

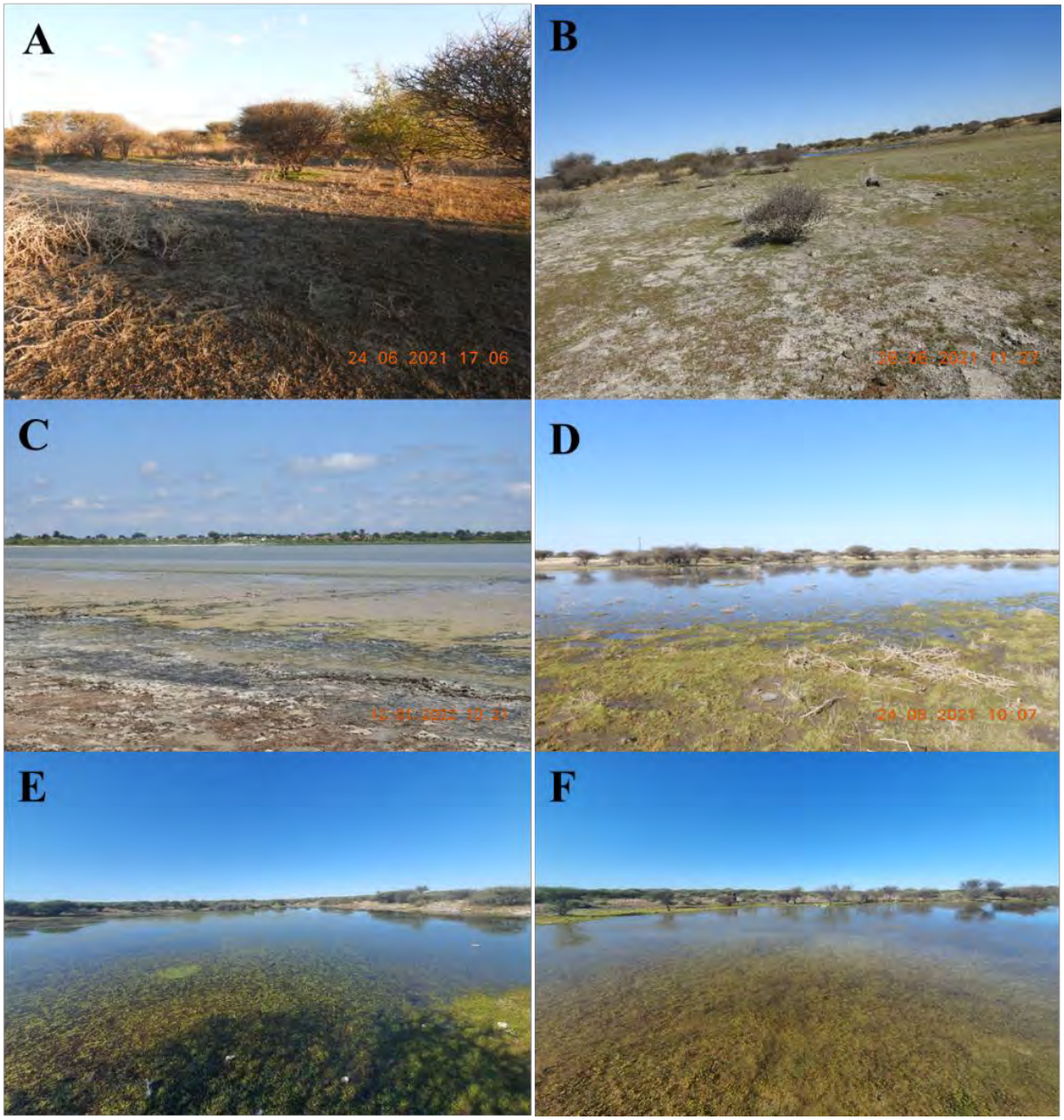


Figure 2.2. Photographs of some of the temporary pans in the Khakhea Bray Transboundary Aquifer region: A and B dry pans in the winter season from where sediment was collected, C – Salt pan, D – a pan with low water levels in winter, E and F – pans with high water levels and filled in the summer season

2.2.2 Environmental variables

2.2.2.1 Water chemistry

Water temperature (°C), pH, conductivity (µS cm^{-1}), turbidity (NTU), total dissolved solids (TDS; mg L^{-1}), salinity (ppm), dissolved oxygen (DO; mg L^{-1}) were measured at three points in each pan using an AquaRead multiparameter meter (Model AP–700 and AP–800, AquaRead Ltd, UK). Three 250 mL water samples were collected from each pan, stored on ice, and analysed within 8 h of sample collection for nutrients to determine nutrients in the water. Nutrients (i.e., phosphates, ammonium) were analysed using a multiparameter benchtop photometer (i.e., Hanna Instruments Model HI83300, Hanna Instruments Inc., Rhode Island) whereby ammonium (photometer range 0–10 ± 0.04 mg L^{-1} accuracy, resolution 0.01 mg L^{-1}) was determined through the adaptation of the ASTM Manual of Water and Environmental Technology (D1426) Nessler method and phosphates (photometer range 0–30 mg L^{-1} ± 1.0 mg L^{-1} accuracy, resolution of 0.1 mg L^{-1}) through the adaptation of the Standard Methods for the Examination of Water and Wastewater, amino acid method (ASTM International, 2003; Rice and Bridgewater, 2012).

Three 250 mL water samples were collected from each pan to determine the chlorophyll–*a* concentrations. The samples were vacuum filtered through a 0.7 µm Whatman glass fibre filter (GF/F), and the GF/F filters were placed individually in small, labelled aluminium foil bags and stored on ice in the field. The filters were then stored at –20 °C until extraction in the laboratory. Each GF/F filter was extracted in 10 mL of 90 % acetone in the dark over 24 h. Chlorophyll–*a* concentration was then determined fluorometrically using a Turner 10AU fluorometer. Absorbances were taken for each sample before and after adding two drops of 1 N HCl (Holm–Hansen and Riemann, 1978; Gusha et al., 2021). Chlorophyll–*a* concentrations

were then calculated following the Environmental Protection Agency (EPA) method 445.0 (Arar and Collins, 1997).

2.2.2.2 Sediment chemistry

During both sampling events, two sediment samples were collected randomly from each freshwater pan. No sediment samples were collected from the salt pan. A plastic hand shovel was used to collect the sediment from the top 5–10 cm of the sediment layer, which was then placed into a new labelled polyethylene Ziplock bag. The Ziplock bags were stored on ice in a cooler box and transported to the laboratory for further analysis. In the laboratory, the sediment samples were oven–dried at 60 °C for 72 h before being crushed using a porcelain mortar and pestle. The sediment was then sieved with a mesh size of 0.05 mm to remove any plant roots and other debris present in the sample. The sediment samples were then sent to BEMLAB, a South African National Accreditation System—certified laboratory in South Africa for further processing. The following were elements analysed: (i) *nutrients*; nitrogen (N) and phosphorus (P) using a SEAL Auto Analyzer, (ii) *cation elements*; potassium (K), calcium (Ca), boron (B), magnesium (Mg), sodium (Na) using the acid digestion method, with the metal content being determined using an ICP–OES optical emission spectrometer (Varian, Mulgrave, Australia) and (iii) *heavy metals*; iron (Fe), zinc (Zn), manganese (Mn) and copper (Cu) were measured using an ICP– OES optical emission spectrometer (Varian, Mulgrave, Australia).

2.2.3 Plankton sampling

Sampling was carried out at each pan across the two seasons. Plankton samples were collected using horizontal hauls with a zooplankton net (40 cm diameter, 64 µm mesh size) and phytoplankton net (40 cm diameter, 20 µm mesh size). Horizontal tows were accomplished by towing the plankton net subsurface through the water for 10 m. The concentrated samples were

collected in small labelled 250 mL polyethylene bottles. To preserve and fix the samples, a solution of 70 % alcohol and Lugol's iodine was added to the zooplankton and phytoplankton sample bottles, respectively. The plankton samples were then taken to the Wetland Ecology Laboratory at Rhodes University for identification and counting. Plankton samples were left to settle for 24 h using the Utermöhl technique (Edler and Elbrächter, 2010). Aliquots of 5–20 mL of samples were taken after sedimentation, depending on the plankton abundances in each sample. Species were determined and enumerated using an inverted Nikon TMS light microscope at ×200 magnification. Plankton was identified using dichotomic keys and guides (i.e., Lund and Lund, 1995; John et al., 2002; Taylor et al., 2007; Elenbaas and Grundel, 1994; Fernando, 2002; van Vuuren et al., 2006) to their lowest taxonomic unit possible based on the literature.

2.2.4 Data analysis

A Kruskal–Wallis analysis was used to determine differences in the environmental variables measured between the different pans sampled in summer and winter in STATISTICA version 14.0.0.15. Distance–based Permutational Analysis of Variance (PERMANOVA; Anderson, 2001) were used to test for significant differences in the environmental variables, phytoplankton and zooplankton community among the sampling sites (i.e., 10 temporary pans) across the two sampling seasons (i.e., winter, summer).

Separate cluster analyses were conducted for the water quality and sediment data (based on Euclidean distance similarity) and also for the phytoplankton and zooplankton communities (based on Bray Curtis similarities) to determine similarities among the samples. Diversity indices (Shannon–Wiener index, Pielou's evenness and Simpson's index) were calculated for the phytoplankton and zooplankton data set to assess diversity and plankton community

structure. The cluster analysis and diversity metrics were done in PRIMER version 6 (Clarke and Warwick, 2001; Anderson et al., 2008). Whittaker–β (β_W) diversity was also calculated to measure species turnover (Whittaker, 1960) across the different seasons. The β_W diversity is hypothesised to mark a substantial change in assemblage composition between two sampling points (i.e. seasons). Mean species turnover in each season, i.e.Whittaker beta diversity (β_W), was calculated. Two–way ANOVA in R software was then used to test for differences in all the diversity metrics among the sampled sites and seasons.

Multivariate analysis was used to explore relationships between the biotic (i.e., phytoplankton and zooplankton communities) and environmental variables in the temporary pans sampled during summer and winter. Before analysis, all the environmental variables except pH data were log (x+1) transformed while the biological data was square root transformed to reduce skewness in the data. A preliminary detrended correspondence analysis (DCA) was applied to the data set to examine gradient lengths. Since both response data were compositional and had a gradient of less than 3.0 units long, the linear ordination method was used (Ter Braak and Verdonschot, 1995). A redundancy analysis (RDA) was thus used to explore the relationship between the phytoplankton and zooplankton species and the environmental variables from the different pans sampled at both sampling times. The RDA is a constrained linear ordination method in which forward selection Monte Carlo permutation tests (9999 unrestricted permutations, $p < 0.05$) are used to test the significance of the axis and, therefore, determine if selected environmental variables can explain much of the plankton species variation. The software CANOCO version 5.02 was used for the analysis (Ter Braak and Šmilauer, 2002).

2.3 Results

2.3.1 Environmental variables

Environmental variables measured from the temporary pans were found to differ significantly between seasons (PERMANOVA: Pseudo–F = 5.247, df = 1, $p = 0.011$) but not among pans (PERMANOVA: Pseudo–F = 1.000, df = 9, $p = 0.501$). For the water chemistry, temperature (26.6 ± 1.4 ºC), conductivity (191.8 ± 40.8 µS cm^{-1}), TDS (125.7 ± 28.6 mg L^{-1}) and chlorophyll–*a* (0.08 ± 0.05 mg L^{-1}) were high in summer. In contrast, pH (10.6 ± 0.6), salinity (0.08 ± 0.03), dissolved oxygen (6.8 ± 0.8 mg L^{-1}), ammonium (0.7 ± 0.6 mg L^{-1}) and phosphates (0.7 ± 0.2 mg L^{-1}) concentrations were high in winter. During summer, sediment pH (7.7 ± 0.3), P (47.3 ± 45.1 mg kg^{-1}), K (139.6 ± 132.2 mg kg^{-1}), Ca (8.3 ± 4.8 mg kg^{-1}), Mg (1.3 ± 0.9 mg kg^{-1}) and S (7.7 ± 4.1 mg kg^{-1}) were high, while Na (0.1 ± 0.03 mg kg^{-1}), Cu (2.0 ± 1.3 mg kg^{-1}), Zn (1.0 ± 0.9 mg kg^{-1}), Mn (135.8 ± 179.6 mg kg^{-1}), B (0.3 ± 0.1 mg kg^{-1}), Fe (51.5 ± 25.9 mg kg^{-1}) and SOC (1.4 ± 0.4 mg kg^{-1}) were high in winter. Significant (Kruskal–Wallis, $p < 0.05$) differences in temperature, pH, dissolved oxygen, water phosphates, soil organic carbon (SOC), sediment phosphorous and Na were observed between the two seasons (Table 2.2).

Table 2.2. Summary of Kruskal–Wallis analysis of measured environmental variables (mean ± standard deviation) in temporary pans sampled in winter and summer in the Khakhea Bray Transboundary Aquifer region. Significant values ($p < 0.05$) are indicated in bold.

Environmental variables	Acronym	Winter ($n = 8$)	Summer ($n = 10$)	*p*
Water				
Temperature (ºC)		20.73 ± 1.1	26.62 ± 1.4	**< 0.001**
pH		10.64 ± 0.6	7.98 ± 0.4	**< 0.001**
Conductivity (µS cm^{-1})		159.35 ± 46.6	191.77 ± 40.8	0.1309

Total dissolved solids (mg L^{-1})	TDS	113.05 ± 33.78	125.7 ± 28.5	0.4772
Salinity (ppm)		0.08 ± 0.03	0.06 ± 0.01	0.2279
Dissolved oxygen (mg L^{-1})	DO	6.83 ± 0.8	4.15 ± 1.4	**< 0.001**
Ammonium (mg L^{-1})		0.68 ± 0.6	0.36 ± 0.1	0.7221
Phosphates (mg L^{-1})		0.71 ± 0.2	0.10 ± 0.1	**< 0.001**
Chlorophyll–*a* (mg L^{-1})		0.05 ± 0.04	0.08 ± 0.05	0.1551
Sediment				
pH		7.68 ± 0.4	7.73 ± 0.3	0.3003
Resistance (Ω)		1835 ± 434.9	1961.5 ± 648.3	0.3281
Stone (%)		3.48 ± 2.2	2.39 ± 3.3	0.1476
Phosphorous (mg kg^{-1})	P	29.20 ± 12.3	47.28 ± 45.1	**< 0.001**
Potassium (mg kg^{-1})	K	133.37 ± 57.7	139.6 ± 132.2	0.3284
Calcium (mg kg^{-1})	Ca	6.91 ± 3.0	8.25 ± 4.8	0.7898
Magnesium (mg kg^{-1})	Mg	1.21 ± 0.5	1.27 ± 0.9	0.4229
Sodium (mg kg^{-1})	Na	0.08 ± 0.03	0.02 ± 0.01	**< 0.001**
Copper (mg kg^{-1})	Cu	1.96 ± 1.3	1.54 ± 1.1	0.3733
Zinc (mg kg^{-1})	Zn	0.99 ± 0.9	0.95 ± 0.5	0.2858
Manganese (mg kg^{-1})	Mn	135.83 ± 179.6	66.85 ± 86.1	0.6569
Boron (mg kg^{-1})	B	0.25 ± 0.1	0.23 ± 0.2	0.2135
Iron (mg kg^{-1})	Fe	51.48 ± 25.9	29.00 ± 14.8	0.0914
Soil organic carbon (mg kg^{-1})	SOC	1.39 ± 0.4	0.67 ± 0.4	**0.0022**
Sulphur (mg kg^{-1})	S	6.51 ± 3.6	7.70 ± 4.1	0.2277

Clustering with the SIMPROF analysis based on similarities showed five groups, with four groups forming significant clusters ($p < 0.05$) from the water quality data analysed. The summer pans were made up of two cluster groups, with *Cluster Group 1* containing pans 1, 5, 6, 9 and 10, while *Cluster Group 2* was made up of pans 2, 3,4 and 7. Winter pans also formed two cluster groups, with *Cluster Group 3* comprising pans 1, 7 and 8 and *Cluster Group 4* made up of 2, 3, 4, 6 and 10. (Figure 2.3).

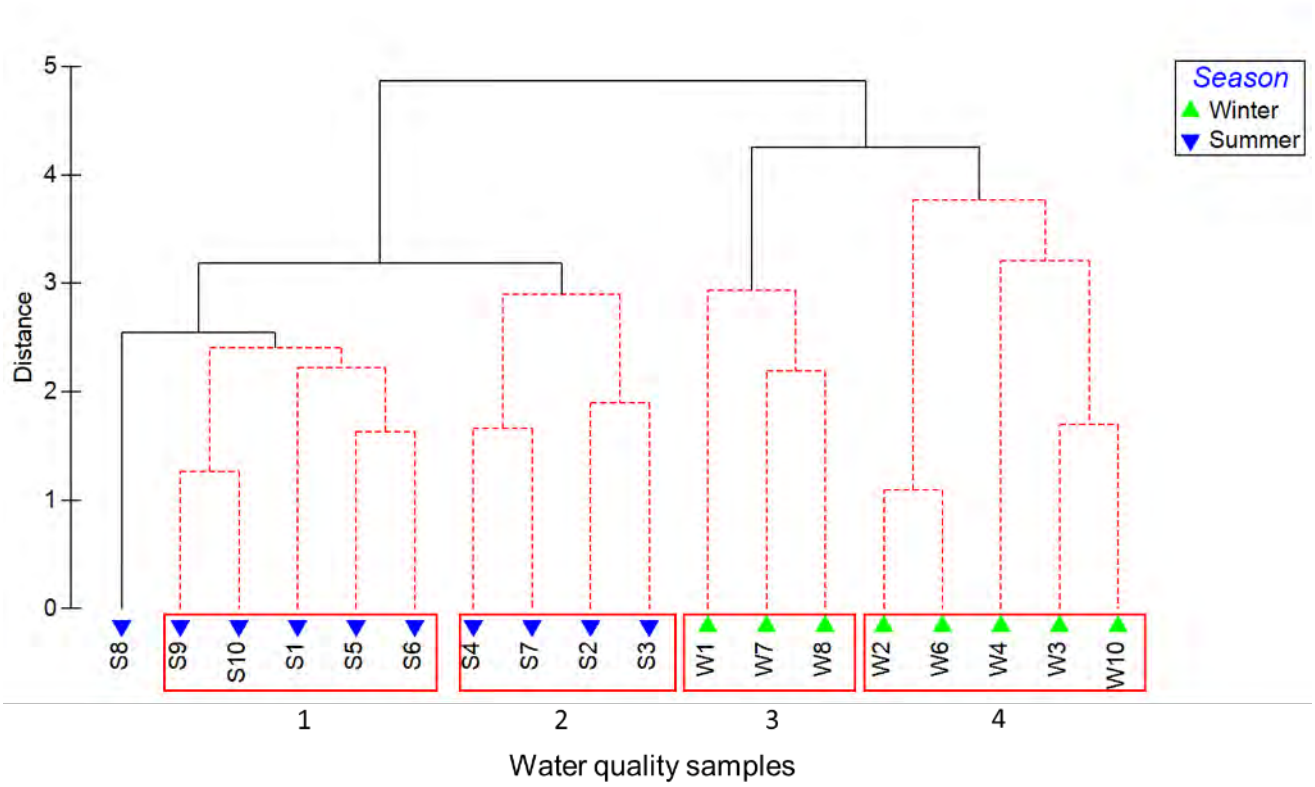


Figure 2.3. Cluster analysis with SIMPROF of water quality variables measured in 10 temporary pans across two seasons in the Khakhea Bray Transboundary Aquifer region. Red boxes denote significant cluster groups. The code per sample denotes the time of sampling and the pan number, where the first letters (S, W) represent the time of sampling (summer, winter), and the numeric values denote the sampled pan number (1 – 10).

Clustering with the SIMPROF analysis based on similarities showed eight groups, with six groups forming significant clusters ($p < 0.05$) from the sediment data analysed. Winter pans 2, 4, 6 and 7 constituted *Cluster Group 1*, while *Cluster Group 2* comprised summer pans 6, 7 and 8. Summer pans 1 and 2 constituted *Cluster Group 3*, while *Cluster Group 4* comprised summer pan 4 and winter pans 8 and 10. *Cluster Group 5* had winter pans 1 and 3, and *Cluster Group 6* had summer pans 9 and 10. (Figure 2.4).

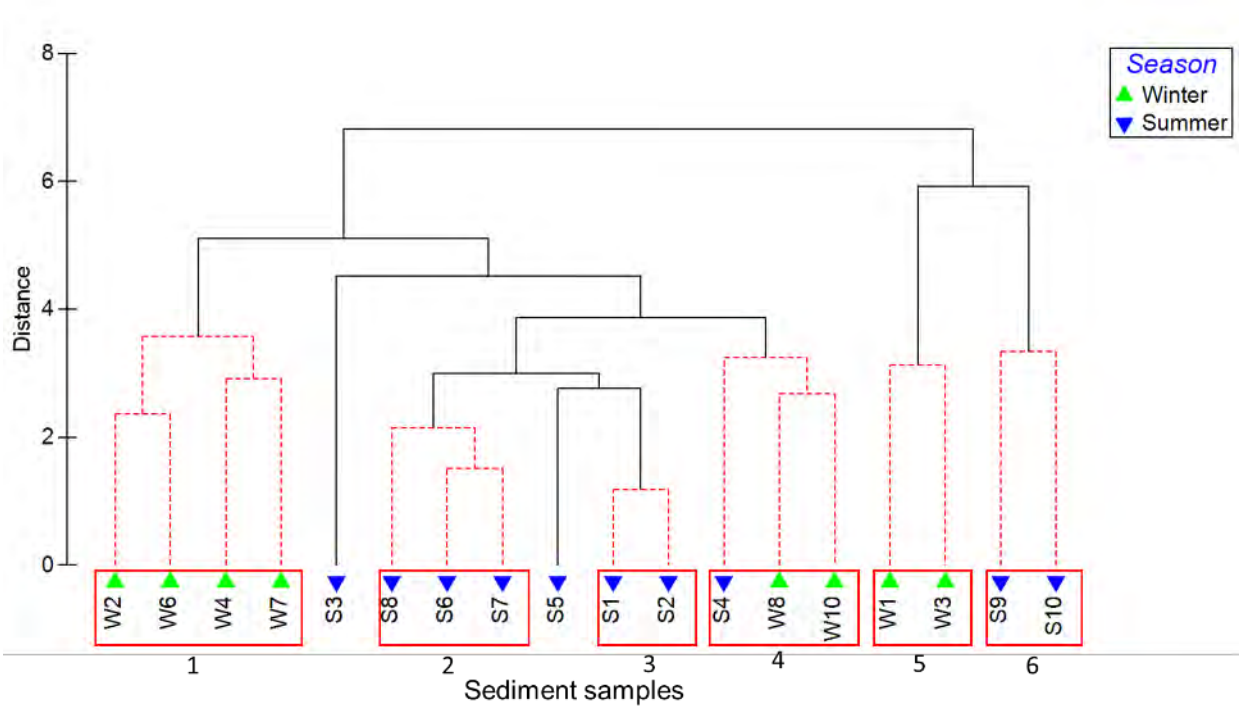


Figure 2.4. Cluster analysis with SIMPROF of sediment variables measured in 10 temporary pans across two seasons in the Khakhea Bray Transboundary Aquifer region. Red boxes denote significant cluster groups. The code per sample denotes the time of sampling and the pan number, where the first letters (S, W) represent the time of sampling (summer, winter), and the numeric values denote the sampled pan number (1 – 10).

2.3.2 Phytoplankton communities

In total, 72 phytoplankton species were identified across both seasons in the KBTA region temporary pans, which belonged to 7 major classes: Bacillariophyceae, Chlorophyceae, Cyanophyceae, Dinophyceae, Euglenoidea, Xanthophyceae and Zygnematophyceae, represented by 39 genera. Phytoplankton community composition was found to differ significantly between seasons (PERMANOVA: Pseudo–F = 14.571, df = 1, p = 0.002) and was similar among the different pans (PERMANOVA: Pseudo–F = 1.282, df = 9, p = 0.092). Winter had a high species richness (37.4 ± 3.8) compared to summer (19.8 ± 4.1). Dinophyceae and

Xanthophyceae were not recorded across all the sites in summer. In summer, Chlorophyceae (79.5 %) was the most dominant in all the pans, followed by Zygnematophyceae (12.1 %) and Bacillariophyceae (5.4 %). In winter, Zygnematophyceae (43.7%) was the most dominant, followed by Chlorophyceae (43.2%) and Bacillariophyceae (8.1%) (Figure 2.5).

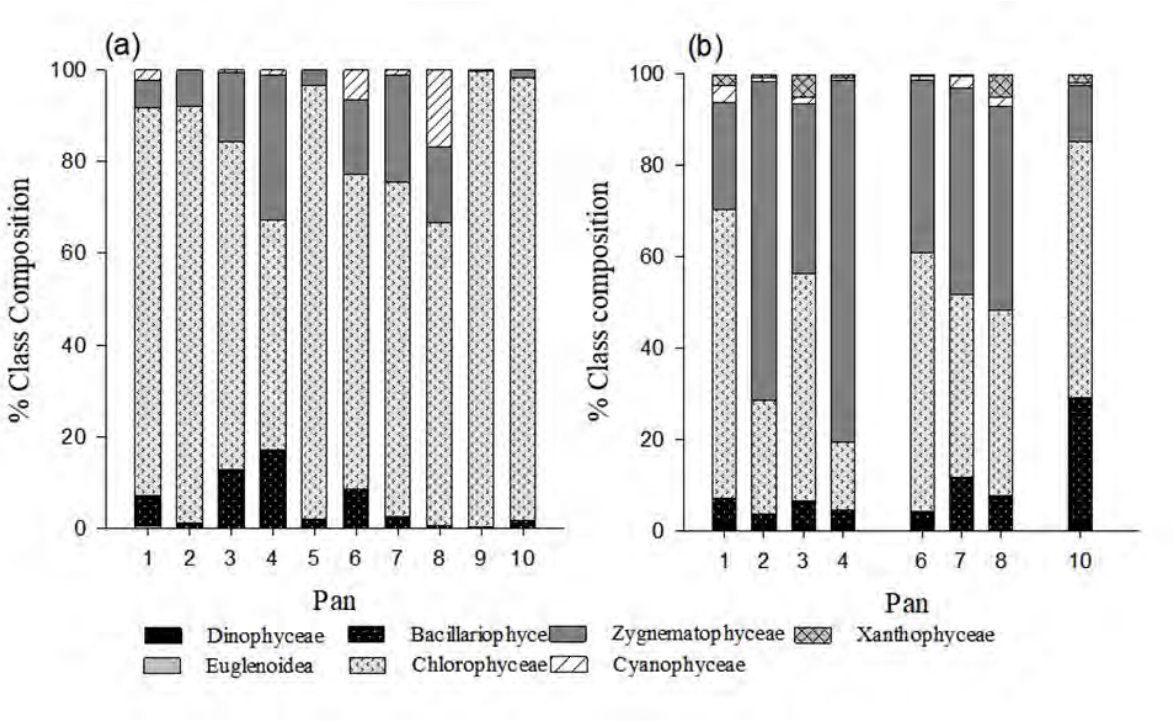


Figure 2.5. Seasonal phytoplankton class composition in temporary pans of the Khakhea Bray transboundary Aquifer region: (a) summer and (b) winter

The diversity metrics indicated no significant differences (two–way ANOVA: all $p > 0.05$) among sites, seasons or their interaction (Table A2.1). However, Shannon–Wiener (2.7 ± 0.1), Pielou's evenness (0.7 ± 0.02) and Simpson's index (0.9 ± 0.02) were all high in winter compared to summer. Species turnover was consistent between summer (β_W: 0.3 ± 0.1) and winter (β_W: 0.3 ± 0.1). Whittaker β–diversity amongst the sampled pans ranged from 0.19 to 0.55 (summer) and 0.13 to 0.53 (winter).

Table 2.3. Phytoplankton species abundance (mean ± standard deviation: individuals L^{-1}) recorded in the sampled temporary pans of the Khakhea Bray Transboundary Aquifer region in summer and winter.

Species	Abbrev.	Winter (n = 8)	Summer (n = 10)
Dinophyceae			
Ceratium hirudinella	CeraH	2 ± 2.6	
Peridinium cinctum	PeriC	39 ± 32.0	
Euglenoidea			
Euglena sp.	Eugle	2 ± 1.6	1 ± 1.7
Bacillariophyceae			
Craticula cuspidata	CratiC	48 ± 54.8	6 ± 7.4
Cymbella sp.	Cymb	4 ± 6.1	
Diatoma vulgaris	DiatV	1 ± 1.3	
Encyonopsis sp.	Encyo	5 ± 13.9	
Fragillaria ulna	FragiU	3 ± 3.9	
Gomphonema affine	GompA	16 ± 20.2	
Gomphonema gracile	GompG	4 ± 8.2	0 ± 0.3
Melosira varians	MeloV	4 ± 11.6	
Navicula sp.	Navi	145 ± 268.0	8 ± 8.7
Nitzschia elegantula	NitzsE	4 ± 2.1	1 ± 1.2
Nitzschia gracilis	NitzsG	2 ± 3.1	
Nitzschia linearis	NitzsL		22 ± 32.9
Pinnularia viridiformis	PinnuV	10 ± 7.3	2 ± 2.9
Synedra ulna	SyneU	1 ± 2.2	
Chlorophyceae			
Ankistrodesmus fusiformis	AnkisF	8 ± 14.1	
Ankistrodesmus spiralis	AnkisS	33 ± 83.7	
Asterococcus superbus	AsteroS	10 ± 26.7	
Chlorella sp.	Chlor	59 ± 59.4	0 ± 0.9
Coelastrum cambricum	CoelaC	40 ± 49.2	172 ± 342.1
Coelastrum microporum	CoelaM	0 ± 1.0	39 ± 59.8
Crucigenia quadrata	CrucigQ	18 ± 21.9	1 ± 3.9
Crucigenia rectangularis	CrucigR	44 ± 46.2	38 ± 37.8
Gleocystis gigas	GleoG	110 ± 198.5	6 ± 9.7
Micractinium pusillum	MicracP	2 ± 3.9	
Microspora flocossa	MicrosF	1 ± 2.0	
Monoraphidium griffithi	MonorG	70 ± 184.9	0 ± 1.0
Oedogonium cymatosporum	OedogC	105 ± 137.0	
Oocystis borgei	OocysB	21 ± 27.1	102 ± 179.1
Oocystis pusilla	OocysP	69 ± 168.6	
Oocystis sollitaria	OocysS	178 ± 246.3	55 ± 50.0
Pediastrum angulosum	PediasA	3 ± 3.4	67 ± 60.9
Pediastrum boryanum	PediasB	5 ± 5.9	273 ± 611.7
Pediastrum duplex	PediasD		17 ± 51.0
Pediastrum gracillimum	PediasG		71 ± 91.0

Species	**Abbrev.**	**Winter** (n = 8)	**Summer** (n = 10)
Pediastrum simplex	PediasS		5 ± 15.0
Scenedesmus arcuatus	SceneAr	235 ± 161.0	14 ± 38.3
Scenedesmus communis	SceneC	3 ± 5.5	41 ± 35.1
Scenedesmus magnus	SceneM	3 ± 4.6	10 ± 10.2
Scenedesmus microspina	SceneMc	127 ± 330.9	10 ± 21.0
Scenedesmus quadricaudata	SceneQ	60 ± 38.3	10 ± 21.0
Tetraedron caudataum	TetraC	11 ± 8.9	
Zygnematophyceae			
Closterium ehrenbergii	ClostE	45 ± 27.0	1 ± 1.8
Closterium gracile	ClostG	2 ± 4.3	2 ± 4.2
Closterium kutzingii	ClostK	13 ± 25.6	0 ± 0.3
Closterium pritchadianum	ClostP		2 ± 4.4
Cosmarium caelatum	CosmC	167 ± 116.8	
Cosmarium depressum	CosmD		2 ± 3.0
Cosmarium laeve	CosmL		3 ± 3.9
Cosmarium sportella	CosmSp	31 ± 21.8	
Cosmarium subcostatum	CosmSb	7 ± 12.9	83 ± 71.7
Cosmarium venustum	CosmV	27 ± 28.1	
Mougeotia sp.	Mouge	12 ± 16.8	1 ± 1.8
Spirogyra sp.	Spirog	1 ± 1.6	1 ± 2.7
Spondylosium moniliforme	SpondyM	2 ± 2.5	
Straurastrum alternans	StrauraAl	39 ± 14.4	1 ± 1.2
Straurastrum anatium	StrauraAn	109 ± 122.3	
Straurastrum arcuatus	StrauraAr	27 ± 40.7	
Straurastrum crenulatum	StrauraC	307 ± 278.3	1 ± 2.4
Straurastrum gracile	StrauraG		2 ± 2.9
Straurastrum lunatum	StrauraL	19 ± 25.4	
Straurastrum planctonica	StrauraP	113 ± 159.0	
Straurastrum vestitum	StrauraV	367 ± 228.2	
Zygnema sp.	Zygne	38 ± 58.7	0 ± 0.7
Cyanophyceae			
Anabaena sp.	Anaba	39 ± 13.5	1 ± 2.7
Merismopedia sp.	Merism		1 ± 1.2
Spirulina major	SpirulM		13 ± 26.2
Xanthophyceae			
Goniochloris mutica	GoniocM	50 ± 38.0	
Goniochloris spinosa	GoniocS	1 ± 3.6	
Number of taxa (N)		37.4 ± 3.8	19.8 ± 4.1
Shannon Wiener index (H')		2.67 ± 0.1	1.95 ± 0.5
Pielou's evenness (J)		0.74 ± 0.02	0.66 ± 0.1
Simpson's index		0.88 ± 0.02	0.75 ± 0.2
Whittaker β diversity		0.40 ± 0.2	0.23 ± 0.1

The cluster analysis with SIMPROF showed ten groups, but only four groups formed significant clusters ($p < 0.05$) for the phytoplankton community sampled. *Cluster Group 1*

comprised winter pans 7 and 8, dominated by the species *Crucigenia quadrata*, *Crucigenia rectangularis* and *Scenedesmus arcuatus,* while *Cluster Group 2* comprised winter pans 2 and 4 dominated by *Cosmarium caelatum*, *Cosmarium venustum*, *Mougeotia* sp., *Straurastrum anatium*, *Straurastrum crenulatum*, *Straurastrum planctonica*, *Straurastrum vestistum* and *Zygnema* sp. Summer pans 9 and 10 constituted *Cluster Group 3,* with *Coelastrum cambricum*, *Pediastrum angulosum* and *Scenedesmus communis* dominating. *Cluster Group 4* constituted summer pans 3, 4, 5, 6, 7 and 8 dominated by *Oocystis borgei*, *Pediastrum boryanum*, *Pediastrum gracillimum*, *Cosmarium subcostatum* and *Spirulina major* (Figure 2.6, Table A2.3).

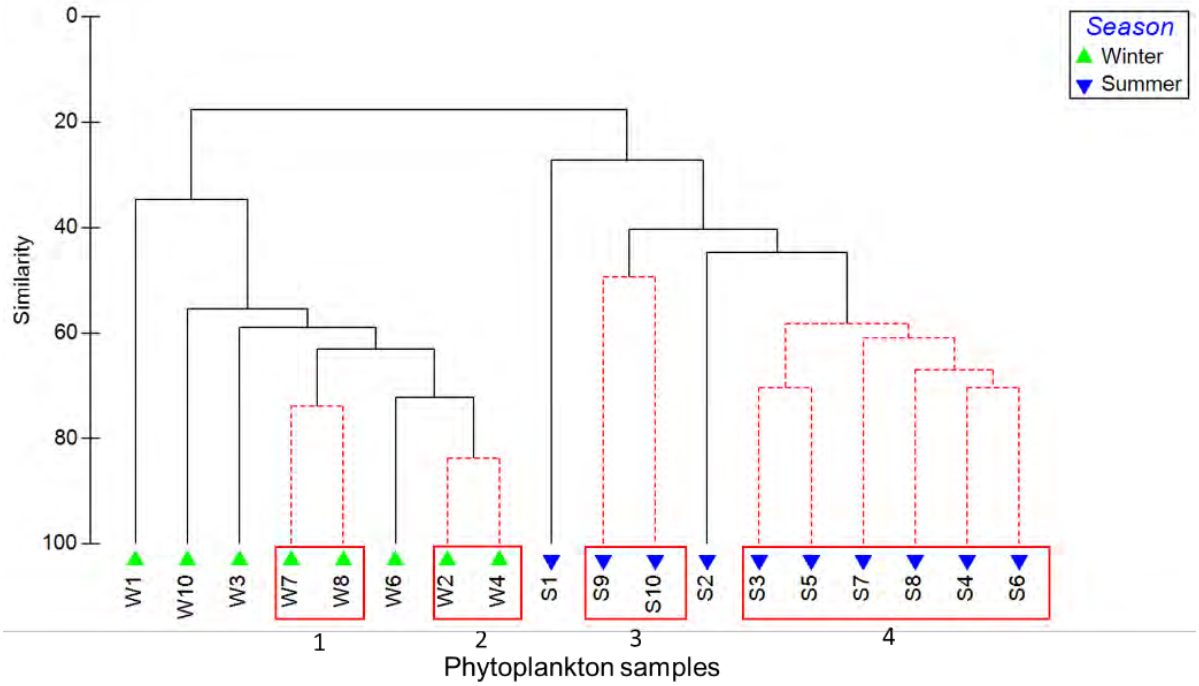


Figure 2.6. Cluster analysis of phytoplankton communities measured in temporary pans across two seasons in the Khakhea Bray Transboundary Aquifer region. Red boxes denote significant cluster groups (*Group 1*, *Group 2, Group 3, Group 4*). The code per sample denotes the time of sampling and the pan number, where the first letters (S, W) represent the time of sampling (summer, winter), and the numeric values denote the sampled pan number (1 – 10).

2.3.3 Zooplankton communities

In total, 21 zooplankton species belonging to the four major taxa, Rotifera, Copepoda, Cladocera and Ostracoda, were recorded in the temporary pans of the Khakhea Bray Transboundary Aquifer region. Significant differences were observed in the zooplankton community structure over the two seasons (PERMANOVA: Pseudo–F = 12.566, df = 1, p = 0.002) but were similar among the sampled pans (PERMANOVA: Pseudo–F = 0.944, df =9, p = 0.599). High species richness was observed in summer (10.5 ± 2.8) compared to winter (8.75 ± 1.5). In summer, the dominant taxa were Copepoda (66.2 %), Rotifera (19.2 %) and Ostracoda (9.1 %), while in winter, the dominant taxa were Copepoda (35.3 %), followed by Rotifera (23.4 %) and Ostracoda (23.4 %), while (Figure 2.7).

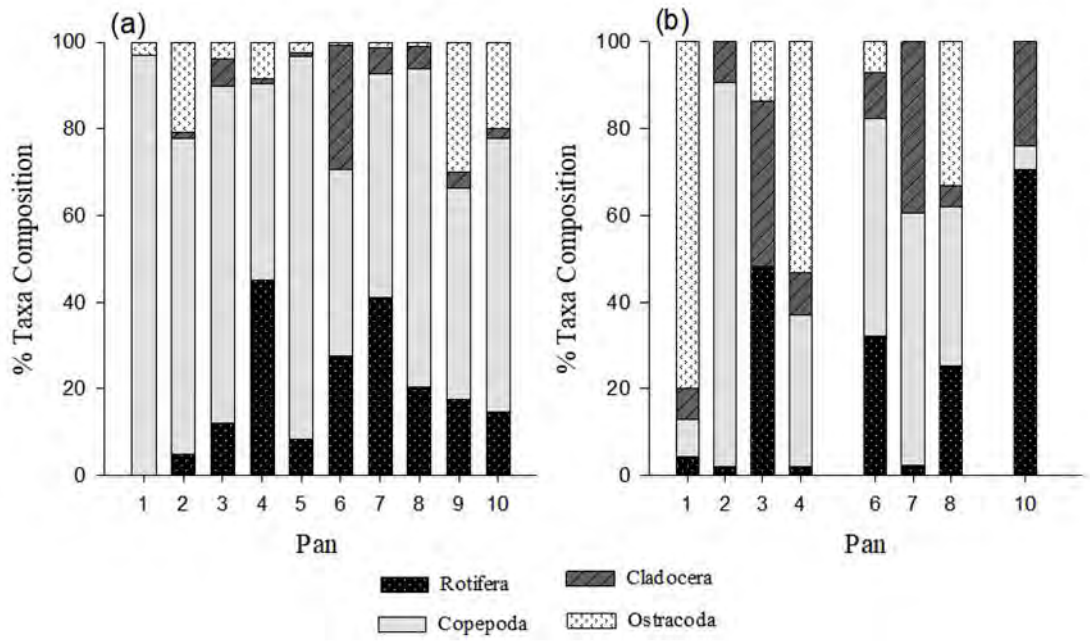


Figure 2.7. Seasonal zooplankton class composition in temporary pans of the Khakhea Bray transboundary Aquifer region (a) Summer (b) Winter

The diversity metrics were found not to be statistically ($p > 0.05$) different amongst the pans, seasons, and interactions (Table A2.2). The Shannon–Wiener index (1.5 ± 0.2), Pielou's evenness (0.7 ± 0.1) and Simpson's index (0.7 ± 0.1) were high in winter. Whittaker β–diversity ranged from 0.1–0.6 (winter) and 0.2–0.5 (summer). Whittaker β–diversity was high in winter (0.4 ± 0.2) and low in summer (0.2 ± 0.1).

Table 2.4. Zooplankton species abundance (mean ± standard deviation: individuals L^{-1}) recorded in the sampled temporary pans of the Khakhea Bray Transboundary Aquifer region in summer and winter.

Species	**Abbrev.**	**Winter** (n = 8)	**Summer** (n =10)
Rotifera			
Anuraeopsis fissa	AnurF	4 ± 2.0	0 ± 0.5
Brachionus quadridentatus f. brevispina	BranQBrev	2 ± 3.4	
Brachionus plicatilis	BranP		75 ± 58.3
Epiphanes senta	EpiphS	0 ± 0.6	0 ± 0.2
Euchlanis dilitata	EuchD	0 ± 0.5	3 ± 5.3
Keratella valga f. heterospina	KeratVH	2.4 ± 4.3	
Lecane luna	LecL	0 ± 0.7	10 ± 18.8
Lepadella ovalis	LepO	1 ± 0.5	
Mytilina sp.	Mytil	0 ± 0.3	
Trichocerca pusilla	TrichoP		16 ± 20.5
Trichocerca elongata	TrichoE		71 ± 83.7
Copepoda			
Cyclops sp.	Cycl	1 ± 1.8	94 ± 104.1
Afrocyclops sp.	Afrocycl	0 ± 0.5	4 ± 6.5
Lovenula falcifera	LovenF	7 ± 11.8	1 ± 1.9
Ectocyclops sp.	Ectocycl	0 ± 0.8	47 ± 34.3
Thermocyclops sp.	Thermocyl	2 ± 4.7	12 ± 15.6
Copepod nauplii	CopepN	29 ± 32.0	416 ± 158.2
Cladocera			
Alona sp.	Alona	1 ± 1.3	0 ± 0.7
Macrothrix spinosa	MacroS	6 ± 7.5	1 ± 2.6
Diaphanosoma excisum	DiaphaE	8 ± 8.7	58 ± 104.1
Ostracoda			
Cyprididae 1	Cypri1	20 ± 29.0	72 ± 100.9
Cyprididae 2	Cypri2	2 ± 3.2	6 ± 8.9
Number of taxa (N)		8.75 ± 1.5	10.5 ± 2.8
Shannon–Wiener index		1.51 ± 0.2	1.42 ± 0.4
Pielou's evenness		0.71 ± 0.1	0.60 ± 0.1
Simpson's index		0.71 ± 0.1	0.63 ± 0.2
Whittaker β diversity		0.40 ± 0.2	0.23 ± 0.1

The cluster analysis showed three significant cluster groups ($p < 0.05$) within the zooplankton communities. *Cluster Group 1* consisted of samples collected in summer pans 1 and 2 dominated by *Afrocyclops* sp., *Thermocyclops* sp., copepod nauplii and Cyprididae 1 and 2.

Cluster Group 2 was made up of summer pans 3, 4, 5, 6, 7, 8, 9 and 10, dominated by *Brachionus plicatilis*, *Euchlanis dilatata*, *Lecane luna*, *Trichocerca elongata*, *Trichocerca pusilla*, *Cyclops* sp., *Ectocyclops* sp., copepod nauplii, *Diaphanosoma excisum* and Cyprididae 1. *Cluster Group 3* comprised all winter pan samples, dominated by *Lovenula falcifera*, *Diaphanosoma excisum*, copepod nauplii and Cyprididae 1 (Figure 2.8, Table A2.4).

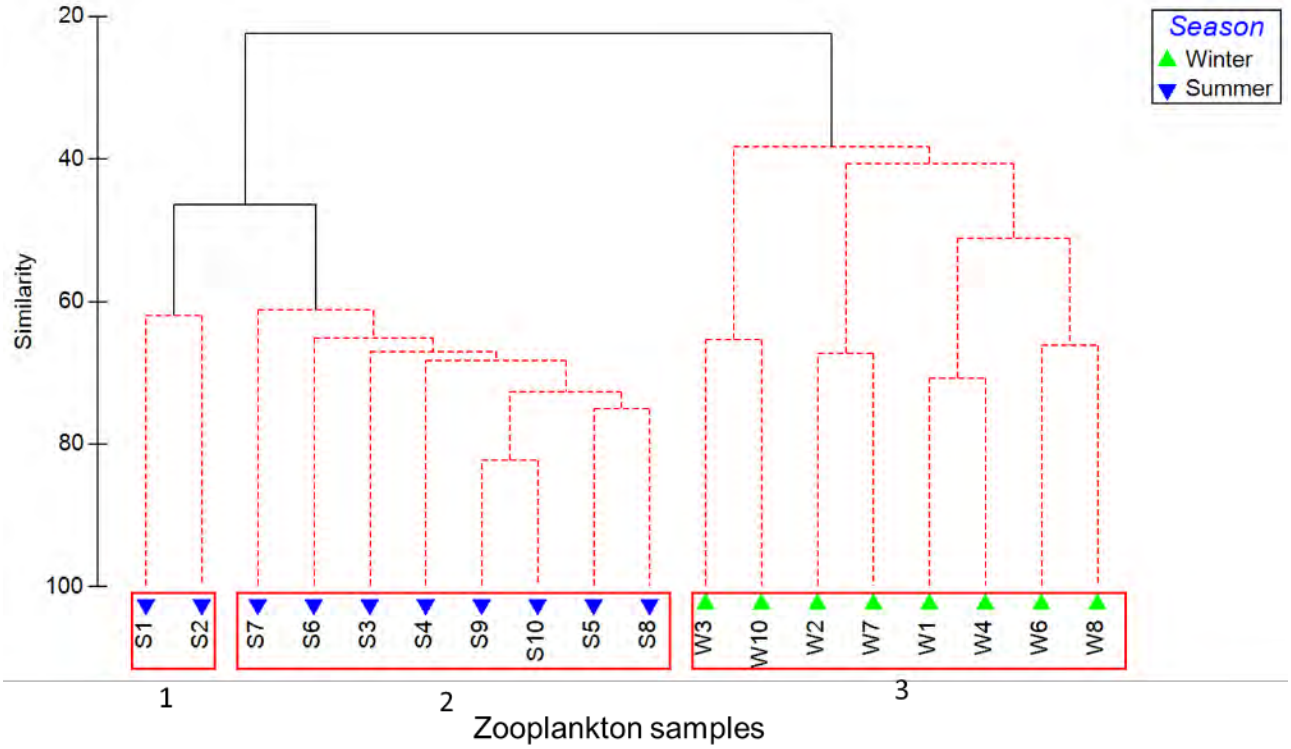


Figure 2.8. Cluster analysis with SIMPROF of zooplankton communities measured in temporary pans across two seasons in the Khakhea Bray Transboundary Aquifer region. Red boxes denote significant cluster groups (*Group 1*, *Group 2* and *Group 3*). The code per sample denotes the time of sampling and the pan number, where the first letters (S, W) represent the time of sampling (summer, winter), and the numeric values denote the sampled pan number (1–10).

2.3.4 Relationships between plankton and environmental variables

2.3.4.1 Phytoplankton communities

Ten environmental variables (i.e., temperature, conductivity, DO, pH, water phosphates, SOC, sediment phosphorous, Fe, sediment Na and zooplankton abundances were identified to have a significant impact (Monte Carlo test, $p < 0.05$) on the phytoplankton community in these temporary pans. The first four axes of the species–environmental variables accounted for 83.2% of the variation in the phytoplankton community, while RDA axes 1 and 2 explained 56.3 % and 11.7 % of the phytoplankton species variation due to environmental variables, respectively. RDA axis 1 separated the sites according to season, where the summer sites were negatively associated with axis 1 and were characterised by high temperatures, sediment phosphorous concentrations, conductivity and zooplankton abundance. The species associated with the summer sites were *Pediastrum angulosum*, *Pediastrum gracillimum* and *Scenedesmus communis*. Winter sites were positively associated with RDA axis 1 and were characterised by high SOC, DO, pH, water phosphates, sediment Na and Fe. The species associated with the winter sites were *Closterium erhernbergii*, *Goniochloris mutica*, *Crucigenia quadrata*, *Peridinium cinctum*, *Mougeotia* sp., *Zygnema* sp., *Oocystis pusilla*, *Cosmarium* species, *Scenedesmus* species and *Straurastrum* species, (Figure 2.9).

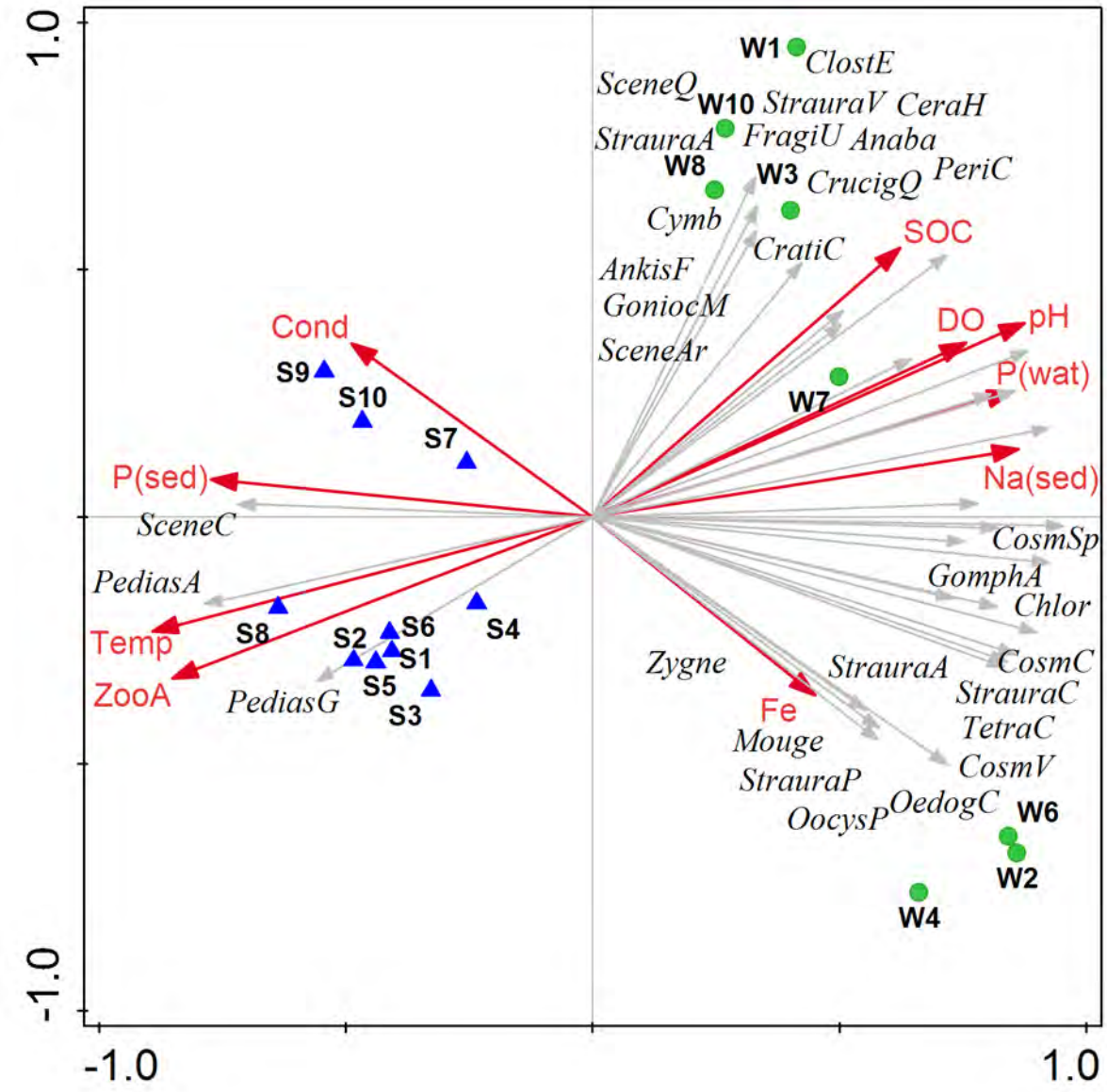


Figure 2.9. Redundancy ordination tri–plot showing the relationship between phytoplankton taxa and associated significant environmental variables collected among temporary pans in the Khakhea Bray Transboundary Aquifer region. Abbreviations: Green circles–winter season pans; blue triangles–summer season pans; W–winter; S–summer; Cond–Conductivity, P(sed) –sediment phosphorous, Temp–temperature; ZooA–zooplankton abundance, SOC–soil organic carbon, DO–dissolved oxygen, P(wat) – water phosphates, Na(sed)– sediment sodium, Fe–Iron. Species abbreviations are provided in Table 2.3.

2.3.4.2 Zooplankton communities

Eight environmental variables (i.e., temperature, DO, pH, water phosphates, SOC, Na and sediment phosphorous and phytoplankton abundances) were identified to have a significant (Monte Carlo test, $p < 0.05$) impact on the zooplankton community in the temporary pans. The first four axes of the species–environmental variables accounted for 99.0 % of the variation in the phytoplankton community, with RDA axes 1 and 2 explaining 75.3 % and 14.2 % of the zooplankton species variation due to environmental variables, respectively. The RDA axis 1 separated the sites according to season, with the axis being negatively associated with winter sites that had high SOC, DO, pH, Na, water phosphates and phytoplankton abundances. The species associated with these winter sites were *Keratella valga* f. *heterospina*, *Epiphanes senta*, *Brachionus quadridentatus* f. *brevispina*, *Lepadella ovalis*, *Anuraepsis fissa*, *Lovenula falcifera*, *Macrothrix spinosa*, *Mytilina* sp. and *Alona* sp. The RDA axis was positively associated with summer sites, which were characterised by high temperature and sediment phosphorous. The species associated with these sites were *Brachionus plicatilis*, *Ectocyclops* sp., *Afrocyclops* sp., *Thermocyclops* sp., copepod nauplii, *Diaphanosoma excisum*, *Euchlanis dilatata*, *Trichocerca elongata*, *Trichocerca pusilla*, *Lecane luna* and *Cyprididae* (Figure 2.10).

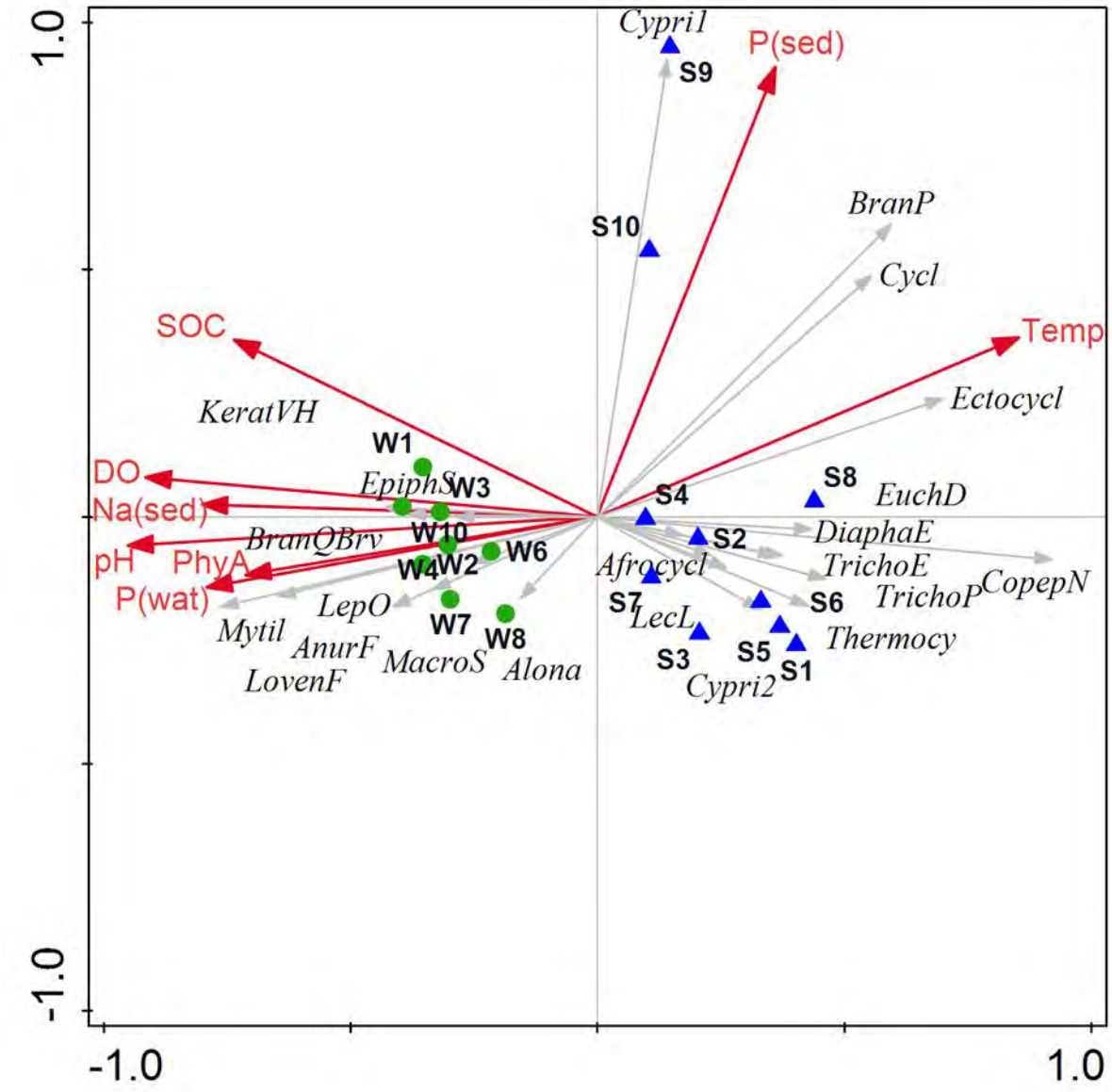


Figure 2.10. Redundancy ordination tri–plot showing the relationship between zooplankton taxa and associated significant environmental variables collected among temporary pans in the Khakhea Bray Transboundary Aquifer region. Abbreviations: Green circles–winter season pans; blue triangles–summer season pans; W–winter; S–summer; SOC–soil organic carbon, DO–dissolved oxygen, Na (sed)–sediment sodium, PhyA–phytoplankton abundance, P(wat)–water phosphates, P(sed)–sediment phosphorous, Temp–temperature. Species abbreviations are provided in Table 2.4.

2.4 Discussion

The present study highlights that plankton composition within temporary pans was coupled with seasonal changes, and in line with the first hypothesis, chlorophytes were dominant in summer but were not solely influenced by temperature but also by sediment phosphorous, conductivity and zooplankton abundance. Bacillariophyceae was not dominant in winter as predicted; instead, Zygnematophyceae was dominant and was influenced not only by pH but also by SOC, DO, water phosphates, sediment Na and Fe. Low phytoplankton species diversity was observed in summer as compared to the winter season; therefore, the second hypothesis was rejected. Additionally, in line with the third hypothesis, zooplankton diversity peaked in the summer season as expected.

2.4.1 Phytoplankton community

The study revealed a total of 72 phytoplankton species across the two seasons which was higher than the 59 species reported by Dalu et al. (2022a) in the Eastern Cape and the 34 species recorded by Anusa et al. (2012) in Zimbabwe. The sampling was conducted with a 20µm phytoplankton net and therefore there could be more picophytoplankton species below this size in these temporary pans. Observations from this study indicate seasonal changes in phytoplankton communities in temporary pans with elevated phytoplankton diversity during winter as compared to summer, which was similar to findings by Chia et al. (2011) in temporary wetlands in Nigeria. The high phytoplankton diversity could be attributed to a concentration effect by evaporation when levels of water were low at later stages of the hydroperiod, which led to an accumulation of nutrients (Nhiwatiwa and Dalu, 2017). Previous studies have shown that phosphorous and nitrogen are essential nutrients for phytoplankton growth within aquatic ecosystems. They not only contribute to increased phytoplankton biomass but also influence the taxonomic composition and abundance (Wetzel, 2001; Ojala et al., 2003; Frost et al., 2023).

The low phosphorous requirement for biomass accumulation in Chlorophyceae provides them with a competitive advantage, especially in oligotrophic conditions as those observed in these semi–arid temporary pans. Indeed, a trend was observed in our study where a heightened peak of Chorophyceae dominance coincided with low phosphate concentrations in the temporary pans in summer (Maberly et al., 2022). The lower species diversity in summer could be attributed to low nutrient concentrations due to a dilution effect because of the rains, as was observed in other studies (Adesalu and Nwankwo, 2008; Bhat et al., 2015). Zooplankton abundance was found to influence the phytoplankton species diversity in summer. Although species diversity was low in summer, the community's predominant taxon was the chlorophytes. Within aquatic ecosystems, these smaller–sized chlorophytes and diatoms have been shown as the preferred food source instead of the toxic, nutritionally inadequate cyanophytes, which often leads to high zooplankton grazing in systems where these taxa are abundant, as was the case in summer (Work and Havens, 2003; Diniz et al., 2022). The pH and conductivity levels were also found to influence the phytoplankton species communities in these pans. The significance of seasonal variation in conductivity and pH in shaping phytoplankton communities is consistent with findings by Mangadze et al. (2017), who found that both these factors drove phytoplankton community composition, particularly diatoms, suggesting that ion concentrations are key in shaping phytoplankton assemblages within these temporary systems.

2.4.2 Zooplankton community

The study recorded a total of 21 species, which was similar to other studies, such as the 24 species recorded in temporary wetlands in the Eastern Cape (Dalu et al., 2022a). The recorded species, however, were higher than the 11 species recorded by Nhiwatiwa et al. (2017) in endorheic pans in the Save Valley Conservancy in Zimbabwe. In line with observations made

by Msiteli–Shumba et al. (2017), zooplankton diversity exhibited a seasonal pattern, high in summer as compared to the winter. The high zooplankton diversity in summer could be attributed to high phytoplankton biomass associated with higher temperatures in this season. The effects of temperature on phytoplankton metabolic processes and physiology have been widely studied, revealing that elevated temperatures increase phytoplankton productivity through enhanced nutrient uptake and photosynthetic carbon assimilation, which ultimately foster increased growth rates (Lewandowska et al., 2012; Trombetta et al., 2019).

In summer, the zooplankton community was dominated by copepods, copepod nauplii, and rotifers. The high diversity of cyclopoid copepods and nauplii in summer suggests that the sampling in the pans was done just after the rains. Frisch and Green (2007) showed that within temporary wetlands, the early hydroperiod, when inundation occurs, is characterised by reduced conductivity, which facilitates high hatching rates and dominance by cyclopoid copepods and nauplii. Dominant rotifer taxa observed during summer, such as *Brachionus plicatilis*, *Trichocerca elongata*, *Trichocerca pusilla* and *Lecane luna,* are generalist feeders that consume a wide variety of food sources (Kostopoulou et al., 2012; Yin et al., 2018). Additionally, the availability of organic detritus from the previous winter season resuspended after the rains, may have provided an additional food source for the rotifers, causing them to thrive in this season (Dong et al., 2022). Species taxa that have been found within temporary freshwater pans in other studies were recorded in this study and included the cladoceran *Macrothrix spinosa*, the ostracods Cyprididae and the rotifer *Mytilina* sp. (Riato et al., 2014). Some of the species dominant in the winter season, such as *Lovenula falcifera*, *Alona* sp. and *Macrothrix spinosa,* are species that are known to tolerate a wide range of salinity levels and, as a result, would be expected to predominate in these wetlands as salinity levels change throughout the hydroperiod (Anderson and Phillips, 2016).

2.5 Conclusions

The findings from this study highlight seasonal variation in phytoplankton and zooplankton communities in temporary pans. They also show that environmental conditions and nutrient dynamics all play crucial roles in shaping the plankton community structure and abundance across different seasons within these systems. This underscores the need for further research on the diversity and distribution of temporary pan plankton communities within the local context and throughout southern Africa. Understanding the seasonal dynamics of plankton in temporary wetlands is crucial for biodiversity conservation. To gain a more comprehensive understanding of the seasonal plankton dynamics of temporary pans, it is recommended to increase the frequency of sampling in each season since temporary pans can exhibit rapid changes, especially during inundation events. More frequent sampling, therefore, allows for the capture of short–term fluctuations in environmental conditions and plankton dynamics. Moreover, the integration of plankton data with other environmental data (e.g., climate data, hydrological data) is recommended to better understand the relationships between plankton dynamics and broader ecological processes. Considering that temporary pans in semi–arid regions are particularly susceptible to environmental changes, especially climate variability, studying plankton dynamics contributes to understanding the resilience of ecosystems in these regions. Insights gained can inform conservation and management strategies to enhance the adaptive capacity of semi–arid temporary ecosystem.

CHAPTER 3: DIVERSITY OF LARGE BRANCHIOPODA (CRUSTACEA) IN SEMI–ARID TEMPORARY PANS, WITH A NEW RECORD FROM THE NORTH WEST PROVINCE, SOUTH AFRICA

"The more clearly we can focus our attention on the wonders and realities of the universe about us, the less taste we shall have for destruction."

– Rachel Carson

Plate 3. Lateral view of male *Streptocephalus namibiensis* head and frontal appendages. Photo by Chipo P. Mungenge

Abstract

Large branchiopods are considered the flagship group for endorheic temporary wetlands globally. Large branchiopod diversity and water quality were assessed from temporary pans in the Khakhea–Bray Transboundary Aquifer region, a previously unexplored area in the North west Province of South Africa. Ten freshwater pans and a salt pan were sampled across two seasons (summer and winter) in 2022. Six large branchiopod species were identified across seasons in the temporary pans in the KBTA region, namely *Streptocephalus namibiensis*, *Streptocephalus ovamboensis*, *Branchipodopsis wolfi*, *Phallocryptus spinosa*, *Triops granarius* and *Ozestheria australis*. The record of *P. spinosa* in the salt pan was a new record for North west province and South Africa. The current data was combined with previous records, revealing that to date, 13 large branchiopod species have been documented in the North west Province. *Triops granarius*, *B. wolfi*, and *O. australis* were recorded in summer only and were the most common recorded species, while *S. ovamboensis* was only recorded in winter. Species richness was high in summer as compared to winter. Large branchiopod diversity was mainly influenced by water temperature and phosphorous in summer, while sediment sodium influenced the diversity in winter. Understanding the large branchiopod communities in temporary pans can help inform conservation managers, decision–makers, and stakeholders about the importance of these understudied temporary systems.

Keywords : *Southern Africa, temporary pans, diversity, large branchiopods, aquatic invertebrates*

3.1 Introduction

Large branchiopods are enigmatic crustaceans that are often considered the "flagship group" of endorheic temporary wetlands globally (Brendonck et al., 2008; Marrone et al. 2017). They have a worldwide distribution, including polar regions such as the Antarctic Peninsula, often reaching maximum abundance and species richness in areas where temporary waterbodies are found (Brendonck et al., 2008). Although large branchiopods occur globally in a wide variety of astatic waters, southern Africa is considered a large branchiopod biodiversity hotspot (Nhiwatiwa et al., 2014; Brendonck et al., 2022a). The large branchiopods are a morphologically diverse group of ecologically important, large freshwater organisms. They are composed of the following taxa; Anostraca (fairy shrimps), Notostraca (tadpole shrimps), Laevicaudata (smooth clam shrimps) and Spinicaudata (spiny clam shrimps) and Cyclestherida (tropical clam shrimp) (Brendonck et al., 2022a).

The intermittent inundation and unpredictable nature of temporary habitats are key factors in driving hydroperiod dynamics and thereby determining the life histories of large branchiopod species within these systems (Williams, 2006). They possess characteristics such as the production of drought–resistant resting eggs, diapause, and risk–spreading strategies coupled with rapid life cycles, which enable them to persist and thrive as permanent residents of temporary waters (Brendonck, 1996). Large branchiopods are predominately planktonic or semi–benthic, show versatile feeding strategies and often outcompete the smaller zooplankton within these temporary systems (Bird et al., 2019; Brendonck et al., 2022b). Additionally, the absence of large aquatic predators reduces the predation pressure on them, allowing populations to grow (Brendonck et al., 2008; Pinceel et al., 2021). Within temporary wetlands, hydroperiod and salinity have been shown to be key factors in influencing the diversity and structure of large branchiopod communities (Waterkeyn et al., 2008; Dalu et al.,2017b;

Mdidimba et al., 2021; Mungenge et al., 2023). Climatic factors that appear to influence anostracan distribution are the annual average rainfall, rainfall season and effective temperature (Hamer and Brendonck, 1997). However, other local factors such as waterbody size, geochemical substrate properties, number of niches, habitat duration, and life history traits also influence the richness of large branchiopod species in temporary systems (Mabidi et al., 2016).

Although Brendonck et al. (2022a) approximated seventy–two large branchiopod species in the Southern African region it is expected that there should more than this since many areas where the fauna occur remain unexplored. Information on the distribution of large branchiopod species is available for diverse localities in the region, including Zimbabwe (Nhiwatiwa et al., 2014; Brendonck et al., 2015), Namibia (Hamer and Brendonck, 1995), Botswana (Brendonck and Riddoch, 1997), and the South African provinces of Free State (Vanschoenwinkel et al., 2007), Mpumalanga (Ferreira et al., 2011, 2012), Northern Cape (Buschke et al., 2012; Meyer–Milne et al., 2020), Western Cape (De Roeck et al., 2007), Eastern Cape (Mabidi et al., 2016) and KwaZulu–Natal (Hamer and Martens, 1998; Dube et al., 2020). The studies for the North west Province are few despite the region having high densities of temporary pans (Henri et al., 2014a; De Necker et al., 2016). These studies in the region help us better understand the distribution patterns of the large branchiopods; however, knowledge of the fauna cannot be considered complete as large areas remain unsampled. Studying these systems is, however, challenging. They are frequently found in remote areas characterised by limited accessibility. These areas are largely unmapped, and unpredictable rainfall patterns hinder the predictability of hydroperiod dynamics (Hamer and Brendonck, 1997). The large branchiopods are temporary specialists, and since their populations are exclusively limited to these habitats, they have suffered dramatically from the significant loss of temporary wetlands in human–modified landscapes (Brendonck et al., 2008; Dalu et al., 2016). This underscores the imperative need to

generate more information on species distributions and diversity, allowing proper conservation of the different large branchiopod species and maybe uncovering of new species. This chapter, therefore, presents insights into the large branchiopod species, physical and chemical characteristics of temporary pans in the Khakhea Bray Transboundary Aquifer (KBTA) region in the North west Province of South Africa. This area had remained largely unexplored until now, and this investigation encompassed sampling of various temporary freshwater pans, as well as a salt pan, over the summer and winter seasons to provide a comprehensive overview of the region's large branchiopod communities.

3.2 Materials and methods

3.2.1 Study area

A detailed description of the study area is provided in Chapter 2. The study was undertaken in January 2022 (summer) and May 2022 (winter) in the KBTA region of the North west Province of South Africa. This study sampled ten freshwater pans and one salt pan of various sizes. In summer, all 10 freshwater pans and the salt pan were sampled; however, in winter, only 8 out of 10 of the pans contained water and were sampled, and the salt pan was not sampled.

3.2.2 Environmental variables

3.2.2.1 Water chemistry

In each freshwater pan during each sampling event, temperature (°C), pH, conductivity (µS cm^{-1}), turbidity (NTU), total dissolved solids (TDS; mg L^{-1}), salinity (ppm), dissolved oxygen (DO; mg L^{-1}) were measured at three points in each pan at a depth of 1 m from the surface using an AquaRead multiparameter meter (Model AP–700 and AP–800, AquaRead Ltd, UK). In the salt pan measurements were taken once at the centre of the pan in summer. Three 250 mL water samples were collected from each freshwater pan and the salt pan, stored on ice and

analysed within 8 h of sample collection for nutrients. Nutrients (i.e., phosphates, ammonium) were analysed using a multiparameter benchtop photometer (i.e., Hanna Instruments Model HI83300, Hanna Instruments Inc., Rhode Island) whereby ammonium (photometer range 0–10 ± 0.04 mg L^{-1} accuracy, resolution 0.01 mg L^{-1}) was determined through the adaptation of the ASTM Manual of Water and Environmental Technology (D1426) Nessler method and phosphates (photometer range 0–30 mg L^{-1} ± 1.0 mg L^{-1} accuracy, resolution of 0.1 mg L^{-1}) through the adaptation of the Standard Methods for the Examination of Water and Wastewater, amino acid method (ASTM International, 2003; Rice and Bridgewater, 2012).

Chlorophyll–*a* concentrations were determined by taking two 250 mL water samples from the freshwater pans and the salt pan in summer. These were vacuum filtered through a 0.7 µm Whatman glass fibre filter (GF/F) and the GF/F filters were placed individually in small labelled aluminium foil bags and stored on ice in the field. The filters were then stored at –20 °C until extraction in the laboratory. Each GF/F filter was extracted in 10 mL of 90 % acetone in the dark over 24 h. Chlorophyll–*a* concentration was then determined fluorometrically using a Turner 10AU fluorometer. Absorbances were taken for each sample before and after adding two drops of 1 N HCl (Holm–Hansen and Riemann, 1978). Chlorophyll–*a* concentrations were then calculated following the Environmental Protection Agency (EPA) method 445.0 (Arar and Collins, 1997).

3.2.2.2 Sediment chemistry

During both sampling events, two sediment samples were collected randomly from each freshwater pan. No sediment samples were collected from the salt pan. A plastic hand shovel was used to collect the sediment from the top 5–10 cm of the sediment layer, which was then placed into a new labelled polyethylene Ziplock bag. The Ziplock bags were stored on ice in a

cooler box and transported to the laboratory for further analysis. In the laboratory, the sediment samples were oven–dried at 60 °C for 72 h before being crushed using a porcelain mortar and pestle. The sediment was then sieved with a mesh size of 0.05 mm to remove any plant roots and other debris present in the sample. The sediment samples were then sent to BEMLAB, a South African National Accreditation System–certified laboratory in South Africa for further processing. The following were elements analysed: (i) *nutrients*; nitrogen (N) and phosphorus (P) using a SEAL Auto Analyzer, (ii) *cation elements*; potassium (K), calcium (Ca), boron (B), magnesium (Mg), sodium (Na) using the acid digestion method, with the metal content being determined using an ICP–OES optical emission spectrometer (Varian, Mulgrave, Australia) and (iii) *heavy metals*; iron (Fe), zinc (Zn), manganese (Mn) and copper (Cu) were measured using an ICP– OES optical emission spectrometer (Varian, Mulgrave, Australia).

3.2.3 Branchiopod sampling

Branchiopod samples were collected from the freshwater pans during both the summer and winter seasons and also from the salt pan during the summer season. At each of the pans, branchiopod samples were collected using zooplankton nets (40 cm diameter, 64 µm mesh size). Horizontal tows were accomplished by towing the plankton net subsurface through the water for 10 m. After collection, the branchiopod samples were immediately preserved in 70 % ethanol. The samples were then taken to the Wetland Ecology Laboratory at the Department of Zoology and Entomology, Rhodes University. Species determination and enumeration were done using an Olympus stereo microscope. Branchiopods were identified to the lowest taxonomic unit possible using dichotomous keys, and for the anostracans, only mature adult males and females could be identified to the lowest taxonomic unit (Day et al., 1999).

3.2.4 Data analysis

A Kruskal–Wallis ANOVA was used to determine differences in the environmental variables measured between freshwater pans in summer and winter in STATISTICA version 14.0.0.15. Multivariate analysis was done to detect and visualise similarities in the large branchiopod samples in relation to environmental variables. Before analysis, all the environmental variables except pH data were log (x+1) transformed to reduce skewness in the data. A preliminary detrended correspondence analysis (DCA) was applied to the data set to examine gradient lengths. Since the response data were compositional and had a gradient of 3.0 SD (Standard Deviation) units long, the unimodal ordination method was therefore used (Ter Braak and Verdonschot, 1995). A canonical correspondence analysis (CCA) was thus used to explore the relationship between the large branchiopod species and the predictor variables from the different pans sampled in both seasons. The CCA is a constrained linear ordination method in which forward selection Monte Carlo permutation tests (9999 unrestricted permutations, $p < 0.05$) are used to test the significance of the axis and therefore determine if selected environmental variables can explain much of the large branchiopod species variation. The software CANOCO version 5.02 was used for the analysis (Ter Braak and Šmilauer, 2002). The salt pan sampled was not included in this analysis.

Distance–based Permutational Analysis of Variance (PERMANOVA; Anderson, 2001) was used to analyse whether the large branchiopod total counts and environmental variables differed between sampling sites (i.e., 10 pans) and the two sampling times (i.e.,summer and winter) using PERMANOVA+ in PRIMER version 6 (Anderson et al., 2008). Euclidean distance and Bray Curtis dissimilarities were employed for environmental and biological data, respectively, and 9 999 permutations were used to test for significance.

3.3 Results

3.3.1 Environmental variables

In the freshwater pans, there were significant differences in the water temperature, pH, dissolved oxygen and sediment Na ($p < 0.001$) between the two seasons (Table 1). During summer, conductivity (191.8 ± 40.8 µS cm^{-1}), turbidity (91.9 ± 266.2 NTU), TDS (125.7 ± 28.5 mg L^{-1}) and chlorophyll–*a* (0.08 ± 0.05 mg L^{-1}) exhibited high values when compared to winter. pH (9.5 ± 1.3), DO (7.1 ± 0.8 mg L^{-1}), ammonium (0.4 ± 0.3 mg L^{-1}) and phosphate (0.5 ± 0.5 mg L^{-1}) concentrations were high in winter in comparison to summer. However, for the sediment P (48.23 ± 61.8 mg kg^{-1}), K (144.6 ± 172.8 mg kg^{-1}), Ca (8.81 ± 7.4 mg kg^{-1}), Mg (1.3 ± 0.9 mg kg^{-1}), Na (0.08 ± 0.04 mg kg^{-1}), Cu (1.86 ± 1.3 mg kg^{-1}), Zn (2.38 ± 2.2 mg kg^{-1}), Mn (122.9 ± 200.6 mg kg^{-1}), B (0.26 ± 0.2 mg kg^{-1}), Fe (82.96 ± 123.5 mg kg^{-1}) and S (21.99 ± 46.6 mg kg^{-1}) were high in winter. In contrast, sediment pH (7.73 ± 0.3) and soil organic carbon (0.67 ± 0.4 mg kg^{-1}) were high in summer (Table 3.1). All the environmental variables measured were found to differ significantly between seasons (PERMANOVA: Pseudo–F = 4.948, $p = 0.007$) and among pans (PERMANOVA: Pseudo–F = 2.071, $p = 0.038$). The salt pan has a pH of 9.68, conductivity of 56.52 (µS cm^{-1}), turbidity of 569 (NTU), TDS of 36.4 (mg L^{-1}), salinity of (36.9 ppm) and dissolved oxygen concentration of 7.81 (mg L^{-1}). Phosphate levels in the pan were too low to be detected however the ammonia concentration in the pan was 1.99 ± 0.6 mg L^{-1} and the chlorophyll–*a* concentration was 0.312 ± 0.1 mg L^{-1}.

Table 3.1. Summary of Kruskal–Wallis ANOVA of measured environmental variables (Mean ± standard deviation) between freshwater pans sampled in January and May 2022 in the Khakhea Bray Transboundary Aquifer region. Significant values ($p < 0.001$) are indicated in bold.

Environmental variables	**Summer** ($n = 10$)	**Winter** ($n = 8$)	*p*
Water			
Temperature (ºC)	26.62 ± 1.4	22.26 ± 0.9	**<0.001**
pH	7.98 ± 0.4	9.45 ± 1.3	**<0.001**
Conductivity (µS cm^{-1})	191.77 ± 40.8	173.38 ± 91.6	0.0914
Turbidity (NTU)	91.88 ± 266.2	69.21 ± 120.8	0.9105
Total dissolved solids (mg L^{-1})	125.7 ± 28.5	111.54 ± 60.4	0.0756
Salinity (ppm)	0.06 ± 0.01	0.06 ± 0.04	0.0674
Dissolved oxygen (mg L^{-1})	4.15 ± 1.4	7.08 ± 0.8	**<0.001**
Ammonium (mg L^{-1})	0.36 ± 0.1	0.42 ± 0.3	0.7897
Phosphates (mg L^{-1})	0.10 ± 0.1	0.54 ± 0.5	0.0084
Chlorophyll– *a* (mg L^{-1})	0.08 ± 0.05	0.04 ± 0.08	0.0209
Sediment			
pH	7.73 ± 0.3	7.26 ± 0.8	0.8583
Resistance (Ω)	1961.50 ± 648.3	2171 ± 1517	0.1549
Stone (%)	2.39 ± 3.3	7.73 ± 5.1	0.0087
Phosphorous (mg kg^{-1})	47.28 ± 45.1	48.23 ± 61.8	0.9292
Potassium (mg kg^{-1})	139.6 ± 132.2	144.6 ± 172.8	0.4239
Calcium (mg kg^{-1})	8.25 ± 4.8	8.81 ± 7.4	0.1826
Magnesium (mg kg^{-1})	1.27 ± 0.9	1.3 ± 0.9	0.4499
Sodium (mg kg^{-1})	0.02 ± 0.01	0.08 ± 0.04	**<0.001**
Copper (mg kg^{-1})	1.54 ± 1.1	1.86 ± 1.3	0.7221
Zinc (mg kg^{-1})	0.95 ± 0.5	2.38 ± 2.2	0.5938
Manganese (mg kg^{-1})	66.85 ± 86.1	122.9 ± 200.6	0.6569
Boron (mg kg^{-1})	0.23 ± 0.2	0.26 ± 0.2	0.1198
Iron (mg kg^{-1})	29.00 ± 14.8	82.96 ± 123.5	0.5940
Soil organic carbon (mg kg^{-1})	0.67 ± 0.4	0.49 ± 0.4	0.2481
Sulphur (mg kg^{-1})	7.70 ± 4.1	21.99 ± 46.6	0.0444

3.3.2 Large branchiopod diversity

Five species of large branchiopods were identified from the freshwater pans in January and May 2022 in the KBTA region, namely the anostracans *Streptocephalus namibiensis* Hamer and Brendonck, 1993, *Streptocephalus ovamboensis* Barnard, 1924 and *Branchipodopsis wolfi* Daday, 1910 (sensu Hamer and Appleton, 1996), the notostracan *Triops granarius* (Lucas, 1864) and the spinicaudatan *Ozestheria australis* (Lovén, 1847). The species collected from each pan are listed in Table 3.2. *Triops granarius*, *B. wolfi* and *O. australis* were recorded in summer only while *S. ovamboensis* was recorded in winter only. *Streptocephalus namibiensis* and unidentified juvenile anostracans were, however, recorded in both summer and winter. In summer *T. granarius* and *B. wolfi* were recorded in 80 % of the sampled pans with *T. granarius* in high numbers in Pan 10 while *B. wolfi* numbers were high in Pan 5. *Streptocephalus namibiensis* was recorded in 40 % of the pans with high numbers being recorded in Pan 7. *Ozestheria australis* was recorded in 80 % of the pans sampled but found in high numbers in Pan 9 while the juvenile anostracans were recorded in all of the pans in high numbers in summer. During winter *S. namibiensis* and juvenille anostracans were found in 10 % of the sampled pans while *S.ovamboensis* was recorded in 20 % with Pan 2 with high numbers. Overall, there was high species richness in summer as compared to winter. Pan 3 and 4 had the highest species richness in summer while Pan 5 had the highest in winter with 2 species recorded. (Table 3.2). The coexistence of at least two or more large branchiopod species was found during both sampling events in the pans. Large branchiopod total counts were found to differ significantly among seasons (PERMANOVA: Pseudo–F = 3.572, $p = 0.010$) but were similar among the sampled pans (PERMANOVA: Pseudo–F = 0.889, $p = 0.783$).

Table 3.2. Occurrence of large branchiopod species collected from temporary pans in the Khakhea Bray Transboundary Aquifer Region. + indicates the taxa was present in a particular pan, and in winter most of the pans did not have large branchiopods

	Species							
	Summer							
Pan	***Triops granarius***	***Anostraca juvenilles***	***Branchipodopsis wolfi***	***Streptocephalus namibiensis***	***Streptocephalus ovamboensis***	***Ozestheria australis***	***Phallocryptus spinosa***	***Total***
1	+	+	+					3
2	+	+	+			+		3
3	+	+	+	+		+		5
4	+	+	+	+		+		5
5	+	+	+			+		4
6		+	+					2
7		+		+		+		3
8	+	+		+				2
9	+	+	+			+		4
10	+	+	+			+		4
Salt pan							+	1
	Winter							
2	–	–	–	–	+	–	–	1
5	–	–	–	+	+	–	–	2
10	–	+	–	–	–	–	–	1

3.3.3 Current distribution records for North west Province

Table 3.3 summarises large branchiopod species recorded in the North west Province, incorporating information from existing literature, prior studies and data obtained from this study. The widespread species *Ozestheria australis* previously recorded in the province was observed in 70 % of the sampled pans during this study. *Branchipodopsis wolfi* a species not previously documented in the province was identified in 80 % of the pans in this study. *Streptocephalus namibiensis* another species not previously recorded in the province was found

in 40 % of the sampled pans. *Streptocephalus ovamboensis* recorded by Wasserman et al. (2022) was also recorded in this study. However, other previously documented large branchiopod species in the species including *Carinophallus ornata*, *Eocyzicus obliquus*, *Gondwanalimnadia costata*, *Streptocephalus cafer*, *Streptocephalus* cf. *indistinctus* and *Streptocephalus macrourus* were not observed in this study. From the salt pan, only the anostracan species *Phallocryptus spinosa* (H. Milne–Edwards, 1840) was recorded (Figure 1; Table 3). This finding represents a new record for both the province and South Africa, as this species had not been previously documented in the country. It was originally classified under the genus *Branchinella* but was later reassigned to the genus *Phallocryptus*, along with other morphologically similar species (Rogers, 2003, 2006). No other large branchiopod species were identified in the salt pan during the study.

Table 3.3. Distribution records for large branchiopods in the North west Province of South Africa. Species that were not previously recorded in the province are indicated with an asterisk.

Species	Locality	Year Collected	Reference
Branchipodopsis sp.	Delareyville	2013	Foster et al. (2015)
			De Necker et al. (2016)
Branchipodopsis wolfi Daday, 1910 *	KBTA region	2022	This study
Carinophallus ornata (Daday, 1910)	Potchefstrom	1994	Hamer (1999)
Eocyzicus obliquus (Sars, 1905)	Potchefstrom	–	Brendonck (1999)
Gondwanalimnadia costata Rogers, Rabet and Weeks 2016	Vryburg	1918	Rogers and Meyer–Milne (2022)
Ozestheria australis (Loven 1847)	KBTA region	–	Brendonck (1999)
		2022	This study
Phallocryptus spinosa (H–Milne Edwards 1840) *	Salt pan	2022	This study
Streptocephalus cafer (Loven 1847)	Batsalano Game Reserve	–	Daniels et al. (2004)
Streptocephalus cf. *indistinctus* Barnard 1924	KBTA region	2022	Wasserman et al. (2022)
Streptocephalus macrourus Daday 1908	North west/Botswana border		Hamer (1999)
Streptocephalus namibiensis Hamer and Brendonck 1993 *	KBTA region	2022	This study
Streptocephalus ovamboensis Barnard 1924	KTBA Region	2022	Wasserman et al.(2022)
		2022	This study
Triops granarius (Lucas 1864)	Delareyville	2013	De Necker et al.(2016)
	KBTA region	2022	This study

Figure 3.1. A photo of a male and female *Phallocryptus spinosa* sampled from a salt pan in the Khakhea Transboundary Aquifer region.

3.3.4 Relationship between large branchiopods and environmental variables in freshwater pans

Based on the CCA analysis, three environmental variables (i.e., sediment Na, water temperature and phosphates) were found to have a significant effect (Monte Carlo permutation test, $p < 0.05$) on the large branchiopod community structure in the freshwater pans (Figure 3.2). The CCA axes 1 and 2 accounted for 38.6 % of the total variation in the large branchiopod composition, with CCA axis 1 explaining 34.6 %. The CCA axis 1 and 2 clearly separated the sampled pans into 2 clear groups based on the two sampling events, summer and winter (Figure 3.2). High sediment Na concentrations and low water phosphates characterised temporary pans in winter. Large branchiopod species associated with these sites were the anostracan *S.*

ovamboensis and juveniles. The second group consisted of the pans sampled in the summer season, which were negatively associated with CCA axis 2 and low water temperatures. The species associated with these sites were *T. granarius*, *O. australis*, *S. namibiensis* and *B. wolfi* (Figure 3.2).

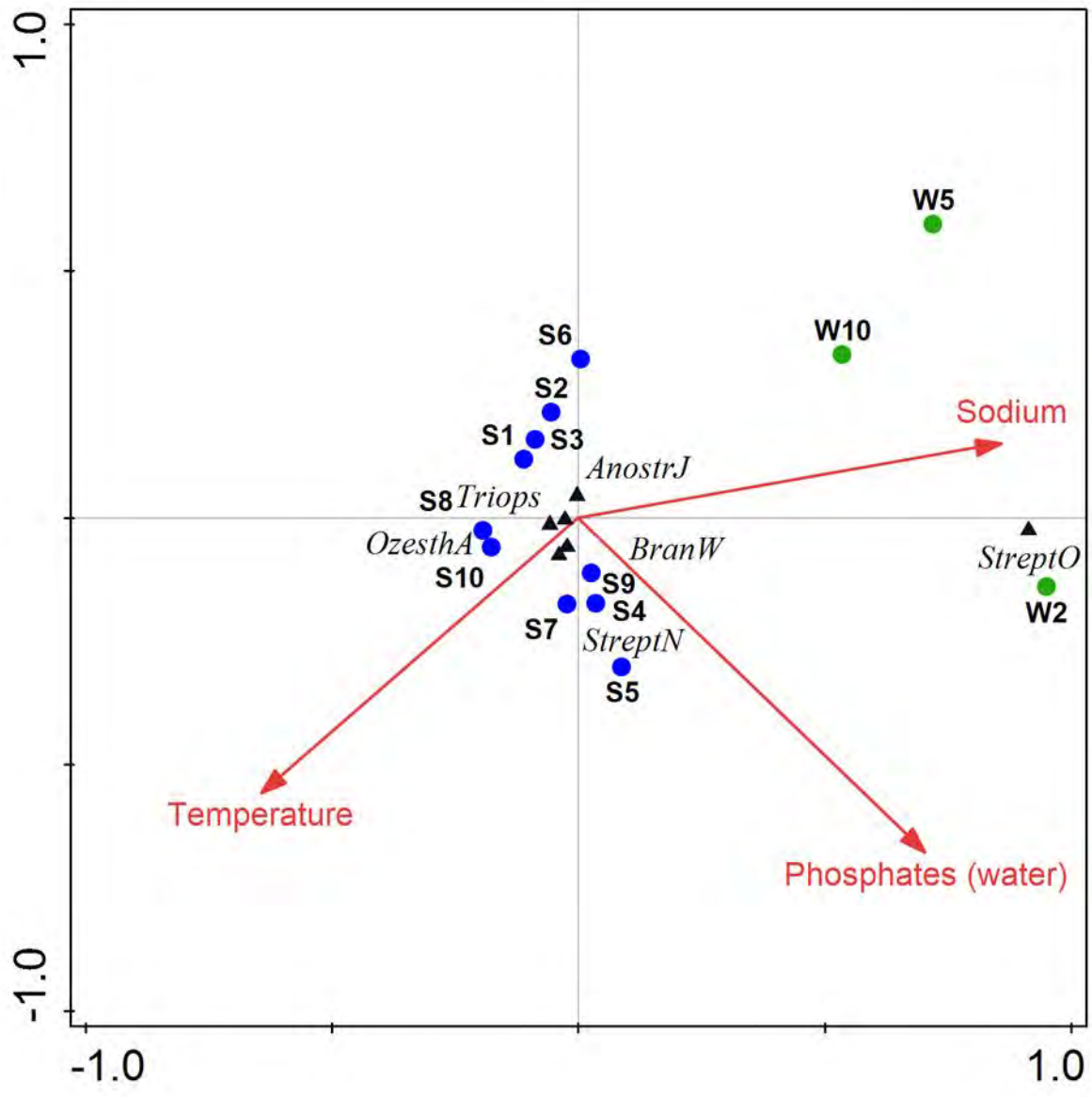


Figure 3.2. CCA ordination plot showing the relationship between measured significant environmental variables with the large branchiopod species. Abbreviations: black triangles – species; blue circles –summer season pans; green triangles – winter season pans; J–January; M–May. Abbreviations: AnostrJ– Anostracan juvenilles; BranW– *Branchipodopsis wolfi* ;

OzesthA– *Ozestheria australis*; StrepN– *Streptocephalus nambibiensis*; StrepO– *Streptocephalus ovamboensis*; Triops–*Triops granarius*.

3.4 Discussion

3.4.1 Previous and current distribution records for the North west province

Previous records in the North west Province documented 10 large branchiopod species, and this current study expands upon this, identifying a total of 6 species, including the noteworthy record of *Phallocryptus spinosa,* bringing the total in the province to 13 species. This high diversity is comparable to other regions such as Lake Manyara Basin in Northern Tanzania, which had 6 species (Kalufa et al., 2023), the Drakensberg region with 11 species (Hamer and Martens, 1998), the Western Cape Province where 14 species were recorded (De Roeck et al., 2007) and the Save Valley Conservancy in Zimbabwe with 16 species (Nhiwatiwa et al., 2014). While slightly less diverse than the Eastern Cape Province, where 22 species were recorded (Mabidi et al., 2016), the North west's 13 species reveal a rich branchiopod fauna.

Gondwanalimnadia costata was last recorded in the North West Province in Vryburg over a century ago. The scarcity of recent records may suggest it may be missed in surveys due to its short adult activity period. However, this species has been recorded in various locations within the Northern Cape Province (Rogers and Meyer–Milne, 2022). Previously, *Streptocepahlus ovamboensis* eggs were documented for the region (Wasserman et al. 2023), but the presence in the province was reconfirmed in this study. Both *Triops granarius* and *Ozestheria australis* had been documented in the province before and were both found in 70 % of the sampled pans. Both species are habitat generalists and have been found in various areas of different physicochemistry (Mabidi et al., 2016).

3.4.2 New distribution records for North West Province

Streptocephalus namibiensis was recorded from this study, a species not previously documented in the province and was initially recorded in the Makgadikgadi pan area in Botswana by Hamer and Brendonck (1993). Subsequent studies have expanded its known distribution to the Transvaal Highveld (South Africa) and central and northern Namibia (Brendonck and Riddoch, 1997). Additionally, this study found the presence of *Branchipodopsis wolfi* in 80% of the temporary pans in summer. Foster et al. (2015) had previously recorded only *Branchipodopsis* sp. in Delareyville. This species exhibits life history adaptations such as rapid growth rates, early maturation and the frequent production of small broods of resting eggs in 4–6 days after hatching. These adaptations likely allow the species to compensate for egg loss from the egg bank due to hatching before pools dry out and contribute to its wide distribution (Hamer and Appleton, 1991; Hamer and Appleton, 1996; Brendonck et al., 2000). Moreover, their flexible feeding behaviour involving both filter feeding and substrate scraping with phyllopods allows them to take up particles which are not in suspension and persist even in low nutrient conditions such as that found in these pans (Hamer and Martens, 1998).

The occurrence of *Phallocryptus spinosa* in the salt pan particularly stands out since the species has mostly been palearctic in its distribution. It has also been recorded from saline waters in Eurasia, Greece, the Mediterranean area and North Africa. This halophilic anostracan is known to inhabit shallow brackish, saline and hypersaline waters with sparse vegetation (Thiéry and Puente, 2002; Amarouayache, 2014; Rais and Amarouayache, 2018). *Phallocryptus spinosa* is a filter feeder, but larger forms were observed by Abatzopoulos et al. (2000) to take in large prey like the cladocerans, copepods and anostracan eggs. *P. spinosa* exhibits a remarkable tolerance to salinity, having been recorded in environments with salinities ranging from 0–65

ppt (Abatzopoulos et al., 2000). The study reveals its occurrence in a salt pan with salinity levels exceeding 36 ppt, a condition where most crustaceans typically experience reduced hatching success beyond 2.5 ppt (Mungenge et al., 2023). This finding suggests a potential competitive advantage for *P. spinosa* in high–salinity environments and may explain why it was the only branchiopod species in the pan. Southern Africa has a wide distribution of inland salt systems that are understudied (Seaman et al., 1991a). The distribution of *P. spinosa* is extremely disjunct, and until this study, the only recorded occurrence of the species south of the Sahara was in the Makgadikgadi Pans in Botswana (Brendonck and Riddoch, 1997; Hulsmans et al., 2006). Unlike observations in other regions where *P. spinosa* co–occurs with *Artemia*, this study did not record any other species alongside *P. spinosa* in the salt pan (Samrayoui et al., 2006; Timms, 2009; Amayourache, 2014). The only other halophilic species documented in the province was Artemiidae, which was recorded in a temporary pan in Delareyville in high numbers (Foster et al., 2015).

3.4.3 Environmental factors and large branchiopod composition

Among the environmental variables examined, only water temperature and phosphate concentrations emerged as significant factors in shaping the large branchiopod assemblages in the KBTA region in the summer season. This was consistent with findings by Waterkeyn et al. (2009), who found that total phosphorus concentration was one of the significant factors explaining large branchiopod species richness in temporary wetlands. The influence of water temperature on the seasonal variation of aquatic invertebrate community structure was shown previously in a study by De Necker et al. (2016), with species such as *T. granarius* specifically favouring higher temperatures (Seaman et al., 1991b). Overall, high species diversity was observed in summer as compared to winter. In contrast, during the winter season, sediment Na concentration was identified as influential in shaping the large branchiopod species. This was

likely due to its impact on habitat salinity and subsequent effects on osmoregulation in large branchiopods (James et al., 2003). The distinct temporal occurrence patterns of *S. nambiensis* in summer and *S. ovamboensis* in winter suggest temporal variations in niche quality related to prevailing weather conditions which influence suitable conditions for hatching of each species (Waterkeyn et al., 2009). Hatching is highly variable within the same batch of mixed dormant egg banks as an adaptation to ensure the persistence of populations in a variable and unpredictable environment. This allows the species to successfully coexist through the storage effect where different species may be favoured at different seasons or years (Brendonck and De Meester, 2003; Vanschoenwinkel et al., 2010b).

Large numbers of juvenile anostracans dominated the summer season samples suggesting that the sampling was done early on in the hydroperiod. Hatching has been shown to occur over several days as long as conditions are favourable (Brendonck, 1996). For the majority of these species, a high peak of hatching occurs around the first three days (Mungenge et al., 2023). The high hatching rates allow a high chance of the successful production of eggs before the pan dries out (Vanschoenwinkel et al., 2010a). This same trend was also observed before by Jocque et al. (2010), and this early recolonisation was attributed to an efficient filtering system of the anostracans, thereby effectively monopolising resources immediately after the flooding of the pans.

3.5 Conclusions

The large branchiopod data generated in this study creates baseline information on the KBTA region while also simultaneously expanding the biodiversity database for the North West Province. However, substantial temporal variability in these habitat conditions poses challenges in predicting large branchiopod species richness throughout the year, with certain

species potentially present in some inundations but not in others. To ensure a comprehensive assessment, future investigations should employ repetitive sampling efforts at each inundation and across various inundations to account for these temporal variations in species occurrence, given the distinct phenologies of large branchiopods. A large number of the sampled temporary pans were in close proximity to human settlements and are susceptible to potential wetland degradation. Consequently, future studies must extend to other areas in the region. These studies will play a crucial role in informing conservation managers, decision–makers, and stakeholders, thereby aiding in the development of informed conservation strategies and sustainable land management practices for these temporary systems where these large branchiopod species are found.

CHAPTER 4: TROPHIC DYNAMICS IN SEMI–ARID TEMPORARY PAN SYSTEMS USING STABLE ISOTOPE ANALYSES

"I didn't want to just know names of things. I remember really wanting to know how it all worked."

–Elizabeth Blackburn

Plate 4. *Triops granarius* recorded in the Khakhea Bray Transboundary Aquifer region temporary pans. Photo by Chipo P. Mungenge

Abstract

Trophic dynamics between organisms underpin the ecological functioning and evolution of biological communities. In aquatic ecosystems, most research has focused on permanent systems resulting in limited knowledge of the food web structures of temporary systems particularly within the global south. In the face of climate change, many permanent systems are projected to become temporary systems. In this study we assessed the overall food web structure in temporary pans in the Khakhea–Bray transboundary aquifer region of South Africa, using stable isotope analyses. We used carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) stable isotope analyses to infer the trophic positions of key organisms in the pans and the overall food web structure in these systems. The dominant consumers were predatory insects (*Sigara* sp., *Anisops* sp., *Lestes* sp., *Rhantus* sp. and *Cybister* sp. adults and larvae) and *Tomopterna* sp. tadpoles. The diets of these taxa were determined using Stable Isotope Mixing Models and trophic niche overlaps were also assessed using Bayesian mixing models in R. The analyses revealed that the food web had four trophic levels with the top predators being the notonetids *Anisops* sp. The primary consumers were the molluscs and tadpoles. The dystiscids occupied the intermediate levels and had generalist diets with a preference for *Streptocephalus* sp. Additionally, high niche overlaps were found between the notonectids and the dytiscids indicating potential competition for limited resources. Overall, the food web structure exhibited mid–stage trophic structural complexity highlighting the importance of temporary systems in sustaining diversity for aquatic communities.

Keywords: *trophic structure, food web, temporary pans, niche, trophic position, invertebrates*

4.1 Introduction

Understanding the food web structure of aquatic ecosystems is highly relevant as trophic dynamics underpin the ecological functioning and evolution of biological communities (Doi et al., 2012; Cuthbert et al., 2022). Ecological research on food web dynamics in African freshwater ecosystems has been primarily focused on permanent systems (O'Reilly et al., 2002; Campbell et al., 2003; Hart, 2013; Peel et al., 2019). Despite some ecological similarities between the permanent and temporary systems, most freshwater studies lack integration with other types of ephemeral systems (O'Neill and Thorp, 2014). Extreme environmental fluctuations in the temporary systems drive community dynamics, with most organisms found in permanent systems being absent from the temporary ecosystems (Williams, 2006). As such, community dynamics in temporary aquatic ecosystems are somewhat unique, with implications for their food web structure and functioning.

These ecosystems support a diverse range of terrestrial and aquatic invertebrates, amphibians, plants, and foraging birds (Ferreira et al., 2012; Dalu et al., 2016; Bird et al., 2019). The varying hydroperiods in these ecosystems result in fluctuations in water levels, rendering them only temporarily available as habitats for aquatic organisms (Cuthbert et al., 2022). The water level fluctuations have implications for the physical and chemical properties of the water and drive environmental heterogeneity, with notable implications on the food web structure and trophic interactions in these systems (De Meester et al., 2005). Ephemeral pans with their small size can serve as critical models for testing wider community ecology theories and understanding the intricacies of trophic interactions within these unique ecosystems. (De Meester et al., 2005). Given the within–system variability of these environments, food web dynamics in temporary pans can be complex and variable, including strong phenological processes associated with hydroperiod dynamics (Wasserman et al. 2018). Most of the aquatic biota associated with these

ecosystems, including zooplankton and macroinvertebrates, have specific adaptations that allow them to persist through the dry phases (Bird et al., 2019; Brendonck et al., 2022a, b; Dube et al., 2020). In the past these temporary pans have been depicted as 'predator–free' but evidence shows that predation in the temporary pans by both amphibians and various invertebrates is a vital mechanism regulating community structure within temporary pans (Brendonck et al., 2002). Food webs in these temporary systems are generally short, however, further into the hydroperiod, the food web lengthens and becomes more complex in response to insect predator arrival (Dalu, 2017a).

Food webs can be intricate maps that offer a simplified depiction of the numerous network of consumer–resource interactions and feeding relationships that exist among living organisms. (Pimm et al., 1991; Polis and Winemiller, 2013). It is important in community ecology to understand the diet composition of organisms as the utilisation of resources by these organisms is key in shaping the population interactions within the community (Shalloof and Khalifa, 2009). To generate data on the food items consumed by an organism and eventually to understand food preferences, traditional approaches such as gut content analysis have been used (Hyslop, 1980). This approach provides short–term dietary data of the recently ingested items. It also requires high sampling frequency and offers a minimal indication of the degree of assimilation (Shalloof and Khalifa, 2009). In certain instances, some food categories which are chitinous in nature may be overemphasized since they are not easy to digest (Baker et al., 2014).

Stable isotope analysis has, however, emerged as an important cost–effective tool for analysing food web structures because it provides an understanding of the trophic interactions between different organisms over time and space which aids in the development of trophic structure

models (Layman et al., 2012; Whiteman et al., 2019). It also provides unbiased information on the food sources that are assimilated by the consumer (De Necker et al., 2020). When all organisms are sampled, stable isotope analysis has the potential to elucidate complex interactions in ecological communities, through the incorporation of trophic level and food web paradigms into food web ecology. Additionally, it also gives a measure of the energy flow and trophic positions of the organisms in different pathways (Post, 2002). In this study, we used the stable carbon ($\delta^{13}C/\delta^{12}C$) and nitrogen ($\delta^{15}N/\delta^{14}N$) isotope ratios (referred to as $\delta^{13}C$ and $\delta^{15}N$, respectively) to examine the temporary pan system communities. Standard $\delta^{15}N$ values can be used to estimate the trophic position of an organism by showing the stepwise enrichment in trophic transfers (Layman et al., 2012). The ratio of carbon isotopes ($\delta^{13}C$) varies so little with trophic transfers therefore, $\delta^{13}C$ is used to determine the original sources of the dietary carbon and the overall diet preferences of the consumers (Post, 2002). The carbon and nitrogen isotopic composition of consumer tissues is thus determined by the following factors: the $\delta^{15}N$ and $\delta^{13}C$ of each prey species; the relative proportions of the prey species assimilated, and the isotopic fractionation associated with converting prey tissue into consumer tissue. Furthermore, the stable isotope signatures of tissues generally reflect the diet during the time the tissue was synthesised (Bearhop et al., 2002). Using both $\delta^{13}C$ and $\delta^{15}N$ values of 'bulk' animal tissues, such as muscle or whole animals, as in the case of small invertebrates, can reflect their diet, trophic position, and physiological state (Whiteman et al., 2019). These values can also be used to calculate individual and population dietary niche metrics, as well as community measures of food chain length and food web structure (Layman et al., 2007b; Perkins et al., 2014).

While globally prevalent, southern Africa has one of the highest densities of temporary pans in the world, with these temporary systems contributing to increased regional diversity (De Necker et al., 2020). Their prevalence in the region offers an opportunity to study food web

dynamics, relevant to existing and future temporary systems around the world given that many permanent freshwater ecosystems are projected to become more temporary in the face of climate change (Tonkin et al., 2019). The purpose of this study was to apply the use of stable isotope analyses at the community level in a previously unexplored part of South Africa, attempting to assess the nature of trophic links and their importance in community structure within temporary pan systems. More specifically, the objectives of this research were (a) to assess the overall food web structure in the temporary pans in the Khakhea Bray transboundary aquifer region in the North west Province in South Africa (b) to assess the trophic interactions and diet proportions in the dominant invertebrates and tadpoles in the pans and c) to assess niche breadth and overlap among these dominant faunal consumer groups in the pans. We predicted that (1) the trophic levels of the food web of these temporary pan systems would be limited as compared to those of permanent systems. We further predicted (2) that the dominant consumers in the pans which include the predaceous insects and tadpoles would adhere to generalist diets because of how prey availability changes with the alternating hydroperiods. We finally predicted that (3) there would be high niche overlap among the dominant consumers because of the limited food resources in the temporary pans.

4.2 Materials and methods

4.2.1 Study area

The study was done in January 2022 in five pans located in the Khakhea Bray transboundary aquifer (KBTA), North west Province of South Africa (Chapter 2). Water and stable isotope samples were collected from Pans 2, 4,5 ,8 and 10.

4.2.2 Physical and chemical variables

From each pan, temperature (°C), pH, conductivity (µS cm^{-1}), turbidity (NTU), TDS (mg L^{-1}), salinity (ppm), dissolved oxygen (mg L^{-1}) were measured at three points in each pan at a depth of 1 m from the surface using an AquaRead multiparameter meter (Model AP–700 and AP–800, AquaRead Ltd, UK). Three water samples (250 mL) were collected at each pan and stored on ice and were analysed within 8 h of sample collection. Nutrients (i.e., phosphates and ammonium) were analyzed using a multiparameter benchtop photometer (Hanna Instruments Model HI83300) (Hanna Instruments Inc., Rhode Island) whereby ammonium (Photometer range 0–10 ± 0.04 mg L^{-1} accuracy, resolution 0.01 mg L^{-1}) was determined through the adaptation of the ASTM Manual of Water and Environmental Technology, D1426, Nessler method, phosphates (Photometer range 0–30 mg L^{-1} ± 1.0 mg L^{-1} accuracy, resolution of 0.1mg L^{-1}) through the adaptation of the Standard Methods for the Examination of Water and Wastewater, amino acid method and nitrates (Photometer range 0–30 ± 0.5 mg L^{-1} accuracy, resolution, resolution 0.1 mg L^{-1}) through an adaptation of the cadmium reduction method (Wood et al., 1967; ASTM International, 2003; Rice et al., 2012) Concentrations were determined within 8h of water sample collection. Additionally, two samples of 250 mL each were collected from each pan and filtered through a 0.7 µm Whatman glass fibre filter (Ø= 47mm). The filters were then placed in plastic Ziplock bags and stored on ice in the field. The filters were then stored at –20 °C until extraction. Each filter was extracted in 10 mL of 90 % acetone in the dark for 24 h. Chlorophyll–*a* concentration was then determined fluorometrically using a Turner 10AU fluorometer. Absorbances were taken for each sample before and after adding two drops of 1 N HCl (Holm–Hansen and Riemann, 1978; Gusha et al., 2021). Chlorophyll–*a* concentrations were then calculated following the Environmental Protection Agency (EPA) method 445.0 (Arar and Collins, 1992):

$$chl - a\ (mg\ L^{-1}) = \left(\frac{a}{V}\right) \times (F_o - F_a)\ \times C$$

Where chl–*a* (mg L^{-1}) is the chl–*a* concentration in mg L^{-1}, "*a*" is the quantity of acetone used for extraction in mL, V is the quantity of water filtered in mL, F_o is the chl–*a* reading before acidification with 1 N hydrochloric acid (HCl), F_a is the chl–*a* reading after acidification with 1 N HCl, and C is the constant value of 0.325.

4.2.3 Stable isotope sample collection

Samples for stable isotope analysis were collected in the rainy season (January 2022) when the pans were filled with water, and we expected that this period coincided with high species diversity. From each of the five pans, three surface water samples (30 – 40 cm depth) of 250 ml, were collected for the determination of the particulate organic matter (POM). The water was filtered through pre–combusted (60 ºC, 12h) 0.7 µm Whatman GF/F filters (Ø= 47mm) with the aid of a vacuum pump. Sediment samples (*n* = 3) were randomly collected from each of the five pans. Sediment was collected using a plastic hand shovel and placed into new labelled polyethylene Ziplock bags. Macrophytes and detritus (*n* = 3) were collected by hand along the littoral zones of each pan. Terrestrial plants were also collected by hand from nearby riparian vegetation and placed in plastic Ziplock bags. At each pan, macroinvertebrates were sampled using a standard D–net (30 × 30 cm aluminium rim, 1 mm mesh size, attached to a 1.5 m handle). The net was submerged in the water, and macroinvertebrates were collected by sweeping along a 10 m transect. The macroinvertebrates were then placed in plastic Ziplock bags. For the frog samples, toe clips from each adult individual and fin clips from the tadpoles were taken and placed in individual 1.5 mL Eppendorf tubes for further processing and analysis in the laboratory. All macroinvertebrate and frog species collected were identified to the lowest taxonomic level using identification keys (Du Preez, 2017; Channing and Rődel, 2019; Fry, 2022). All samples were then stored separately for each pan, placed in labelled Ziplock bags, and kept on ice in a cooler box.

4.2.4 Stable isotope sample processing and analysis

All samples were oven dried at 60 ºC for 48 h and then ground into a fine homogenous powder using a pestle and mortar. The carbon and nitrogen ratios of the samples were analysed at the Stable Isotope Laboratory, Mammal Research Institute, University of Pretoria, South Africa using a Flash EA 1112 Series coupled to a Delta V Plus stable light isotope ratio mass spectrometer via a ConFlo IV system (Thermo Fischer, Bremen, Germany). Two laboratory running standards (Merck Gel: $\delta^{13}C$ = – 20.26 ‰, $\delta^{15}N$ = 7.89 ‰, C% = 41.28, N% = 15.29 and DL–Valine: $\delta^{13}C$ = – 10.57 ‰, $\delta^{15}N$ = – 6.15 ‰, C% = 55.50, N% = 11.86) were used, and a blank sample was run after every 12 unknown samples. Standard delta notation (δ) was used to express stable carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$). The isotope ratios in parts per thousand (‰) differences from a standard material were expressed as follows:

$$\delta^{13}C \text{ or } \delta^{15}N \text{ (‰)} = \left[\left(\frac{R_{sample}}{R_{standard}}\right) - 1\right] \times 1000$$

Where R is $^{13}C/ ^{12}C$ or $^{15}N/ ^{14}N$ respectively. The average analytical precision was <0.15 ‰ for δ^{13} C and <0.1 ‰ for $\delta^{15}N$.

For the baseline $\delta^{15}N$ values we used the molluscs in the pans, represented by the snail *Lymnaea truncatula*. The mollusc was used since it has a relatively long–life span and falls under trophic level 2, as a primary consumer. This is assuming that primary producers will then fall under trophic level 1. The calculated average baseline $\delta^{15}N_{baseline}$ was 5.1 ± 0.9 ‰ and –17.9 ± 3.2 ‰ for $\delta^{13}C_{baseline}$. The trophic positions of consumers in the pans were then estimated using the following equation:

$$Trophic\ position = \frac{\delta^{15}N_{organism} - \delta^{15}N_{baseline}}{\Delta^{15}N} + 2$$

Where $\delta^{15}N_{organism}$ is the isotope ratio of the organism, $\delta^{15}N_{baseline}$ is the isotope ratio of the primary consumer used as the baseline, $\Delta^{15}N$ is the fractionation factor calculated as 3.23 ‰ and the 2 represents the trophic position of the baseline organism (Post, 2002).

4.2.5 Data analysis

The data was tested for normality and homogeneity of variance and a one–way ANOVA test was used to compare the $\delta^{13}C$ and $\delta^{15}N$ values of the basal sources and the consumers using the software package STATISTICA version 14.0.0.15 (TIBCO Software Inc., 2020). Values of $\delta^{13}C$ and $\delta^{15}N$ were used from selected different basal sources and prey based on literature for the potential food sources (Table 4.1). In this study five invertebrates from different orders i.e. Hemiptera (*Sigara* sp., *Anisops* sp.), Coleoptera (*Rhantus* sp., *Cybister* sp., *Rhantus* sp. and *Cybister* sp. larvae) and Odonata (*Lestes* sp.,) together with *Tomopterna* sp. tadpoles in the 5 pans were considered. Adult frogs were not included in the models as they are semi–aquatic and a great proportion of species feed on land. Terrestrial anthropods constitute a great proportion of their diets (Du Preez, 2017), and as they were not sampled in this study, adult frogs could not be included. To estimate the relative contribution of prey to the diet of the dominant consumers (i.e., *Sigara* sp., *Anisops* sp., *Rhantus* sp., *Cybister* sp., *Lestes* sp., *Rhantus* sp. larvae, *Cybister* sp. larvae) and *Tomopterna* sp. tadpoles, we used an R package SIMMR (Stable Isotope Mixing Models in R, Parnell and Inger, 2019). The trophic enrichment factors were set at 1.3 ± 0.4 ‰ for carbon and 3.4 ± 1 ‰ nitrogen, respectively (McCutchan et al., 2002). A comparison of resources used by consumers was conducted by comparing the 95 % credibility limits of each prey source. The model was run with a Markov chain Monte Carlo (MCMC) parameter of three chains of 300 000 iterations, a burn–in phase of 200 000 and a thinning of 100. Both residual and process error and individuals as a random effect were

included in the model. The Bayesian model was used to generate potential dietary solutions as true probability distributions by incorporating families of multiple consumer sources (Parnell et al., 2010).

Table 4.1. Most common food sources for the dominant consumers sampled from the Khakhea Bray Transboundary Aquifer

Dominant consumer	**Potential food sources**	**References**
Sigara sp.	Algae, POM, detritus, culicid larvae	Fernando, 1959; Reynolds, 1975; Popham et al., 1984; Nam et al., 2000; Chuanren and Lizhen , 2005;Alahmed et al., 2009
Anisops sp.	Zooplankton, culicid larvae, corixids, anostracans	Scott and Murdoch, 1983; Streams 1992; Gilbert and Burns, 1999; Brendonck et al., 2002; Shaalan et al., 2007;Buxton et al. 2020;
Rhantus sp.	Zooplankton, aquatic invertebrates, tadpoles, culicid larvae	Aditya and Saha, 2006; Aditya et al., 2006; Culler et al., 2014
Cybister sp.	Zooplankton, aquatic invertebrates, tadpoles, culicid larvae	Ohba and Takagi, 2010; Culler et al., 2014
Lestes sp.	Culicid larvae	Lee, 1967; Kaunisto et al., 2017
Dytiscidae larvae (*Rhantus* and *Cybister* sp. larvae)	Zooplankton, Aquatic invertebrates, tadpoles, culicid larvae	Ohba, 2009; Yee, 2010
Tomopterna tadpoles	Algae, detritus	Altig et al., 2007; Ocock et al., 2019

To examine the trophic niche dynamics of the dominant invertebrate consumers and the tadpoles in the temporary pans, we used two common metrics: the TA (total area of the convex hull, i.e., the convex hull area enclosing all the individual points) and the SEA (standard ellipse

area). We calculated the TA and the SEA using the $\delta^{13}C$ and $\delta^{15}N$ values of the dominant consumers with the stable isotope Bayesian ellipses in R (SIBER) model in SIAR (Layman et al., 2007a; Jackson et al., 2011; Jackson et al., 2012) to determine the trophic niche width and breadth of each species at each pan. We also calculated estimates for SEA small sample size corrected (SEA_c) to improve the accuracy of the SEA values with a small sample size correction that was done as indicated by the small letter '*c*' (Jackson et al., 2011). This SEA_c area provides a bivariate measure of the mean isotopic niche. The calculated isotopic niche breadths and widths for the consumers were represented by convex hulls and ellipses. The metrics were not calculated for Pan 5 since it only contained *Tomopterna* sp. tadpoles from the selected dominant consumers considered in this study.

4.3 Results

4.3.1 Physical and chemical variables

Temperatures in the pans were warm with low and high temperatures being observed in Pan 2 (25.15 ± 2.3 ºC) and Pan 10 (29.14 ± 0.5 ºC), respectively. The pH in all the pans was generally slightly alkaline during the sampled period (Table 4. 2). Turbidity was low across all the pans. The measured conductivity and total dissolved solids were high in Pan 8 (Table 4.2). Low salinity levels (< 0.1 ppm) were recorded across all five pans. Dissolved oxygen was high in pan 4 (6.32 ± 0.4 mg L^{-1}). Ammonium concentrations were high in Pan 10 (0.43 ± 0.05 mg L^{-1}) and Pan 2 (0.40 ± 0.04 mg L^{-1}). Pan 5 had a high phosphate concentration of 0.35 mg L^{-1}. Chlorophyll–*a* concentration in the pans was low with Pan 8 having a high concentration (0.14 ± 0.03 mg L^{-1})

Table 4.2. Mean ± SD summary of physical and chemical variables in the five pans in January 2022

Variable	Pan 2	Pan 4	Pan 5	Pan 8	Pan 10
Temperature (°C)	25.15 ± 2.3	26.23 ± 0.9	26.70 ± 0.6	28.31 ± 1.4	29.14 ± 0.5
pH	7.95 ± 0.5	8.22 ± 0.2	7.82 ± 0.1	7.91 ± 0.2	8.19 ± 0.1
Conductivity (µS cm^{-1})	153.67 ± 13.0	174.67 ± 20.9	188.33 ± 47.6	233.33 ± 3.4	224.33 ± 10.0
Turbidity (NTU)	0	0	0	9.33 ± 1.2	19.20 ± 2.1
Total dissolved solids (mg L^{-1})	99.33 ± 8.3	114.0 ± 14.2	122.0 ± 26.8	151.0 ± 2.2	145.67 ± 7.4
Salinity (ppm)	0.05 ± 0.0	0.06 ± 0.009	0.06 ± 0.008	0.07 ± 0.02	0.07 ± 0.005
Dissolved oxygen (mg L^{-1})	2.86 ± 0.9	6.32 ± 0.4	3 ± 1.0	2.25 ± 0.07	5.45 ± 0.3
Ammonium (mg L^{-1})	0.40 ± 0.04	0.19 ± 0.03	0.26 ± 0.01	0.36 ± 0.04	0.43 ± 0.05
Phosphates (mg L^{-1})	0	0	0.35 ± 0.35	0	0
Chlorophyll–*a* (mg L^{-1})	0.02 ± 0.003	0.08 ± 0.003	0.07 ± 0.004	0.14 ± 0.03	0.06 ± 0.009

4.3.2 Stable isotope composition

A total of 283 samples were collected for stable analysis within the 5 temporary pans. Among these samples, 105 comprised the basal sources, while the remaining samples represented consumers (Table 4. 3). The basal sources consisted of POM, sediment, detritus, macrophytes and terrestrial vegetation. The $\delta^{13}C$ of POM and detritus was –20.63 ± 2.61 ‰ and –24.01±1.62 ‰, respectively, while sediment had a $\delta^{13}C$ of –20.95 ± 1.91 ‰. Sediment had a high $\delta^{15}N$ of 8.44 ± 1.03 ‰ followed by detritus (8.12 ± 0.81 ‰) and POM (6.44 ± 2.19 ‰). The macrophytes had values of $\delta^{13}C$ ranging from – 30.4 to –27.9 ‰ with relatively low $\delta^{15}N$ values of 4.5–8 ‰. *Lagarosiphon* sp. had a high $\delta^{13}C$ of 8.0 ± 1.4 ‰, while *Potamogeton* sp. had a high $\delta^{15}C$ value of –27.9 ± 0.5 ‰. Among the terrestrial plants, *Trifolium repens* had a high $\delta^{13}C$ of –18.8 ± 7.4 ‰ and $\delta^{15}N$ of 9.6 ± 0.5 ‰. *Vachellia* sp. had low $\delta^{13}C$ of –30.7 ± 0.3 ‰ and *Vachellia grandicornuta* had low $\delta^{15}N$ of 0.9 ± 0.5 ‰.

A one–way ANOVA revealed significant differences between the $\delta^{13}C$ ($F = 77.494$, $df = 1$, $p < 0.05$) and the $\delta^{15}N$ ($F = 41.789$, $df = 1$, $p < 0.05$) values of the basal sources and the consumers in the 5 pans. Macroinvertebrates exhibited $\delta^{13}C$ values ranging from –23.6 to –17.8‰ and $\delta^{15}N$ ranging from 5.1–11.2 ‰. *Streptocephalus* sp. had the lowest $\delta^{13}C$ of –24.7 ± 1.0 ‰ and *Lymnaea truncatula* had the lowest $\delta^{15}N$ of 5.1 ± 0.9 ‰. Among all the macroinvertebrates, *Anisops* sp. had the highest $\delta^{13}C$ of –17.8 ± 4.7 ‰. Amphibians collected from the pans had $\delta^{13}C$ values ranging from –23.5 to –18.6 ‰ and the $\delta^{15}N$ ranged from 6.0–11.9 ‰. *Pyxicephalus adspersus* and *Tomopterna* sp. had high $\delta^{13}C$ of –18.6 ‰. *Tomopterna* sp. tadpoles had high $\delta^{15}N$ of 11.9 ± 0.3 ‰.

4.3.3 Trophic positions

Four trophic levels were identified from the pans with adult frogs and the notonectids at the top of the sampled food web at trophic position 4. The tadpoles and the macroinvertebrates had a wide range of values but mostly occupied trophic position 3. The clam shrimps, ostracods, molluscs and mosquito larvae were in trophic position 2 (Table 4.3; Figure 4.1).

Table 4.3. Carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) stable isotope values (mean ± standard deviation) of basal sources, invertebrate groups and frog species sampled in five pans in the Khakhea Bray Transboundary Aquifer in January 2022. TP = Trophic position, *n* = number of samples.

	Pan 2				Pan 4			Pan 5				Pan 8				Pan 10		
Group	***n***	**TP**	**δ13C**	**δ15N**	***n***	**TP**	**δ13C**	**δ15N**	***n***	**TP**	**δ13C**	**δ15N**	***n***	**TP**	**δ13C**	**δ15N**	***n***	**TP**	**δ13C**	**δ15N**
Basal Sources																				
POM	3		–23.5 ± 0.1	5.6 ± 0.4	3		–22.9 ± 1.4	4.9 ± 0.7	3		–18.6 ± 1.5	5.6 ± 0.1	3		–18.0 ± 2.6	5.5 ± 0.6	3		–20.9 ± 0.6	10.9 ± 0.7
Detritus	3		–25.0 ± 2.2	8.2 ± 1.3	3		–23.1 ± 0.6	8.0 ± 0.5	–		–	–	–		–	–	–		–	–
Sediment	2		–22.9 ± 1.6	10.3 ± 1.8	3		–20.3 ± 1.1	7.7 ± 0.8	3		–18.7 ± 1.8	8.1 ± 0.7	3		–21.7 ± 0.5	8.5 ± 0.4	3		–21.9 ± 0.4	8.4 ± 0.3
Potamogen sp.									5		–27.9 ± 0.5	4.5 ± 0.5	–		–	–	–		–	–
Lagarosiphon sp.	3		–30.4 ± 0.1	8.0 ± 1.4	3		–29.3 ± 0.6	7.0 ± 2.6	–		–	–	–		–	–	–		–	–
Trifolium repens	3		–27.4 ± 0.4	9.6 ± 2.2	4		–25.3 ± 0.2	8.2 ± 1.5	3		–25.5 ± 0.4	7.3 ± 0.3	3		–25.4 ± 1.2	6.8 ± 1.1	7		–18.8 ± 7.4	9.6 ± 0.5
Trifolium sp.	–		–	–	–		–	–	–		–	–	–		–	–	3		–27.0 ± 0.1	6.2 ± 1.4
Eragrostis sp.	–		–	–			–	–	–		–	–	2		–13.3 ± 0.4	4.6 ± 0.1	1		–26.7 ± 0.1	7.9 ± 0.1
Vachellia grandicornuta	–		–	–	3		–27.9 ± 0.6	0.9 ± 0.5	3		–26.5 ± 0.5	3.8 ± 0.3	–		–	–	3		–27.3 ± 0.2	4.3 ± 0.5
Senegalia nigrescens	4		–29.4 ± 0.3	4.0 ± 0.1	–		–	–	–		–	–	–		–	–	–		–	–
Vachellia sp. 1	3		–30.7 ± 0.3	4.8 ± 0.3	–		–	–	–		–	–	–		–	–	–		–	–
Vachellia sp. 2	3		–28.6 ± 0.4	4.2 ± 2.3	–		–	–	–		–	–	–		–	–	–		–	–
Diospyros lycioides	–		–	–	–		–	–	3		–28.0 ± 0.6	7.1 ± 0.7	–		–	–	–		–	–
Scolopia zeyheri	–		–	–	–		–	–	3		–25.4 ± 1.0	7.26 ± 0.1	–		–	–	–		–	–
Senegalis nigrescens	–		–	–	–		–	–	–		–	–	–		–	–	2		–26.8 ± 0.7	9.1 ± 2.9
Stylosanthes humilis	–		–	–	–		–	–	–		–	–	–		–	–	4		–26.5 ± 0.7	6.5 ± 0.4
Ziziphus mucronata	–		–	–	2		–26.6 ± 0.0	5.7 ±0.6	–		–	–	–		–	–	–		–	–
Macroinvertebrates															–	–	–		–	–

	Pan 2				Pan 4				Pan 5				Pan 8				Pan 10		
Group	***n***	**TP**	**δ13C**	**δ15N**	***n***	**TP**	**δ13C**	**δ15N**	***n***	**TP**	**δ13C**	**δ15N**	***n***	**TP**	**δ13C**	**δ15N**	***n***	**TP**	**δ13C**	**δ15N**
Branchipodopsis sp.	3	3.00	–22.7 ± 0.7	8.3 ± 1.3	2	2.47	–23.0 ± 0.4	6.5 ± 0.4	4	2.52	–21.5 ± 0.6	6.8 ± 0.5	–		–	–	–		–	–
Ozestheria sp.	–		–	–	–	–	–	–	–		–	–	3	2.31	–22.3 ± 0.4	6.1 ± 0.4	–		–	–
Cyprididae	–		–	–	–	–	–	–	–		–	–	3	2.32	–18.8 ± 1.0	6.1 ± 0.4	–		–	
Sigara sp.	5	3.72	–19.7 ± 1.7	10.7 ± 2.1	4	2.44	–19.7 ± 0.4	6.5 ± 0.6	–		–	–	4	2.66	–20.4 ± 0.2	7.3 ± 0.5	3	3.33	–19.9 ± 0.7	9.4 ± 1.3
Culex sp. larvae	6	2.64	–22.1 ± 0.5	7.2 ± 0.6	–	–	–	–	4	2.42	–21.9 ± 0.7	6.5 ± 0.2	–	–	–	–	5	3.35	–18.3 ± 0.2	9.5 ± 0.3
Cybister sp. larvae	–		–	–	–		–	–	–		–	–	5	3.00	–21.9 ± 2.0	8.1 ± 1.5	–	–	–	–
Rhantus sp. larvae	–		–	–	–		–	–	–		–	–	–		–	–	4	3.71	–19.8 ± 0.1	10.6 ± 0.2
Cybister sp.	–		–	–	–		–	–	–		–	–	–		–	–	6	3.85	–21.7 ± 1.0	11.1 ± 2.6
Rhantus sp.	–		–	–	–		–	–	–		–		3	2.42	–21.7 ± 0.3	6.5 ± 1.0	3	3.86	–20.0 ± 1.0	8.0 ± 0.9
Lestes sp.	–		–	–	–		–	–	–		–		4	2.63	–24.3 ± 0.5	7.1 ± 0.2	4		–	–
Lymnaea truncatula	–		–	–	–		–	–	–		–		6	2.00	–17.9 ± 3.2	5.1 ± 0.9	–		–	–
Anisops sp.	2	3.15	–19.9 ± 0.3	8.8 ± 0.4	6	2.77	–21.0 ± 1.0	7.6 ± 1.0	–		–		6	3.23	–21.9 ± 1.4	9.1 ± 2.2	4	3.89	–17.8 ± 4.7	11.2 ± 5.4
Streptocephalus sp.	3	3.07	–21.3 ± 0.7	8.6 ± 0.7	9	2.65	–24.7 ± 1.0	7.2 ± 0.4	–		–		8	2.44	–23.6 ± 0.5	6.5 ± 1.1	8	3.75	–21.0 ± 0.8	10.8 ± 0.8
Triops sp.	7	3.16	–22.6 ± 0.6	–	3	2.79	–23.2 ± 0.9	7.7 ± 0.3	6	2.57	–20.6 ± 0.1	6.9 ± 0.7	5	2.67	–22.3 ± 1.3	7.3 ± 0.5	4	3.67	–20.4 ± 0.5	10.5 ± 0.6
Amphibians																				
Kassina senegalensis	3	3.56	–20.9 ± 0.2	10.1 ± 1.3	–		–	–	3	3.30	–19.4 ± 0.8	9.3 ± 0.5	10	3.27	–19.9 ± 0.9	9.2 ± 2.0	–		–	–
Pyxicephalus adspersus	–		–	–	4	3.86	–18.6 ± 1.5	11.1 ± 0.6	–		–		2	3.87	–19.1 ± 0.7	11.2 ± 0.1	–		–	–
Tomopterna tadpoles	4	3.13	–23.5 ± 0.9	8.8 ± 2.0	4	2.29	–22.9 ± 0.2	6.0 ± 0.3	4	3.25	–20.1 ± 1.8	9.2 ± 0.3	6	2.55	–21.9 ± 0.8	6.9 ± 0.3	4	4.10	–20.4 ± 0.4	11.9 ± 0.3
Tomopterna adiastola	–		–	–	1	3.09	–21.0 ± 0.1	8.6 ± 0.1	–	–	–	–	–		–	–	–		–	–
Tomopterna sp.	1	3.52	–20.7 ± 0.1	10.0 ± 0.1	3	3.58	–20.2 ± 1.3	10.2 ± 0.7	–	–	–	–	–		–	–	3	3.66	–18.6 ± 0.4	10.5 ± 0.8
Sclerophrys poweri	–	–	–	–	1	3.31	–19.9 ± 0.1	9.4 ± 0.1	2	3.34	–19.5 ± 0.3	9.5 ± 0.3	–		–	–	–		–	–

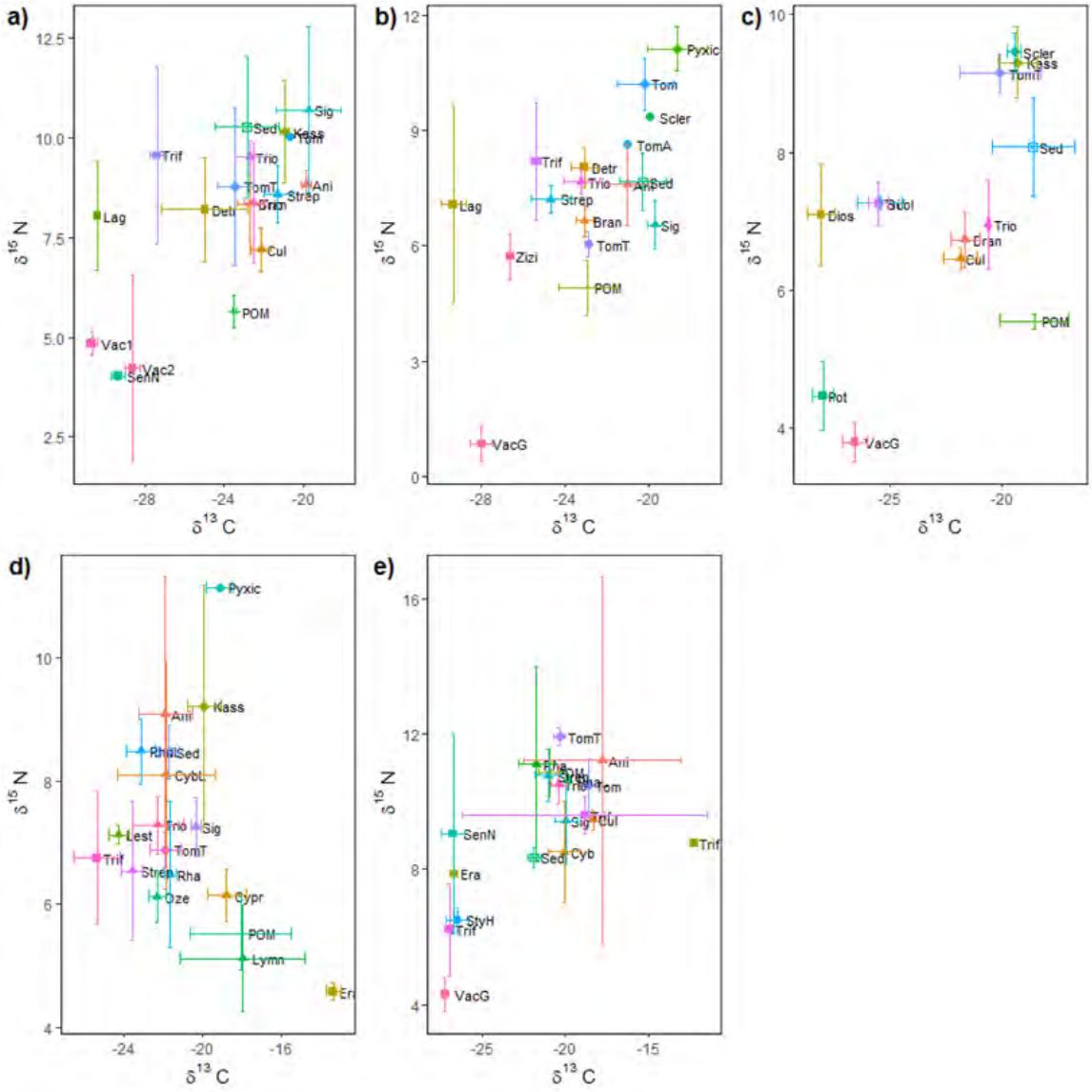


Figure 4.1. Biplot indicating mean $\delta^{13}C$ and $\delta^{15}N$ isotopic signatures (± standard deviation) of the different trophic components sampled from the five pans in the Khakhea Bray Transboundary Aquifer System (a) Pan 2 (b) Pan 4 (c) Pan 5 (d) Pan 8 and (e) Pan 10 in January 2022. Abbreviations: Ani–*Anisops* sp., Bran–*Branchipodopsis* sp., Cul–*Culex* sp. larvae,Cyb–*Cybister* sp., CybL–*Cybister* larvae, Cypr–Cyprididae, Detr–Detritus, Dios–*Diospyros lycioides*, Era–*Eragrostis* sp., Kass–*Kassina senegalensis*, Lag–*Lagarosiphon* sp., Lest–*Lestes* sp., Lymn–*Lymnaea truncatula*, Not–Notonectidae, Oze–*Ozestheria* sp., POM–particulate

organic matter, Pot–*Potamogeton* sp., Pyxic–*Pyxicephalus adspersus,*Rha–*Rhantus* sp., RhaL–*Rhantus* sp. larvae, Scler–*Sclerophrys poweri*,Sco–*Scolopia zeyheri*, Sed–sediment, SenN–*Senegalia negresens*, Sig–*Sigara* sp., Strep–*Streptocephalus* sp.,StyH–*Stylosanthes humilis*, Tom–*Tomopterna* sp., TomA–*Tomopterna adiastola*, TomT–*Tomopterna* sp. tadpoles, Trif–*Trifolium repens*, Trio–*Triops* sp., Vac1–*Vachellia* sp. 1, Vac2–*Vachellia* sp. 2, VacG–*Vachellia grandicornuta*, Zizi–*Ziziphus mucronata.*

4.3.4 Dominant consumer diet proportions

The mixing model output revealed the primary dietary composition of the different species. For *Anisops* sp. in Pan 2, the main components of the diet were *Streptocephalus* sp. (24.5 %) *Sigara* sp. (20.4 %) and *Culex* sp. larvae (18.9 %) (Figure A4.1a). In contrast, *Sigara* sp. predominantly consumed *Culex* sp. larvae (38.1 %), sediment (24.1 %) and POM (23.1 %) (Figure A4.1b). For the *Tomopterna* sp. tadpoles their diet mainly comprised POM (40.8 %), detritus (29.2 %) and sediment (21.5 %) (Figure A4.1c).

In Pan 4, *Anisops* sp. had its diet made up of *Sigara* sp. (22.6 %) while *Tomopterna* sp. tadpoles (19.8 %) and *Triops* sp. (19.3 %) made almost equal contributions (Figure A4.2a). A substantial proportion of the diet of *Sigara* sp. consisted of sediment (58.1 %) and POM (29.4 %) (Figure A4.2b). *Tomopterna* sp. tadpoles primarily relied on detritus (50.3 %) and POM (38.9 %) as their main food sources (Figure A4.2c). The *Tompterna sp.* tadpoles' diet in Pan 5 consisted mostly of POM (62.5 %) and sediment (32.0 %) (Figure A4.3a).

In Pan 8, the diet of *Anisops sp.* was made up mostly of *Streptocephalus* sp. (21.5 %), *Ozestheria* sp. (14.5 %) and *Rhantus* sp. (12.7 %) larvae and an almost equal contribution of

Tomopterna sp. tadpoles (11.8 %) and *Rhantus* sp. (12.2 %) (Figure A4.4a). The POM (30.9 %) and sediment (69.1 %) contributed primarily to the diet of *Sigara* sp. (Figure A4.4b). *Lestes* sp. diet was mainly composed of the larvae of *Rhantus* sp. (55.5 %) and *Cybister* sp. (44.5 %) (Figure A4.4c). The diet of *Rhantus* sp. was mainly composed of *Streptocephalus* sp. (40.3 %) but also included *Ozestheria* sp. (13.5 %) and *Triops* sp. (12.3 %) in high proportions (Figure A4.4d). Similar to the adults *Rhantus* sp. larvae had a large proportion of *Streptocephalus* sp. (31.1 %) in their diet followed by *Ozestheria* sp. (17.8 %) and *Tomopterna* sp. tadpoles (12.5 %) (Figure A4.4e). *Cybister* sp. larvae diet was also composed mainly of *Streptocephalus* sp. (19.5 %) and *Ozestheria* sp. (16.6 %) but *Triops* sp. (14.8 %) also contributed to the diet (Figure A4.4f). *Tomopterna* sp. tadpoles in Pan 8 like tadpoles from the other pans had their diet mainly composed of POM (57.4 %) and sediment (42.6 %) (Figure A4.4g).

The diet of *Anisops* sp. in Pan 10 was composed of *Streptocephalus* sp. (12.7 %), *Culex* sp. larvae (12.6 %) and *Sigara* sp. (12.6 %) (Figure A4.5a), while *Sigara* sp. fed mostly on sediment (63.4 %) and POM (26.8 %) (Figure A4.5b). The diet of *Rhantus* sp. was mainly composed of *Sigara* sp. (20.9 %), *Streptocephalus* sp. (20.1 %) and *Triops* sp. (18.5 %) (Figure A4.5c). *Cybister* sp. diet included *Streptocephalus* sp (27.1 %). The diet also included *Sigara* sp. (18.8%) and *Triops* sp. (18.7 %) almost in the same proportions. *Tomopterna* sp. tadpoles consumed mostly POM (50.6 %) and sediment (49.4 %) (Figure A4.5e).

4.3.5 Dominant consumers niche breadths and overlaps

The TA and SEA_c estimates representing the trophic niche revealed high trophic niche widths and a wide range of resources exploited for the three predator consumer insects *Anisops* sp., *Rhantus* sp. and *Cybister* sp. in all the pans. *Tomopterna* sp. tadpoles and the larvae for *Rhantus*

sp. and *Cybister* sp. had low Layman metrics in these pans (Table 4.4). The model estimated the TA values which ranged from 0.1 (*Rhantus* sp. larvae) – 12.4 (*Anisops* sp.). The TA was high for *Anisops* sp. in pans 8 (12.4) and 10 (10.5) followed by *Rhantus* sp. (9.3) and *Cybister* sp. (5.3) and was narrow in *Rhantus* sp. larvae (0.1) in Pan 10 and *Tomopterna* sp. tadpoles (0.1) in Pan 4. The SEA_c values varied among the species and ranged from 0.1 (*Rhantus* sp. larvae) to 25.5 (*Anisops* sp.). The estimated SEA_c values which were less influenced by extreme values than the TA values were high for *Anisops* sp. (25.5) followed by *Rhantus* sp. (10.9). Low SEA_c values were found for the *Rhantus* sp. larvae (0.1) and the *Tomopterna* sp. tadpoles (0.3) (Table 4.4).

Table 4.4. Trophic niche metrics of dominant invertebrate consumers and tadpoles in the 5 pans. Total area (TA), standard ellipse area (SEA), ellipse area corrected for small sample size (SEA_c), *n* = number of samples.

Species	*n*	TA	SEA	SEA_c
Pan 2				
Sigara sp.	5	3.21	3.08	4.11
Tomopterna sp. tadpoles	4	1.66	2.21	3.32
Pan 4				
Sigara sp.	4	0.24	0.30	0.45
Anisops sp.	6	3.24	3.26	4.07
Tomopterna sp. tadpoles	4	0.12	0.17	0.25
Pan 8				
Sigara sp.	4	0.29	0.31	0.47
Rhantus sp.	3	0.72	1.30	2.60
Rhantus sp. larvae	4	1.15	1.20	1.60
Cybister sp. larvae	5	0.16	0.29	0.58
Lestes sp.	4	0.18	0.23	0.35
Anisops sp.	6	10.47	8.40	10.50
Tomopterna sp. tadpoles	6	0.80	0.69	0.86
Pan 10				
Sigara sp.	3	0.52	0.94	1.87
Rhantus sp.	3	9.30	8.69	10.86
Cybister sp.	6	5.30	4.12	5.15
Rhantus sp. larvae	4	0.07	0.09	0.13
Anisops sp.	4	12.40	16.98	25.47

Tomopterna sp. tadpoles	4	0.22	0.24	0.37

In Pan 2, there was a low trophic niche overlap observed between *Tomopterna* sp. tadpoles and *Sigara* sp. (2.4) (Figure 4.2; Table A4.2). While in Pan 4 there was a niche overlap between *Anisops* sp. and *Sigara* sp. (4.0) (Figures 4.2 and A4.6; Table A4.3). In Pan 8 high trophic niche overlap was found between the predator consumer insects *Anisops* sp. and *Rhantus* sp. larvae (27.8) and *Rhantus* sp. adults (7.9). There was also intermediate niche overlap between *Tomopterna* sp. tadpoles and *Rhantus* sp. larvae (6.3) and *Anisops* sp. (6.6). Intermediate niche overlap was also observed between *Rhantus* sp larvae and *Rhantus* sp. adults (5.3) and *Cybister* sp. (5.3). However, there was no overlap observed between *Lestes* sp. and *Sigara* sp. and *Rhantus* sp. This could be attributed to them utilizing different resources in the pans (Figure A4.6; Table A4.4). Lastly, in Pan 10 there was a high niche overlap between *Anisops* sp. and *Rhantus* sp. (27.2) and *Cybister* sp. (11.9). High niche overlap was also found between *Cybister* sp. and *Rhantus* sp. adults (9.1). Low niche overlap was observed between *Tomopterna* sp. tadpoles and *Sigara* sp., *Cybister* sp., and *Rhantus* sp. larvae, where overlap was very small (<0.001) (Figures 4.2 and A4.6; Table A4.5).

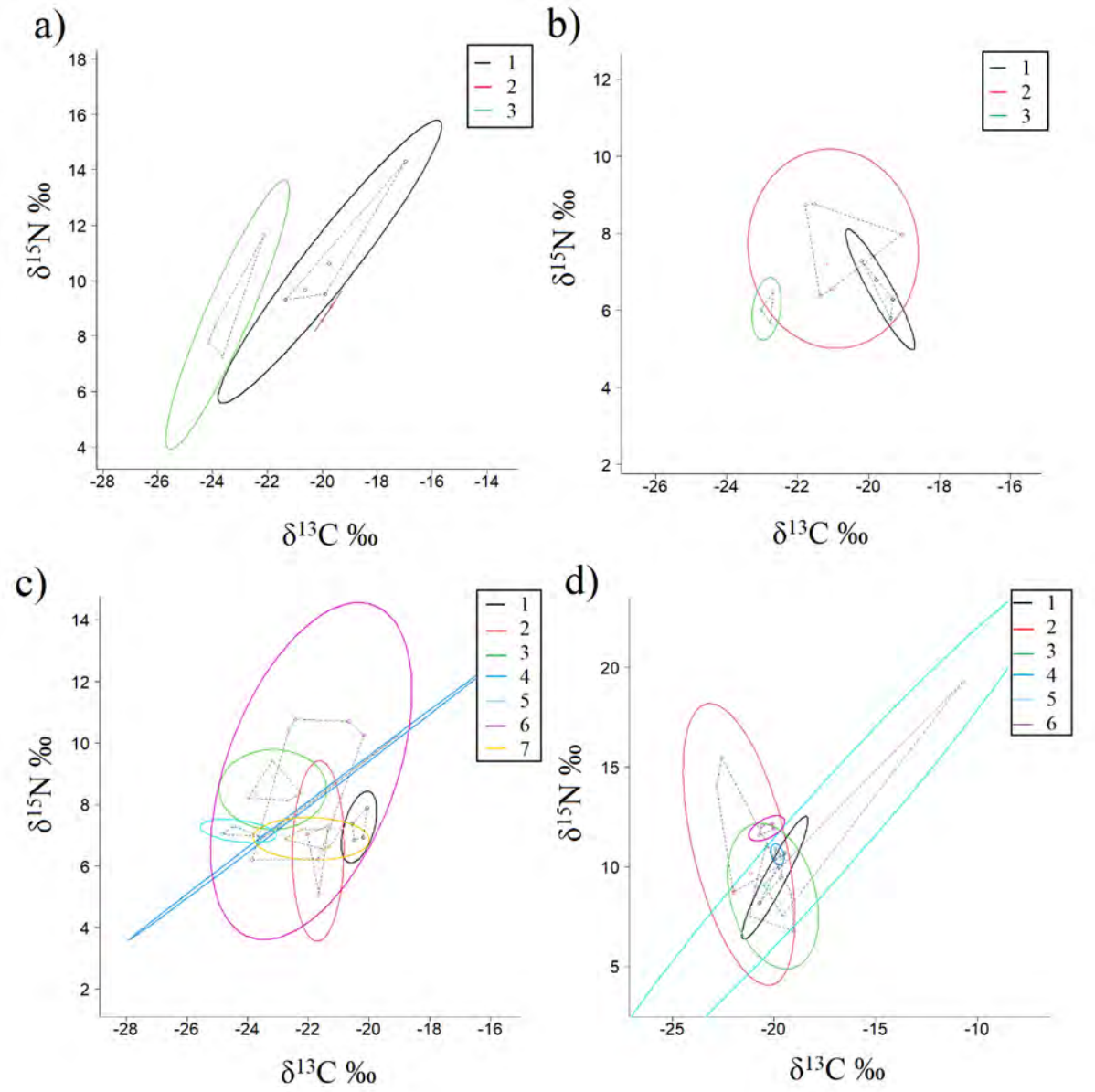


Figure 4.2. SIBER convex hull areas (solid colored lines) representing the calculated trophic niche width in (a) Pan 2: 1 = *Sigara* sp.; 2 = *Anisops* sp.; 3 = *Tomopterna* sp. tadpoles (b) Pan 4: 1 = *Sigara* sp.; 2 = *Anisops* sp.; 3 = *Tomopterna* sp. tadpoles (c) Pan 8: 1 = *Sigara* sp.; 2 = *Rhantus* sp.; 3 = *Rhantus* sp. larvae; 4 = *Cybister* sp. larvae; 5 = *Lestes* sp.; 6 = *Anisops* sp.; 7 = *Tomopterna* sp. tadpoles (d) Pan 10 1 = *Sigara* sp.; 2 = *Rhantus* sp. 3 = *Cybister* sp.; 4 = *Rhantus* sp. larvae; 5 = *Anisops* sp.; 6 = *Tomopterna* sp. tadpoles.

4.4 Discussion

This study provides insights into food web structure and dynamics in arid zone temporary pans. The results showed that the food web length of temporary pans in the KBTA in the rainy season had less than four trophic levels, which supported our first hypothesis. This was likely driven by the variability in the environmental conditions in these endorheic systems, precluding the presence of large–bodied predators like fish. The primary consumers in the food web structures for these pans were mainly supported by aquatic macrophytes and algae in the form of POM and detritus. The primary consumers were mainly composed of filter feeders of the specialist taxa of large branchiopods *Branchipodopsis* sp., *Streptocephalus* sp., Cyprididae and *Ozestheria* sp. Other primary consumers within these temporary pans also included molluscs and tadpoles. Omnivores such as the corixid *Sigara* sp. were also part of the food web structure. Insect diversity drove trophic structure complexity with the secondary consumers in trophic level 3 comprising mainly of predaceous insects such as the dytiscids *Rhantus* sp. and *Cybister* sp. At the top of this food web occupying the highest trophic positions were the notonectid *Anisops* sp. and the adult frog *Pyxicephalus adspersus*. The findings also showed that most of the dominant invertebrate consumers that are found in the temporary pans, such as the dytiscids and the notonectids, had generalist diets as they fed on a diverse array of smaller prey, which were present in the temporary pans during the time of sampling. This is in agreement with our hypothesis. However, the diets of most of these predator invertebrates consisted of significant proportions of *Streptocephalus* sp. We also found considerable niche overlap among the dominant consumers in these temporary pans.

The food chains from this study were relatively short and this is in agreement with what is generally known about temporary systems (Williams, 2006). These simple food web structures,

as supported by the theory of dynamic constraints hypothesis in ephemeral systems, have also been observed in other studies (Dalu et al., 2016; Mdidimba et al., 2021; O'Neill and Thorp, 2014; de Necker et al., 2020). One of the key environmental gradients structuring communities in these temporary systems is the hydroperiod gradient (Brendonck and Williams, 2000). Dalu et al. (2017b) showed that as the hydroperiod progresses the trophic level structure of temporary systems becomes increasingly complex, with a gradual decline in diversity as the water source dries out. There is also a significant association between this heightened complexity later in the hydroperiod and the insect diversity within these systems. Species–specific traits exhibited by these insects play a vital role in extending the food chain length contributing to the intricate trophic structures observed later in the hydroperiod. Thus, systems with higher insect diversity often have more complex trophic structures than those dominated by large branchiopods, which are often considered trophically redundant since all the different branchiopod species have similar diets, feeding behaviours, and ecological functions (O'Neill and Thorp, 2014).

The temporary nature of the pans resulted in smaller–bodied predators being dominant, as compared to the large predators that are found occupying the top levels of food webs in permanent water bodies (Dalu, 2017a; Champion and Downs, 2017). Many temporary water habitats have food webs that are detritus–based, with the detritus being composed of leaf–fall from surrounding vegetation and decaying plants, which grow in sediments during the dry phase (Williams, 2006). Additionally, the food webs in this study could not be clearly described as being either green or brown food webs. There was an interaction between both the green and brown food webs, driving the ecosystem to function, with them being supported by algae,

macrophytes, POM, and detritus (Zou et al., 2016). The coupling of both the green and brown food webs is crucial for ecosystem stability (Mougi, 2020).

In the absence of fish in these temporary pans, macroinvertebrate predators such as the notonectids are often abundant in the littoral zones and have a significant influence on the zooplankton and other components of the community (Scott and Murdoch, 1983). From this study the notonectid *Anisops* sp. was at the top of the food web. This was consistent with findings of other studies on small freshwater systems that showed the importance of other notonectid species such as *Notonecta* sp. as top predators in these systems (Dalu et al., 2016). The African bull frog *Pyxicephalus adspersus* also occupied a higher trophic position in the pans. The bullfrogs breed in ephemeral pans and are voracious carnivores who feed on insects, other invertebrates, small rodents, reptiles, small birds, fish, and smaller amphibians (Thomas et al., 2014). The corixids, dytiscids and large branchiopods in this study exhibited a shift in trophic positions, ranging from 2 to 3 across various pans. This indicates their adaptation to a broader diet, enabling them to consume a greater variety of food items, thereby enhancing their chances of survival and achieving maturity before the hydroperiod concludes (Dalu et al., 2017b).

Bayesian mixing models revealed that the diet of both the Dytiscidae adults and larvae consisted of a significant proportion of *Streptocephalus* sp., and that *Rhantus* sp. adults ate corixids and mosquito larvae. In pans where mosquito larvae were absent, the dytiscids fed on clam shrimps. Invertebrate predators in these pans were found to be polyphagous, feeding on a broad range of prey species (generalist predator). Both adults and larvae of Dytiscidae are common predators in both permanent and temporary ponds (Shaalan and Canyon, 2009) and

both are generalist predators (Lundkvist et al., 2003). The dytiscid larvae are strictly predatory while the adults may be partly scavengers, with larval prey choice largely correlated with body size (Nilsson, 2005). This was revealed here with *Cybister* sp. larvae feeding on a larger proportion of *Triops* sp. than the adults. Apart from predation on other invertebrates, large dytiscid larvae may also feed on small vertebrates. This was also the case in these pans with *Rhantus* sp. larvae feeding on a higher proportion of tadpoles than the adults. A study by Pearman (1995) showed that increased densities of dytiscid larvae of the genera *Dytiscus* resulted in higher predation pressure on the tadpoles present. From the evidence presented by Aditya et al. (2006) predation rate of *Rhantus* on mosquito larvae was high and ranged between 22–87 larvae per day depending on prey–predator densities. *Anisops* sp. in these pans also had a high proportion of *Streptocephalus* sp. and *Sigara* sp. in their diet. Their diet did however also consist of mosquito larvae and tadpoles in other pans. In depressions where *Anisops assimilis* were located, mosquitoes were absent (Kumar and Hwang, 2006). In Saha et al., (2007), a single adult of *Anisops bouvieri* consumed 2–34 mosquito larvae. Munga et al. (2007) identified seven families (Hydrophilidae, Dytiscidae, Corixidae, Nepidae, Notonectidae, Belostomatidae, and Corduliidae) of larval mosquito predators from different natural habitats in Western Kenya Highlands.

Findings from this study also highlighted that in the diet of both the dytiscid adults and larvae, and the notonectids there was a preference of *Streptocephalus* sp.. High predation rates on anostracans such as *Branchipodopsis wolfi* were observed by Brendonck et al. (2002). From their findings, there was a preference for female prey. The possession of a conspicuous brood pouch may have made the female anostracans more vulnerable to visual predators. Additionally, we can only speculate that the high predation rates on *Streptocephalus* sp. in this

study may be related to their slow locomotion, which inhibits their ability to avoid predation. Predator–prey dynamics are influenced by various factors such as catchability and availability. When prey is abundant and easily accessible, predators are more likely to focus on those options as they require less effort to capture, while also providing a reliable food source (Underwood et al., 2004). This was seen in our study by the swarms of *Streptocephalus* sp. during sampling. Although prey availability may have played a significant role in shaping these predator invertebrates' diets, predation could also have been affected by other environmental factors such as the presence of aquatic vegetation (Shaalan et al., 2007).

The food web structure of these temporary pans is 'interval' in nature as indicated by the niche overlaps between predators, which agreed with observations from intermittent ponds (Williams, 2006). Our findings revealed that these temporary freshwater systems exhibit significant trophic redundancy, shown by the high niche overlap between the predator invertebrates and the dominance of generalist feeders. Both of these mitigate the negative impacts of disturbances on the systems (Layman et al., 2007a; Klecka and Boukal, 2012; Dalu et al., 2016). Niche overlap was observed between the Dytisicidae adults and larvae and the Notonectidae. This was also the case between the tadpoles and the corixids. This suggests that the predators in these temporary pans have similar prey preferences and rely on the same food sources, which could lead to competition for the limited resources. However, there was low overlap observed between the Lestidae and the other predator insects, which suggests niche segregation and distinct dietary preferences is more likely. It must however be noted that the incorporation of isotopes into an organism's tissues can be influenced by physiological and ecological factors such as metabolism and tissue turnover rates. These factors are not always accounted for in SIBER and SIAR models.

4.5 Conclusions

Our findings provide valuable insights into the food web structures of temporary pans. The food web structures in these temporary pans show mid–stage trophic structural complexity with four trophic levels and the notonectids and frogs at the top of the food web structure. The findings highlight the overall food web structures in temporary pans. They also show that the dominant invertebrate predators had generalist diets with a preference for *Streptocephalus* sp. at the time of sampling, highlighting the key role of these organisms. High niche overlaps were also found between the dominant predator invertebrates. To get a finer resolution on the trophic links in these food webs, further studies in temporary pan systems should include quantitative analyses of the role of changes in keystone species (species whose roles are so critical in supporting the food web, that their elimination causes the food web to collapse) result in changes in the local ecosystem. Extended monitoring of food web dynamics over multiple wet–dry cycles is also necessary for these temporary systems to help identify patterns and drivers of change, which would allow for a more comprehensive understanding of how food webs respond to different environmental conditions. This can also shed some light on the intricacies of the predator–prey interactions within temporary pan systems, particularly during the transitions between the dry phases. This information can contribute to a better understanding of the ecological processes that govern these unique and understudied ecosystems.

CHAPTER 5: ASSESSING CHLOROPHYLL–*A* AND WATER QUALITY DYNAMICS IN ARID–ZONE TEMPORARY PAN SYSTEMS ALONG A DISTURBANCE GRADIENT

"We owe it to ourselves and to the next generation to conserve the environment so that we can bequeath our children a sustainable world that benefits all."

–Wangari Maathai

Plate 5. Pollution in some of the temporary pans sampled in the Khakhea Bray Transboundary Aquifer region. Photo by Dr Chad Keates.

Abstract

Temporary pans are susceptible to various anthropogenic effects such as pollution, resource extraction, and land use intensification. However, given their small endorheic nature, they are almost entirely influenced by activities close to their internally drained catchments. Human–mediated nutrient enrichment within the pans can lead to eutrophication, resulting in increased primary productivity and decreased associated alpha diversity. The Khakhea–Bray Transboundary Aquifer region pan systems are a major water source for the people in these areas. This study assessed differences in nutrients (i.e., ammonium, phosphates) and their effect on chlorophyll–*a* (chl–*a*) concentrations in pans along a disturbance gradient in the Khakhea–Bray Transboundary Aquifer region, South Africa. Physical and chemical variables, nutrients, and chl–*a* were measured from 33 pans representing variable anthropogenic exposure during winter in May 2022. Five environmental variables (i.e., temperature, pH, dissolved oxygen, ammonium, and phosphates) showed significant differences between the undisturbed and disturbed pans. The disturbed pans generally had elevated pH, ammonium, phosphates and dissolved oxygen compared to the undisturbed pans. A strong positive relationship was observed between chl–*a* and temperature, pH, dissolved oxygen, phosphates and ammonium. Chlorophyll–*a* concentration increased as surface area, and the distance from kraals, buildings and latrines decreased. Anthropogenic activities were found to have an overall effect on the pan water quality within the Khakhea–Bray Transboundary Aquifer region. Therefore, continuous monitoring strategies should be established to better understand the nutrient dynamics through time and the effect that this may have on productivity and diversity in these small endorheic systems.

Keywords: *Anthropogenic activities, chlorophyll–a, nutrient dynamics, temporary pans*

5.1 Introduction

Anthropogenic effects such as pollution, resource extraction and land use intensification (Dube et al., 2020; Wasserman and Dalu, 2022) are increasingly prevalent with implications for primary and secondary productivity, and associated ecosystem functioning within temporary pan systems. Many physical and biological factors determine water quality within these temporary systems (Henri et al., 2014a; Park and Hwang, 2016; Nhiwatiwa et al., 2017). The physical and chemical conditions within these pan systems reflect the land use patterns and physical characteristics of the landscapes in which they are found (Morrice et al., 2008). Human disturbances around the pan systems can lead to changes in the water quality, and in turn, this can change the diversity within these systems (Khudair et al., 2019). The endorheic nature of these pans, and reduced water flow (in and out), coupled with the shallowness of these pan systems, make them more vulnerable and susceptible to anthropogenic impacts (Henri et al., 2014b). Human–mediated nutrient enrichment within the pans can lead to adverse effects such as eutrophication, which promotes plant and algal growth. Nutrient enrichment degrades aquatic ecosystems and impairs water viability for consumption, industry, agriculture, recreation, and other purposes (Carpenter et al., 1998). Water nutrient analyses provides information on the ecological integrity of the water resources at a specific time. The water quality and trophic status of these ecosystems can be monitored using chlorophyll–*a,* as an algal parameter in conjunction with other environmental indicators (Omar, 2010).

Although a water quality classification system was developed for pans (De Klerk et al., 2016), very few studies (e.g., Bird and Day, 2014; Dube et al., 2020) have specifically looked at the effects that anthropogenic activities have on water quality within temporary pans in South Africa. The current study aimed to assess how differences in nutrient (i.e., ammonium,

phosphates) concentrations and anthropogenic activities affect chl–*a* concentration variation among pans in the Khakhea–Bray Transboundary Aquifer region. Overall, we hypothesized that anthropogenic activities near human settlement areas would result in significantly higher chl–*a* and nutrient concentrations. Generating information on the effect of anthropogenic activities on water quality of pans in temporary wetlands will help develop assessment tools to better monitor human impacts across temporary wetland ecosystems and aid in the prioritization of temporary wetlands for conservation.

5.2 Methods

5.2.1 Study area

The study was carried out in the Khakhea–Bray Transboundary Aquifer region during winter in May 2022. Site selection was based on the presence of high densities of pans near human settlements (Figure 5.1). Within the chosen study region, a line transect of 1.5 km along the main road was measured. Two perpendicular transects 1.3km long each from the road were also measured (Figure 5.1). All human structures, namely buildings, latrines, and kraals, were identified within 200m of the transect line. Within the area, 33 pans were identified and designated as either undisturbed or disturbed. Undisturbed pans were all pans located more than 500m from the road transect that did not have any human structure observed within a 400m buffer. Disturbed pans were found less than 500m from the road and had human structures within a 400m buffer. The GPS coordinates of the 33 pans (14 undisturbed, 19 disturbed) and all associated human structures were collected in the field. These coordinates were then used to measure the distance of each of the pans from the nearest human structure and anthropogenic activity. The distances and the surface area of each pan were measured using the Google Earth Pro Desktop version 9.172.0.0. As such, three measurements (distance to the

nearest anthropogenic disturbance type) were associated with each pan based on the nearby human structures.

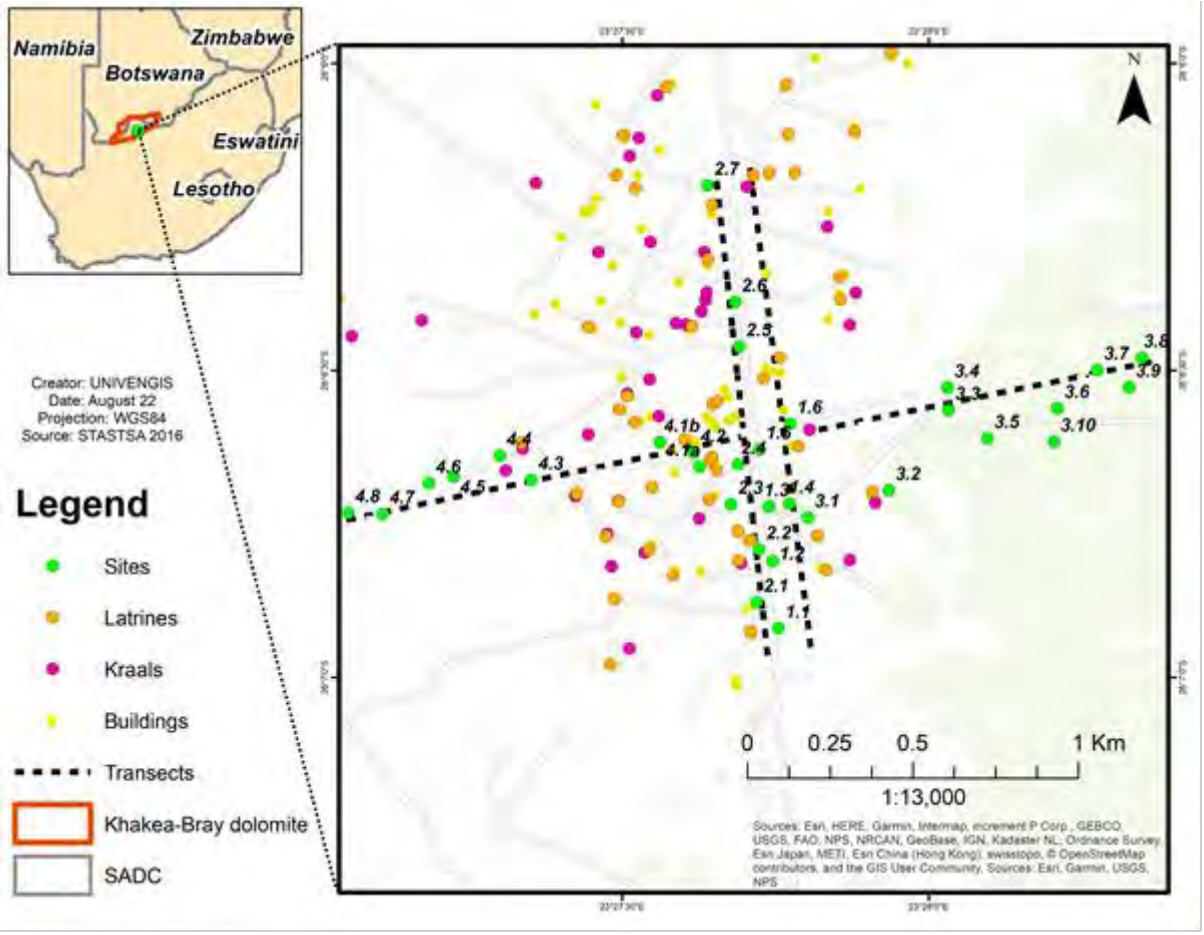


Figure 5.1. Map showing the 33 sampled pans (in green) in May 2022 in the southern section of the Khakhea–Bray Transboundary Aquifer region and the various anthropogenic activities around the pans

5.2.2 Physical and chemical analysis

At the centre of each pan, temperature (°C), pH, conductivity (µS cm^{-1}), turbidity (NTU), TDS (mg L^{-1}), salinity (ppm), percentage oxygen saturation and dissolved oxygen (mg L^{-1}) were

measured at a depth of 1m from the surface using an AquaRead multiparameter meter (Model AP–700 and AP–800, AquaRead Ltd, UK. 250ml of water was collected in plastic containers from each pan for nutrient analysis and stored on ice. Nutrients (i.e., phosphates and ammonium) were analyzed using a multiparameter benchtop photometer (Hanna Instruments Model HI83300) whereby ammonium (Photometer range 0–10 ± 0.04 mg L^{-1} accuracy, resolution 0.01 mg L^{-1}) was determined through the Nessler method, phosphates (Photometer range 0–30mg L^{-1} ± 1.0 mg L^{-1} accuracy, resolution of 0.1mg L^{-1}) through the amino acid method and nitrates (Photometer range 0–30 ± 0.5 mg L^{-1} accuracy, resolution, resolution 0.1mg L^{-1}) through the cadmium reduction method (Dalu et al.,2019).

5.2.3 Chlorophyll–a analysis

Chlorophyll–concentrations were determined by taking 250 mL of each water sample filtered through a 0.7 µm Whatman glass fiber filter (Ø= 47mm). The filters were placed in plastic zip–lock bags and stored on ice in the field. The filters were then stored at –20 °C until extraction. Each filter was extracted in 10 mL of 90 % acetone in the dark for 24 h. Chlorophyll–*a* concentration was then determined fluorometrically using a Turner 10AU fluorometer. Absorbances were taken for each sample before and after adding two drops of 1 N HCl (Holm–Hansen and Riemann, 1978; Gusha et al., 2021). Chlorophyll–*a* concentrations were then calculated following the Environmental Protection Agency (EPA) method 445.0 (Arar and Collins, 1992):

$$\boldsymbol{chl-a\ (mg\ L^{-1}) = \left(\frac{a}{V}\right) \times (F_o - F_a)\ \times C}$$

Where chl–*a* (mgL^{-1}) is the chl–*a* concentration in mg L^{-1}, "a" is the quantity of acetone used for extraction in mL, V is the quantity of water filtered in mL, F_o is the chl–*a* reading before

acidification with 1 N HCl (hydrochloric acid), F_a is the chl–*a* reading after acidification with 1 N HCl (hydrochloric acid), and C is the constant value (0.325).

5.2.4 Data analysis

ESRI ArcGIS 10.8 software was used for symbolization using proportional circles for the pH, ammonium, phosphate and chl–*a* concentrations in the 33 pans that were sampled. A two–sample *t*–test was used to determine the environmental variables' differences between the Undisturbed and disturbed pans. All the measured variables had skewed distributions except for pH. Therefore, all the other variables were log (x + 1) transformed, excluding percentage oxygen saturation, which was square root transformed. A correlation analysis was then done to compare the relationships between chl–*a* and all the other variables measured. The test and analysis were carried out using the software package STATISTICA version 14.0.0.15 (TIBCO Software Inc, 2020).

5.3 Results

Environmental variables varied between the undisturbed and disturbed pans. Nitrate concentrations in all sampled pans were all below detectable limits, highlighting that nitrate levels in the pans are low. As such, nitrates were not included in further analyses. The *t*–tests comparing environmental variables measured for the two groups of pans showed that temperature, pH, dissolved oxygen, ammonium, and phosphate levels were significantly different between the undisturbed and disturbed pans. Disturbed pans generally had higher pH (mean ± standard deviation: 9 ± 0.5), ammonium (1.9 ± 0.6 mg L^{-1}), phosphates (0.74 ± 0.4 mg L^{-1}) and dissolved oxygen (6.9 ± 1.5 mg L^{-1}) levels compared with the undisturbed pans (Fig. 2). Salinity levels were low across all the pans (< 0.05 mg L^{-1}). The undisturbed pans

showed higher total dissolved solids (107.6 ± 96.6 mg L^{-1}) and turbidity (46.8 ± 85.2 NTU) levels. The disturbed pans had slightly higher chl–*a* concentrations (mean ± standard deviation: 0.11 ± 0.2 mg L^{-1}) than the undisturbed pans (0.08 ± 0.1 mg L^{-1}) but this was not found to be significant ($p > 0.05$) (Table 5.1, Figure 5.2).

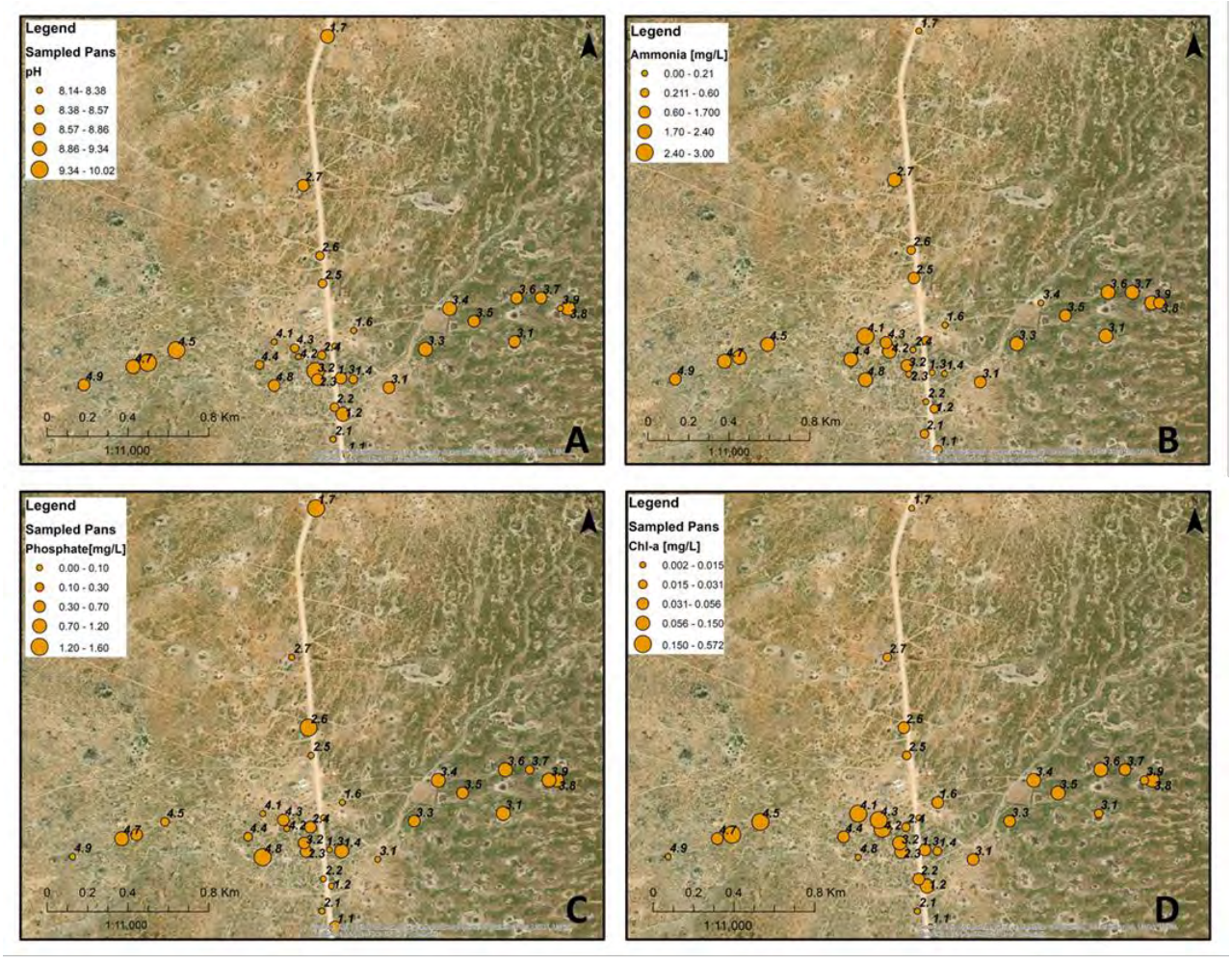


Figure 5.2. Maps showing different environmental variables measured across the 33 pans in May 2022: (A) pH, (B) ammonium, (C) phosphates (D) chlorophyll–*a* concentration

Table 5.1. Summary of pairwise comparisons (*t*–test) of environmental variables (mean ± standard deviation) between the undisturbed and the disturbed pans. Significant values ($p < 0.05$) are indicated in bold

Variable	Undisturbed pans	Disturbed pans	*p*
Temperature (°C)	20.8 ± 3.1	22.9 ± 1.9	**<0.001**
pH	8.6 ± 0.4	9.0 ± 0.5	**<0.001**
Conductivity (µS cm^{-1})	173.2 ± 160.3	144.6 ± 33.0	0.145
Turbidity (NTU)	46.79 ± 85.2	37.8 ± 68.6	0.319
Total dissolved solids (mg L^{-1})	107.6 ± 96.6	93.4 ± 19.7	0.193
Salinity (ppm)	0.04 ± 0.02	0.05 ± 0.01	0.217
Percentage oxygen saturation	66.0 ± 17.2	85.2 ± 23.2	**<0.001**
Dissolved oxygen (mg L^{-1})	5.3 ± 1.2	6.90 ± 1.5	**<0.001**
Ammonium (mg L^{-1})	0.86 ± 0.9	1.90 ± 0.6	**<0.001**
Phosphates (mg L^{-1})	0.39 ± 0.5	0.74 ± 0.4	**0.020**
Chlorophyll–a (mg L^{-1})	0.08 ± 0.1	0.11 ± 0.2	0.209
Total surface area (m2)	816.5 ± 735.6	1092.7 ± 735.1	0.154

In all 33 pans, there was a strong significant positive relationship between chl–*a* and temperature, pH, turbidity and ammonia. Strong negative relationships were observed between chl–*a* and phosphates, distance to the nearest latrines, kraals and buildings and surface area of each pan (Table 5.2). Conductivity, TDS, salinity, and dissolved oxygen were not significantly correlated ($p > 0.05$) with chl–*a* concentrations in the pans (Table 5.2).

Table 5.2. Summary of correlation analysis comparing relationships between chlorophyll–*a* and all the other environmental variables. Significant relationships ($p < 0.05$) are highlighted in bold

Pair of variables	Spearman R	*p*
Chlorophyll–*a vs* temperature	**0.529**	**0.029**
Chlorophyll–*a vs* pH	**0.571**	**0.017**
Chlorophyll–*a vs* conductivity	–0.051	0.844
Chlorophyll–*a vs* turbidity	**0.593**	**0.012**
Chlorophyll–*a vs* total dissolved solids	–0.049	0.852
Chlorophyll–*a vs* salinity	–0.424	0.090
Chlorophyll–*a vs* %dissolved oxygen	0.019	0.474
Chlorophyll–*a vs* dissolved oxygen	0.328	0.198
Chlorophyll–*a vs* ammonium	**0.534**	**0.027**
Chlorophyll–*a vs* phosphate	**–0.502**	**0.040**
Chlorophyll–*a vs* distance to the nearest latrines	**–0.566**	**0.018**
Chlorophyll–*a vs* distance to the nearest Kraals	**–0.730**	**<0.001**
Chlorophyll–*a vs* distance to the nearest buildings	**–0.551**	**0.022**
Chlorophyll–*a vs* surface area	**–0.617**	**0.008**

5.4 Discussion

The present study found significant differences in the pH, temperature, dissolved oxygen, ammonium, and phosphate concentrations overall between the undisturbed and disturbed pans. All five of these variables were higher in the disturbed pans than in the undisturbed pans. The higher temperatures recorded in the disturbed pans, when compared to the undisturbed pans, are likely attributed to anthropogenic activities within the temporary pan systems of the Khakhea Bray Transboundary Aquifer Region.

Most of the disturbed pans were surrounded by bare ground since the land had been cleared for construction and domestic activities, exposing the terrestrial environment to higher levels of solar radiation. In comparison, the undisturbed pans were bordered by a matrix of trees and shrubs, that overshadowed the banks of the pans. The slightly higher pH in the disturbed pans

may also indicate pollution from the various human activities around the pans. Although the disturbed pans had a slightly high value of dissolved oxygen, the oxygen levels measured in all the pans, in general, were considered good since, for most natural freshwater systems, any concentration above 5 mg L^{-1} is considered healthy (Dalu et al., 2013).

The below–detectable levels of nitrates after the inundation of sediment from a semi–arid area have been observed previously in a study by Arce et al. (2015). For this study, the pans were sampled soon after the rains when they contained water. It is likely that nitrate levels that accumulate in sediment during the periods of desiccation are low and are immediately processed after inundation via denitrification rather than being released into the water. The reduction of nitrates in the water stimulates the production of ammonium, which can be preferably assimilated by organisms in the water (Dortch, 1990). Ammonium was detectable in the pans, and it was found that ammonium, phosphate and chl–*a* concentrations were higher in the disturbed pans than in the undisturbed pans. These results show a similar pattern to Dube et al. (2020), where chl–*a* and ammonium concentrations were found to be high in pans in the Ndumo communal area, indicating different levels of anthropogenic activities in close proximity to pans. It is more likely that the high ammonium levels in the disturbed pans could be related to direct input from human activities especially in the presence of latrines and cattle.

The present study also examined the relationship between several environmental variables and chl–*a* concentrations to elucidate potential indicators for the impacts of anthropogenic activities. Temperature, pH and ammonium were found to drive the chl–*a* concentrations in the pan systems, with strong positive relationships between these three variables and chl–*a*. The strong relationship between chl–*a* and temperature has been shown in other studies (Pieterse et

al., 1997) since temperature influences the rate of chemical reactions within aquatic systems. This may also explain why the smaller pans had higher concentrations of chl–*a,* as the smaller surface area resulted in the pan heating up faster, thereby facilitating higher algal production. The chl–*a* concentration is highly dependent on the nutrient concentration as these nutrients (i.e., nitrogen, phosphorous) are essential for primary plant production. In this study, the strong relationship between ammonium and chl–*a* may suggest that these pans are nitrogen–limited aquatic ecosystems. Further studies over an extended period should be conducted to validate these findings.

In this study, chl–*a* concentrations in the pans increased with decreasing phosphates and decreasing distances from each pan to the identified associated anthropogenic activity, namely latrines, kraals, and buildings. This meant that the pans influenced by the various anthropogenic disturbances showed higher chl–*a* concentrations and, higher algal growth when compared to the undisturbed pans. Proximity to kraals was the major factor driving the chl–*a* concentrations in the pans. Most of the pans observed in the Khakhea Bray Transboundary Aquifer region were frequented by livestock. Cattle typically enter water systems to drink, and the activity is often associated with urination and defecation within or near these temporary systems, with implications for primary productivity dynamics (Buxton et al. 2020). The cattle have unrestricted access to the temporary pans and tend to be attracted to the water and the presence of forage material (Hughes et al., 2016). Faecal contamination from grazing livestock results in higher ammonium concentrations in the pans closer to the homesteads (Collins and Rutherford, 2004). The pit latrines represented the primary means for human–waste disposal within the study site. The leaching of ammonium and nitrates from the pit latrines into the groundwater that feeds the pans results in high levels of nitrogen–containing compounds

(ammonium) in the disturbed pans close to the homesteads and thus represents a major threat to these systems. Similar studies have also shown that contamination is particularly high (particularly with nitrates and coliform bacteria) when the aquifer is located five meters below the pit latrines. (Love et al., 2005; Templeton et al., 2015).

Chlorophyll–*a* concentration was also driven by the proximity of the pans to buildings and homesteads. Pollution problems from domestic point sources, which lead to excessive nutrient enrichment, are an ever–growing threat (Dudgeon et al., 2006). For this study, waste dumping was prevalent in the sampled area, given the lack of appropriate waste disposal facilities. Dry pans are used as dumping sites with plastics and even baby diapers. Once inundation occurs after the wet season, the waste is seen within these pans (C.P. Mungenge, *pers. observ.*). The endorheic nature of the pans allows no outward drainage or flushing, which can cause an accumulation of nutrients from the latrines, livestock waste and waste pollution within these systems. (Henri et al., 2014b; Nhiwatiwa et al., 2019).

Freshwater systems are threatened globally by overexploitation, pollution, and destruction and/or degradation of habitat (Dudgeon et al., 2006; De Villiers, 2007; Reid et al.,2019). This is problematic as wetlands are often biodiversity hotspots that support high densities and diversities of wildlife (Calhoun, 2017). Nutrient enrichment (particularly phosphorous and nitrogen) associated with anthropogenic activities can, therefore alter algal production within aquatic ecosystems and have detrimental consequences on these systems (Chislock et al., 2013). Anthropogenic–mediated changes to temporary wetlands can alter hydroperiods (Eullis and Mushet, 2004), leading to compositional change in biotic communities, altered predation

pressure, invasions and altered biogeochemical cycles which can disrupt ecosystem functioning and of the loss of ecosystem services.

5.5 Conclusions

As we hypothesised, the pans with human disturbances had higher nutrient concentrations, and these anthropogenic activities did impact the nutrient concentration dynamics and chl–*a* concentrations of temporary pans. Proximity to kraals had the strongest influence on the chl–*a* in the pans. Continuous monitoring strategies need to be established to better understand the nutrient dynamics over time, especially since the Khakhea Bray Transboundary Aquifer Region pan systems are an understudied area and a major water source for the people in the area.

CHAPTER 6: SALINISATION EFFECTS ON BENTHIC PHYTOPLANKTON COMMUNITIES IN TEMPORARY PANS

"Science makes people reach selflessly for truth and objectivity; it teaches people to accept reality, with wonder and admiration."

–Lise Meitner

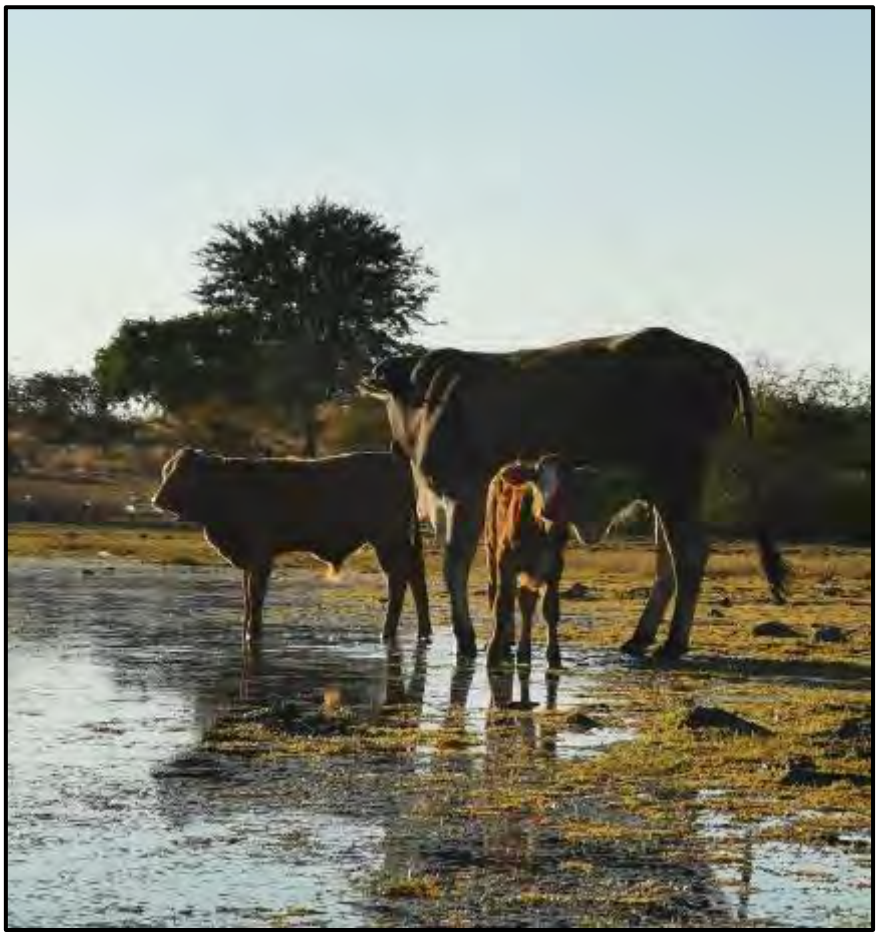


Plate 6. Cattle observed from nearby homesteads utilising the temporary pans as a source of water

Abstract

Freshwater salinisation is rapidly accelerating globally and posing a threat to biodiversity within freshwater ecosystems. Freshwater salinisation, primarily driven by land use changes altering hydrological and hydrogeological balances and ecological integrity, will negatively impact the quality and provision of water and related ecosystem services globally. Despite its profound implications, limited research has been done on the impacts of freshwater salinisation on phytoplankton in temporary pan systems. This study employed a laboratory–based approach to investigate how benthic phytoplankton community structure in semi–arid temporary pans changes with increased salinity over time using sediment from the Khakhea–Bray Transboundary Aquifer region. Sediment containing phytoplankton propagules from dry pans was exposed to water with varying salinities (0–10 ppt), and emergent benthic phytoplankton were assessed over a 35–day hydroperiod. Elevated salinity levels not only caused notable shifts in abiotic factors but also led to shifts in the benthic phytoplankton community, favouring the proliferation of saline–tolerant species such as the diatoms *Melosira varians*, *Navicula radiosa* and *Navicula zanonii* at the cost of more sensitive taxa. The study also revealed that in interaction with salinisation, time also exerted a notable influence on shaping the benthic phytoplankton community.

Keywords: *freshwater salinisation, saline–tolerance, diatoms, temporary pans, community structure*

6.1 Introduction

Freshwater ecosystems are experiencing declines in biodiversity worldwide as species losses continue at alarming rates (Dudgeon et al., 2006). Amongst the global threats to biodiversity is freshwater salinisation, which is occurring at an exceptional rate and geographic scale (Herbert et al., 2015). Freshwater salinisation will impact the quality and provision of water and related ecosystem services globally (Reid et al., 2019). The primary cause of freshwater salinisation is land use change within catchments, which alters hydrological and hydrogeological balances and ecological integrity (Williams, 1999). These anthropogenic modifications, coupled with climate change, are expected to additionally increase the severity of freshwater salinisation, with profound consequences for wetlands in arid and semi–arid regions (Cunillera–Montcusí et al., 2022).

The impacts of freshwater salinisation are adverse, diverse and, in most cases, irreversible (Williams, 1999). Salinisation levels can exceed the tolerance levels of sensitive biota (Nielsen et al., 2003b). Osmotic stress due to changes in the water and sediment chemistry in osmoconformers can then interfere with the ecological and physiological functions of the biota in these systems, thus adversely affecting life histories, fitness and food supply (Brock et al., 2005). Sustained periods of increased salinity can reduce productivity within aquatic ecosystems (Reid et al., 2019). Over time, the biological effects of salinisation will include the replacement of salt–intolerant species with those that can tolerate elevated concentrations (Radke et al., 2003). These effects lead to the loss of species and modifications to the community structure, thereby significantly altering trophic systems by reducing consumer food sources (Nielsen et al., 2007).

The ecological importance of temporary wetlands is enhanced by their ephemeral hydroperiod and small size (Calhoun et al., 2017), with hydroperiod as the primary driver of community and population dynamics in these wetlands (Boix and Batzer, 2016). Temporary wetlands are highly variable ecosystems with regularly changing physical and chemical characteristics, depending on the local climate, soil characteristics, and hydrology (De Roeck et al., 2007). However, their small size and depth make them vulnerable to anthropogenic impacts, including salinisation (Jiménez–Melero et al., 2023). They often contain distinct and sometimes endemic species with structural, behavioural and physiological adaptations to living within such habitats (Williams, 2000).

Over the hydroperiod, evaporative conditions and leaching from sediments lead to elevated ion concentrations in the water (Wasserman et al., 2018). Therefore, organisms that inhabit these systems are naturally exposed to a variable abiotic environment over the hydroperiod, including exposure to salinisation (Jolly et al., 2008). Throughout the hydroperiod, species richness and trophic complexity also vary, often predictably (O'Neill and Thorp, 2014; Dalu et al., 2017b), suggesting that temporary wetland communities evolve to deal with this variability. However, unnatural and inflated increases in salinity likely have implications for these phenological patterns in species turnover, as well as overall patterns in diversity and community structure.

Benthic phytoplankton are unicellular microscopic organisms which play a pivotal role at the base of food webs in aquatic ecosystems as primary producers (D'Costa and Naik, 2019). Within temporary pans, they are also a significant food source for benthic fauna such as zooplankton and crustaceans from various functional feeding groups, including filter feeders, deposit feeders and scrapers (Brendonck et al., 2022). In addition to their contribution to food webs, they also play an essential role as biological indicators of human impacts such as

pollution and eutrophication within freshwater environments (Williams, 2006). The diversity of benthic phytoplankton is influenced by sediment chemistry, which is a function of the quality and quantity of organic matter transferred from the pelagic to the benthic zone (Bergamino and Richoux, 2015). Within temporary wetlands, phytoplankton abundance and diversity are also driven by local environmental factors such as hydroperiod, area, nutrients, ionic composition (i.e., pH, conductivity, hardness, calcium) and temperature (Hope et al., 2020; Dalu et al., 2022b).

Like the invertebrate fauna, phytoplankton in temporary wetlands have developed various strategies to survive during the dry periods. In an unpredictable environment, phytoplankton produce dormant propagules that stay in the sediment until inundation (Brock et al., 2003). The benthic phytoplankton form through secondary succession from phytoplankton propagules, such as resting spores preserved in the bottom sediment (Celewicz and Goldyn, 2021). These phytoplankton propagules can be dispersed over substantial distances via different dispersal agents such as wind, water and zoochory (Dalu et al., 2022b). Depending on the prevailing conditions, they can remain dormant for several months to years and germinate when conditions become favourable (Timoney et al., 1997). While such features inevitably have implications for phytoplankton community dynamics in all ecosystems, the role of dormant propagules in driving community dynamics is paramount in temporary wetlands, with an impact on productivity. Most metabolically active phytoplankton communities are driven by emergent dormant propagules, particularly during early hydroperiod stages in temporary wetlands. This is an aspect of temporary wetland ecology that is poorly understood. In particular, specific dormancy cues for different phytoplankton species are underexplored.

Numerous studies have assessed the effects of increased salinisation on invertebrate fauna in temporary wetlands (e.g., Nielsen et al., 2003b; Waterkeyn et al., 2010; Waterkeyn et al., 2011; Mabidi et al., 2018; Mungenge et al., 2023), however despite their crucial role in aquatic food webs, phytoplankton have received much less attention (Bisson and Bartholomew, 1984; Hart et al., 1991). Increased awareness towards primary producers, which are essential food sources for the invertebrates and play a key role in nutrient cycling, will help us understand the emergent effects of freshwater salinisation, from a bottom–up perspective, on ecosystem functioning.

To better understand how salinisation affects benthic phytoplankton in freshwater temporary pans, a laboratory–based approach was used whereby sediment collected from dry pans in the North west Province of South Africa was submerged and exposed to varying salinity concentrations. The present study aimed to experimentally examine how benthic phytoplankton community structure in semi–arid temporary pans changes with increased salinity over time. It was hypothesised that (i) salinisation would cause substantial shifts in abiotic factors, causing a decline in the overall biodiversity (ii) salinisation would disrupt the benthic phytoplankton phenology dynamics in these pans and ultimately (iii) salinisation would exert an influence and alter both benthic phytoplankton community structure and diversity metrics throughout the hydroperiod.

6.2 Materials and methods

6.2.1 Sediment collection

In June 2021, sediment samples were collected from 10 temporary pans in the semi–arid Khakhea–Bray Transboundary Aquifer region, South Africa. These sites were specifically

selected to increase the likelihood of collecting sediment that contains phytoplankton propagules, as various phytoplankton species had been observed in the previous wet season. A more detailed description of the study area and sampling sites is given in Chapter 1. Approximately 15 kg of dry sediment was collected using a shovel from around the centre of each of the ten pans during a dry phase and placed in 20 L plastic buckets. In each pan, the top 5 cm of soil was used. The collected sediment samples were taken back to the Wetland Ecology Laboratory at Rhodes University, Makhanda, where they were kept at room temperature under dry conditions for eight months until February 2022, when the experiments commenced.

6.2.2 Experimental set–up and design

Experiments were conducted in controlled environment (CE) rooms (25 ºC; 12:12 light/dark regime) at the Department of Zoology and Entomology, Rhodes University, Makhanda. These conditions were similar to those measured at the collection site in the field at the time of sampling. The hatching experiments were conducted over 35 days from 21 February to 28 March 2022.

In the laboratory, 3 kg of sediment from each pan was mixed to make one composite sediment sample in a 110 L plastic storage box with a lid and homogenised by repeatedly shaking. This was done to minimise variability and maximise the diversity of phytoplankton propagules (i.e., representative of the region) available for use across treatments. The mixed sediment was then serially sieved through a 5 mm mesh and 500 µm mesh sieve to remove stones, roots and other debris. A 400 g sample of the sieved sediment was placed into each of the 30 separate 2.3 L (19 cm × 19 cm × 9 cm) polypropylene containers (5 replicates × 6 salinity treatments). In each container with sediment, 1.6 L of the treatment salt solution [i.e., distilled water (control), 0.5

ppt, 1 ppt, 2.5 ppt, 5 ppt and 10 ppt] was added. The treatment solutions were made by adding the appropriate amount of natural unrefined Oryx desert salt (crystal white salt harvested from the Kalahari Desert, South Africa, free from additives and preservatives) per litre of distilled water, and the salinity measurement was verified by an AquaRead multiparameter meter (Model AP–700 and AP–800, AquaRead Ltd, UK). The initial water level within each container was marked and maintained throughout the experiment by topping up with distilled water daily. On every third day, zooplankton hatchlings were removed using a 64 µm handheld scoop net to remove grazing pressure. The 30 containers were arranged in a randomised manner within the CE room to remove potential spatial confounds.

6.2.3 Sample processing

Following inundation, all containers were sampled every 7 days for the duration of the 35–day experiment (i.e., 150 samples over 5 weeks of sampling). Before each sample was collected, pH, temperature (ºC), dissolved oxygen (DO: mg L^{-1}), conductivity (mS cm^{-1}), turbidity (NTU) and total dissolved solids (TDS: mg L^{-1}) were measured in each container, using the same AquaRead multiparameter. For the benthic phytoplankton samples, the top 1 cm layer was collected from each treatment container using a Perspex sediment corer (20 mm internal diameter) and transferred into a labelled container before distilled water (20 mL) was added to each sample, followed by 10 drops of Lugol's iodine solution. Qualitative and quantitative analysis of the benthic phytoplankton was done using the Utermöhl method. Before counting, the samples were shaken firmly and left for an hour to allow large soil particles to settle. Then, the supernatant was taken out of the polyethylene containers and allowed to sediment overnight in 50 mL phytoplankton settling chambers. After sedimentation, the chimney of the sedimentation chamber was gently slid off from the bottom plate and replaced by a cover. The

bottom plate was placed on the inverted microscope, and the benthic phytoplankton cells were identified and enumerated (Edler and Elbrächter, 2010). Cells were approximately counted up to 400 cells to achieve a precision of ± 10 % (Andersen and Throndsen, 2003) under an inverted Nikon TMS light microscope at ×400, based on identification guides by John et al. (2002), van Vuuren et al. (2006) and Taylor et al. (2007).

6.2.4 Data analysis

Linear Mixed–effects Models were used to determine the effect of time, salinity and their interaction on the environmental variables measured over the 35 days of the experiment. The models were fit using the "*lmer*" function from the "*lme4*" package in R software (Bates et al., 2015; R Core Team, 2023) with replicate number as the random effect to account for repeated measures in the experiment. Day and salinity were used as the fixed effects in the model. A Type III Analysis of Variance (ANOVA) was used from the "car" package to ascertain the statistical significance of the fixed effects. Diagnostic plots were examined to check the model assumptions and fit.

Multivariate techniques were employed to characterise benthic phytoplankton community assemblages across time and salinity treatments. An analysis of similarities (ANOSIM) was then used to test for differences in similarity or dissimilarity of the benthic phytoplankton communities emerging from the sediment between sampling days and salinity treatments. An ANOSIM R statistic was computed by comparing rank similarities within and among sample groups. The significance of group dissimilarity was assessed through permutation tests. An R–value of 1 denotes complete dissimilarity among groups, whereas an R–value of 0 signifies a high degree of community similarity among salinity treatments and days. The similarity

percentages–species contributions (SIMPER) analysis was employed to assess the contribution of each taxon to group similarity across salinity treatments and days. Taxa with contributions exceeding 5% to within–group similarity were identified. Distance–based Permutational Analysis of Variance (PERMANOVA; Anderson, 2001) was used to test for significant effects of time and salinity treatments on the benthic phytoplankton community. To explore for interactions between treatment and time, a cluster analysis with similarity profile (SIMPROF) was then done with the sample averages per species, using the Bray–Curtis distance to quantify the similarity between samples collected over the experiment period and identify significant cluster groups. Group average linking was then applied to create a dendrogram showing the relationship between the samples collected. These analyses were carried out in PRIMER version 6.1.16 (Clarke and Warwick, 2001; Clarke and Gorley, 2006).

To determine the changes in the benthic phytoplankton species diversity over time at different salinities, the Shannon–Wiener index and Pielou's evenness were calculated for each sample in PRIMER version 6.1.16 (Clarke and Warwick, 2001). Whittaker–beta (β_W) diversity measured species turnover (Whittaker 1960) over time and was also calculated. Whittaker's beta diversity captures species identity and considers species lost, gained and shared between consecutive points in time (Koleff *et al.* 2003). The β_W diversity is hypothesised to mark a substantial change in assemblage composition between two sampling points (i.e. days). β_W diversity was calculated as (re–expressed equation from Koleff et al., 2003):

$$\beta w = \frac{a + b + c}{(2a + b + c)/2} - 1$$

Where a is the number of species shared between sampling days, b is the number of species recorded on the first sampling day and c is the number of species recorded on the next sampling

day. For the calculation, sampling days were regarded as the adjacent pairs. As such, in each replicate container β_W diversity was calculated between days 7 – 14, 14 – 21, 21 – 28 and 28 – 35.

Calculations and statistical analyses were performed in Excel and Sigmaplot version 12. The differences in the diversity metrics among the treatments were then compared using repeated measures ANOVA in R with the "*aov*" function within–subject "*Error*" terms (salinity and time as categorical variables). Tukey–adjusted pairwise comparisons using the "*emmeans*" package were employed to assess the significance of differences between groups (Searle et al., 1980).

6.3 Results

6.3.1 Environmental variables

Environmental variables measured over the 35–day experiment at different salinity treatments showed different patterns. Significant changes in temperature, pH, dissolved oxygen and turbidity ($p < 0.001$) occurred over time. Salinity significantly affected pH, conductivity and TDS across the different salinity treatments ($p < 0.001$). There were no significant effects of the interaction of time and salinity on all the environmental variables measured (all $p > 0.05$), and therefore, the effects of salinity on these parameters were consistent over time (Table 6.1).

Table 6.1. Linear Mixed–effects Model results of effects of time, salinity treatment and their interaction on environmental variables measured. Significant *p*–values are indicated in bold.

Variables	**Day**			**Salinity**			**Day × Salinity**		
	Df	**χ2**	***p***	**Df**	**χ2**	***p***	**Df**	**χ2**	***p***
Temperature	4	170.665	**<0.001**	5	1.979	0.852	20	21.253	0.382
pH	4	54.097	**<0.001**	5	35.846	**<0.001**	20	9.158	0.981
DO	4	12.589	**0.014**	5	2.251	0.813	20	9.796	0.972
Conductivity	4	0.326	0.988	5	218.390	**<0.001**	20	20.551	0.424
Turbidity	4	16.658	**0.002**	5	9.188	0.102	20	22.889	0.294
TDS	4	0.323	0.988	5	218.587	**<0.001**	20	20.406	0.433

6.3.2 Benthic phytoplankton community structure

Throughout the 35 days of the experiment, 63 benthic phytoplankton species belonging to 26 genera were identified (Table A6.1). The Bacillariophyta (diatoms) was the most diverse group, with 49 species identified, representing 77.8% of all species encountered in the study. The second most diverse group comprised 9 Chlorophyta species, comprising 14.3% of all species identified in the study. Four Charophyta and one Cyanophyta species were also recorded. The samples were dominated in abundance by cosmopolitan diatom species such as *Melosira varians*, *Nitzschia linearis*, *Nitzschia pusilla* and *Navicula radiosa*. Other tropical and less cosmopolitan species were also present (Table A6.1).

ANOSIM similarly revealed significant differences in the composition of benthic phytoplankton species across the different salinity treatments (Global $R = 0.229$, $p = 0.01$). Significant pairwise differences were found between 0–1 ppt ($R = 0.239$, $p = 0.03$), 0–2.5 ppt ($R = 0.369$, $p = 0.01$), 0–5 ppt ($R = 0.389$, $p = 0.01$), 0–10 ppt ($R = 0.496$, $p = 0.01$), 0.5–2.5 ppt ($R = 0.261$, $p = 0.04$), 0.5–5 ppt ($R = 0.269$, $p = 0.01$), 0.5–10 ppt ($R = 0.413$, $p = 0.01$), 1–10 ppt ($R = 0.362$, $p = 0.01$). SIMPER analysis showed the predominant phytoplankton species across different salinity treatments. Table 6.2 shows the dominant species and the percentage contribution of each species across the different salinity treatments. At low salinities of 0 ppt and 0.5 ppt, the dominant species were identified to be *Melosira varians*, *Nitzschia pusilla* and *Nitzschia linearis*. However, a salinity increase to 1 ppt resulted in a compositional shift, with *Melosira varians*, *Nitzschia pusilla* and *Navicula radiosa* as the dominant species. At 2.5 and 5 ppt salinity levels, the dominant species comprised *Melosira varians*, *Navicula radiosa* and *Nitzschia linearis,* including *Navicula zanonii.* This species had not been previously recorded at lower salinity treatments. The dominant species at 10 ppt were *Melosira varians*, *Navicula*

radiosa and *Navicula zanonii* (Table 6.2). The benthic phytoplankton species that contributed to the observed differences between the salinity treatments were primarily the diatom species *Nitzschia elegantula*, *Nitzschia pusilla*, *Melosira varians*, *Nitzschia palea*, *Nitzschia linearis* and *Pinnularia viridiformis* (Table 6.3).

There were significant differences in the phytoplankton species composition over time (ANOSIM: $R = 0.576$, $p = 0.01$). Pairwise tests indicated significant differences among all pairs of sampling days (7–14, $R = 0.424$; 7–21, $R = 0.639$; 7–28, $R = 0.8$; 7–35, $R = 0.901$; 14–21, $R = 0.387$; 14–28, $R = 0.669$; 14–35, $R = 0.846$; 21–28, $R = 0.609$; 21–35, $R = 0.779$; 28–35, $R = 0.321$, $p < 0.01$ in all cases). Over time, distinct patterns were observed in the benthic phytoplankton composition. On day 7, the dominant species included *Melosira varians*, *Nitzschia linearis* and *Pinnularia viridiformis*. By day 14, the dominant species shifted to *Melosira varians*, *Nitzschia linearis* and *Craticula cuspidata*. *Navicula radiosa* was also dominant, a species not previously recorded on day 7. There was a shift on day 21 again with *Melosira varians*, *Navicula zanonii* and *Nitzschia pusilla* as the dominant species. Following day 21, composition stabilised with *Melosira varians* , *Navicula radiosa* and *Navicula zanonii* remaining as the dominant species from day 28 to 35 as indicated in Table 6.2. The differences between the different sampling days were related to the high average dissimilarity distances among all the pairs of days. The species that most contributed to the observed differences between the sampling days were the diatom species *Navicula radiosa*, *Navicula zanonii*, *Craticula cuspidata*, *Melosira varians, Pinnularia viridiformis* and *Nitzschia pusilla* (Table 6.3).

Table 6.2. Results of SIMPER analysis indicating benthic phytoplankton species contributions to the average similarity among different salinity treatments and different sampling days.

Group	Average similarity	Main contributing species
Salinity treatment		
0ppt	64.11	*Melosira varians* (29.69 %), *Nitzschia pusilla* (10.70 %), *Nitzschia linearis* (8.63 %), *Craticula cuspidata* (7.90 %)
0.5ppt	67.09	*Melosira varians* (26.79 %), *Nitzschia linearis* (12.58 %), *Nitzschia pusilla* (10.52 %), *Nitzschia elegantula* (7.63 %)
1ppt	69.12	*Melosira varians* (27.85 %), *Nitzschia pusilla* (10.92 %), *Navicula radiosa* (10.23 %), *Nitzschia linearis* (9.44 %)
2.5ppt	66.99	*Melosira varians* (27.81 %), *Navicula radiosa* (11.29 %), *Nitzschia linearis* (9.66 %), *Navicula zanonii* (8.92 %)
5ppt	65.86	*Melosira varians* (22.65 %), *Nitzschia linearis* (12.15 %), *Navicula radiosa* (10.32 %), *Navicula zanonii* (9.54 %)
10ppt	69.35	*Melosira varians* (24.95 %), *Navicula zanonii* (11.40 %), *Navicula radiosa* (9.85 %), *Nitzschia linearis* (9.17 %)
Sampling day		
Day 7	63.42	*Melosira varians* (26.50 %), *Nitzschia linearis* (12.14 %), *Pinnularia viridiformis* (11.23 %), *Craticula cuspidata* (10.04 %)
Day 14	71.29	*Melosira varians* (28.00 %), *Nitzschia linearis* (15.11 %), *Craticula cuspidata* (9.59 %), *Navicula radiosa* (9.36 %)
Day 21	72.69	*Melosira varians* (26.61 %), *Navicula zanonii* (11.81 %), *Nitzschia pusilla* (10.35 %), *Nitzschia linearis* (9.60 %)
Day 28	64.48	*Melosira varians* (25.72 %), *Navicula radiosa* (14.22 %), *Navicula zanonii* (14.14 %), *Nitzschia pusilla* (12.22 %)

Day 35	63.55	*Melosira varians* (26.06 %), *Navicula radiosa* (16.22 %), *Navicula zanonii* (14.20 %), *Nitzschia pusilla* (7.56 %)

Table 6.3. Summary of results of two–way crossed ANOSIM and SIMPER to identify benthic phytoplankton species contributing to differences observed between different days and salinity treatments from sediment from the Khakhea Bray Transboundary Aquifer region

Groups	Global test R	Pairwise test R	Pairwise test p–value	Dissimilarity distance	Main contributing taxa to dissimilarity
Salinity	**0.229**				
0 ppt vs 0.5 ppt		0.178	0.5	37.20	*Nitzschia linearis* (6.39 %), *Melosira varians* (6.24 %), *Nitzschia elegantula* (5.92 %), *Navicula zanonii* (5.71 %)
0 ppt vs 1 ppt		0.239	0.03	39.49	*Nitzschia elegantula* (7.00 %), *Nitzschia pusilla* (6.57 %), *Craticula cuspidata* (6.49 %), *Nitzschia linearis* (6.22 %)
0 ppt vs 2.5 ppt		0.369	0.01	40.55	*Nitzschia pusilla* (6.68 %), *Nitzschia linearis* (6.45 %), *Nitzschia elegantula* (6.26 %), *Navicula radiosa* (5.81 %)
0 ppt vs 5 ppt		0.389	0.01	41.99	*Melosira varians* (7.50 %), *Nitzschia linearis* (6.57 %), *Navicula radiosa* (6.16 %), *Nitzschia pusilla* (6.14 %)
0 ppt vs 10 ppt		0.496	0.01	42.24	*Melosira varians* (7.44 %), *Nitzschia linearis* (6.31 %), *Nitzschia pusillla* (5.87 %), *Nitzschia elegantula* (5.86 %)
0.5 ppt vs 1 ppt		0.105	4.8	33.74	*Nitzschia pusilla* (7.04 %), *Pinnularia viridiformis* (6.24 %), *Melosira varians* (5.99 %), *Nitzschia elegantula* (5.98 %)
0.5 ppt vs 2.5 ppt		0.261	0.04	35.65	*Nitzschia pusilla* (6.93 %), *Nitzschia palea* (6.50 %), *Nitzschia elegantula* (6.23 %), *Navicula radiosa* (6.17 %)
0.5 ppt vs 5 ppt		0.269	0.01	36.80	*Melosira varians* (7.60 %), *Nitzschia palea* (7.49 %), *Navicula zanonii* (6.48 %), *Nitzschia pusilla* (6.47 %)
0.5 ppt vs 10 ppt		0.413	0.01	37.27	*Nitzschia palea* (7.18 %), *Melosira varians* (7.05 %), *Nitzschia pusilla* (6.46 %), *Nitzschia elegantula* (6.41 %)

Groups	Global test R	Pairwise test R	Pairwise test p–value	Dissimilarity distance	Main contributing taxa to dissimilarity
1 ppt vs 2.5 ppt		0.014	39.3	32.43	*Nitzschia palea* (7.60 %), *Nitzschia pusilla* (6.73 %), *Nitzschia linearis* (6.48 %), *Nitzschia elegantula* (6.47 %)
1 ppt vs 5 ppt		0.186	0.2	34.75	*Nitzschia palea* (7.70 %), *Melosira varians* (7.68 %), *Navicula zanonii* (6.67 %), *Navicula radiosa* (6.35 %)
1 ppt vs 10 ppt		0.362	0.01	35.28	*Melosira varians* (7.47 %), *Nitzschia pusilla* (7.29 %), *Nitzschia palea* (7.11 %), *Pinnularia viridiformis* (6.71 %)
2.5 ppt vs 5 ppt		0.009	42.2	34.23	*Melosira varians* (7.24 %), *Nitzschia pusilla* (6.73 %), *Nitzschia palea* (6.59 %), *Navicula zanonii* (6.52 %)
2.5 ppt vs 10 ppt		0.194	0.07	34.09	*Nitzschia pusilla* (8.25 %), *Melosira varians* (6.83 %), *Nitzschia linearis* (6.66 %), *Navicula zanonii* (6.61 %)
5 ppt vs 10 ppt		0.14	0.2	34.63	*Melosira varians* (8.59 %), *Nitzschia linearis* (6.81 %), *Nitzschia pusilla* (6.68 %), *Navicula zanonii* (6.65 %)
Days	**0.576**				
7 vs 14		0.424	0.01	41.03	*Navicula radiosa* (8.26 %), *Melosira varians* (7.13 %),*Nitzschia elegantula* (6.40 %), *Nitzschia palea* (6.18 %),
7 vs 21		0.639	0.01	43.62	*Navicula zanonii* (10.36 %), *Melosira varians*(6.70 %), *Diatoma vulgaris* (6.37 %), *Navicula radiosa* (6.05 %)
7 vs 28		0.8	0.01	54.74	*Navicula radiosa* (8.28 %), *Craticula cuspidata* (7.81 %), *Navicula zanonii* (7.68 %), *Melosira varians* (6.65 %)
7 vs 35		0.901	0.01	59.10	*Navicula radiosa* (8.12 %), *Craticula cuspidata* (7.42 %), *Navicula zanonii* (7.26 %), *Nitzschia elegantula* (6.27 %)
14 vs 21		0.387	0.01	32.87	*Navicula zanonii* (11.66 %), *Nitzschia linearis* (6.85 %), *Melosira varians* (6.43 %), *Nitzschia filiformis* (6.34 %)

Groups	Global test R	Pairwise test R	Pairwise test p–value	Dissimilarity distance	Main contributing taxa to dissimilarity
14 vs 28		0.669	0.01	43.10	*Craticula cuspidata* (9.20 %), *Navicula zanonii* (7.98 %), *Melosira varians* (7.73 %), *Nitzschia linearis* (7.57 %)
14 vs 35		0.846	0.01	48.68	*Craticula cuspidata* (9.64 %), *Melosira varians* (8.25 %), *Nitzschia linearis* (8.24 %), *Navicula zanonii* (6.98 %)
21 vs 28		0.609	0.01	40.18	*Craticula cuspidata* (8.23 %), *Melosira varians* (7.67 %), *Nitzschia elegnatula* (7.57 %), *Nitzschia linearis* (7.08 %)
21 vs 35		0.779	0.01	46.15	*Craticula cuspidata* (8.86 %), *Melosira varians* (8.68 %), *Nitzschia elegantula* (7.12 %), *Nitzschia linearis* (6.52 %)
28 vs 35		0.321	0.01	40.03	*Nitzschia pusilla* (8.33 %), *Melosira varians* (6.67 %), *Nitzschia linearis* (6.52 %), *Nitzschia palea* (6.38 %)

Significant effects of salinity (PERMANOVA: Pseudo–F = 2.259; df = 5; p = 0.001) , time (PERMANOVA: Pseudo–F = 19.985; df = 4; p = 0.001) and the interaction time × salinity (PERMANOVA: Pseudo–F = 2.118; df = 20; p = 0.001) were found on the benthic phytoplankton community. The cluster analysis with SIMPROF revealed five significant cluster groups, clearly further highlighting the interaction between salinity treatment and time effects (Figure 6.1). The Control treatment on Day 0 was the most significantly different group from all other sample clusters. *Melosira varians*, *Oscillatoria* sp., *Pinnularia gibba*, *Pinnularia* and *Cymbella* species, which were dominant in Group 1, represented the pioneer species in the early days of the experiment. *Group 2* comprised the intermediate salinity treatments (0.5, 1 and 2.5 ppt) from early on in the experiment (Day 7). *Group 3* included a combination of all salinities but at intermediate points (days 14–21) except for the Salinity 10 treatment on Day 7 and the Control treatment on Day 28. *Group 4* was comprised of later–stage samples (days 28–

35) from lower salinity treatments (0, 0.5, 1 and 2.5), while *Group 5* was comprised of a combination of later–stage samples (days 28–35) from higher salinity treatments (5 and 10) and final day treatments (Day 35) from all salinities other than the control. Species dominant in Gropus 2–5 were *Melosira varians*, various *Nitzschia* and *Navicula* species.

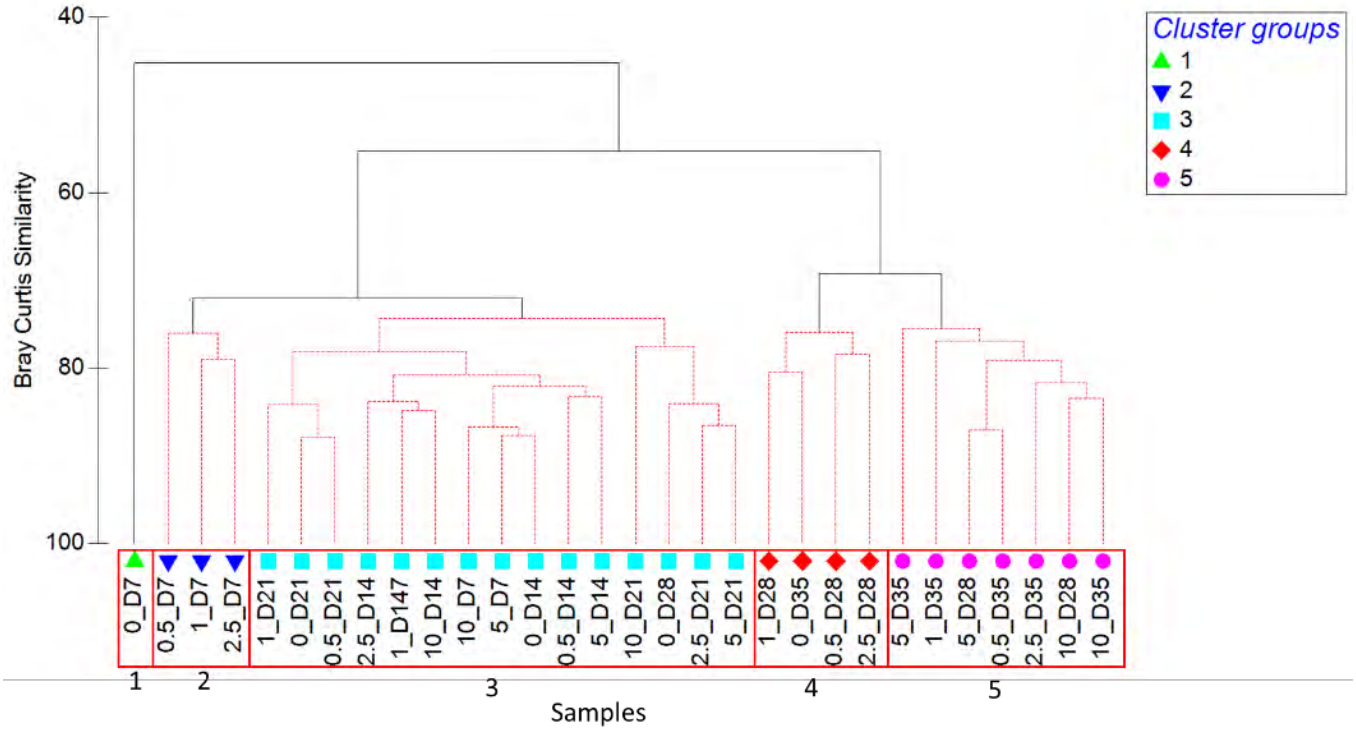


Figure 6.1. Cluster analysis with SIMPROF of benthic phytoplankton communities emerging over the 35 days from sediment. Red boxes denote significant cluster groups. The code per sample denotes the treatment and time, whereby the first letter (0, 0.5, 1, 2.5, 5, 10) represents the salinity treatment (in ppt) and the final two alphanumeric values denote the sampling day (D7, D14, D21, D28, D35).

6.3.3 Diversity metrics

Phytoplankton diversity metrics varied over time and across salinity treatments. The Shannon–Wiener diversity did not differ significantly over time and across salinity treatment ($p > 0.05$

in both cases), but there was a significant time × salinity interaction effect ($p = 0.009$). This interaction was driven by significant differences between the control group on day 7 at 0 ppt and treatments at 0ppt from later days [day 7:0 ppt – day 14:0 ppt ($p = 0.011$); day 7:0 ppt – day 28:0 ppt ($p = 0.022$)] and also differences of the same control group with other days with high salinities [day 7:0 ppt – day 28:1 ppt ($p = 0.033$); day 7:0 ppt–day 7:5 ppt ($p = 0.031$) and day 7:0 ppt–day 28:5 ppt ($p = 0.013$)] These findings highlight distinct variations in the benthic phytoplankton species community on day 7 at 0 ppt, not only compared to samples in later days at low salinities but also in comparison to samples from high salinities at later stages of the experiment. No significant salinity, time and salinity × time interaction effects were evident for Pielou's evenness ($p > 0.05$ in all cases).

On day 7, high species diversity was observed at 0 ppt (1.97 ± 0.5) and 0.5 ppt (1.97 ± 0.2) according to the Shannon–Wiener index. Between days 14–21, species diversity at lower salinities declined, followed by an increase from days 28–35 (Figure 6.2a, b). In these lower salinity treatments (0 ppt and 0.5 ppt), species composition remained relatively even with a decrease in Pielou's evenness on day 21 at 0 ppt (0.74 ± 0.03) and 0.5 ppt (0.74 ± 0.08) (Figure 6.3a, b). At 1 ppt and 2.5 ppt, a noticeable community shift led to reduced diversity on Day 14 (Fig 6.2c,d). Species distribution, however, remained even from day 21 to day 35, as shown by high Pielou's evenness (Figure 6.3c,d). At 5 ppt salinity, diversity fluctuated, peaking on day 21 (2.02 ± 0.1) but decreasing on day 28 (1.79 ± 0.2) before rising again on day 35 (2.1 ± 0.1) (Figure 6.2e). At a salinity level of 10 ppt, the Shannon Wiener index slightly decreased on day 14 (1.81 ± 0.3) (Figure 6.2f), but Pielou's evenness increased on day 21 (0.84 ± 0.08). Species diversity remained stable while Pielou's evenness peaked on day 28 (0.85 ± 0.03) and decreased on day 35 (0.82 ± 0.04) (Figure 6.3f).

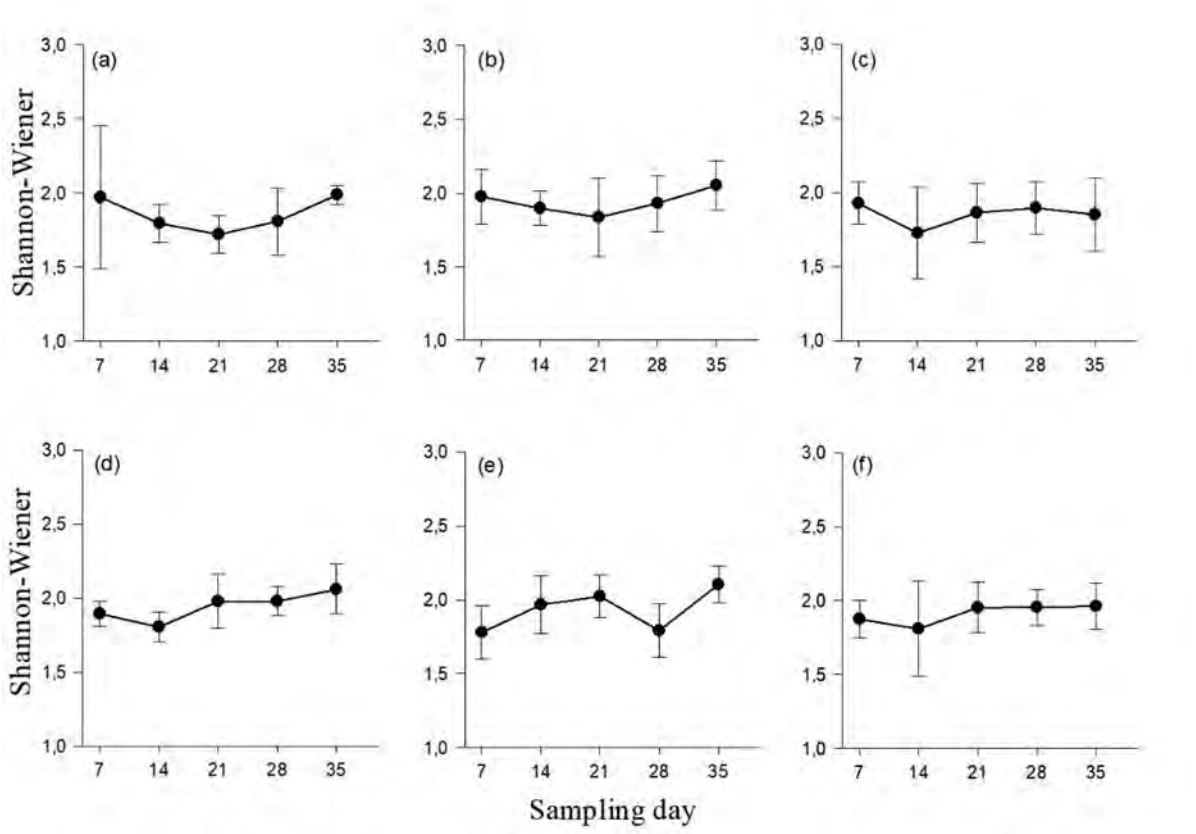


Figure 6.2. Shannon–Wiener index trends over the 35 days at salinities of (a) 0 ppt (b) 0.5 ppt (c) 1 ppt (d) 2.5 ppt (e) 5 ppt and (f) 10 ppt.

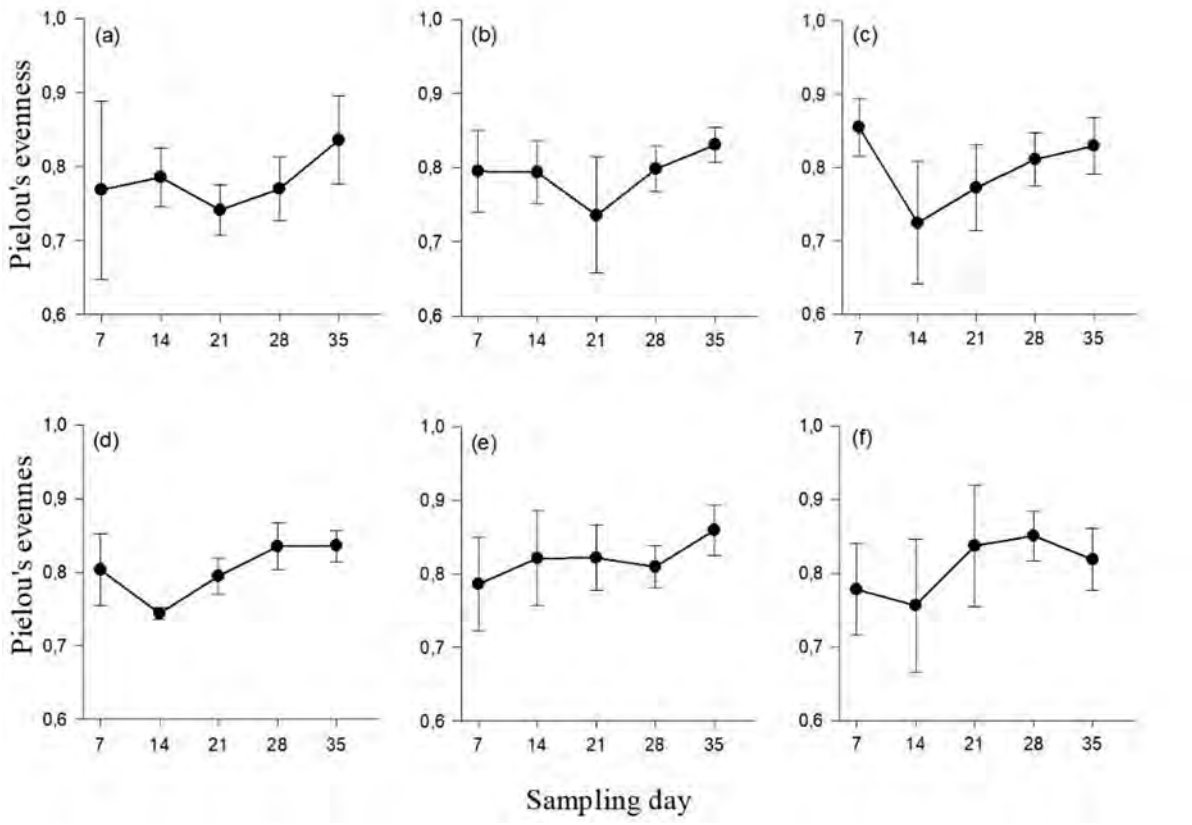


Figure 6.3. Pielou's evenness trends over the 35 days at salinities of (a) 0 ppt (b) 0.5 ppt (c) 1 ppt (d) 2.5 ppt (e) 5 ppt and (f) 10 ppt.

Significant differences in β_W diversity were observed for the main effects of time and the interaction between time and salinity (all $p < 0.05$) (Table 6.2). Post–hoc analysis further revealed significant effects of time × salinity were found for β_W at 0 ppt for days 7–14 and days 14–21 ($p = 0.016$). Additionally, at 0.5 ppt, significant effects were noted for days 7–14 and 14–21 ($p = 0.002$); day 7–14 and day 21 –28 ($p = 0.019$); day 7–14 and day 28–35 ($p = 0.026$). From days 7–14, a pronounced shift was observed at low salinities of 0 ppt (β_W–diversity: 0.65 ± 0.06) and 0.5 ppt (β_W–diversity: 0.5 ± 0.1). However, from day 14–21, these low salinities exhibited high similarity in species composition, reflected by the low β_W–diversity at 0 ppt

(0.20 ± 0.1) and 0.5 ppt (0.26 ± 0.03). Species turnover increased from day 28 to 35 at 0 and 0.5 ppt. (Figure 6.3a,b). In contrast, species composition remained similar from day 7–21, with a slight increase in turnover from day 21–28 and day 28–35 (Figure 6.3c). At 2.5 ppt, species composition remained consistent from days 7–35 with low β_W–diversity values ranging from 0.29–0.36 (Figure 6.3d). For the high salinities of 5 ppt and 10 ppt, similarity in species composition persisted from day 7–21. However, substantial turnover occurred from day 21–28 at 5 ppt (0.43 ± 0.04) and 10 ppt (0.46 ± 0.02). This high species turnover persisted from day 28 to 35 at 5 ppt (0.43 ± 0.07). Species composition at 10 ppt between days 28–35 was similar, as indicated by the drop in the β_W–diversity (0.26 ± 0.07) (Figure 6.2e,f).

Table 6.4. Repeated measures analysis of variance (ANOVA) results of effects of time, salinity and time × salinity on diversity metrics. Significant *p*–values are indicated in bold.

Diversity metric	Day			Salinity			Day × Salinity		
	Df	**F**	***p***	**Df**	**F**	***p***	**Df**	**F**	***p***
Shannon–Wiener	4	1.805	0.135	5	0.429	0.827	20	2.115	**0.009**
Pielou's evenness	4	0.625	0.646	5	0.760	0.581	20	1.622	0.064
Beta diversity	3	3.756	**0.015**	5	1.370	0.246	15	2.190	**0.014**

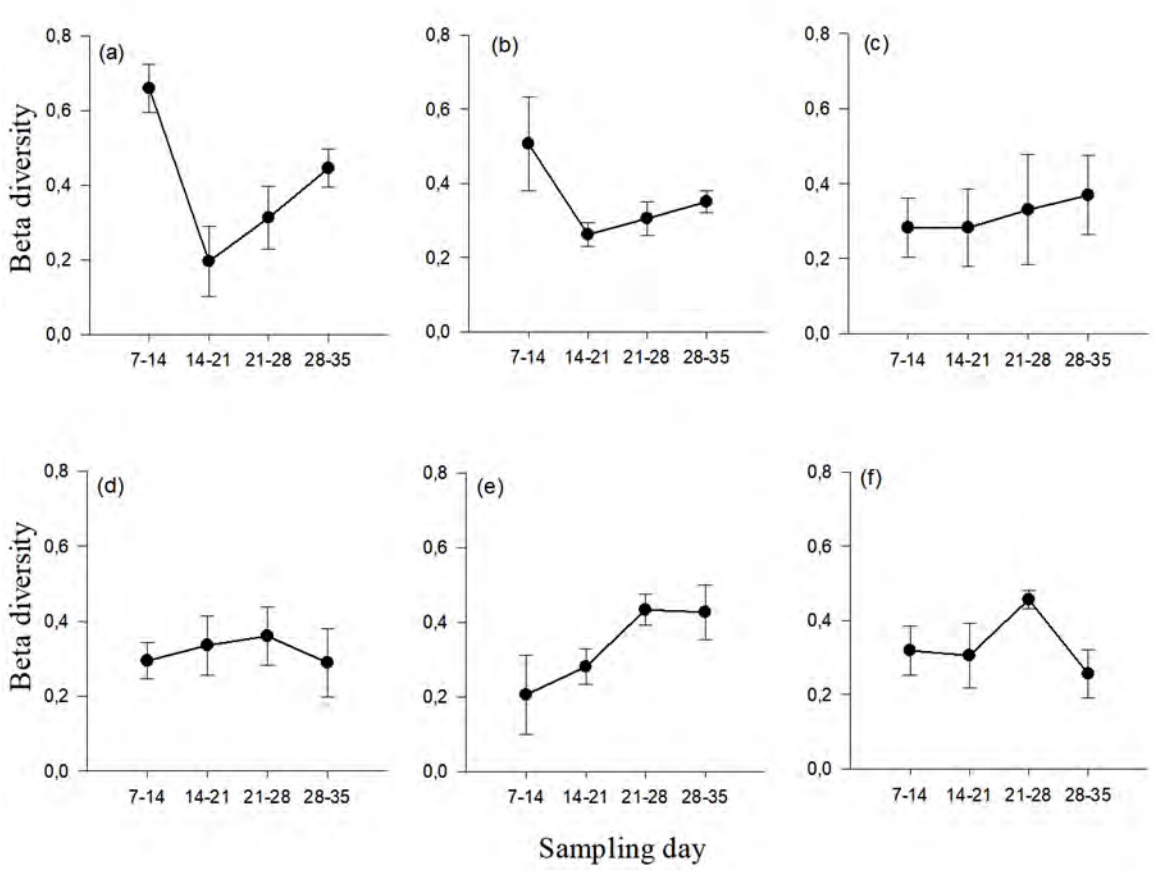


Figure 6.4. Temporal variation in β_W–diversity (Mean ± standard deviation) of benthic phytoplankton communities at salinities of (a) 0 ppt (b) 0.5 ppt (c) 1 ppt (d) 2.5 ppt (e) 5 ppt and (f) 10 ppt.

6.4 Discussion

The impact of salinisation on the abiotic factors, benthic phytoplankton community and diversity metrics were found to be significant as predicted. Additionally, this study's findings also revealed that in interaction with salinisation, time also exerted a notable influence on shaping the benthic phytoplankton community. In line with the first hypothesis, there were significant salinisation effects on the environmental variables pH, conductivity and TDS. Significant temporal effects were also observed for other environmental variables such as temperature, pH, dissolved oxygen and turbidity. Parallel with expectations in the second

hypothesis, salinisation did disrupt phenology dynamics with increased salinisation, causing the accelerated development of later–stage phytoplankton communities during the early stages. Consistent with the third hypothesis, salinisation led to shifts in the benthic phytoplankton community, favouring the proliferation of saline–tolerant species at the cost of more sensitive taxa. At lower salinity levels of 0 ppt and 0.5 ppt, the diatom species *Melosira varians*, *Nitzschia pusilla* and *Nitzschia linearis* dominated the community. Most of the Chlorophyta species emerging from the sediment were found only at salinities of 2.5 ppt and below, indicating their susceptibility to elevated salinity levels. However, a slight increase to 1 ppt immediately caused a shift with *Navicula radiosa* dominating. Subsequent increases in salinity exceeding 2.5 ppt caused *Melosira varians* to dominate and saw the co–dominance of two other diatom species, *Navicula radiosa* and *Navicula zanonii*. Benthic phytoplankton responses to salinisation were also associated with statistically significant changes in the diversity metrics Shannon–Wiener index and the Whittaker beta diversity, with changes in these metrics over time being conserved at lower salinities.

Species dominant at elevated salinities, such as *Melosira varians*, various *Nitzschia* species and *Navicula* species, are taxa generally known for their ability to survive in variable water quality conditions. For example, *Melosira* taxa are known to inhabit a wide range of ecosystems, spanning from marine, brackish to freshwater benthic ecosystems, with *Melosira varians* primarily favouring alkaline conditions (pH 7–8.5) in oligotrophic to heterotrophic environments (Soltanpour–Gargari et al., 2011). Similarly, *Navicula* sp. and *Nitzschia* sp. are euryhaline species that can regulate their intracellular osmotic pressure and grow at different salinity conditions (Ziemann et al., 2001; Bharathi et al., 2022; Moreno et al..,2021). The co–occurrence of both *Navicula radiosa* and *Navicula zanonii* can be attributed to their preference for alkaline waters, and results from this study identified a significant influence of salinity on

pH, leading to a slight alkaline shift that favoured these two *Navicula* species. Our findings revealed intra–species salt tolerance among the *Nitzschia* species. This intra–species variation among diatoms buffers the immediate effects of environmental changes in highly variable environments such as temporary habitats and may help explain the plankton paradox (Godhe and Rynearson, 2017). Trobajo et al. (2011) also showed the broad salinity tolerance of *Nizschia* species. On the other hand, the elimination of chlorophyte species when there are subtle changes in salinity was shown by Chakraborty et al. (2011) and diatom species such as *Cymbella* spp. are often found in waters characterised by low ionic composition.

The significant temporal changes observed in this study show that the benthic phytoplankton community is not static but changes over time due to drivers occurring over the hydroperiod in temporary pans. Anusa et al. (2012) showed that following inundation, phytoplankton communities are initially dominated by short–residence green algae and then subsequently transition to blue–green algae in the later stages of the hydroperiod, while Dalu et al. (2022) showed that fast–growing phytoplankton species which are adapted to short hydroperiod phases colonise first in the temporary pans while large slow–growing species that are adapted to more stable conditions develop later. Therefore, the dominance of different groups varies over the hydroperiod, with certain species prevalent at specific times while others are not. Aside from salinity, other environmental factors, such as nutrient levels, dissolved oxygen, temperature and turbidity, may also influence phytoplankton community dynamics (Dalu et al., 2022b).

This study's significant time and salinity interaction effects on the benthic phytoplankton community, Shannon–Wiener index and Whittaker beta diversity show that time's influence on phytoplankton structure depends on the specific salinity levels and vice versa. This

interaction emerged because salinity effects differed mainly over time at low salinities below 0.5 ppt, enhancing temporal changes' impact compared to higher salinities. Salinity levels vary throughout the hydroperiod, and the impact of time on phytoplankton was more pronounced and evident during the initial days and later days at high salinity. Hydroperiod dynamics dictate that as temporary pans dry out, a concentration effect of existing salts in the water ensues (Wasserman et al., 2018). This dynamic environmental setting suggests that benthic phytoplankton species inhabiting these temporary pans may have evolved and adapted to the continual shifts in salt concentrations. The heightened salt tolerance and adaptation allow them to persist and remain the dominant species even as salinity levels increase. As such, saline–tolerant phytoplankton taxa comprised an important component of these systems. These results reiterate that variability is indeed key in temporary wetland phytobenthic community ecology (O'Neill and Thorp 2014), and further suggest that threats to these systems may result in the development of more homogenous communities (less variability) over time, as has been found for invertebrate groups (Dalu et al. 2017b). In particular, the greater the treatment salinity, the more similar the early hydroperiod community was to the later–stage hydroperiod community. These results suggest that saline–tolerant species eventually dominate in the late hydroperiod stages. However, when salinity is artificially increased, these late–stage communities seem to develop more rapidly, altering the phenology dynamics of the benthic phytoplankton community. Species turnover (Whittaker beta–diversity) and Shannon –Wiener index revealed significant shifts due to salinity and time interaction effects. The two metrics were important in reiterating significant species turnover effects where communities from early on in the experiment differed from the species later. Combining both metrics enhances understanding of community assemblage, as demonstrated in a previous study (Shil and Thakare, 2023).

6.5 Conclusion

Our study highlights the role of salinisation in shaping the benthic phytoplankton community structure within temporary pan ecosystems and that salinisation causes changes in the temporal dynamics as well. Salinisation significantly impacted abiotic factors, leading to a shift in the benthic phytoplankton composition favouring saline–tolerant taxa. Temporal dynamics revealed distinct species dominance in the early and late stages of hydroperiod phytoplankton community development. These findings carry broader implications, suggesting that elevated salinisation when a certain threshold of salt concentration is exceeded will have implications for the phytoplankton community itself and zooplankton grazing dynamics and overall secondary productivity within temporary systems, and could eventually alter the structure and dynamics of food webs within temporary pans. This is particularly important in early hydroperiod stages where pioneer communities are affected. This knowledge is crucial for predicting species succession dynamics with increased freshwater salinisation resulting from anthropogenic activities that alter water quality. Future studies should further explore the salinity tolerance dynamics of benthic phytoplankton species, particularly in relation to the grazing preference of crustaceans in temporary pans.

CHAPTER 7: SALINISATION OF ARID TEMPORARY POOLS ALTERS CRUSTACEAN HATCHING SUCCESS BUT NOT PHENOLOGY DYNAMICS

"I am among those who think that science has great beauty. A scientist in his laboratory is not a mere technician: he is also a child confronting natural phenomena that impress him as though they were fairy tales."

– Marie Curie

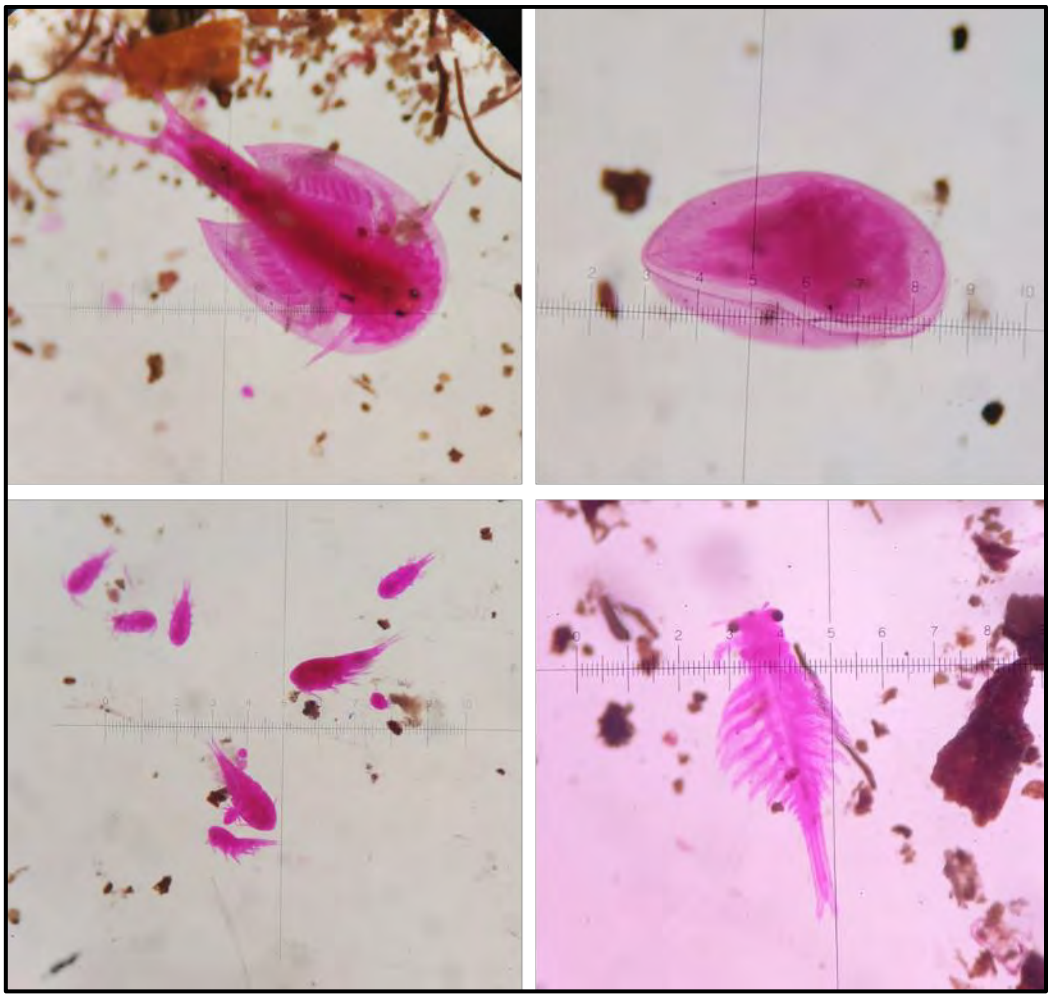


Plate 7. Crustacean hatchlings emerging from sediment collected from the Khakhea Bray Transboundary Aquifer. Top row (Notostraca, Ostracoda), Bottom row (Copepoda, Anostraca). Photos by Chipo P. Mungenge

Abstract

The widespread acceleration of freshwater salinisation due to human activities, such as pollution, resource extraction and urbanization coupled with climate change, poses a significant threat to aquatic ecosystems. Limited work has been directed towards salinisation effects in temporary wetland systems. These systems are characterised by unique crustacean communities reliant on dormant egg production. We assessed salinisation effects on temporary wetland crustacean communities from semi–arid pans in the Khakhea–Bray Transboundary Aquifer region of South Africa using a laboratory–based approach. Sediment from pans containing crustacean resting eggs was exposed to water with varying salinities (0–10 ppt), and emergent hatchlings were assessed over a 30–day hydroperiod. At salinities of 2.5ppt and above, there were significant decreases in emergent taxa richness and abundance. Spinicaudata and Ostracoda were the most sensitive taxa to high salinities. Cladocera, Copepoda, Notostraca and Anostraca hatchlings had shallower decreases with salinity, but hatchability still fell rapidly. There was a limited effect on community hatching phenology dynamics from salinity, with all taxa showing reduced hatchability over time overall, with the exception of Cladocera which exhibited a clear unimodal response, peaking around 20 days post–inundation. This suggests that the main impact of salinisation in these systems will be reductions in hatching success and hence reduced recruitment, leading to changes in predation pressures, food web structure and functioning of these ecosystems, with implications for associated ecosystem services.

Keywords: *agricultural intensification, branchiopods, copepods, human activities, temporary wetlands, zooplankton*

7.1 Introduction

Globally, the salinisation of freshwater ecosystems and projected shifting climates have been identified as major threats to aquatic ecosystems, particularly in arid and semi–arid regions (Engelbrecht et al., 2015; Cunillera–Montcusí et al., 2022). Various anthropogenic activities such as agriculture, resource extraction and urbanisation can act as drivers of freshwater salinisation (Cunillera–Montcusí et al., 2022). Climate change can further intensify this process, where increased temperatures and alterations in the hydrological cycle can lead to greater salt concentrations within freshwater systems (Williams, 2001).

Salinisation can lead to low survival rates, mortalities and shifts in species composition in freshwater ecosystems (Pinder et al., 2005). For many aquatic taxa, increases in salinisation and changes in ion concentrations of the external medium force the organisms to regulate their internal ion concentrations accordingly (osmoregulation) (Cunillera–Montcusí et al., 2022). This increase in osmoregulation has energetic costs that can lead to reduced performance of the organism in the long term, resulting in increased stress and/or mortalities (Kaushal, 2017). This may lead to changes in species diversity, modifying community composition, structure and function.

Temporary depression wetlands, also known as pans, are intermittently inundated systems that have varying hydroperiods (Goudie and Wells, 1995). Their key defining feature is that they dry unpredictably or annually, with hydroperiod being the main driver of population dynamics in these systems (Calhoun et al., 2017). Often found in arid and semi–arid areas, they support highly specialized assemblages of often rare species that are adapted to living in these waters (Brendonck et al., 2022). In many regions, these systems represent much of the available

standing surface water (Williams, 2000), and are therefore heavily utilised by local communities for household and agricultural activities. Temporary wetland systems are vulnerable and at a high risk of the effects of salinisation due to their endorheic nature (Nicolet et al., 2004). These pans also have no outflow to external waterbodies, and this allows no outward drainage or flushing, facilitating an accumulation of salts (Mabidi et al., 2018). Given a lack of baseline studies in certain parts of the world, there is limited knowledge on the extent of salinisation which these systems have been exposed to, and how key specialist taxa are likely to respond to this environmental pressure.

The large branchiopods form the 'flagship' group of aquatic taxa for temporary wetland systems (Brendonck et al., 2007). These branchiopod crustaceans are specialised for life in these systems, with strategies to cope with variable hydrological regimes and disturbances as temporary pans cycle between wet and dry phases (Bird et al., 2019). This group relies on banks of resting eggs to bridge the dry phases. These eggs lie dormant in the substrate in a diapause stage, also called the resting stage, and can remain viable for years if rehydration does not occur (Brendonck et al., 1996). The dormant egg bank is comprised of eggs from various species and is also a means of maintaining genetic, phenotypic, species and community diversity (Brock et al., 2003). The external egg morphology is very diverse and species–specific, while constituting a key taxonomic tool to allow the description of communities even when there is no water present (Brendonck et al., 2007). The egg morphology is also functionally important in mediating the dispersal of the large brachiopods and contributes to the distribution patterns observed in the group (Bilton et al., 2001; Meyer–Milne et al., 2021). In addition to large branchiopods, microcrustaceans such as copepods, cladocerans and

ostracods form part of the crustacean community and similarly produce resting eggs that facilitate population persistence across hydroperiods (Bird et al., 2019).

Crustacean egg dormancy facilitates reproductive "bet–hedging" or risk spreading strategies, where the proportion of eggs that hatch with each inundation should correspond with the likelihood of completing the life cycle with the successful formation of resting eggs (Simovich and Hathaway, 1997; Pinceel et al., 2017). The large branchiopod eggs show both quiescence and diapause dormancy, with the quiescence dormancy initiated by external factors but terminated as soon as conditions become favourable (Brendonck, 1996; Cáceres, 1997). Some diapause stage eggs in the egg bank will, however, not hatch irrespective of environmental cues, as this is internally controlled (Brendonck, 1996). This ensures a fraction of eggs remain dormant in each growing season, thereby buffering against the probability of total reproductive failure in any one inundation event (Pinceel et al., 2017). Nevertheless, for those eggs that are responsive to initiation by external factors, light, temperature, and salinity have all been identified as important cues for effective hatching (Brendonck et al., 1996; Vanschoenwinkel et al., 2009; Pinceel et al., 2013).

There are knowledge gaps on crustacean hatching phenology with salinisation effects, with only a few studies (Nielsen et al., 2007; Satangelo et al., 2014; Mabidi et al., 2018) that have assessed the hatching success of crustaceans with changes in salinity. Understanding hatching phenology for crustacean species in these systems is important since the first colonizers' maturation and quick accumulation of an egg bank inside a population may, due to founder effects, reduce the likelihood of other genotypes establishing (Brendonck et al., 2017). This is

particularly pertinent given predicted shifting climate dynamics, altered hydroperiods and increased disturbances in temporary pan systems (Waterkeyn et al., 2008).

Large branchiopods, inhabiting temporary wetlands, play a pivotal role in the food web structure due to their remarkable diversity and composition of diverse feeding functional groups (Brendonck et al., 2022). This group, which occurs specifically in temporary systems, plays an important role in these environments and is key to maintaining ecosystem stability, functioning and services. In addition to these specialist taxa, the pans contain other micro–crustacean groups which are often ubiquitous habitat generalists found in both permanent and temporary water bodies (Brendonck et al., 2002; Williams, 2006). Aquatic organisms in the pans which include the crustaceans may, however, have varied hatching dynamics, facilitating temporal community variability and phenology.

This study aims to contrast dormant egg hatching dynamics between specialist large branchiopod and microcrustacean components in relation to salinisation, with sediment collected from pan systems in the semi–arid North west Province in South Africa, using a laboratory–based approach. It was hypothesized that (1) the hatching success of the resting eggs of the large branchiopods would be reduced by increased salinities more than the other micro–crustacean taxa, and that (2) salinisation would influence the hatching phenology of the crustaceans through delayed hatching. The hatching phenology aspects considered for this study were the asynchrony of initial hatching and the time of hatching peaks in relation to salinity and time among the different taxa.

7.2 Methods

7.2.1 Sediment collection

In June 2021, samples were collected from temporary pans in the semi–arid Khakhea–Bray Transboundary Aquifer region, South Africa ((–25.978254) – (–26.143701) S, and 23.47345–23.78585 E) (See Chapter 2 for site details). The pans from which the sediment was collected during the wet season generally had low conductivity (mean ± standard deviation: 135.47 ± 39.1µS cm^{-1}), total dissolved solids (129.33 ± 33.9mg L^{-1}) and salinity (0.07 ± 0.02 ppt). Using a shovel, approximately 15 kg of dry sediment was collected in 20 L plastic buckets from different points at the centre of each of the 10 dry pans. In each pan, the top 5cm of soil was used, as it has been shown to contain much of the egg bank in the system (Vanschoenwinkel et al., 2013). Pan selection was based on observing large numbers of crustaceans, including large branchiopods, during a site visit in the previous wet season (T. Dalu, *pers. comm.*). The collected sediment samples were taken back to the laboratory, where they were kept at room temperature under dry conditions until February 2022, when the hatching experiments commenced.

7.2.2 Hatching experiment

Hatching experiments were conducted in controlled environment (CE) rooms (25 ºC; 12:12 light/dark regime) at the Department of Zoology and Entomology, Rhodes University. These conditions were similar to those measured at the collection site in the field at the time of sampling. The hatching experiments were conducted over a 30–day period from 21 February to 23 March 2022.

In the laboratory, 3 kg subsamples from each pan were transferred into a 110 L plastic storage box with a lid and homogenised by repeatedly shaking. All the sediment subsamples were mixed to make one composite sediment sample for the pan system, which was used in the

experiments. Samples from the different pans were mixed in this way to minimize variability and maximize the diversity of crustacean eggs available for use across treatments. The mixed sediment was then serially sieved through a 5 mm mesh and 500 µm mesh sieve to remove stones, roots and other debris. A 200g sample of the sieved sediment was placed into each of the 30 separate 2.3 L (19cm × 19cm × 9cm) polypropylene containers. In each of the containers with sediment, 2 L of the treatment salt solution (i.e., distilled water (control), 0.5 ppt, 1 ppt, 2.5 ppt, 5 ppt and 10 ppt) was added. The treatment solutions were made by adding the appropriate amount of natural unrefined Oryx desert salt (crystal white salt harvested from the Kalahari Desert, South Africa, free from additives and preservatives) per litre of distilled water, and the salinity measurement was verified by an AquaRead multiparameter meter (Model AP–700 and AP–800, AquaRead Ltd, UK). The initial water level within each container was marked. The water level was then topped up to this original mark every two days, with distilled water, to keep the containers from drying and to account for the loss by evaporation, as that will cause changes in ion concentrations. Five replicates were each used for the control and treatments (5 replicates × 6 treatments), and all containers were arranged in a randomised manner within the CE room to remove potential spatial confounds.

All containers were sampled every 3 days after inundation (Henri et al., 2014a). Prior to each sample being collected, pH, temperature (ºC), dissolved oxygen (mg L^{-1}), conductivity (mS cm^{-1}), turbidity (NTU) and total dissolved solids (mg L^{-1}) were measured in each container, using the same AquaRead multiparameter. To count hatchlings, all water in each container was carefully decanted and filtered over an 84 µm mesh sieve. The filtrate was then poured back into the container. Hatchlings on the sieve were washed into labelled containers and fixed with 5 % formalin for 10 days. The hatchlings were then stained with 0.25% w/v Rose Bengal

Solution and preserved in 70 % ethanol, and later identified to order level using an Olympus stereo microscope using identification guides by Day et al. (2001) and Olesen (2007). Abundances were determined for the following crustacean groups: Anostraca (fairy shrimps), Notostraca (tadpole shrimps) and Spinicaudata (clam shrimps), Cladocera (water fleas), Copepoda (copepods), and Ostracoda (seed shrimps).

7.2.3 Data analysis

Linear models were used to test for differences in the environmental variables measured at different salinities [pH, temperature (ºC), dissolved oxygen (mgL^{-1}), conductivity ($mScm^{-1}$), turbidity (NTU) and total dissolved solids (mgL^{-1})], (averaged over time). A mixed generalized linear model (GLMM) was fit with a negative binomial family (accounting for residual overdispersion and/or zero inflation) to examine the total numbers of individuals hatched among taxonomic groups over the total experimental period. A quadratic term was included for the experimental period term (i.e., sampling day), as this improved the model fit, as evidenced by lower AIC (Akaike Information Criterion) ($\Delta > 2$). Models were fit as a function of the taxonomic group and salinity, and their interaction, as well as over time. The individual experimental unit was included as a random effect to account for repeated measures of multiple taxa per replicate and over time. Analysis of deviance was used to infer the main effects with Chi–square tests, and Tukey comparisons were used for post–hoc tests as needed. All analyses were carried out in the R software package (R Core Team, 2021).

7.3 Results

The mean pH values across all the treatments ranged from 7.57–7.97, with pH, significantly decreasing with rising salinity. The mean dissolved oxygen in the containers was similar across

all the treatments, ranging from 3.74–3.94 mg L^{-1}. Conductivity levels and total dissolved solids increased significantly with rising salinity, (Table 7.1). Turbidity levels were lowest in the 2.5 ppt and 0 ppt treatment solutions and were higher in all the other treatments, albeit non–significantly. Temperature was also statistically similar ($p > 0.05$) among salinity groups (Table 1).

Table 7.1. Environmental variables (mean ± standard deviation) measured in the containers at the five different salinity treatments. p–values are from linear models of each variable across salinities, with significant terms in bold

Variable	0 ppt	0.5 ppt	1 ppt	2.5 ppt	5 ppt	10 ppt	t	*p*
Temperature (ºC)	23.80 ± 0.4	23.23 ± 0.5	23.26 ± 0.6	23.21 ± 0.5	23.26 ± 0.6	23.24 ± 0.5	0.192	0.849
pH	7.97 ± 0.2	7.85 ± 0.2	7.75 ± 0.3	7.75 ± 0.2	7.64 ± 0.3	7.57 ± 0.2	5.685	**<0.001**
Dissolved oxygen (mg L^{-1})	3.92 ± 0.7	3.97 ± 0.7	3.89 ± 0.7	3.98 ± 0.7	3.97 ± 0.7	3.74 ± 0.5	1.935	0.063
Conductivity (mS cm^{-1})	0.81 ± 0.3	3.14 ± 0.8	5.54 ± 1.2	12.22 ± 2.9	22.31 ± 4.4	43.81 ± 4.4	66.682	**<0.001**
Turbidity (NTU)	606.98 ± 364.0	802.79 ± 428.6	715.17 ± 534.9	507.66 ± 285.7	772.18 ± 527.3	824.48 ± 444.2	1.204	0.239
Total Dissolved Solids (mg L^{-1})	0.52 ± 0.2	2.04 ± 0.5	3.60 ± 0.8	8.15 ± 1.3	14.50 ± 2.8	28.56 ± 2.9	64.581	**<0.001**

Hatching of crustaceans from six different taxa occurred over the 30–day period of the experiment. By day 3, all the taxa present in the study had hatched in the lower salinity treatments. Taxon richness was highest in the treatment solutions of 1 ppt and below, with 6 taxa emerging (Anostraca, Notostraca, Spinicaudata, Cladocera, Copepoda and Ostracoda). The large branchiopod taxa that emerged from the sediment were of the genera *Branchipodopsis*, *Ozestheria*, *Streptocephalus* and the Notostracan genus *Triops*. The micro–crustacean taxa emergent were *Daphnia, Ceriodaphnia, Eucyclops, Ectocyclops* and ostracods of the family Cyprididae. Copepoda were by far the most abundant taxa across salinities, with 71 384 individuals recorded overall, followed by Cladocera with 2 776 individuals and lastly Ostracoda (1 664 individuals). These three taxa had significantly higher numbers than the Anostraca (521 individuals), Notostraca (71 individuals) and Spinicaudata (433 individuals) (Figure 7.2).

For the large branchiopods, the hatchlings exhibited a single early peak, with the highest densities on day 3 across all treatments, except for Anostraca at 0.5 ppt, which were highest on day 6. Notostraca in 0 ppt exhibited a second peak in hatching activity on day 12 and day 15, while no hatchlings were observed for Notostraca at salinities of 5 and 10 ppt throughout the 30–day period. The Spinicaudata did not hatch over the 30–day period for salinities of 2.5 ppt and above. For the Anostraca no hatching was observed from day 18–30 at 5 ppt and 10 ppt treatment solutions. Small changes in salinity concentration from 1 ppt to 0.5 ppt caused a large drop in hatching for the Anostraca (Figure 7.1)

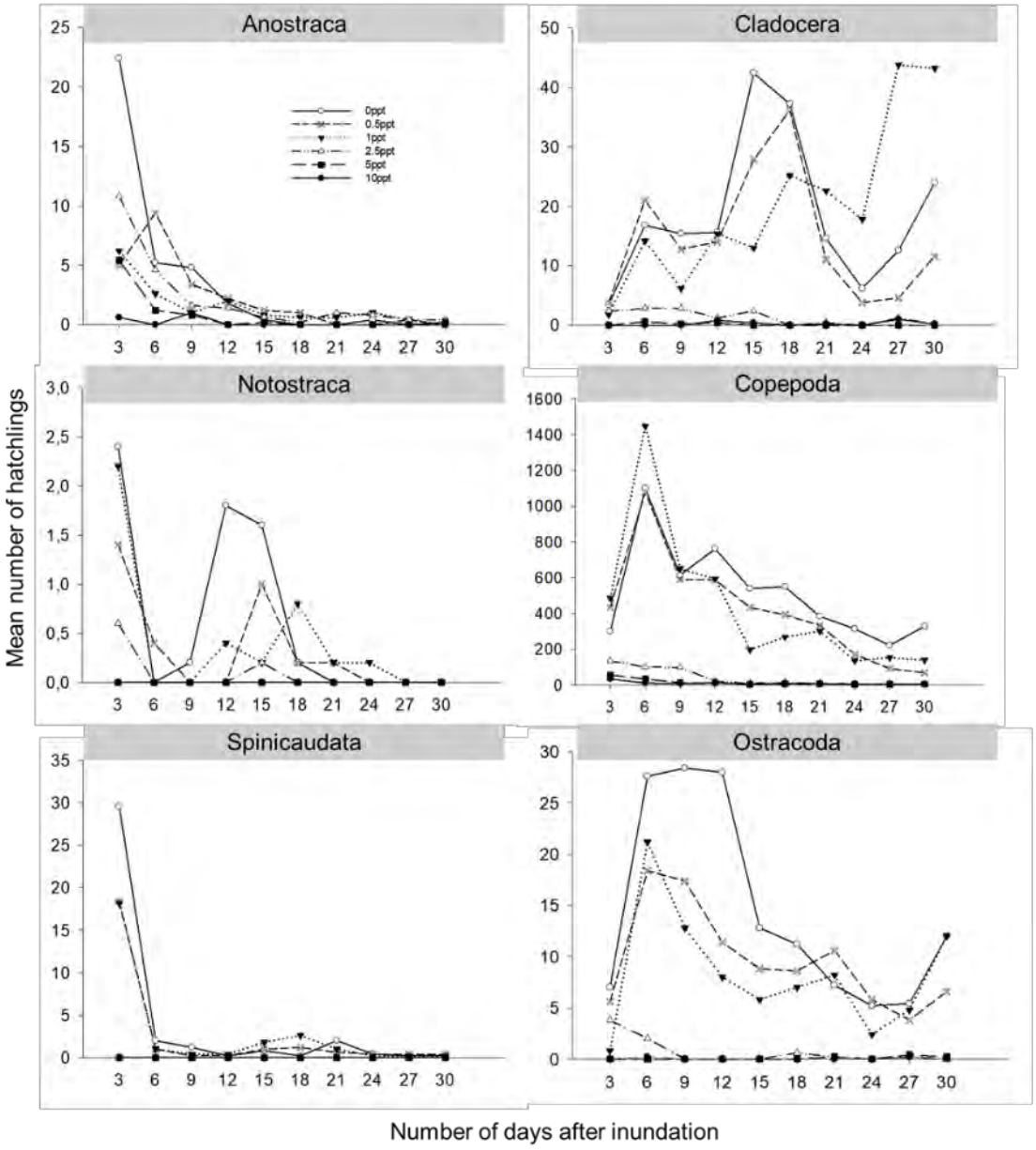


Figure 7.1. Mean number of hatchlings of large branchiopods (left panel) and other crustacean taxa (right panel) that emerged from sediment from the Khakhea Bray Transboundary Region at five different salinity treatment

The highest hatchling abundance for the microcrustacean taxa, however, generally occurred later and were more varied. Copepoda hatchlings peaked on day 6 for the lower salinity

treatments (0 ppt, 0.5 ppt, 1 ppt) and on day 3 for the higher salinities (2.5 ppt, 5 ppt, 10 ppt), although at very low numbers. The Ostracoda hatchling peak at 0 ppt spanned days 6 – 12. At 0.5 ppt and 1 ppt, Ostracoda hatchlings peaked on day 6, while at 2.5 ppt and 5 ppt peaked on day 3 (at very low numbers). Cladocera hatchlings peaked on day 15 at 0 ppt, day 18 at 0.5 ppt and day 30 at 1 ppt. Very few Cladocera emerged at salinities above 1 ppt (Figure 7.1)

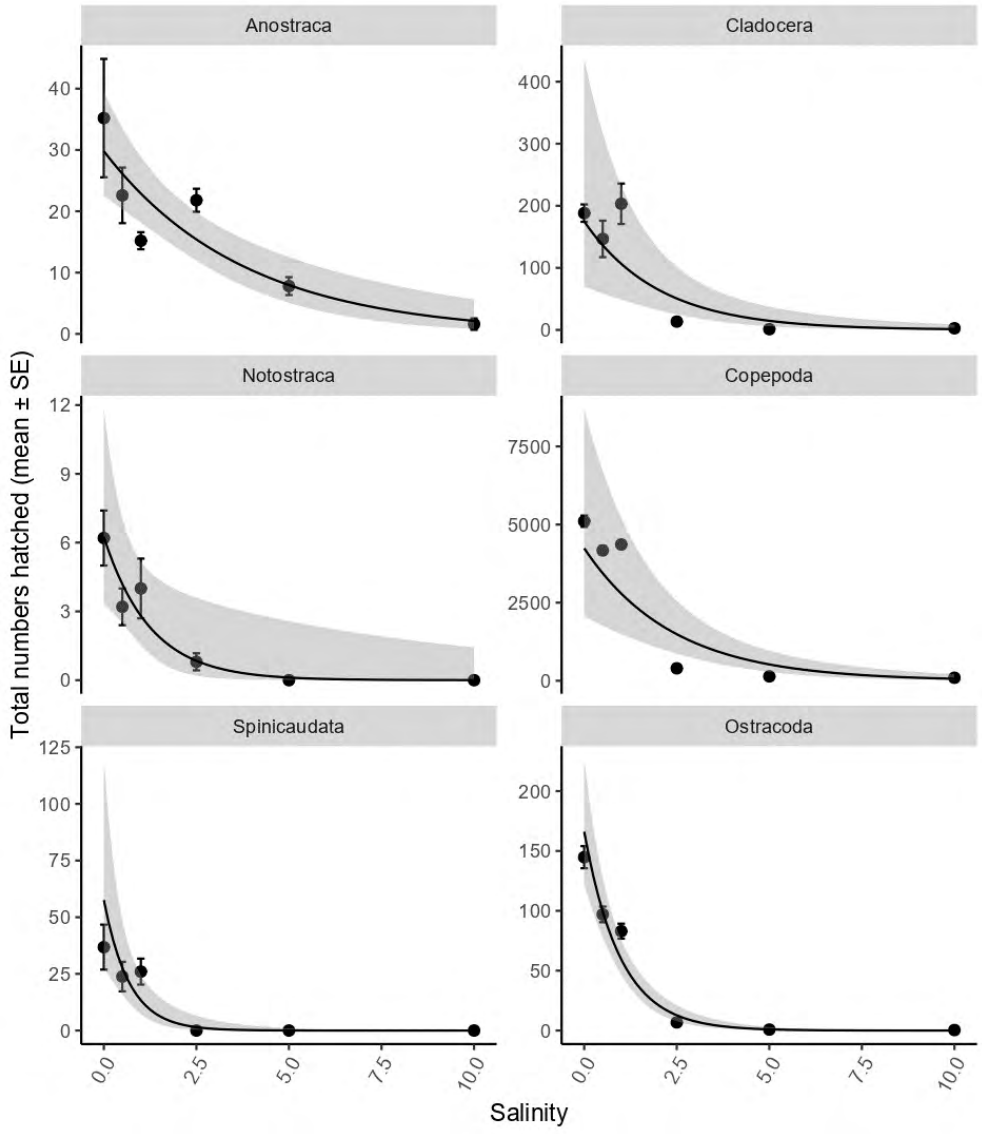


Figure 7.2. Mean numbers of individuals hatched per taxon over the entire experimental period across salinities. Means are shown alongside standard errors. Lines correspond to the negative

binomial generalized linear model fit with confidence intervals shaded in grey. Note y–axes differ among panels

The number of hatchlings differed significantly among taxonomic groups in two–way interactions with salinity regime (GLMM: $\chi^2 = 70.576$, df = 5, $p < 0.001$) and time (GLMM: $\chi^2 = 212.776$, df = 10, $p < 0.001$) (Figures 7.2 and 7.3). The responses of the various taxa differed with salinity reductions as well as over time. However, overall responses to salinity over time among taxa were similar owing to a non–significant interaction (GLMM: salinity × time: $\chi^2 = 2.272$, df = 2, $p = 0.321$; taxon × salinity × time: $\chi^2 = 16.245$, df = 10, $p = 0.093$). Therefore, temporal responses among taxa were not mediated by the salinity regime. All taxa displayed a negative relationship with increasing salinity in terms of abundance. For the large branchiopods, hatchability responses were similar, but Anostraca hatchability responses to salinity were significantly different to the micro–crustaceans, which showed steeper declines (Cladocera, Copepoda and Ostracoda) (Tukey: all $p < 0.05$). Copepoda and Cladocera hatching trends significantly differed from Ostracoda, which had the steepest decline with rising salinity (Tukey: both $p < 0.05$) (Figure 7.2). Over time, Anostraca showed the strongest reductions in hatchability, with stronger negative trends than the Cladocera, Copepoda and Ostracoda, which tended to continue hatching later (all $p < 0.001$). Cladocera was the only taxon with non–linear hatchability trends over time, which initially rose before falling, and this was also significantly different to Copepoda (Tukey: $p < 0.001$), which in turn differed to Ostracoda (Tukey: $p < 0.05$) (Figure 7.3). Other trend comparisons were not statistically clear (Tukey: all $p > 0.05$).

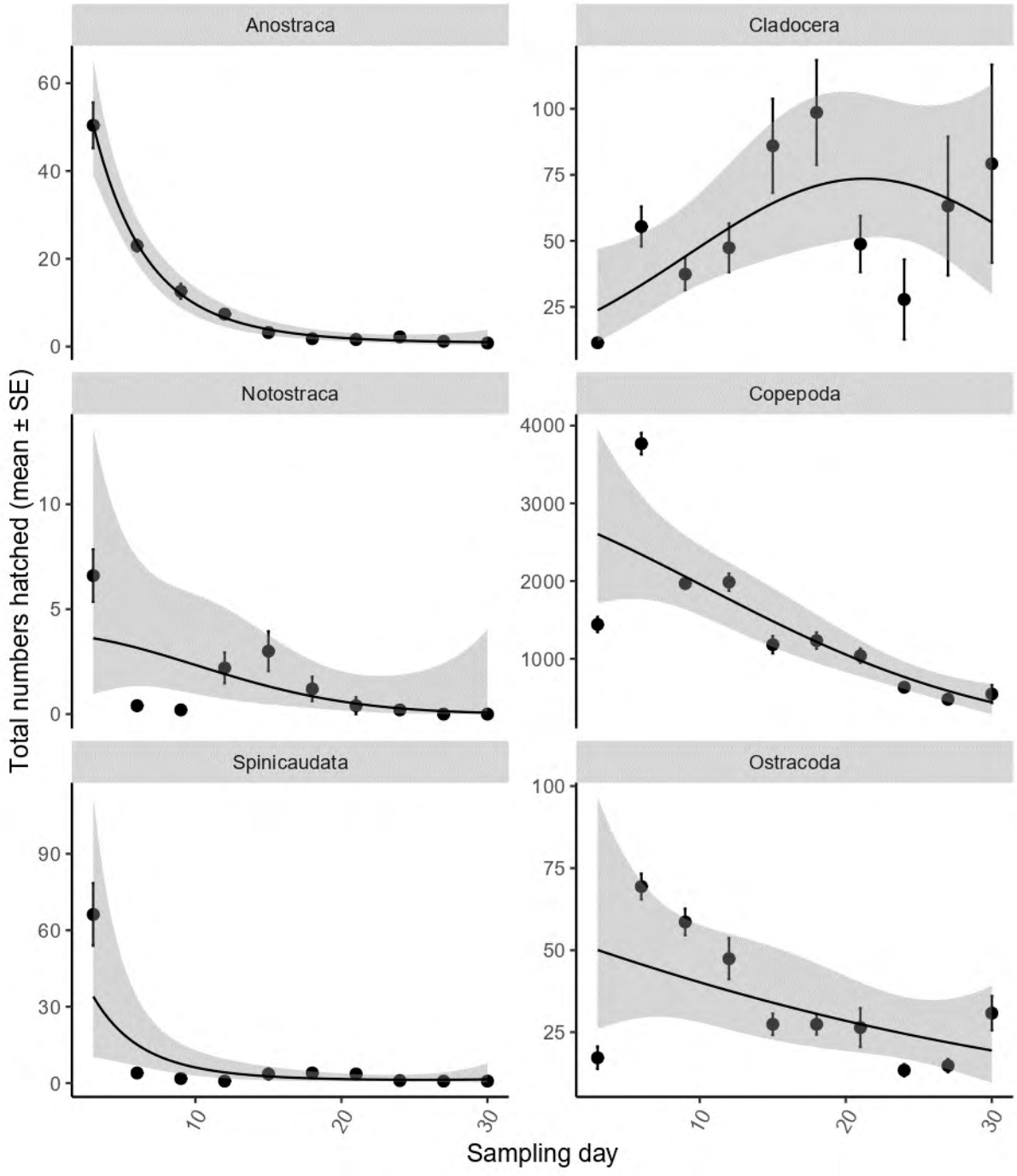


Figure 7.3. Mean numbers of individuals hatched per taxon over all salinities over time. Means are shown alongside standard errors. Lines correspond to the negative binomial generalized linear model fit with confidence intervals shaded in grey. Note y–axes differ among panels

7.4 Discussion

The present study highlights that salinisation of temporary wetland ecosystems alters the hatching success of crustacean communities in terms of overall community structure but has very little effect on the phenological hatching dynamics. The results showed that the Spinicaudata and Ostracoda were particularly sensitive to high salinities with reduced hatching success as salinity increased. The other taxa were found to be less sensitive to high salinities, but hatching rates still reduced rapidly with rising salinity levels. Over time, hatching rates generally declined for all taxa, but for the Cladocera in particular there was a later hatching peak irrespective of the salinity, which manifested unimodally in time. Overall, all the taxa were negatively affected by salinisation, therefore rejecting the first hypothesis. Larger crustaceans generally hatched earlier in the hydroperiod compared to smaller groups across salinities, therefore rejecting our second hypothesis. While these results offer little insight into the specifics of how crustacean communities may shift with increasing salinization, it still successfully highlights how broad taxonomic contributions may vary.

High salinities have been shown to inhibit growth and lead to high mortalities in freshwater crustacean taxa in other studies (Thiéry and Puente, 2002; Hulsmans et al., 2006; Vanschoenwinkel et al., 2009; Castro–Mejía et al., 2011; Mabidi, 2018). Here, all taxa were adversely affected by high salinities, with hatching success decreasing to variable extents. Changes in salinisation can have direct effects, such as physiological changes, which can lead to species extirpation (Nielsen et al., 2003a). Increases in salinity can also influence the hatching of large branchiopod crustaceans from eggs, through either reducing the viability of the egg bank or blocking the cues that trigger hatching. Mortality soon after hatching may also occur due to high salinities (Nielsen et al., 2003b; Bailey et al., 2004). At the individual level,

increases in salinisation can affect osmoregulation, leading to high metabolic costs, which can affect the general fitness of the organisms (James et al., 2003). Therefore, high salinities disturb growth, abundance, and overall viability (Nielsen et al., 2003b).

The results show that for all the large branchiopods taxa, the highest hatching occurred in the first six days. The peak hatching observed in the early inundation period has been shown in other studies (Brendonck ,1996; Waterkeyn et al., 2009). Within these temporary pan systems with varying hydroperiods, the associated desiccation can act as a selective force that can lead to differentiation in the life history stages in the populations that have experienced disturbances (Vanschoenwinkel et al., 2010a). In this study, the start of inundation corresponded with the hatching of a higher fraction of the large branchiopod eggs, which allows for a higher probability of completing the life cycle (Vanschoenwinkel et al., 2010b; Brendonck et al., 2022). Even within a single batch of dormant eggs, hatching frequently varies greatly, and this variation is regarded to be an adaptation to maintain population stability in an unstable environment (Brendonck, 1996). Shifting climatic patterns can lead to erratic and shorter hydroperiods (Waterkeyn et al., 2008), and early hatching of the dormant eggs can lead to a smaller fraction of the 'reserve' fraction left in the dormant egg bank. This may lead to the local extinction of some of the populations (Bhusnale et al., 2016). However, no shifting patterns of hatching with increases in salinity were observed, and therefore, salinisation did not have effects on the hatching phenology of the crustacean dormant eggs overall.

From this study, the micro–crustaceans Cladocera and Copepoda and the Notostraca and Anostraca (large branchiopods) had a shallower decrease with salinity. The Copepoda and Cladocera likely show higher tolerance to higher salinity levels due to them being generalist

species found across a range of temporary and permanent systems of varying physical and chemical composition. Cladocerans have been shown to be tolerant of a broad range of salinities (De Deckker, 1982; Hall and Burns, 2002; Nielsen et al., 2003a; Gonçalves et al., 2007; Satangelo et al., 2014). These micro–crustacean taxa are found not only in freshwater habitats but also in high-saline waters such as coastal and brackish habitats. Some calanoid copepods have also been found to have wide salinity tolerance (Horváth et al., 2014). Similarly, Nielsen et al. (2003a) found that copepods are adapted to tolerate a wide range of salinities, while Halls and Burn (2001) showed that larger copepod females exhibited higher hatching success at high salinity levels. These generalist and salt–tolerant species may benefit from the reduction of competition and predation pressures in the temporary pan systems that otherwise impedes larger taxa from hatching. Anostraca did hatch at high salinities here. The anostracan *Artemia salina* has been recorded in a salt pan in South Africa in a previous study (De Roeck et al., 2007). This tolerance may reflect that they developed salt tolerance in order to survive the hydro chemical fluctuations associated with them inhabiting ephemeral water bodies where salt concentrations change in relation to the rates of evaporation (Sarma et al., 2005).

Increased anthropogenic activities in semi–arid regions, particularly agricultural intensification to support growing human population levels (Dalu et al., 2017a), are likely to compromise temporary pan systems through associated salinisation dynamics. The evolutionary consequences of salinisation may increase with longer exposure time and may have detrimental implications for the more sensitive taxa (i.e., due to reduced genetic diversity). Salinity is a strong evolutionary pressure that can alter functional aspects related to trait diversity, food web structures and trophic dynamics (Latta et al.,2012). Across trophic chains, the intolerant taxa may disappear, with the tolerant species having a competitive advantage towards destabilizing

the food web structure (James et al., 2003). This can have cascading effects on the ecosystem functioning (e.g., bottom–up and top–down control) (Cullinera–Montcusi et al., 2021), thereby affecting human societies relying on them for various ecosystem services.

7.5 Conclusions

Overall, the results showed that the hatchling success of resting eggs of all the taxa, both the large branchiopods and the micro–crustacean taxa, were inhibited by high salinities. The increase in salt concentrations and exceeding concentrations of 2.5 ppt has implications for the more sensitive biota, such as the Spinicaudata and Ostracoda, when it exceeds the tolerance level of these biota. There were, however, limited effects of salinity on the hatching phenology of the crustaceans in the temporary wetlands since no obvious temporal shifts in the hatching patterns were observed with increases in salinity. The current study provides some understanding of the impacts of freshwater salinisation on the hatching success of crustaceans in temporary pan systems. The findings of this study also highlight which broad taxonomic groups are affected, potentially stimulating autecological assessments at finer taxonomic resolutions for key groups. Future studies might include species–level assessments of salinity tolerances within each taxon (e.g., Copepoda/Cladocera) and further refinement of the taxonomy of their juvenile phases. The endorheic nature of temporary pans, coupled with climate change and the agricultural landscapes in which they dominate, likely favour salinization increases at landscape levels. Effective monitoring systems and research in these temporary pan systems can, therefore, provide information that will contribute to significantly advancing and developing better conservation management strategies.

CHAPTER 8: CONCLUSION AND SYNTHESIS

"We must have perseverance and above all confidence in ourselves. We must believe that we are gifted for something and that this thing must be attained."

– Marie Curie

Plate 8. A temporary pan in the Khakhea Bray Transboundary Aquifer region filled in the rainy season, January 2022. Photo by Dr Chad Keates

8.1 Synthesis and Conclusions

Temporary wetland degradation is a global concern, exacerbated by various anthropogenic activities. The small endorheic nature of these temporary wetlands makes them particularly vulnerable even to minor disturbances (Boix et al., 2016; Calhoun et al., 2017). Within Southern Africa, these temporary wetlands form the most abundant aquatic features in the semi–arid or arid climate (Bird et al., 2019). Despite their ecological significance, especially in contributing to the region's aquatic biodiversity, there has been a bias towards larger aquatic invertebrates, and smaller taxa have received less attention. It is, therefore, imperative to monitor these ecosystems and generate information on taxon distributions, composition and abundances coupled with understanding the associated environmental drivers, as this is informative if conservation measures are to be taken. Conserving these systems requires not only monitoring but also anticipating and predicting responses of the organisms in these systems to potential stressors, uncovering physiological and ecological processes at play. This study assessed the plankton and macroinvertebrate dynamics in temporary pans of the Khakhea Bray Transboundary Aquifer region in the North west Province of South Africa. The study highlights that the complex interplay between hydroperiod, environmental variables, food web structure and anthropogenic impacts drivers of plankton and macroinvertebrate dynamics in these temporary wetland ecosystems.

The study involved the assessment of numerous freshwater temporary pans and a salt pan (Chapter 2), and sampling was conducted in June 2021 (winter), January 2022 (summer) and May 2022 (winter). This represented a significant step in the establishment of a comprehensive dataset that characterises baseline physical, chemical and ecological conditions of temporary pans in this region. The results showed that there were significant differences in water quality

composition across seasons but not among the pans sampled. Temperature was significantly higher in summer, while pH, DO, and phosphates were higher in winter (Chapter 2). The pH in these pans was slightly alkaline, ranging from 7.2 to 11.6, which could be attributed to the dolomitic landscape in which these pans occur, which has calcite rocks predominant. According to DWAF (2002) standards, the physicochemistry of these temporary pans across the seasons was characteristic of oligotrophic systems, given that the chlorophyll–*a* concentrations observed were below 10 µg L^{-1}. However, there were no clear seasonal patterns in the sediment chemistry in these pans (Chapter 2).

Plankton dynamics encompassing both phytoplankton and zooplankton communities are known to play a pivotal role in the ecological functioning of temporary wetlands. In Chapter 2, the findings revealed high plankton diversity in the KBTA region, comprising 72 phytoplankton species and 21 zooplankton species, comparable to other regions studied (Anusa et al., 2012; Msiteli–Shumba et al., 2017; Nhiwatiwa et al., 2017; Dalu et al., 2022a). The study also revealed seasonal variations in the plankton communities in the region, highlighting the hydrological dynamics inherent in these systems. During summer, increased water availability resulted in low phosphate concentrations, leading to the dominance of Chlorophyceae such as *Coelastrum cambricum*, *Oocystis borgei*, *Pediastrum boryanum* and filamentous forms like *Oedogonium cymatosporum* in this season. The abundance of chlorophytes in summer suggests that the temporary pans were sampled early in the hydroperiod, which is similar to findings by Williams et al. (2005) and Anusa et al. (2012), who also found chlorophytes dominating early in the hydroperiod. Conversely, as water levels decreased in winter, nutrient concentrations increased due to an evapo–concentration effect, as also observed in other studies (Boone et al., 2006; Chia et al., 2011), leading to high phytoplankton diversity in this season. The phytoplankton diversity in winter was then dominated by Zygnematophyceae species such as

Cosmarium caelatum, *Straurastrum anatium*, *Straurastrum crenulatum*, *Straurastrum planctonica* and *Straurastrum vestitum* (Chapter 2). The zooplankton community assemblages also exhibited seasonal changes, with high diversity in summer. The dominant species were *Trichocerca elongata*, *Brachionus plicatilis*, *Cyclops* sp., copepod nauplii, *Diaphanosoma excisum* and Cypridídae, while in winter, the dominant species were *Lovenula falcifera*, copepod nauplii, *Diaphanosoma excisum* and Cypridídae (Chapter 2). The high zooplankton diversity in summer could have been attributed to increased resource availability in the form of the Chlorophyceae in the hydrologically active phase.

Previous records for the North west Province reported 10 large branchiopod species. This study expands this to 13 species, including the first record of *Branchipodopsis wolfi*, *Streptocepahlus namibiensis* and *Phallocryptus spinosa* in the province. These findings indicate a high large branchiopod diversity for the region, similar to other areas such as the Lake Manyara Basin in Northern Tanzania (6 species; Kalufa et al., 2023), the Drakensberg region (11 species; Hamer and Martens, 1998), the Western Cape Province (14 species; De Roeck et al., 2007) and the Save Valley Conservancy in Zimbabwe with (16 species; Nhiwatiwa et al., 2014) (Chapter 3). The record of *Phallocryptus spinosa* (Chapter 3) is the first record not only for the province but also for the country. Prior to this study, south of the Sahara, *P. spinosa* had only been recorded in the Makgadikgadi pans in Botswana and had not been recorded in the province or the country. In line with findings from Chapter 2, there were seasonal variations in the large branchiopod diversity in these pans, with less diversity in the winter season. The dominant species in summer were *Triops granarius*, *Branchipodopsis wolfi*, *Ozestheria australis*, *Streptocephalus namibiensis* and juvenile anostracans, while *Streptocephalus namibiensis* was dominant in winter.

Trophic dynamics within these temporary pan ecosystems help us understand the response of food web structures to hydroperiod fluctuations. Results from the stable isotope analyses in Chapter 4 highlighted the trophic positions of organisms in these pans and revealed that the food web structure in these temporary pan systems had less than four trophic levels. These short and simple food web structures have been shown in other temporary systems and support the theory of dynamic constraints hypothesis (Williams, 2006; Dalu et al., 2016; Mdidimba et al., 2021; O'Neill and Thorp, 2014; de Necker et al., 2020). The food webs in the KBTA region could not be clearly defined as green or brown; instead, it can be concluded that there is an interaction of both green and brown food webs driving the functioning and stability of the temporary wetland ecosystems with the base of the food web supported by aquatic macrophytes and algae in the form of POM and detritus (Shrama et al., 2012; Mougi, 2020). It can, therefore, be suggested that the temporary wetland ecosystem persists through alternative food web configurations with the green food web dominating early in the hydroperiod, but later on, the hydroperiod, as water levels decrease and macrophytes dry, then the brown food web becomes dominant as organic material input increases.

The dominant primary consumers in summer were mainly the filter feeders *Branchipodopsis* sp., *Streptocephalus* sp., Cyprididae and *Ozestheria* sp. (Chapter 4). Massina et al. (2023) recorded high diversity of macroinvertebrates in summer in this region, and the colonisation by aquatic insects increased the trophic complexity of the food web structure. At the top of the food web was the notonectid *Anisops* sp, which was similar to findings by Dalu et al. (2016), who also found the notonectid *Notonecta* sp as the top predator in a temporary pond in the Eastern Cape. In the diets of both the dytiscid adults and larvae and the notonectids, there was a preference for *Streptocephalus* sp. (Chapter 4). These high predation rates in summer also align with high abundances of prey in this season (Chapter 3), suggesting that predator –prey

dynamics in these temporary pans may be influenced by availability and catchability, where predators are more likely to focus on the more abundant and easily accessible prey. While inconclusive, the predation dynamics could also be influenced by the presence of aquatic vegetation, which was observed in these pans (Chapter 1). The large branchiopods considered as “flagship species” in these systems shifted between positions 2 and 3, which is indicative of habitat generalists. Many dominant invertebrate consumers also exhibited generalist diets, leading to high trophic niche overlap. The presence of these generalists may be beneficial for the organisms, mitigating the negative impacts in a highly variable environment vulnerable to disturbances. Although high niche overlap was observed amongst most of the invertebrates, the findings also highlight niche segregation, specifically between the Lestidae and the other predator insects showing distinct dietary preferences (Chapter 4).

Temporary wetlands are vulnerable to environmental fluctuations, which are largely driven by hydrodynamics. Contrary to other studies, such as Batzer et al. (2004) and Mabidi et al. (2016), which found no significant influence of environmental variables on the fauna of temporary wetlands, the results of this study indicate that the influence of the hydroperiod and environmental variables on the plankton and macroinvertebrate dynamics is strong, as this finding was consistent throughout Chapters 2, 3 and 4. The environmental variables temperature, conductivity, sediment phosphorous and zooplankton abundance were found to influence the phytoplankton community assemblage in summer, while pH, SOC, DO, water phosphates, sediment Na, Fe and SOC influenced the phytoplankton community in winter. The zooplankton community was influenced by SOC, DO, sediment Na, pH, phytoplankton abundance and water phosphates in winter, while sediment phosphorous and temperature were found to influence zooplankton community assemblage in summer (Chapter 3). Phosphorous and water temperature were found to significantly influence the large branchiopod community

assemblage in summer, while sediment Na influenced the species in winter. The influence of the sediment Na was not unexpected as this directly impacts habitat salinity and subsequently affects the zooplankton and large branchiopod communities therein (Chapter 6).

Anthropogenic activities, especially those influencing nutrient dynamics, pose a significant threat to the ecological integrity of temporary wetlands. Findings from Chapter 5, where nutrient differences and chlorophyll–*a* were assessed along a disturbance gradient, revealed the vulnerability of temporary pans to human–mediated nutrient enrichment. Elevated levels of pH, ammonium and phosphates were found in disturbed pans compared to undisturbed pans. Proximity to kraals was found to be the major driver of chlorophyll–*a* concentrations in the pans. Strong positive relationships were found between temperature, ammonium, pH and chlorophyll–*a* indicating the potential for eutrophication, impacting primary productivity and alpha diversity as anthropogenic activities increase in the area (Chapter 5).

Freshwater salinisation driven by anthropogenic activities and climate change is an emerging global threat to biodiversity in temporary wetlands, which is a major concern for the organisms found within these systems. The laboratory–based approach to investigating the impacts of salinisation on benthic phytoplankton and crustacean communities presented in Chapters 6 and 7 offers critical insights. The findings from Chapter 6 revealed that increased salinisation disrupted phenology dynamics, causing the accelerated development of later–stage phytoplankton communities during the early stages. Contrary to initial expectations, time in interaction with salinity was found to influence the benthic phytoplankton communities significantly. Additionally, increased salinisation also led to shifts in the benthic phytoplankton community, favouring the proliferation of saline–tolerant diatom species at the expense of more sensitive taxa such as the chlorophytes. Findings from Chapter 7 build on these observations,

highlighting the cascading effects of salinisation on the functioning of these ecosystems. Elevated salinities were found to reduce hatching success but had minimal effects on the phenology dynamics. Spinicaudata and Ostracoda emerged as the most sensitive taxa to high salinities, while Cladocera exhibited a late hatching peak, irrespective of the salinity levels. The impact of salinisation on benthic phytoplankton and crustacean communities, as shown in Chapters 6 and 7, highlights their vulnerability to changing physicochemical conditions. Significant declines in emergent taxa richness and abundance at higher salinities emphasise the potential threats posed by altered environmental conditions on these communities in temporary wetland ecosystems.

8.2 Future research

The findings presented in this thesis contribute to a better understanding of the plankton and macroinvertebrate community assemblages and the factors structuring them in semi–arid temporary wetlands (Chapters 2, 3 and 4). Furthermore, it also shows the impact of anthropogenic activities on water quality, phytoplankton and crustacean community assemblages in these systems (Chapters 5, 6 and 7). This knowledge is relevant for the region and globally, where these systems are found to inform conservation strategies. Despite recent efforts in the past decade to study these systems, further research studies must be done to fill knowledge gaps on these systems:

1. The current study only sampled once in each season for the specific sampling event. Long–term monitoring is required in these systems to comprehensively capture the total diversity and variation occurring in these systems in a robust dataset that captures the nuances of temporal changes in plankton and macroinvertebrate dynamics, environmental variables, and ecosystem responses. This entails the implementation of

more frequent sampling across multiple inundation events to ensure a more comprehensive representation. Building on this initial sampling, sustained sampling will help us understand how these systems evolve and provide practical insights for conservation managers and decision–makers striving to preserve the ecological integrity of these unique environments in the region.

2. To fully understand these temporary wetland ecosystems, there is also a compelling need to assess the response of these wetlands to climate change. Modelling approaches that simulate potential impacts such as changes in temperature, precipitation patterns, and extreme weather events can be used. These modelling techniques will determine the potential projected shifts in the hydroperiod and overall ecological functioning of these systems.
3. The current study assesses the effects of freshwater salinisation on community structure. Future studies should expand on this and look at functional aspects, especially with a focus on trait diversity, to find which traits are susceptible to freshwater salinisation. Focusing on traits may help determine impacts on food web structures and trophic dynamics in these systems.
4. It may also be necessary to explore the connectivity between these temporary wetlands and the surrounding landscape, particularly other water bodies, both permanent and temporary, in the region. This may also involve exploring the potential of landscape features (i.e. topography, soil characteristics, vegetation corridors) to influence the dispersal of species and species composition. The genetic diversity of plankton and macroinvertebrate populations between connected wetlands can then be explored to understand implications for population resilience and adaptability.

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APPENDICES

Table A2.1. Two–way ANOVA results of the influence of sampled pans, season and the interaction of both on diversity metrics measured for the phytoplankton community in the KBTA region

	Pan			**Season**			**Pan × Season**		
Diversity metric	**df**	**F**	***p***	**df**	**F**	***p***	**df**	**F**	***p***
Pielou's evenness	9	0.254	0.922	1	0.145	0.768	6	0.165	0.951
Shannon–Wiener	9	0.229	0.934	1	1.140	0.479	6	0.073	0.990
Simpson's index	9	0.181	0.957	1	0.390	0.645	6	0.101	0.980
Whittaker beta diversity	8	144.13	0.0643	1	13.23	0.1708	5	60.19	0.0975

Table A2.2. Two–way ANOVA results of the influence of sampled pans, season and the interaction of both on diversity metrics measured for the zooplankton community in the KBTA region

	Pan			**Season**			**Pan × Season**		
Diversity metric	**df**	**F**	***p***	**df**	**F**	***p***	**df**	**F**	***p***
Pielou's evenness	9	0.845	0.695	1	0.761	0.543	6	0.518	0.786
Shannon–Wiener	9	0.488	0.814	1	0.008	0.944	6	0.208	0.929
Simpson's index	9	0.638	0.758	1	0.068	0.837	6	0.221	0.922
Whittaker beta diversity	8	1.030	0.647	1	10.688	0.189	5	1.101	0.616

Table A2.3. Phytoplankton species abundance (indL^{-1}) at the different sampled pans across summer and winter seasons in the KBTA region.

	Winter								Summer									
	Pan 1	**Pan 2**	**Pan 3**	**Pan 4**	**Pan 6**	**Pan 7**	**Pan 8**	**Pan 10**	**Pan 1**	**Pan 2**	**Pan 3**	**Pan 4**	**Pan 5**	**Pan 6**	**Pan 7**	**Pan 8**	**Pan 9**	**Pan 10**
Dinophyceae																		
Ceratium hirudinella	+	+	+	–	–	–	+	–	–	–	–	–	–	–	–	–	–	–
Peridinium cinctum	+	+	+	+	+	–	–	+	–	–	–	–	–	–	–	–	–	–
Euglenoidea																		
Euglena sp.	+	–	+	–	+	–	+	–	+	+	–	–	–	–	–	–	–	–
Bacillariophyceae																		
Craticula cuspidata	+	+	+	+	+	+	+	+	+	–	+	+	+	+	+	+	+	+
Cymbella sp	+	–	+	–	–	+	–	–	–	–	–	–	–	–	–	–	–	–
Diatoma vulgaris	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Encyonopsis sp.	–	–	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Fragillaria ulna	+	–	+	–	–	–	+	+	–	–	–	–	–	–	–	–	–	–
Gomphonema affine	–	+	+	+	+	+	+	+	–	–	–	–	–	–	–	–	–	–
Gomphonema gracile	–	–	+	–	–	–	+	–	+	–	–	–	–	–	–	–	–	–
Melosira varians	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Navicula sp	–	+	+	+	+	+	+	+	–	–	–	–	–	–	–	–	–	–
Nitzschia elegantula	–	+	+	+	+	+	+	+	–	–	–	–	+	–	–	–	–	–
Nitzschia gracilis	–	–	+	–	+	–	+	–	–	–	–	–	–	–	–	–	–	–
Nitzschia linearis	–	–	–	–	–	–	–	–	+	+	+	+	+	+	–	–	–	+
Pinnularia viridiformis	+	+	–	+	+	+	+	+	+	–	+	+	+	+	–	–	–	–
Synedra ulna	–	–	+	–	–	–	+	–	–	–	–	–	–	–	–	–	–	–
Chlorophyceae																		
Ankistrodesmus fusiformis	+	–	+	–	–	+	+	–	–	–	–	–	–	–	–	–	–	–

	Winter								Summer									
	Pan 1	**Pan 2**	**Pan 3**	**Pan 4**	**Pan 6**	**Pan 7**	**Pan 8**	**Pan 10**	**Pan 1**	**Pan 2**	**Pan 3**	**Pan 4**	**Pan 5**	**Pan 6**	**Pan 7**	**Pan 8**	**Pan 9**	**Pan 10**
Ankistrodesmus spiralis	+	–	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Asterococcus superbus	+	–	–	–	–	–	–	+	–	–	–	–	–	–	–	–	–	–
Chlorella sp.	–	+	–	+	+	+	+	+	+	–	–	–	–	–	–	–	–	–
Coelastrum cambricum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Coelastrum microporum	+	–	–	–	–	–	–	–	–	+	+	+	+	–	–	+	+	+
Crucigenia quadrata	+	+	+	–	+	+	+	+	–	+	–	–	–	–	–	–	–	–
Crucigenia rectangularis	–	+	–	+	+	+	+	+	–	+	+	+	+	+	+	+	–	+
Gleocystis gigas	+	+	+	+	+	–	+	+	–	–	+	–	+	–	+	+	+	+
Micractinium pusillum	+	–	–	–	–	–	+	–	–	–	–	–	–	–	–	–	–	–
Microspora flocossa	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Monoraphidium griffithi	+	–	–	–	–	–	–	–	–	+	+	–	–	–	–	–	–	–
Oedogonium cymatosporum	–	+	+	+	+	+	+	+	–	–	–	–	–	–	–	–	–	–
Oocystis borgei	–	+	+	+	+	+	–	+	–	+	+	+	+	+	+	+	–	–
Oocystis pussilla	–	+	–	+	+	+	–	–	–	–	–	–	–	–	–	–	–	–
Oocystis sollitaria	–	+	+	+	+	+	+	+	–	+	+	+	+	+	+	+	–	+
Pediastrum angulosum	–	+	–	+	–	+	–	+	+	+	+	+	+	+	+	+	+	+
Pediastrum boryanum	–	+	+	+	+	+	+	–	+	+	+	+	+	+	+	+	+	+
Pediastrum duplex	+	–	–	+	–	–	–	–	–	+	–	–	–	–	–	–	–	–
Pediastrum gracillium	–	–	–	–	–	–	+	–	–	+	+	+	+	+	+	+	–	–
Pediastrum simplex	–	–	–	–	–	–	–	–	–	+	–	–	–	–	–	–	–	–

	Winter								Summer									
	Pan 1	**Pan 2**	**Pan 3**	**Pan 4**	**Pan 6**	**Pan 7**	**Pan 8**	**Pan 10**	**Pan 1**	**Pan 2**	**Pan 3**	**Pan 4**	**Pan 5**	**Pan 6**	**Pan 7**	**Pan 8**	**Pan 9**	**Pan 10**
Scenedesmus acuminatus	–	+	–	–	–	–	+	–	–	+	+	+	+	–	–	–	–	–
Scenedesmus arcuatus	–	+	+	+	+	+	+	+	–	+	–	–	–	–	+	+	–	+
Scenedesmus communis	+	–	–	–	–	–	+	+	–	+	+	+	+	+	–	+	+	+
Scenedesmus magnus	–	+	+	–	–	+	+	+	–	+	+	+	+	+	0	+	+	+
Scenedesmus microspina	–	–	+	–	–	–	–	+	–	–	–	–	–	–	–	–	+	+
Scenedesmus quadricauda	+	+	+	+	+	+	+	+	–	–	–	–	–	–	–	–	–	–
Tetraedron caudatum	–	+	+	+	+	+	+	+	–	–	–	–	–	–	–	–	–	–
Zygnematophyceae																		
Closterium ehrenbergii	+	+	+	+	+	+	+	+	+	–	–	+	–	–	–	–	–	–
Closterium gracile	–	–	+	–	–	–	–	–	+	–	–	–	–	+	+	–	–	–
Closterium kutzingii	+	–	–	–	–	–	+	–	–	+	–	–	–	–	–	–	–	–
Closterium pritchadianum	–	–	–	–	–	–	–	–	–	+	–	–	+	–	+	+	–	–
Cosmarium caelatum	+	+	+	+	+	+	+	+	–	–	–	–	–	–	–	–	–	–
Cosmnarium depressum	–	–	–	–	–	–	–	–	+	+	+	+	–	–	+	–	–	–
Cosmarium laeve	–	–	–	–	–	–	–	–	+	+	+	–	–	–	+	–	–	–
Cosmarium sportella var subnudum	–	+	+	+	+	+	+	+	–	–	–	–	–	–	–	–	–	–
Cosmarium subcostatum	+	–	+	–	–	–	–	–	–	+	+	+	+	+	+	+	–	+
Cosmarium venustum	–	+	+	+	+	+	+	+	–	–	–	–	–	–	–	–	–	–
Mougeotia sp.	–	+	–	+	+	+	+	–	+	+	–	–	–	–	+	–	–	–
Spirogyra sp.	+	–	+	–	+	+	–	–	+	+	–	–	–	–	–	–	–	–

	Winter								Summer									
	Pan 1	**Pan 2**	**Pan 3**	**Pan 4**	**Pan 6**	**Pan 7**	**Pan 8**	**Pan 10**	**Pan 1**	**Pan 2**	**Pan 3**	**Pan 4**	**Pan 5**	**Pan 6**	**Pan 7**	**Pan 8**	**Pan 9**	**Pan 10**
Spondylosium moniliforme	+	–	–	–	+	+	+	–	–	–	–	–	–	–	–	–	–	–
Straurastrum acuminatus	–	–	+	–	–	+	–	–	–	–	–	–	–	–	–	–	–	–
Straurastrum alternans	+	+	+	+	+	+	+	+	–	–	–	+	–	–	+	–	–	–
Straurastrum anatinum	+	+	+	+	+	+	+	+	–	–	–	–	–	–	–	–	–	–
Straurastrum arcuatus	–	+	+	–	–	+	–	–	–	–	–	–	–	–	–	–	–	–
Straurastrum crenulatum	+	+	+	+	+	+	+	+	–	–	–	+	–	–	+	+	–	–
Straurastrum gracile	–	–	+	–	–	–	+	–	–	–	–	+	–	+	+	+	–	–
Straurastrum lunatum	–	–	+	–	+	+	+	–	–	–	–	–	–	–	–	–	–	–
Straurastrum planctonica	+	+	–	+	+	–	–	+	–	–	–	–	–	–	–	–	–	–
Straurastrum vestitum	+	+	+	+	+	+	+	+	–	–	–	–	–	–	–	–	–	–
Zygnema sp.	–	+	–	+	–	+	+	–	–	–	+	–	–	–	+	+	–	–
Cyanophyceae																		
Anabaena sp.	+	+	+	+	+	+	+	+	+	–	–	–	–	–	–	–	–	–
Merismopedia sp.	–	–	–	–	–	–	–	–	–	–	–	+	–	+	–	–	–	–
Spirulina major	–	–	–	–	–	–	–	–	+	–	+	+	+	+	+	+	+	+
Xanthophyceae																		
Goniochloris mutica	+	+	+	+	+	+	+	+	–	–	–	–	–	–	–	–	–	–
Goniochloris spinosa	–	–	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–

Table A2.4. Zooplankton species abundance (ind L^{-1}) at the different sampled pans across summer and winter seasons in the KBTA region.

	Winter								Summer									
	Pan 1	**Pan 2**	**Pan 3**	**Pan 4**	**Pan 6**	**Pan 7**	**Pan 8**	**Pan 10**	**Pan 1**	**Pan 2**	**Pan 3**	**Pan 4**	**Pan 5**	**Pan 6**	**Pan 7**	**Pan 8**	**Pan 9**	**Pan 10**
Rotifera																		
Anuraeopsis fissa	+	+	+	+	+	+	+	+	–	–	–	–	–	0	+	–	–	–
B. quadridentatus f. brevispina	–	–	+	–	–	–	+	+	–	–	–	–	–	+	0	–	–	–
Brachionus plicatilis	–	–	–	–	–	–	–	–	–	+	+	+	+	+	+	+	+	+
Epiphanes senta	+	–	–	–	–	–	+	–	–	–	–	+	–	–	–	–	–	–
Euchlanis dilatata	–	–	–	–	–	–	+	–	–	+	+	+	0	0	+	+	0	0
K. valga f. heterospina	–	–	+	–	–	–	–	+	–	–	–	–	–	–	–	–	–	–
Lecane luna	–	–	–	–	+	–	+	–	–	+	+	+	+	+	+	+	0	+
Lepadella ovalis	+	+	–	+	+	–	+	+	–	–	–	–	–	–	–	–	–	–
Mytilina sp.	–	–	–	–	–	–	+	–	–	–	–	–	–	–	–	–	–	–
Trichocerca pusilla	–	–	–	–	–	–	–	–	–	–	+	+	+	+	+	+	+	0
Trichocerca elongata	–	–	–	–	–	–	–	–	–	–	+	+	+	+	+	+	+	+
Copepoda																		
Cyclops sp.	+	+	–	–	–	–	–	–	–	–	–	+	+	+	+	+	+	+
Afrocyclops sp.	–	+	–	–	–	–	–	–	–	+	–	+	+	–	–	–	–	–
Lovenula falcifera	+	+	–	+	+	+	+	+	–	–	–	+	+	+	–	–	–	–
Ectocyclops sp.	–	–	–	–	–	+	–	–	+	+	+	+	+	+	+	+	+	+
Thermocyclops sp.	–	+	–	–	–	+	–	–	+	+	+	+	+	–	–	+	–	–
Copepod Nauplii	+	+	–	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Cladocera																		
Alona sp.	–	+	–	–	–	+	–	+	–	–	–	–	–	+	+	–	–	+
Macrothrix spinosa	+	+	+	+	+	+	–	+	–	–	–	–	+	–	+	–	–	+

	Winter								Summer									
	Pan 1	**Pan 2**	**Pan 3**	**Pan 4**	**Pan 6**	**Pan 7**	**Pan 8**	**Pan 10**	**Pan 1**	**Pan 2**	**Pan 3**	**Pan 4**	**Pan 5**	**Pan 6**	**Pan 7**	**Pan 8**	**Pan 9**	**Pan 10**
Diaphanosoma excisum	+	+	+	+	+	+	+	+	–	+	+	+	+	+	+	+	+	+
Ostracoda																		
Cyprididae 1	+	–	+	+	+	–	+	–	–	+	+	+	+	–	+	+	+	+
Cyprididae 2	+	–	–	+	–	–	–	–	+	+	–	+	–	+	–	–	–	–

Table A4.1. Mean ± standard deviation (Mean ± SD) of food resources contribution to the diet of dominant invertebrate consumers and tadpoles sampled in temporary pans in the KBTA; confidence intervals (CI)

***Anisops* sp.**				***Sigara* sp.**				***Tomopterna* tadpoles**			
Pan 1	**Mean ± SD**	**2.5 CI**	**97.5 CI**	**Pan 1**	**Mean ± SD**	**2.5 CI**	**97.5 CI**	**Pan 1**	**Mean ± SD**	**2.5 CI**	**97.5 CI**
Branchipodopsis sp.	0.13 ± 0.11	0.01	0.42	*Culex* sp. larvae	0.38 ± 0.22	0.05	0.83	Detritus	0.29 ± 0.22	0.02	0.76
Culex sp. larvae	0.19 ± 0.15	0.02	0.58	Detritus	0.15 ± 0.13	0.02	0.53	*Lagarosiphon* sp.	0.08 ± 0.08	0.01	0.30
Sigara sp.	0.20 ± 0.12	0.02	0.47	POM	0.23 ± 0.18	0.02	0.68	POM	0.41 ± 0.28	0.03	0.91
Streptocephalus sp.	0.25 ± 0.18	0.02	0.67	Sediment	0.24 ± 0.19	0.02	0.69	Sediment	0.22 ± 0.18	0.02	0.66
Tomopterna tadpoles	0.11 ± 0.09	0.01	0.34								
Triops sp.	0.13 ± 0.11	0.01	0.41								
***Anisops* sp.**				***Sigara* sp.**							
Pan 2	**Mean ± SD**	**2.5 CI**	**97.5 CI**	**Pan 2**	**Mean ± SD**	**2.5 CI**	**97.5 CI**				
Branchipodopsis sp.	0.20 ± 0.13	0.02	0.51	Detritus	0.13 ± 0.13	0.02	0.55				
Sigara sp.	0.23 ± 0.14	0.03	0.55	POM	0.29 ± 0.20	0.03	0.78				
Streptocephalus sp.	0.19 ± 0.13	0.02	0.49	Sediment	0.58 ± 0.22	0.10	0.90				
Tomopterna tadpoles	0.20 ± 0.13	0.03	0.51								
Triops sp.	0.19 ± 0.13	0.03	0.51								
***Tomopterna* tadpoles**											
Pan 3	**Mean ± SD**	**2.5 CI**	**97.5 CI**								
POM	0.63 ± 0.24	0.04	0.66								
Potamogeton sp.	0.06 ± 0.08	0.02	0.53								
Sediment	0.32 ± 0.24	0.02	0.83								
***Anisops* sp.**				***Sigara* sp.**				***Lestes* sp.**			
Pan 4	**Mean ± SD**	**2.5 CI**	**97.5 CI**	**Pan 4**	**Mean ± SD**	**2.5 CI**	**97.5 CI**	**Pan 4**	**Mean ± SD**	**2.5 CI**	**97.5 CI**
Cybister sp. larvae	0.11 ± 0.11	0.01	0.42	POM	0.31 ± 0.32	0.01	0.93	*Cybister* sp. larvae	0.45 ± 0.26	0.05	0.93
Cyprididae	0.09 ± 0.08	0.01	0.30	Sediment	0.69 ± 0.32	0.07	0.99	*Rhantus* sp. larvae	0.56 ± 0.26	0.07	0.95
Ozestheria sp.	0.15 ± 0.14	0.01	0.53								
Rhantus sp.	0.12 ± 0.12	0.01	0.46								
Rhantus sp. larvae	0.13 ± 0.12	0.01	0.46								
Sigara sp.	0.08 ± 0.07	0.01	0.28								
Streptocephalus sp.	0.22 ± 0.18	0.02	0.67								
Tomopterna tadpoles	0.12 ± 0.11	0.01	0.44								
***Rhantus* sp.**				***Rhantus* larvae**				***Cybister* larvae**			

Pan 4	**Mean ± SD**	**2.5 CI**	**97.5 CI**	**Pan 4**	**Mean ± SD**	**2.5 CI**	**97.5 CI**	**Pan 4**	**Mean ± SD**	**2.5 CI**	**97.5 CI**
Anisops sp.	0.09 ± 0.09	0.01	0.36	*Anisops* sp.	0.08 ± 0.08	0.01	0.32	*Anisops* sp.	0.11 ± 0.11	0.01	0.42
Cyprididae	0.07 ± 0.07	0.01	0.25	Cyprididae	0.10 ± 0.10	0.01	0.37	Cyprididae	0.12 ± 0.11	0.01	0.41
Ozestheria sp.	0.14 ± 0.13	0.01	0.51	*Ozestheria* sp.	0.18 ± 0.17	0.01	0.65	*Ozestheria* sp.	0.17 ± 0.15	0.01	0.57
Sigara sp.	0.07 ± 0.06	0.01	0.25	*Sigara* sp.	0.09 ± 0.11	0.01	0.32	*Sigara* sp.	0.12 ± 0.11	0.01	0.42
Streptocephalus sp.	0.40 ± 0.22	0.03	0.77	*Streptocephalus* sp.	0.31 ± 0.24	0.02	0.81	*Streptocephalus* sp.	0.20 ± 0.17	0.02	0.64
Tomopterna tadpoles	0.11 ± 0.10	0.01	0.40	*Tomopterna* tadpoles	0.13 ± 0.12	0.01	0.47	*Tomopterna* tadpoles	0.14 ± 0.13	0.01	0.51
Triops sp.	0.12 ± 0.12	0.01	0.46	Triops sp.	0.12 ± 0.12	0.01	0.47	*Triops* sp.	0.15 ± 0.14	0.01	0.53

***Tomopterna* tadpoles**			
Pan 4	**Mean ± SD**	**2.5CI**	**97.5CI**
POM	0.57 ± 0.3	0.03	0.96
Sediment	0.43 ± 0.3	0.04	0.97

***Anisops* sp.**				***Sigar*a sp.**				***Rhantus* sp.**			
Pan 5	**Mean ± SD**	**2.5 CI**	**97.5CI**	**Pan 5**	**Mean ± SD**	**2.5 CI**	**97.5CI**	**Pan 5**	**Mean ± SD**	**2.5CI**	**97.5CI**
Culex sp. larvae	0.13 ± 0.11	0.01	0.41	*Culex* sp. larvae	0.10 ± 0.16	0.01	0.61	*Anisops* sp.	0.12 ± 0.11	0.01	0.42
Cybister sp.	0.12 ± 0.10	0.01	0.40	POM	0.27 ± 0.24	0.02	0.86	*Culex* sp. larvae	0.14 ± 0.12	0.01	0.48
Rhantus sp.	0.13 ± 0.10	0.01	0.40	Sediment	0.63 ± 0.28	0.06	0.97	*Sigara* sp.	0.21 ± 0.18	0.02	0.66
Rhantus sp. larvae	0.12 ± 0.10	0.01	0.41					*Streptocephalus* sp.	0.20 ± 0.17	0.02	0.66
Sigara sp.	0.13 ± 0.10	0.01	0.40					*Tomopterna* tadpoles	0.15 ± 0.13	0.01	0.51
Streptocephalus sp.	0.13 ± 0.11	0.01	0.41					*Triops* sp.	0.19 ± 0.16	0.02	0.60
Tomopterna tadpoles	0.12 ± 0.11	0.01	0.41								
Triops sp.	0.13 ± 0.11	0.01	0.41								

***Cybister* sp**				***Tomopterna* tadpoles**			
Pan 5	**Mean ± SD**	**2.5 CI**	**97.5 CI**	**Pan 5**	**Mean ± SD**	**2.5 CI**	**97.5 CI**
Anisops sp.	0.09 ± 0.08	0.01	0.31	POM	0.51 ± 0.23	0.09	0.92
Culex sp. larvae	0.11 ± 0.10	0.01	0.40	Sediment	0.49 ± 0.23	0.08	0.92
Sigara sp.	0.19 ± 0.17	0.01	0.63				
Streptocephalus sp.	0.27 ± 0.22	0.02	0.80				
Tomopterna tadpoles	0.16 ± 0.14	0.01	0.56				
Triops sp.	0.19 ± 0.17	0.02	0.64				

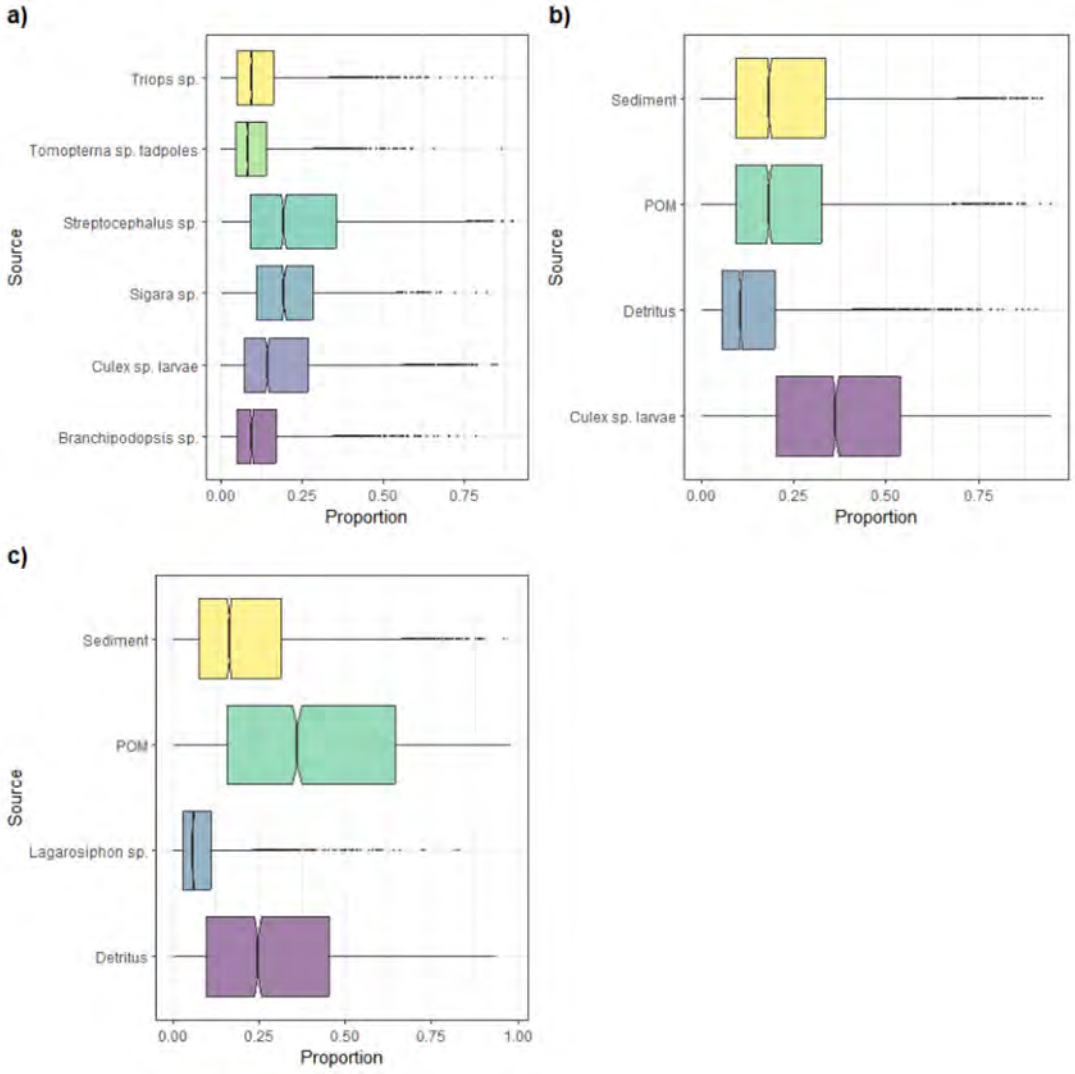

Figure A4. 1. Proportional resource contributions to (a) *Anisops* sp.(b) *Sigara* sp. (c) *Tomopterna* sp. tadpoles as determined by Stable Isotope Mixing Models (SIMMR) for Pan 1.

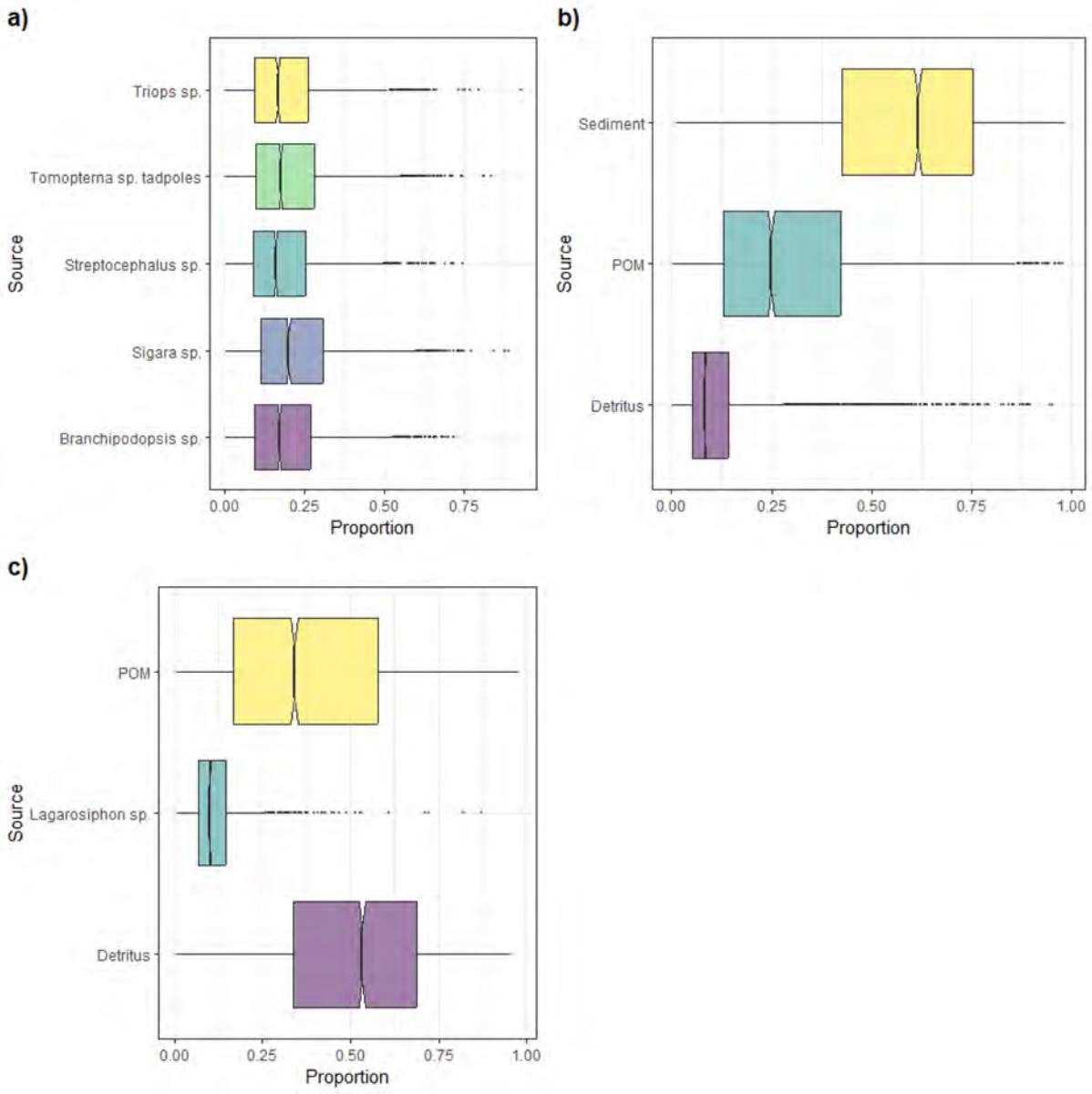

Figure A4. 2. Proportional resource contribution to (a) *Anisops sp.* (b) *Sigara sp.* (c) *Tomopterna sp.* tadpoles as determined by Stable Isotope Mixing Models (SIMMR) for Pan 2

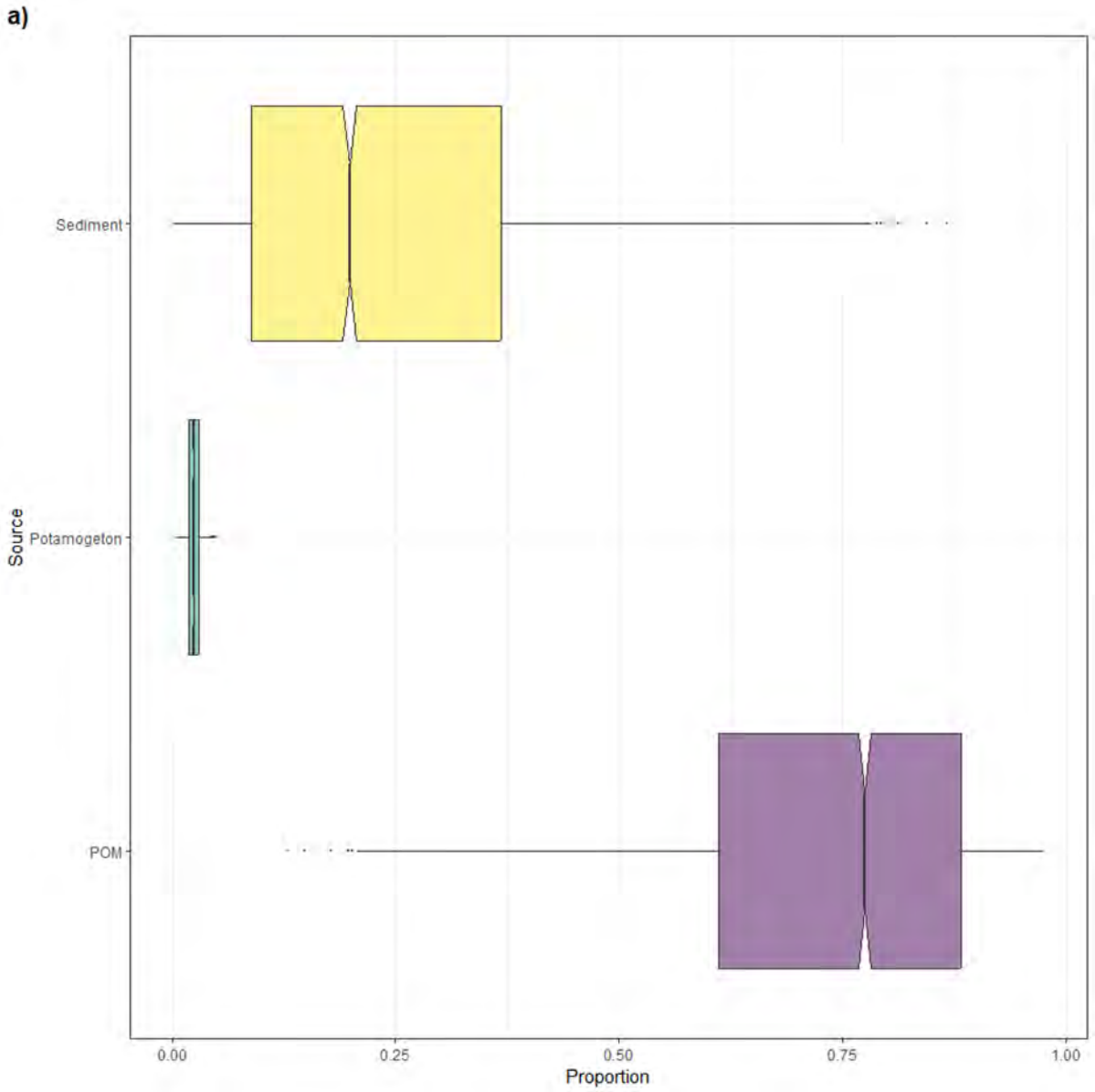

Figure A4. 3. Proportional resource contributions to (a) *Tomopterna sp.* tadpoles as determined by Stable Isotope Mixing Models (SIMMR) for Pan 3

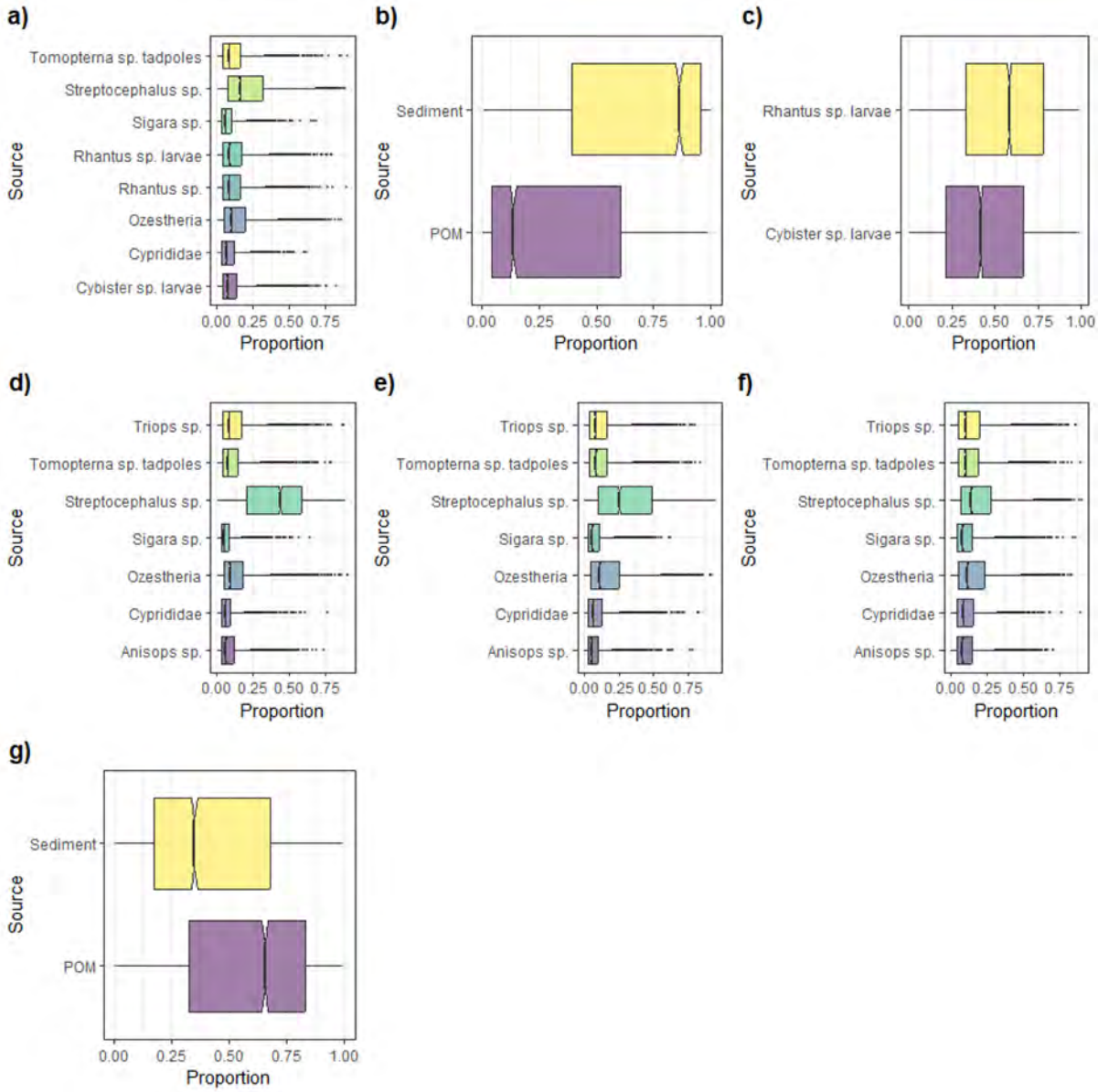

Figure A4. 4. Proportional resource contributions to (a) *Anisops sp.* (b) *Sigara sp.* (c) *Lestes sp.* (d) *Rhantus sp.* (e) *Rhantus sp.* Larvae (f) *Cybister* sp. larvae (g) *Tomopterna sp.* tadpoles as determined by Stable Isotope Mixing Models (SIMMR) for Pan 4.

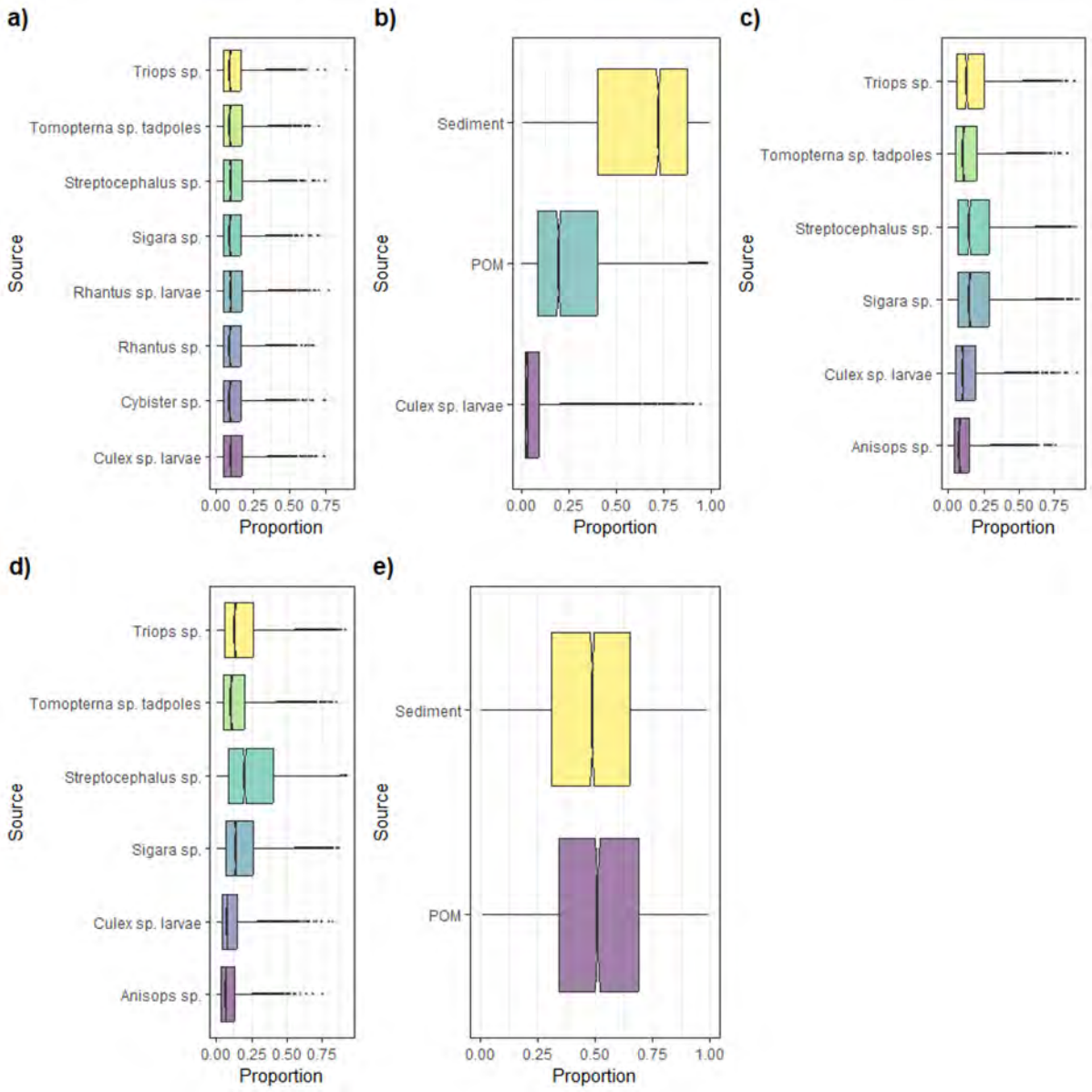

Figure A4. 5. Proportional resource contributions to (a) *Anisops* sp. (b) *Sigara* sp. (c) *Rhantus* sp. (d) *Cybister* sp. (e) *Tomopterna* sp. tadpoles as determined by Stable Isotope Mixing Models (SIMMR) for Pan 5.

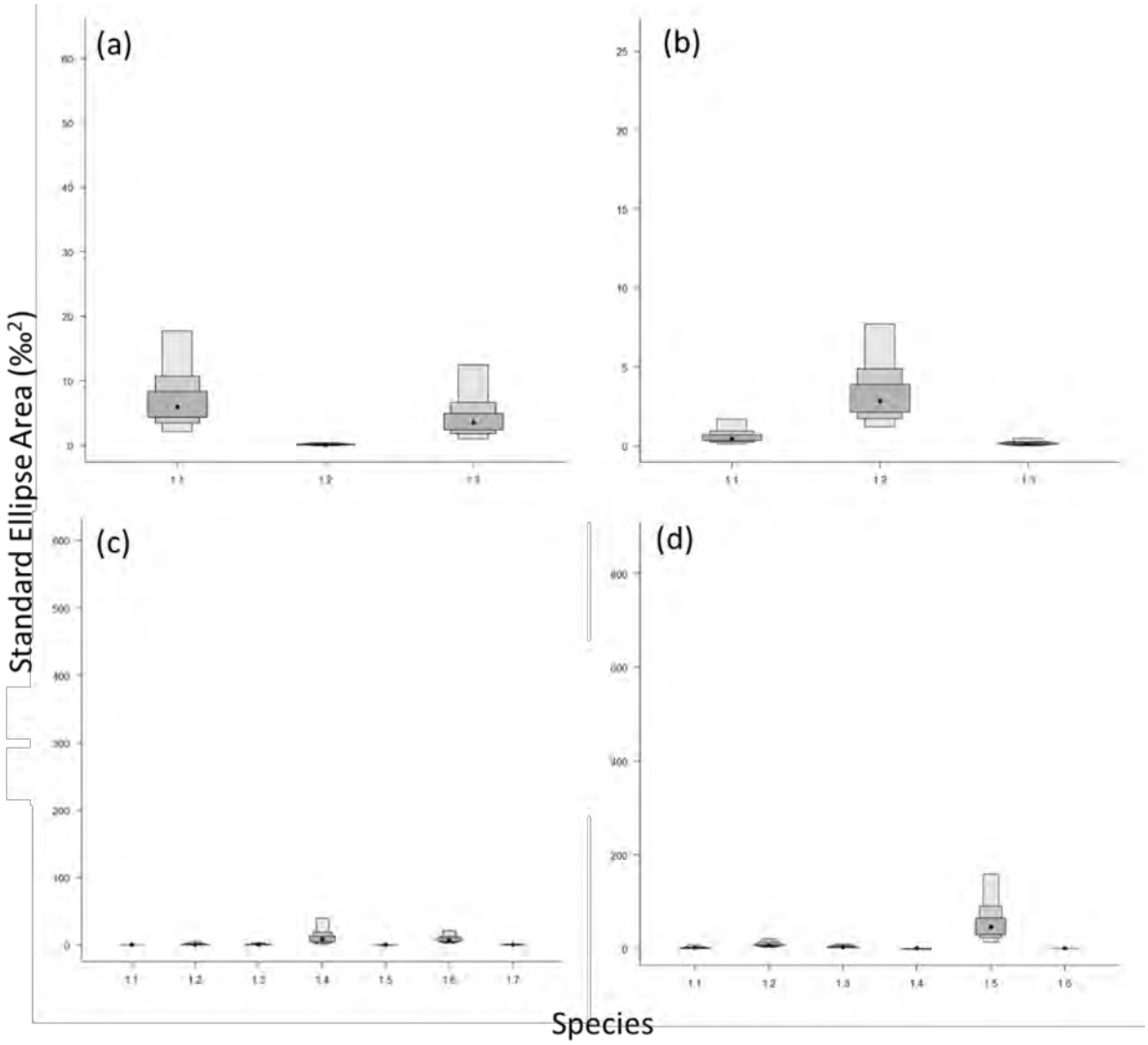

Figure A4. 6. SIBER Ellipse area plots for (a) Pan 1: 1.1 = *Sigara* sp.; 1.2 = *Anisops* sp.;1.3 = *Tomopterna* sp. tadpoles (b) Pan 2: 1.1 = *Sigara* sp.; 1.2 = *Anisops* sp.; 1.3 = *Tomopterna* sp. tadpoles (c) Pan 4: 1.1 = *Sigara* sp.;1.2 = *Rhantus* sp.; 1.3 = *Rhantus* sp. larvae; 1.4 = *Cybister* sp. larvae; 1.5 = *Lestes* sp.; 1.6 = *Anisops* sp.; 1.7 = *Tomopterna* sp. tadpoles (d) Pan 5 1.1 = *Sigara* sp.; 1.2 = *Rhantus* sp. 1.3 = *Cybister* sp.; 1.4 = *Rhantus* sp. larvae; 1.5 = *Anisops* sp.; 1.6 = *Tomopterna* sp. tadpoles.

Table A4. 2. Niche breadth overlaps of the standard ellipse areas with small sample corrections (SEAc) for dominant consumer insects and tadpoles in Pan 1 pans using SIBER.

	Sigara sp.	*Anisops* sp.	*Tomopterna* sp. tadpoles
Sigara sp.	–	1.812	2.418
Anisops sp.	–	–	0

Table A4. 3. Niche breadth overlaps of the standard ellipse areas with small sample corrections (SEAc) for dominant consumer insects and tadpoles in Pan 2 pans using SIBER.

	Sigara sp.	*Anisops* sp.	*Tomopterna* sp. tadpoles
Sigara sp.	–	3.984	0
Anisops sp.		–	0.72

Table A4. 4. Niche breadth overlaps of the standard ellipse areas with small sample corrections (SEAc) for dominant consumer insects and tadpoles in Pan 4 pans using SIBER.

	Sigara sp.	*Rhantus* sp.	*Cybister* sp.	*Rhantus* sp. larvae	*Lestes* sp.	*Anisops* sp.	*Tomopterna* sp. tadpoles
Sigara sp.	–	1.078	<0.001	2.133	0	1.524	0.302
Rhantus sp.		–	0.125	5.270	0	7.888	1.849
Cybister sp.			–	5.252	0.103	5.322	2.353
Rhantus sp. larvae				–	1.008	27.785	6.283
Lestes sp.					–	0.763	0.145
Anisops sp.						–	6.573

Table A4. 5. Niche breadth overlaps of the standard ellipse areas with small sample corrections (SEAc) for dominant consumer insects and tadpoles in Pan 5 pans using SIBER

	Sigara sp.	*Rhantus* sp.	*Cybister* sp.	*Rhantus* sp larvae	*Anisops* sp.	*Tomopterna* sp tadpoles
Sigara sp.	–	4.272	3.436	0.654	7.810	<0.001
Rhantus sp.		–	9.089	0.201	27.205	0.427
Cybister sp.			–	0.299	11.904	<0.001
Rhantus sp. larvae				–	0.742	<0.001
Anisops sp.					–	0.680

Table A6. 1. Benthic phytoplankton species identified at different salinities. + = present, – = absent.

Species	0ppt	0.5ppt	1ppt	2.5ppt	5ppt	10ppt
Amphora veneta	+	+	+	+	–	+
Asterococcus superbus	+	+	+	+	+	–
Craticula cuspidata	+	+	+	+	+	+
Closterium erhenbergii	+	–	–	–	–	+
Closterium littorale	+	–	–	+	+	–
Closterium navicula	–	–	–	+	+	–
Closterium pritchadianum	+	+	+	+	+	+
Cosmarium laeve	+	+	+	–	–	+
Cosmarium subcostatum	+	–	–	–	–	–
Cosmarium venustum	+	+	+	–	–	–
Cosmarium sp.	+	–	–	–	–	–
Craticula cuspidata	+	+	+	+	+	+
Cyclotella sp.	–	–	–	+	–	–
Cymatopleura sp.	+	+	+	–	–	+
Cymbella sp.	+	+	–	–	–	–
Diatoma vulgaris	+	+	+	+	+	+
Encyonopsis sp.	–	–	–	+	–	–
Eunotia minor	–	–	–	+	–	+
Fragillaria ulna	–	+	–	–	+	+
Frustulia vulgaris	+	+	+	–	–	+
Gomphonema affine	+	–	–	–	+	–
Gomphonema gracile	–	+	–	–	–	–
Gomphonema venusta	–	+	–	+	+	+
Goniochloris mutica	+	–	–	–	–	–
Goniochloris spinosa	+	–	–	–	–	–
Gyrosigma acuminatum	+	+	+	+	–	–
Gyrosigma attenuatum	–	–	–	+	+	–
Gyrosigma sp.	+	–	–	–	–	–

Species	0ppt	0.5ppt	1ppt	2.5ppt	5ppt	10ppt
Hantzchia amphioxys	+	+	+	+	–	+
Melosira varians	+	+	+	+	+	+
Mougeotia sp.	–	+	–	+	–	–
Pinnularia borealis	+	+	+	+	–	+
Pinnularia gibba	+	–	–	–	–	–
Pinnularia viridiformis	+	+	+	+	+	+
Navicula angusta	–	+	–	+	–	–
Navicula cryptonella	–	+	+	+	+	–
Navicula placentula	+	–	–	–	–	–
Navicula radiosa	+	+	+	+	+	+
Navicula symmetrica	+	–	–	–	–	–
Navicula trivialis	+	+	+	+	+	+
Navicula veneta	+	+	+	+	+	+
Navicula zanonii	+	+	+	+	+	+
Navicula sp.	+	+	–	–	+	–
Nitzschia aurariae	+	+	+	+	+	+
Nitzschia capitellata	–	+	+	+	+	–
Nitzschia dissipata	+	+	+	–	+	–
Nitzschia elegantula	+	+	+	+	+	+
Nitzschia filiformis	+	+	+	+	+	+
Nitzschia frustulum	+	–	+	–	+	–
Nitzschia gracilis	+	+	–	+	+	–
Nitzschia heufleriana	+	+	+	+	+	+
Nitzschia linearis	+	+	+	+	+	+
Nitzschia littorea	+	+	+	+	–	–
Nitzschia palea	+	+	+	+	+	+
Nitzschia perspicua	+	+	+	+	+	+
Nizschia pura	+	+	+	+	+	+
Nitzschia pusilla	+	+	+	+	+	+
Nizschia recta	–	+	–	+	+	–
Nitzschia sigma	–	+	+	+	+	+

Species	0ppt	0.5ppt	1ppt	2.5ppt	5ppt	10ppt
Nitzschia umbonata	+	+	–	+	+	+
Nitzschia sp.	+	–	–	–	+	–
Oscillatoria sp.	+	+	–	+	+	–
Surirella angusta	+	+	–	+	–	–
Zygnema sp.	+	+	–	–	–	–