Phytoplankton and Aquatic Macroinvertebrate Assemblages from Coastal and Inland Lakes of South Africa

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ABSTRACT

Freshwater lakes are generally defined as permanent natural standing water bodies, with some of them having a direct and indirect connection with groundwater, rivers, and the ocean. Freshwater lakes provide essential socio-economic and ecological goods and services including recreation, aesthetic, support aquatic biodiversity, food in a form of fisheries and water for domestic use. Given their critical role in sustainability in providing socio-economic services, freshwater lakes are among the most threatened ecosystems globally due to intense human impacts over the last decades. South Africa has limited freshwater lakes, which according to the National Biodiversity Assessment (NBA 2018), we know little about their current biology except historic aquatic biodiversity studies conducted in the early 1940s. There are no management strategies in place to protect and conserve freshwater lake biodiversity and important ecosystem services. This thesis aims to: (1) produce a biodiversity inventory of phytoplankton and aquatic macroinvertebrate species and, (2) investigate important environmental drivers responsible for phytoplankton and aquatic macroinvertebrate species composition from six South African freshwater lakes. It was hypothesized that the three lake types will show different phytoplankton and aquatic macroinvertebrates species composition attributed by the geographical region (coastal and inland lake) and related physico-chemical parameters.

Study sites consisted of two Northern KwaZulu-Natal coastal lakes (hereafter Coastal Lakes, CL) *i.e.,* Lake Sibaya, Lake Mzingazi; two fresh inland lakes (hereafter Fresh Inland Lakes, FIL) *i.e.,* Lake Banagher fresh and Lake Tevrede Se Pan; and two inland salt lakes (hereafterSalt Inland Lakes, SIL) *i.e.,* Lake Banagher salt and Lake Chrissiesmeer, all inland lakes are situated in Mpumalanga province, together with other Pans making up the Mpumalanga Lake District of South Africa. The study sites were categorized basedon their geographical position *i.e.,* coastal vs inland and physico-chemical characteristics *i.e.,* the presence and absence of aquatic vegetation, dominate substrate, salinity and different physico-chemical concentration *i.e*., Temperature, Dissolved Oxygen. Aquatic macroinvertebrates were collected from four littoral zone sites (< 1-meter depth) around each lake, whereas phytoplankton samples were collected from four water column sites (> 5-meters depth) and (0.5-meter depth) from the water surface at each lake during summer and winter season. The results were consistent with our hypothesis that bothphytoplankton and aquatic macroinvertebrate species composition were influenced by physico-chemical parameters and that the differences in salinity concentration and aquatic vegetation between CL, FIL, and SIL were the driving factors for phytoplankton and aquatic macroinvertebrate species composition.

In summary, one hundred and twenty-two phytoplankton taxa were collected and identified during this study, belonging to seven Phyla which included Chlorophyta, Bacillariophyta, Cyanophyta, Chrysophyta, Dinophyta, Euglenophyta, and Cryptophyta. The most abundant phytoplankton groups were Bacillariophyta and Chlorophyta. Phytoplankton relative taxa abundance, Pielou's evenness, taxa richness, and Shannon diversity were significantly different between lake types. Aquatic macroinvertebrates, on the other hand, summed up to10 orders, 67 families, and 80 taxa. The most abundant group were the order Coleoptera, Hemiptera, Odonata, and Gastropoda. Aquatic macroinvertebrate relative taxa abundance, taxa richness, and Shannon diversity were also significantly different between lake types. Aquatic macroinvertebrate relative taxa abundance, Pielous evenness, and Shannon diversity index were not significant between seasons, and only taxarichness was significant. Canonical analysis of principal coordinates (CAP) results further showed unique and distinct phytoplankton and aquatic macroinvertebrates community composition between lake types.

The present study provides baseline biodiversity inventory (or species list) for important lake ecosystems biological indicators *i.e.,* phytoplankton and aquatic macroinvertebrates and species composition in relation to lake type for six freshwater lakes in South Africa. Furthermore, the study provides empirical evidence that will inform policy and the development of management strategies for freshwater lakes in South Africa which is currently missing. The current study will also contribute to the next National Biodiversity Assessment Report (2024), concerning the freshwater lakes biological data deficiency noted in theprevious NBA (2018) report. The study will also fill up the gaps to better understand species composition in lake systems and how they function which is currently limited.

Keywords: Aquatic biodiversity, biological indicators, natural standing waterbodies, physico-chemical variables, salinity gradient.

TABLE OF CONTENT

LIST OF FIGURES

LIST OF TABLES

LIST OF APPENDICES

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''Take your place in the sun, you are the hope, you are the one... Okumhlophe kuwe ke, makube chosi, kube hele''

DECLARATION

I declare that this thesis has not been submitted to any university other than Rhodes University (Makhanda, South Africa). The work presented here is that of the author, unless otherwise stated.

Esethu Nkibi
13/09/2023

CHAPTER 1: GENERAL INTRODUCTION

1.1 PROBLEM STATEMENT

Freshwater systems provide essential socio-economic and ecological goods and services to humans and wildlife (Wantzen *et al.,* 2016). However, these systems are among the most threatened ecosystems globally due to the ever-growing human activities within catchments (Costanza *et al.*, 2014; Gozlan *et al*., 2019). Human-driven impacts, *i.e.,* habitat modification, alien species invasion, pollution, over-exploitation, and climate change, are among the most damaging threats negatively impacting aquatic biodiversity in freshwater systems globally. South Africa has limited diversity of natural standing freshwater systems, excluding wetlands.As a result, the South African National Biodiversity Assessment (NBA 2018) observed that the few available and unique inland and coastal freshwater lakes in Southern Africa are among the highly threatened, unprotected, and understudied ecosystems, with five of them not Protected (Lake Fundudzi, Barberspan, Tevredenpan, Lake Banagher and Chrissiesmeer) and three are Poorly Protected (Groenvlei, De Hoop, Lake Sibaya). Furthermore, the NBA (2018)identified eight freshwater lakes that are considered ecologically unique and important, thus needing special conservation measures (van Deventer, 2019), but less is known about their aquatic invertebrates and lower taxa inventories. Research in major lake systems is lagging with the last comprehensive research on these systems done during the 1980s (Bruton & Cooper, 1980; Allanson *et al*., 1990). Investment in freshwater lake research must be made to meet immediate ecological, social, and economic needs and maintain the lake's long-termfunctioning to meet the needs of future generations. Consequently, contemporary inventoriesof biodiversity in South African lakes *e.g.,* Miranda *et al*., 2014 are highly needed.

1.2 FRESHWATER LAKES AND THEIR IMPORTANCE

Freshwater lakes are generally defined as permanent and natural standing water bodies (Schertzer, 1997), with some having a direct and/or indirect connection to groundwater, rivers, and the ocean (Suring, 2020). However, freshwater lakes are a limited resource that forms approximately 0.01% of Earth's surface water (Davies & Day, 1998). As a result, overtwo-thirds of the liquid surface freshwater on Earth is contained in a handful of large lakes (Hereunder, 1990; Gâştescu, 2009). South Africa, in particular, as a dryland country, is notgifted with many natural lakes and as such has created many man-made dams and reservoirs(Allanson *et al*., 1990). Freshwater lakes play an important socio-economic role, as they havea positive impact on controlling natural disasters such as floods and droughts (Acreman, 2004). They are an important resource that is essential for the survival of many organisms (Kamika & Momba, 2012). Therefore, making them home to many faunas and flora with highbiodiversity value and vital ecosystem services (Martens, 1997; Salzburger *et al*., 2002; Schelly & Stiassny, 2004; Sanilkumar & Thomas, 2007; Walthan *et al*., 2014). Furthermore, societies frequently depend on lakes for many life-sustaining activities (Kamika & Momba, 2012). These activities include freshwater supply for drinking and domestic use, sanitation, and agricultural and industrial use (Van Zyl *et al*., 2007; Hobbs *et al*., 2008; DWA, 2011). Freshwater lakes have been shown to have major socio-economic values by improving the tourism industry and increasing property value and recreation (Walsh, 2009; Wen *et al*., 2014).

Most African inland lakes play a considerable role in contributing to food security (Koeberl & Reimold, 2005; National Research Council, 2005; Ajake & Amalu, 2012), the livelihood of rural communities, and help national economies through direct exploitation of fisheries for food and providing water for hydrogeneration and irrigation (Amalu & Ajake, 2019). Lakes have been contributing significantly to the local economy because they promote an increasein economic activities such as agriculture fishing pastoralism (Turner *et al*., 2000; Dontwi *et al*., 2002; Nindi, 2007). For example, their contribution to the economy is evident in Lake Naivasha in Kenya, the lake has contributed about 7% of the country's Gross Domestic Product (GDP) by supporting export-oriented agricultural products that are valued at US$ 613–640 million (Lambert & Turpie, 2003; NSO, 2008). In addition, the lake has also createdjob opportunities, ultimately contributing up to 10% of Kenya's foreign exchange (UNEP, 2006).

Freshwater lakes offer support services such as oxygen provision, and nutrient cycling, and provide benefits through the regulation of life and ecosystem processes and cycles (Apostolaki *et al*., 2019). This then helps improve air quality, protection from shore erosion, climate regulation, carbon dioxide (CO_2) sequestration, flow attenuation, and flood reduction (Fierro *et al*., 2019). In addition to direct support for livelihoods, lakes also play an important socio-economic role in regulating annual water supply, recharging groundwater, and helping control/avoidance of flooding (Araoye, 2000; Jamu *et al*., 2011). Their societal and economic importance is linked to international shipping, commercial fisheries, subsistence fisheries, drinking water supply, waste disposal, and recreation (Beaton, 2002). They can retain, store, clean, and even provide water, making them an essential component of the hydrological and biogeochemical water cycles, and influencing many aspects of ecological relationships and human well-being(Leaner & Doll, 2004). Some lakes have persisted for millions of years, acting as living museums of evolutionary processes like Lake Baikal (Vadeboncoeur *et al*., 2011).

Lakes support a substantial proportion of unique aquatic organisms globally (Turner *et al*., 2007; Suring, 2020). However, littoral communities in large lakes are poorly described, and ecosystem-scale studies in the nearshore zone are few (Niemiet *et al*., 2007). At the same time, littoral zones in lakes are affected by many anthropogenic activities (Edgemont &Verschuren, 2003; Vadeboncoeur *et al*., 2011). Therefore, the lack of biodiversity studies onthe littoral zones is unfortunate (Howard-Williams & Lenton, 1975; Sandergaard & Jeppesen,2007). This study will try to fill this gap by studying macroinvertebrates in six lakes in South Africa. Aquatic macroinvertebrates are an essential part of aquatic food (Vannote *et al*., 1980; Vaughn & Hakenkamp, 2010; Butkas *et al.,* 2011) and play a huge role in ecosystem services(Covich *et al*., 1999; Covich *et al.,* 2004; Cai *et al*., 2017).

1.3 FRESHWATER LAKES IN SOUTHERN AFRICA

Southern African countries have few natural standing freshwater lakes, particularly permanent systems like lakes (Van Deventer *et al*., 2020a). South Africa, however, has several unique coastal lakes which are found on the eastern and south-western coastal line and according to literature, most of these lakes were once or still linked to the sea or estuaries(Hill, 1975). Many coastal lakes are shallow, for example, the average depth of St Lucia andUMhlathuze was 1 to 2 meters before dredging. However, some lakes like Swartvlei are 12 meters deep, with most of the northern KwaZulu-Natal lakes, *i.e.,* Lake Sibaya with a maximum depth of 40 meters, Lake Nhlange 30 meters and Lake Mzingazi 14 meters (Hill, 1975). Research about theorigin of these lakes has been done. Martin (1962), in their study, suggested that some of these lakes were formed by the flooding of existing depressions by a raised sea level. While according to King (1951), UMhlathuze was formed as a coastal lagoon behind an offshore bar,and Nhlange and St Lucia were drowned valleys. However, Day *et al*. (1954) study reported that St Lucia was formed as a depression of the seabed. On the contrary, Hill (1969) claimed that Lake Nhlange and Lake Sibaya were formed from drowned valleys which were originallycut during periods of lowered sea level. Furthermore, these lakes are of estuarine origin, andmost of the lakes are being modified by engineering works.

Salt Lakes are widely distributed in Southern Africa, and most of them are ubiquitous in South Africa (Harrison, 1962; Hugo, 1974), but many occur in Namibia and Botswana (Seaman *et al*., 1991). This includes some floodplain lakes which are associated with the Phongolo River *i.e.,* Nyamithi, Mhlolo, and Tete Rivers. These lakes are of riverine origin and almost all are ephemeral, shallow, and not very salinity rich. These water bodies are commonly referred to as pans and vleis (Seaman *et al*., 1991). Salt lakes are geographically widespread and numerous in Southern Africa (De Klerk *et al*., 2016),but they have attracted considerably low limnological attention (Seaman *et al*., 1991). Hutchinson (1929), Pickford & Schuurman (1932) are the only ones who attempted to investigate those in South Africa, in what was then known as Transvaal (Seaman *et al*., 1991; Lawrie & Stretch, 2011).

Salt Lakes are formed by an increase in land-use changes particularly land cultivation and cattle farming (George *et al*., 1999; Thorburn, 1999). This is evidence of an increase in the amount of salt in the Darling pans of the Western Cape Province. Furthermore, salt in thosepans is the result of prolonged or heavy rainfall in the granite catchment dissolved salts (Smith& Compton, 2004). These dissolved salts are then flushed from the soil and are transported into the pans via the process of throughflow and run-off and underground water. In addition,Salt Lakes may be formed by continuous feeding of older groundwater that has been modifiedby weathering of granitic colluvial sands (Smith & Compton, 2004).

One of the well-known salt pans in South Africa is the Pretoria salt pan, Lake Tswaing (Ashton& Schoeman, 1983; 1988). Lake Tswaing, which is located 60 km north-west of the city of Pretoria in the Gauteng province, South Africa (Partridge*et al*.,1993). According to Gold & Gurney (1993), this pan is of volcanic origin. However, according to Reimold *et al*. (1992), Lake Tswaing was formed by the flooding of a crater thatwas created by the high-velocity impact of a large meteorite approximately 220 000 years ago. Furthermore, a recent study conducted by Oberholster *et al.* (2009a) highlighted that, this lake has been undergoing several important changes due to the drilling of boreholes for domestic water use in the area. These new boreholes provide inflows that have different chemical qualities *i.e.,* the inflowing water to the lake has a higher salinity, where during 1978-1980 the salinity was 3.5 ppt and in 2006 increased to 32.5 ppt. They indicated that Lake Tswaing is fed by water inflow that is from different geological sources. These changes in the lake may influence the survival of aquatic organisms and thus causing low biotic diversity in the lake (Wehr & Seath, 2003). Furthermore, there have recently been studies in some of the southern African pans, for example, a study by McCulloch *et al*. (2008) on the Salt Lake (Sau Pan) in Makgadikgadi, Botswana. In which they were investigating hydrochemical fluctuationand crustacean community composition in the pan. The study showed that change in water chemistry in Sua Pan in Makgadikgadi, Botswana was caused by flood that occurred over the duration of two years. The change in water chemistry and dissolution of mineral efflorescence on the pan surface and the influx of saline groundwater resulted in the formation of this pan.

1.4 FRESHWATER LAKE THREATS

1.4.1 Land use or habitat modification

One of the main drivers for the loss of aquatic biodiversity and the freshwater ecosystem's structure and function collapse is habitat loss or transformation (Hoyer & Chang, 2014). With sixty-eight percent of the global population expected to live in cities by 2050 and with high growth in human population and many cities growing into mega-cities, many aquatic systemsare being threatened (Vorosmarty *et al.,* 2010). Urbanization alters the physical and chemical environment of rivers, streams, ponds, and lakes (Allan, 2004, Heino & Tolonen*,* 2017). This is with evident to the study by Sitaram (2014) on a case study on Ashtamudi Lake Kollam in India on how urbanization impact physico-chemical variables. Physico-chemical variables such as DO, pH and Heavy metals were analyzed in the lake for a period of 7 years. Results from the study showed that physico-chemical variables in the lakes are deteriorating at a faster rate, due to untreated sewage from Kollam city. For example, DO was found decreasing over the years, as a result DO levels were 3.2 mg/l on average on a non-polluted water and 5 mg/l in a healthy tropical water body.

Urbanization does not only alter the physico-chemical environment, but it also has a negative impact on the biology of lakes. This is evident from the study by Gál *et al*. (2019), where they assessed the impact of urbanization on freshwater macroinvertebrates diversity. Results from the study showed thatin most freshwater systems aquatic macroinvertebrates in lakes were ignored entirely. This then emphasizes that freshwater ecologist gives more focus and attention to rivers and streams than lakes (Cereghino *et al.,* 2008). This may be caused by comparing lakecommunities under clear natural and urban conditions that could be challenging (Schmera *etal*., 2017). Therefore, well-documented case studies are needed in the lake, pond, and wetland habitats about aquatic macroinvertebrates communities in these freshwater systems (Gál *et al.,* 2019).

Several studies around the world have been done on the relationship between habitat transformation in freshwater systems and physico-chemical variables (Brooks *et al*., 2002; Carrino-Kyker & Swanson, 2007), but in South Africa, studies on habitat transformation effects on freshwater systems are lacking. This is because South Africa has limited lakes, therefore most studies have been done on wetlands. One of the studies includes one by Rhazi *et al*. (2001) where they found that there were higher nutrient levels in ten temporarywetlands which were surrounded by agricultural fields than those in natural areas. Another study by Bird & Day (2014), investigated the type of habitat transformation around temporarywetlands and their water physico-chemical characteristics. In their study, they concluded thathabitat transformation affects physico-chemical variables. Furthermore, this indicates that inmost freshwater systems, turbidity increase with the transformation of the surrounding landscape, due to sedimentation from agriculture (Crosbie & Chow-Fraser, 1999). Also, the replacement of natural vegetation often results in the de-stabilization of soil (Declercq *et al*., 2006). Another study by Bird *et al.* (2013) investigated 12 temporary wetlands that have landscapes invaded by Kikuyu grass within the Sand fynbos ecosystem in Cape Town. In their study, they found that the replacement of indigenous habitat with alien invasive vegetation has altered physico-chemical variables, which then affect diversity within the wetlands, by raising soil and surface water pH. In addition, urban land-use affects aquatic macroinvertebrates by often leading to a decrease in diversity and a shift in relative abundance among the functional feeding groups (FFGs) (Mangadze *et al*., 2019; Gholizadeh& Heydarzadeh, 2020).

The NBA (2018) emphasized land use change, *e.g.,* commercial forestry within catchments where lakes, are found, as one of the major threats to lake water quantity and quality. Even worse, according to DWAF (1996), commercial forestry was predicted to be in demand, and this has been observed to be rising since then, thus putting pressure on both surface and groundwater reserves. However, several studies done on commercial plantation in South Africa has suggested that a change in land use by pine plantation might result in a reductionof groundwater loss (Salama *et al*., 2002; Vaeret *et al*., 2009).

1.4.2 Alien Species Invasions

The introduction of alien species in freshwater systems has been declared the second leading threat to loss of biodiversity after habitat modification (Vitousek *et al.,* 1997; Wilcove *et al*., 1998; Walker *et al*., 1999; Richardson & van Wilgen, 2004). Alien species are introduced into a habitat in various ways *i.e.,* deliberately, and accidentally (Keller *et al*., 2011). Furthermore, their introduction has been an enormous threat to freshwater systems for the last five decades (Craig *et al*., 2017; van Wilgen *et al*., 2020). In South Africa five of the world worse invasive alien aquatic weeds are responsible for transforming and negatively impacting the aquatic biodiversity and ecosystems (Motitsoe *et al.*, 2022). These invasive alien aquatic weeds included *Eichhornia crassipes Pistia stratiotes, Salvinia molesta, Myriophyllum aquaticum,* and *Azolla filiculoides* (Hill *et al*., 2020). The introduction of these alien invasive species in the absence of natural enemies and productive habitats results in an exponential increase of alien invasive species population, to an extent that they out-competelocal fauna or flora by competing for space and resources (Richardson & van Wilgen, 2004; Simberloff, 2013).

Furthermore, alien species have been introduced for recreation *i.e.,* the fisheries industry but later were noticed to have negative impact on biodiversity. For example, in 1897, Rainbow Trout (*Onchorhynchus mykiss*) were introduced to South Africa (Weyl *et al*., 2020; van Wilgen*et al*., 2020), and hatcheries were established at Jonkershoek (Western Cape) and Boschfontein (KwaZulu-Natal) to breed and distribute trout for recreational fishing (van Wilgen *et al*., 2020; van Wilgen *et al*., 2021). The establishment of *Oncorhynchus mykiss*, Largemouth Bass *(Micropterus salmoides*), Common Carp (*Cyprinus carpio*), Brown Trout (*Salmo trutta*), and Smallmouth Bass (*Micropterus dolomieu*) by government, societies and private individuals for hatchery has caused these species to be declared as conflict species, or rather declared more invasive due to their impact in conservation (Ellender *et al*., 2017; Weyl *et al*., 2020). This matter has complicated the management of some alien fish species in the country, because of how society views the benefits vs impacts (Ellender *et al*., 2014; Woodford *et al*., 2016; Zengeya *et al*., 2017). Apart from fish and macrophytes, freshwater snails are continuously being introduced in many countries via the aquarium industry (Madsen & Frandsen 1989; Cowie, 1998),and South Africa is no exception (Weyl *et al*., 2020; Miranda *et al*., 2022). Two alien freshwater gastropods species *Radix rubiginosa* (Michelin, 1831) (Lymnaeidae) and *Gyraulus chinensis* (Dunker, 1848) (Planorbidae) with an Asian origin, have been reported inKwaZulu-Natal, South Africa (Appleton & Miranda, 2015). Recent studies have shown that *Tarebia granifera* can be extremely abundant to an extent that they affect the functioning of the environment (Miranda *et al*., 2011; Miranda *et al*., 2014). Therefore, with all the threats that affect freshwater systems more work to monitor freshwater systems is needed to avoid loss of biodiversity.

1.4.3 Eutrophication and water pollution

Eutrophication is characterized by nutrient enrichment which results in excessive plant and algal growth in freshwater bodies (Schindler, 2006; Chislock *et al*., 2013). It is a chronic globalthreat that affects humans, water security, and the ecosystem (Smith & Schindler, 2009). As aresult, eutrophication is a severe environmental problem in Southern African countries (VanGinkel, 2011) and according to DWS (2015), over the past 20 years, pollution in freshwaterecosystems has accelerated, leaving about 60% of South African freshwater systemsthreatened, and 25% critically endangered, particularly by eutrophication (Harding, 2015; Mathews & Bernard, 2015). In South Africa, the cyanobacterial toxin is in magnitude higher thanthose reported in northern hemisphere countries (Harding & Paxoton, 2001; Melone & Newton, 2020)).

Harding & Paxton, (2001) reported that severe eutrophication in South Africa has caused livestock and wild animal death. For example, several deaths of mammals and crocodiles in Kruger National Park were reported between 2005 and 2007 (Oberholster *et al.,* 2009b; Ferreira *et al*., 2012). These animals died because of excessive-high exposure to water contaminated by cyanotoxin which resulted in highly eutrophic water in the park. Furthermore, in Southern Africa, a high amount of pollution in freshwater systems has been caused by different sources *i.e.,* a high increase in urbanization, agriculture, industry, and sewage (Oberholster & Ashton 2008; Thornton *et al.*, 2013). These sources contribute to high concentrations of nitrogen, ammonium, and phosphate in freshwater systems, which preventthe system from decomposing or turning over excess nutrients (Luo *et al*., 2012). This then creates toxic cyanobacterial blooms that reduce water clarity, change water quality, and reduces light penetration to underwater life, thus disrupting growth and causing die-offs of submerged macrophytes (Landsberg, 2002; Doster & Zitomer, 2020). This cyanobacterial bloom threatens the ability to supply safe and enough drinking water in large parts of the semi-arid South Africa (Van Ginkel, 2004).

1.4.4 Climate Change

Anthropogenic activities and poor management of freshwater systems have caused irreversible damage to aquatic biodiversity and ecosystem structure and function (Cheng Ouazar, 2004).

Despite these obvious impacts, climate change has further exacerbated the deterioration of freshwater systems even further (Været *et al*., 2009). Climate change has a negative and long-term impact on water resources globally (Urama & Ozor, 2010; Bhave *et al.,* 2020), *i.e.,* flooding, rise in sea levels and salt water in estuaries, drying up of rivers and lakes water level drop (Urama & Ozor, 2010). Studies done in Africa showed that air temperatures have increased by 1-2ºC between 1970 and 2004, and Bhave *et al*. (2020) further predicted a rise of 1.4 to 5.8 ºC in the next two decades. Further increases in temperatures will result in a gradual increase in sea level near coastal areas leading to elevated groundwater boundaries which will cause an inland shift of the mixing zone betweenfresh and salt groundwater (Cheng & Ouazar, 2004; IPCC, 2007).

In South Africa, the effects of sea-level rise due to climate change on the groundwater have previously not been analysed (Været *et al.,* 2009; Wündsch *et al*., 2016). However, a review paper by Været *et al.* (2008) on climate and sea-level changes on the South African East Coast has predicted thatby 2100 South Africa will experience a high rise in sea levels and a warmer and wetter climate. This is evident with the study carried out at the Cape St Lucia weather station for theyears 1928-2006. The analysis suggested that for the past 80 years St Lucia has become wetter rather than drier (Været *et al*., 2009). However, the lake water level has been posing risks to the countries' water, food, and energy security (Nash *et al*., 2018). This is caused bythe recent severe 2015/16 drought (Kolusu *et al*., 2019). The multi-year dry period (the 2015/16 El Niño event) has led to a gradual decrease in lake water levels and has reduced outflows, causing socio-economic disruption through impact on hydropower generation (Conway *et al*., 2017). Lake Sibaya, for an example has lost almost 5 meters of its surface water when compared to the last decade, resulting in reduced water level due to changes in the catchments including settlements encroachment and commercial *Eucalyptus* sp. plantations. Furthermore, this has resulted in the southern basin detaching (or isolating) from the main basin, and this could affect the lake aquatic life. Not only does climate change affect the rise in sea levels, but it also has a negative impact on phytoplankton dynamics, directly and indirectly by changing water temperature (stratification) and resource availability (nutrients and light), and intensified grazing byheterotrophs (Behrenfeld *et al*., 2006; Paerl & Huisman, 2008; Winder & Sommer, 2012). Any change at the base of the aquatic food web can result to negative impact on the ecosystem. For example, if primary produces are compromised, resource supply or subsidies to higher trophic level consumers will be affected. Dynamics in phytoplankton are linked to annual fluctuations of water column stratification, temperature, dissolved oxygen concentration, and light availability (Sommer *et al*., 1986; Cloern, 1996). Due to climate change, these environmental factors can be modified, and phytoplankton structures, taxonomic composition, and seasonal dynamics can be altered (Winder & Sommer, 2012).

1.5 FRESHWATER LAKES MANAGEMENT

In response to finding about how freshwater systems are being threatened, priority actions according to NBA (2018) have been taken, for example control of invasive species. Some ofthe management actions include monitoring, early detection, and rapid responses to new invasive species to avoid the threat of indigenous species. Furthermore, education programslike WESSA Green Coast Tourism are initiated, which are for the protection of traditional andcultural values. More management actions, including improving management to rebuild depleted stocks of overfished species are implemented to avoid loss of fish biodiversity in freshwater systems. These management strategies will help strengthen the resilience and ability of species and ecosystem types to climate change and their capacity to deliver benefits to people *i.e.,* food security. Lakes found in areas that are populated or near places that are intensively cultivated experience increased nutrient loads. These nutrients are caused by theoveruse of pesticides and fertilizers in agriculture, together with poor water waste management (Ansara-Ross *et al*., 2012), which later results in turbid and phytoplankton dominated waters and eventually loss of biodiversity (Wetzel, 2001; Lin *et al*., 2011).

Many urban lake management strategies have been done such as shoreline management by banning construction activity *i.e.,* building roads, and factories to specific heights above the lake periphery, as a result, some of the lake peripheries are declared as protected areasor wildlife sanctuaries (Axon & Whitehurst, 1985; Redy & Char, 2006). For example, in countries like India, conservation trusts have been established, with assistance from the World Wildlife Fund for Nature (WWF)-India, to undertake conservation measures for the lakes (Redy & Char, 2006). In South Africa, scientists are studying the biological and integrative control of several invasive aquatic weed species. Therefore, the management andconservation of freshwater lakes could help in restoration efforts and help with sustainable development over the long run (Arthington *et al*., 2016).

1.6 AIMS OF THE STUDY

This study aims to investigate the species composition and diversity of phytoplankton and aquatic macroinvertebrates from six South African coastal and inland lakes during the wet and dry seasons.

Specific aims:

I. To investigate the phytoplankton diversity and species composition between CL (Lake Sibaya and Lake Mzingazi), FIL (Lake Banagher fresh and Lake Tevrede Se Pan), and SIL (Lake Banagher salt and Lake Chrissiesmeer) of South Africa. The hypothesis is that phytoplankton species composition will be different between lake types due to their geographical location and physico-chemical parameters such as salinity, aquatic vegetation and substrate composition found.

II. Establish a baseline species list of littoral aquatic macroinvertebrates from coastal and inland lakes of South Africa during dry and wet seasons and assess biodiversity dynamics and species composition. The expectation was that lake types will exhibit different aquatic macroinvertebrates composition and that FIL with both aquatic and marginal vegetation will be more diverse and species rich as compared to the rest of the lake types.

1.7 THESIS OVERVIEW

This study comprises four chapters. **Chapter 1** is the general introduction. **Chapter 2** will investigate phytoplankton species composition from coastal and inland lakes of Southern Africa during dry and wet seasons. **Chapter 3** will investigate aquatic macroinvertebrates community structure from coastal and inland lakes of Southern Africa during dry and wet seasons. **Chapter** 4 will synthesize the research findings and provides recommendations and future research opportunities on monitoring and lake research in South Africa.

CHAPTER 2: PHYTOPLANKTON SPECIES COMPOSITION FROM COASTAL, FRESH INLAND AND SALT INLAND LAKES OF SOUTH AFRICA

2.1 INTRODUCTION

Phytoplankton communities occupy an important position in freshwater ecosystems (Loick-Wilde *et al*., 2016), as primary producers together with periphyton and aquatic macrophytes, and they are responsible to fuel aquatic food webs (Emmanuel & Onyema, 2007; Jakhar, 2013). They drive ecosystem productivity by maintaining oxygen levels to sustain essential aquatic processes like respiration and photosynthesis (Dalu *et al*., 2014). This then results in phytoplankton being transducers and acting as a food source for aquatic environments (Gopinathan, 1971; Nair *et al*., 1983; Burchardt, 2014). Phytoplankton produces food by converting solar energy and nutrients intochemical energy for food (Miller *et al.,* 1996; Jakhar, 2013; Burchardt, 2014), in which the majority of higher trophic level organisms are dependent on including filter feeding zooplankton, aquatic macroinvertebrates, and some fish (Ezekiel *et al*., 2011; Wu *et al*., 2011; Jakhar, 2013; Dalu *et al*., 2014; Stevenson, 2014; Sun *et al.,* 2014; Rumin *et al*., 2020).

This makes phytoplankton suitable for investigating trophic interactions such as top-down and bottom-up effects (Kock *et al*., 2019; Peng *et al*., 2021). The bottom-up effect influencesthe development, density, and species composition of phytoplankton through the availabilityof nutrients, light, and temperature (Li *et al*., 2020). While size, abundance, and distribution of phytoplankton are influenced by the top-down effects including grazing pressure from zooplankton and other filter feeders (Carignan & Neiff, 1992; Nicolle *et al.,* 2011). As a result, slight changes in biotic and/or abiotic characteristics in freshwater ecosystems, particularly lakes, will result in a change in phytoplankton biomass and species composition (Carpenter *et al*., 1985; Steve, 2014; Harding, 2015). A greater understanding of the top-down and bottom-up effects on phytoplankton regulation in lakes would result in a betterunderstating of lake ecosystem dynamics and improved management interventions.

Phytoplankton occupies a critical position or trophic level within aquatic systems, assisting with the understanding of various environmental interactions and disturbances (Chutter, 1998; Ndiritu *et al*., 2006). They are organisms whose exposure to pollution is continuous, as a result, they reflect both the past and present water quality history (Stevenson *et al*., 2010; LaHée & Gaiser, 2012; Dalu & Froneman, 2016), making them reliable biological indicators foraquatic ecosystems health in lakes (Dell'Uomo & Torrisi, 2011; Dalu & Froneman, 2016). Özkan *et al.* (2013) reported that phytoplankton richness was primarily affected by water chemistry *i.e.,* temperature and pH in 395 lakes in Denmark, and they concluded that phytoplankton richness was strongly impacted by physico-chemical (Liao *et al*., 2016). This is because physico-chemical parameters like temperature (and light) for example promote the metabolic rate and cell division of phytoplankton and increase their productivity (Cha *et al*., 2017; Kim *et al*., 2019).

The global use of phytoplankton for biomonitoring, for present and past conditions, has increased drastically (Haberyn & Hecky, 1987; Stager & Johnson, 2000; Talbot & Lærdal, 2000; Ndiritu *et al*., 2003; Ndaragu *et al*., 2004; Taylor *et al*., 2005; Ndiritu *et al*., 2006; Bere & Tundisi, 2010; Harding *et al*., 2015). This is because phytoplankton is abundant, diverse and widely distributed in freshwater, brackish, and marine ecosystems (Lane & Brown, 2007; Bere & Tundisi, 2010; Omar, 2010; Chakraborty *et al.,* 2014; Stevenson, 2014). This makes phytoplankton ideal focus taxa for monitoring aquatic environments.

A study was done by Nakanishi *et al.* (2004) and Kumari *et al.* (2008), showed that phytoplankton is useful for biomonitoring in freshwater systems. In their studies, they documented that in less polluted water there was high abundance and diversity of phytoplankton than in heavily polluted waters. This is because phytoplankton responds directly to growth stimulants such as nutrients, physical factors *i.e.,* habitat, light, and stressors, which can be used to monitor changes within aquatic systems (Lobo *et al*., 1995; Bere et al., 2014; Dalu *et al*., 2014; Dalu & Froneman, 2016). The global use of phytoplankton for biomonitoring is that they are time-integrated, allowing the detection of environmental changes that might otherwise be overlooked using physico-chemical assessments (Bere & Tundisi, 2010).

This chapter aims to investigate phytoplankton dynamics *i.e.,* diversity and species composition between Coastal Lakes (CL), Fresh Inland Lakes (FIL), and Salt Inland Lakes (SIL) of South Africa during winter and summer seasons. It was hypothesized that phytoplankton species composition will be different between lake types driven by differences in lake type geographical locations (coastal and inland) and related physico-chemical parameters.

2.2 MATERIALS AND METHODS

2.2.1 Study sites

This study was conducted in six South African freshwater lakes and these included two CL *i.e.,* Lake Sibaya and Lake Mzingazi found in Northern KwaZulu-Natal, and four inland lake systems, two FIL *i.e.,* Lake Tevrede Se Pan and Lake Banagher fresh, and two SIL *i.e.,* LakeBanagher salt and Lake Chrissiesmeer found in Mpumalanga Lake District, South Africa (Figure 2.1).

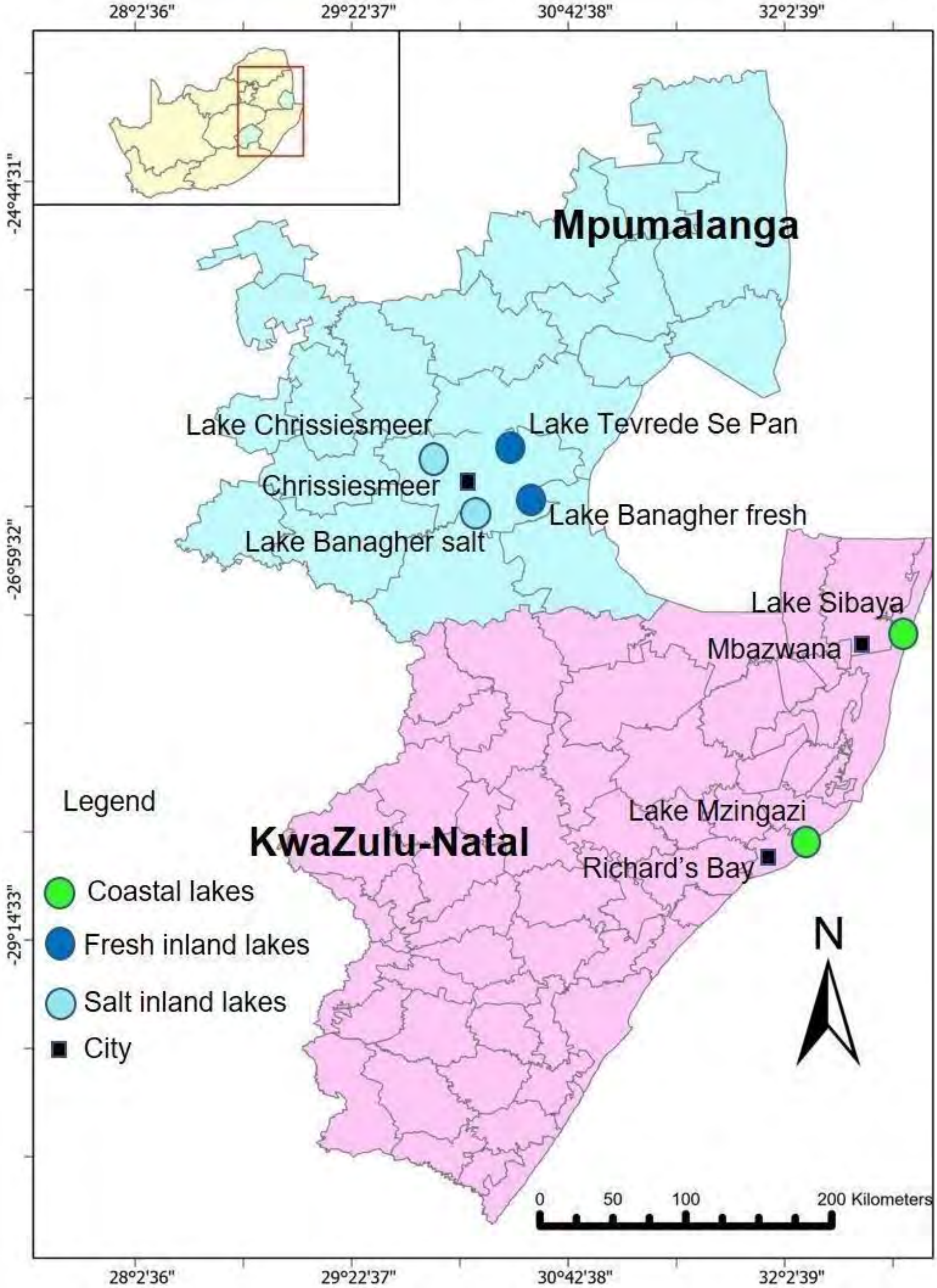

Figure 2.1: A map showing the six freshwater lakes sampled from Kwa Zulu-Natal and Mpumalanga Provinces, South Africa.

Coastal Lakes (CL)

Lake Sibaya (27°20'26.1" S, 32°40'32.7" E; 18 m.a.s.l, Figure 2.1), is the largest freshwater lake in Southern Africa, located in the north-eastern Maputaland (Kyle & Ward, 1995). It is one of theRamsar wetlands of international importance (Combrink *et al*., 2011), and forms part of the Isimangaliso Wetland Park, a World Heritage Site. Lake Sibaya covers a catchment area of ca. 540 km^2 (Bowen, 1979; Ward & Kyle, 1990; Malherbe *et al*., 2017) and has a surface area of 12.16 km^2, a maximum depth of 40 meters, and an average depth of 13 meters (Bruton, 1980; Ward & Kyle, 1990; Humphries & Benitez-Nelson, 2013). Unfortunately, due to landscape developments including an increase in commercial plantation *i.e., Eucalyptus* species in the vicinity, Lake Sibaya water level is seriously threatened resulting to 5 metres reduction (Carnie, 2020). The lake is composed of five main basins namely the main basin, the southern basin (which is now isolated from the main basin due to water level drop), the south-western basin, the western arm, and the northern arm (Allanson, 1979; Bruton, 1979, Combrink *et al*., 2011; DWS, 2015). Average rainfall across the coastal plain varies at 1120 mm/annum, and 43% of the annual rainfall occurs from January to March (Hill, 1979; Miller, 2001; Meyer & Godfrey,2003). Evidence from the geological evolution of this system and the presence of estuarine and marine organisms in this system shows that it was once connected to the Indian Ocean (Bruton, 1980).

Lake Mzingazi (28°45'47.7" S, 32°04'50.5" E, 5 m.a.s.l, Figure 2.1) is found near the coastalcity of Richard's Bay in the warm, humid, subtropical KwaZulu-Natal coastal belt. The lake issituated near Richards Bay Harbour, which is one of the largest exporting ports in Africa. In the catchment region of Lake Mzingazi, there are formal and informal residential developments, subsistence farming in the informal settlements, and industrial and mining operations (Van der Wateren, 2012). The lake has a surface area of 11 km^2 and a catchmentof 164 km^2 (Harding, 2000). A 2 km wide coastal dune barrier separates the lake from the Indian Ocean (Wepener *et al*., 1995). Lake Mzingazi consists of two main compartments, a rounded southerly deep basin, and a narrow northerly section. The two sections are separated by a very shallow and narrow section which is exposed during extreme drought conditions (Cyrus, 1993). The southern compartment at its deepest point is approximately 14meters with a maximum depth of 6 meters above sea level, and therefore susceptible to saltwater penetration under drought conditions (Kelbe *et al.,* 2001). Lake Mzingazi receives between 900 and 1 300 mm of precipitation annually, the majority of which falls in the summer(December to March). Lake Mzingazi is the only suitable source of domestic water supply forthe Richards Bay community (DWAF, 2002).

Thus, due to their geographical position and sea or estuarine influence, Lake Sibaya and Lake Mzingazi were categorized as CL.

Fresh Inland Lakes (FIL) and Salt Inland Lakes (SIL)

Lake Banagher fresh (26°20'21.7" S, 30°21'29.8" E, 1668 m.a.s.l), Lake Tevrede Se Pan (26°12'46.1" S 30°11'58.1" E, 1737 m.a.s.l), Lake Chrissiesmeer (26°19'21.4" S, 30°12'38.9" E, 1674 m.a.s.l) and Lake Banagher salt (26°21'15.9" S, 30°21'29.0" E, 1661 m.a.s.l) systemsare situated in the Mpumalanga Lake District (MLD) in Mpumalanga Province, near Chrissiesmeer town, South Africa (Figure 2.1). The MLD district is located between the Usutu,Vaal, Olifants, Komati, and Mbuluzi catchment areas (McCarthy *et al*., 2007). The MLD is subjected to warm, wet summers (December to May) and cold, dry winters (April to July). MLD is situated in a relatively humid area with a mean annual rainfall of approximately 800–1 000 mm/annum (Goudie & Thomas 1985; Van Deventer *et al.,* 2020a; Van Deventer *et al*.,2020b), and a mean annual potential evaporation of approximately > 1 000 mm and mean annual precipitation of < 500 mm (Goudie & Thomas, 1985; Schulze, 1997). The region is well known for being unique, it is the only part of the African Panveld that was possibly preserved, because of less erosion compared to other parts of South Africa (Partridge & Maud, 1987; McCarthy *et al*., 2008). There is no industrial activity in the vicinity instead the land is greatly used for agriculture and farming livestock (Emery *et al*., 2002). These lakes are usually devoid of vegetation except around their shorelines (Ferreira *et al*., 2012), if present they are highly different in terms of vegetation structure, surface area covarage, and above all physico-chemical parameters and yet they are in close vicinity (Victor, 2013). For example, the SIL were situated on bedrock, both lakes had no vegetation and they weremore saline than other lakes. Lake Banagher salt was populated by large flocks of flamingos feeding in the water and the lake had white salt encrustation from the limestone. Due to physical and chemical characteristics *i.e.,* water quality and vegetation structure, Lake Chrissiesmeer, and Lake Banagher salt were similar, thus categorized as SIL (Table 2.1). While FIL, Lake Tevrede Se Pan, and Lake Banagher Fresh both had submerged and emerged vegetation (Table 2.1). Thus, Lake Tevrede Se Pan and Lake Banagher freshwaterwere categorized as FIL.

Table 2.1: A summary of site description and landuse around the six sampled South African freshwater lakes in the Mpumalanga and KwaZulu-Natal province, South Africa**.**

Study site	Size and depth	Lake type	Site description and land use	Dominant plant species (Aquatic and riparian)	
				Indigenous	Invasive
Lake Mzingazi	The surface areaof 12.16 km^2 and the max depth is 14 m and average depth is 6 m.	Coastal lake	Coastal freshwater lake, area is used as a harbour and there is urbanization. It is situated near formal and informal residential developments, large industries, and Richards Bay Minerals.	*Cyperus* sp. *Ceratophyllum* sp.	
Lake Sibaya	The surface areaof 60-70 km^2, the max depth is 43 m, and the min depth is 13 m.	Coastal lake	The coastal freshwater lake in a rural setting was part of the Isimangaliso Wetland Park, area is largely dominated by plantations *i.e.,* Eucalyptus sp.	*Ceratophyllum* sp. *Cyperus* sp.	
Lake Tevrede Se Pan	The surface areaof 1.8 km^2, with a max depth of 3m.	Fresh inland lake	Inland freshwater lake, the land is used for agriculture and farming, intensive irrigation, and livestock.	*Lagarosiphon muscoides* *Phragmites* sp.	
Lake Banagher fresh	The surface areaof 1.6 km^2, with a max depth of 3m.	Fresh inland lake	Inland freshwater lake, the land is used for agriculture and farming, intensive irrigation, and livestock.	-	-
Lake Banagher Salt	The surface areaof 1.5 km^2, with a max depth of 3m.	Salt inland lake	Inland freshwater lake, the land is used for agriculture and farming, intensive irrigation, and livestock. The Lake is also dominated by Flamingos.	-	-
Lake Chrissiesmeer	The surface areaof 9 km^2, with a max depth of 6m.	Salt inland lake	Inland freshwater lake, the land is used for agriculture and farming, recreation, and livestock.	-	-

2.2.2 Data collection

An integrated water sample (1 000 ml) was collected from the water surface to 3 meters depth using a Horizontal Niskin water sampler (1.7 L volume) at each lake, at four demarcated water column sites (>5 meters depth) during the winter (August 2020) and summer (January2021) seasons. Four sub-samples were extracted; (1) 500 ml for measuring physico-chemical variables; (2) 500 ml water for nutrients analysis; (3) 500 ml for determining Chlorophyll-*a* concentration and (4) 350 ml for phytoplankton community analysis. Detail of the sampling procedure and sample preparationare provided below.

Physico-chemical variables

The following physico-chemical variables were measured; pH, electrical conductivity (EC; µS/cm), water temperature (ºC), and dissolved oxygen (DO; mg/L) concentration at each sampling occasion using a waterproof, handheld multi-parameter probe Eutech (PCS Tester35 model) and a DO Pen Sper-Scientific (85004) meters respectively. A 500 ml of water samples were taken to measure water nutrients including ammonium [NH_4^+; mg/L] (Range: 0.00 to 10.00 mg/L), nitrate [NO_3^-; mg/L] (Range: 0 to 150 mg/L), and phosphate [PO_4^{3-}; mg/L] (Range: 0.0 to 30.0 mg/L), using the Hanna 83200 Multiparameter Photometer within 24 hours after collection.

Another, 500 ml water sample was transferred into an opaque polyethylene container and immediately stored on ice, to estimate chlorophyll-*a* concentration (Chl-*a*). Prior to Chl-*a* analysis, water samples were homogenized by agitating the containers by hand for 5 seconds, and thereafter, 60 ml of the water sample was filtered through a Millipore nylon netfilter (50 mm diameter, 20 µm mesh size) using a vacuum pump (InstruvacR Rocker 300) at20 kiloPalscas (kPa). The nylon filter was then folded in half and placed into a 20 ml reactiontube with a screw and a total volume of 10 ml 90% acetone solution was added to extract Chl-*a* pigment under -20 °C (freezer) for a minimum of 48 hours in the dark (Mdidimba *et al.,*2021). This allowed acetone to break down the chlorophyll lipid bonds and suspend the liquidin the solution for extraction (Ritchie, 2006). Thereafter, Chl-*a* wavelength reading was determined using 10AU Field and Laboratory fluorometer (Turner Designs), noting the wavelength reading before and after the sample was acidified by adding 2/3 drops of 0.1 M hydrochloric acid. The final Chl-*a* concentration was estimated using the formula adopted from Lorenzen (1967) and Daemen (1986):

$$Chl\text{-}a\ (mg/m^3) = \left(\frac{Acetone\ volume}{Filtered\ sample\ volume}\right) X\ (Reading\ before\ acidification - Reading\ after\ acidification)\ X\ 0.325$$

Phytoplankton community samples

Phytoplankton community sub-samples were transferred into a 350 ml polyethylene container and immediately fixed with 5 ml of lugol's iodine solution. Prior to phytoplankton cell identification and counting, samples were allowed to sediment on a flat bench surface for 72 hours (LeGresley & McDermott, 2010). Thereafter, about 300 ml of the sample's supernatant was discarded using a top-down siphoning system leaving a concentrated phytoplankton sample of 50 ml for community analysis.

Before cell identification and enumeration, the concentrated sample was homogenized by hand for 30 seconds. Thereafter, using a Pasture pipette, 0.1 ml of the sample was drawn out and placed onto the hemocytometer counting chamber (Neubauer improved; 9 mm^2 totalgrid area and a standard depth of 0.1 mm) (LeGresley & McDermott, 2010). Thereafter, phytoplankton cell counting, and identification were done using a light phase microscope (Olympus CX21) at 400X magnification using various identification guides including John *et al*. (2002), Taylor *et al*. (2007a). From each phytoplankton sample (n=4) per lake per season,four counting chamber grids (9 mm^2) = 36 mm^2 were rendered sufficient to represent phytoplankton community per sample (LeGresley & McDermott, 2010). The relative phytoplankton abundance cells were calculated using an equation by LeGresley & McDermott (2010):

$$\frac{Cells}{ml} = \frac{cells\ counted\ X\ concentrated\ sample\ volume}{Area\ counted\ X\ 0.1(chamber\ depth)} X\ Total\ sample\ volume$$

2.2.3 Data analysis

All statistical analyses were performed in R studio 4.2.0 (R Development Core Team 2022),and data visualization was completed in R studio and PRIMER version 6.1.16 (Clark & Gorley, 2006).

Physico-chemical variables between lake types and seasons

To test for significant differences in physico-chemical variables between lake types and seasons, water chemistry parameters were first tested for normality and homogeneity of variances in RStudio using the Shapiro-Wilks and Leven's test. All physico-chemical variables were found not to be normally distributed (Shapiro-Wilks's test, $p < 0.05$), nor were the variances homogeneous(Leven's test, $p > 0.05$). Thus, a non-parametric test, in this case, Kruskal-Wallis was employed.

Log-transformed log (x+1) physico-chemical variables data in PRIMER was used to perform Principal Component Analysis (PCA) to visualize physico-chemical variables similarities between lake types and seasons. Furthermore, a permutational multivariate analysis of variance (PERMANOVA) test was performed to test for significant differences in physico-chemical variable patterns as observed from the PCA output. This analysis was performed in PRIMER version 6.1.16 and PERMANOVA+ version 1.0.6 (PRIMER-E Ltd, Plymouth; Clark & Gorley, 2006).

Phytoplankton diversity and species composition

To estimate phytoplankton biological diversity between lake types and seasons, relative phytoplankton abundance (N), taxa richness (S), Shannon's diversity index (H), H'= -$\Sigma^s i$ = 1 p_i ln P_i (where *pi* is the proportional abundance of taxa *i* in the sample given *s* taxa), and Pielou's evenness index: $J' = \frac{H'}{In(s)}$ Indices were computed in PRIMER 6 version 6.1.16 and PERMANOVA+ version 1.0.6 using the DIVERSE function (PRIMER-E Ltd, Plymouth; Clark & Gorley, 2006). Then to test for a significant difference in phytoplankton biodiversity indices, the Shapiro-Wilk test and Levene's test were again employed to test for normality and homogeneity of variances in RStudio. The data was not normally distributed (Shapiro-Wilk, $p < 0.05$)and the variances were not homogenous (Levene test, $p > 0.05$). Kruskal-Wallis test was again used to test for significant differences in phytoplankton biodiversity indices with lake types and seasons as a factor. Furthermore, to compare phytoplankton percentage composition between lake types and seasons, phytoplankton species abundances were categorized into Phyla (Appendix 1), including Cyanophyta, Bacillariophyta, Euglenophyta, and Chlorophyta following John *et al*. (2002) and Taylor *et al*. (2007a). Thereafter, community composition percentages between lake types and seasons were computed.

Canonical Analysis of Principal Coordinates (CAP) was conducted to visualize phytoplankton species assemblage patterns between lake types and seasons. Thereafter, to support the CAP results, PERMANOVA was used to test for significant differences in overall phytoplankton species composition between lake types and seasons.

Relationship between phytoplankton biodiversity indices and physico-chemical variables

Multiple linear regression analysis was used to investigate which physico-chemical variables influenced phytoplankton biodiversity patterns. Physico-chemical variables included pH, DO, water temperature, EC, NH_4^+, PO_4^{3-}, NO_3^-, and Chl-*a* were used as predictor variables, and phytoplankton relative abundance, species richness, Pielous evenness, and Shannon diversity were used as response variables.

2.3 RESULTS

2.3.1 Physico-chemical variables

Water temperature was expectedly high in CL (26.2°C ±4), while SIL recorded low temperatures (16.3°C ±2.4) which was within the Target Water Quality Range (TWQR) by DWAF (1996) (Table 2.2). The temperature for all the lake types was significantly higher in CL during summer (28°C) and significantly lower in SIL during winter (14°C) ($p < 0.05$) (Figure 2.2). A non-parametric Kruskal-Wallis test was conducted between lake types and seasons returned significant results for temperature between lake types ($p < 0.05$) and were also significantly different between seasons ($p < 0.05$) (Table 2.2).The Dunn post hoc test identified CL as being significant to the other lake types ($p < 0.001$; see Appendix 1).

Dissolved oxygen (DO) was on average high in CL (7.2 mg/L ±2.2) and FIL (6.3 mg/) but at values below TWQR by DWAF (1996) (Table 2.2). Kruskal-Wallis, a non-parametric test conducted between lake types and seasons, showed that between seasons DO was significantly different ($p < 0.05$), but was not significant between lake types ($p > 0.05$) (Table 2.2).

The pH was exceptionally high in SIL (10.3 ±0.8) and FIL (9.3) but was low in CL (8.6) (Figure 2.2),and on average higher than the upper limit of TWQR by DWAF (1996). According to the non-parametric test done by Kruskal-Wallis, pH was significantly different for both seasons ($p < 0.05$) and lake type ($p < 0.05$) (Table 2.2 and Figure 2.2). The *post hoc* Dunn test revealed that the significant difference was between CL and FIL, and between CL and SIL ($p < 0.001$) but was not significantly different between FIL and SIL (see Appendix 1).

Electrical Conductivity (EC) was high in FIL and SIL (Table 2.2 and Figure 2.2), and on average (5.3 µS/cm ±3.9) was higher in SIL (Table 2.2). A non-parametric test, Kruskal-Wallis showed that for EC there was a significant difference between lake type ($p < 0.05$) but not between seasons ($p > 0.05$) (Table 2.2). The *post hoc* Dunn test was conducted, and it revealed that in lake types the significant difference was between CL and SIL ($p < 0.001$; seeAppendix 1).

Nitrate (NO_3^-) was expectedly high in FIL and SI, the highest concentration was recorded in SIL, with an average of (17.9 mg/L ±33.6) (Table 2.2). Kruskal-Wallis test was conducted, and it showed that NO_3^- was only significant between lake types ($p < 0.05$; see Table 2.2). The *post* *hoc* Dunn test showed that the significant difference was between CL and FIL ($p < 0.001$) and between CL and SIL ($p < 0.001$) (Figure 2.2 and Appendix 1).

Ammonium (NH_4^+) was high in CL with a concentration of (10.8 mg/L ±32.8) and was low in FIL (1.8 mg/L ±1.1) and SIL (1.4 mg/L) (Table 2.2). Ammonium concentration in CL exceeded TWQRby DWAF (1996), and for FIL and SIL was within TWQR. Non-parametric test, Kruskal-Walllisconducted between lake types and seasons showed that NH_4^+ was significantly different between seasons ($p < 0.05$) but was not significantly different between lake types ($p > 0.05$) (Table 2.2).

Phosphate (PO_4^{3-}) concentration was high in CL and FIL (Table 2.2). According to the non-parametric test, Kruskal-Wallis PO_4^{3-} showed no significant difference for both seasons ($p > 0.05$) and lake types ($p > 0.05$) (Table 2.2 and Figure 2.2).

Chlorophyll-a highest concentration was found in SIL, with a concentration of (1.1 mg/m^3 ±3.3) (Table 2.2). Chl-*a* was significantly different only between lake types ($p < 0.05$) butnot between seasons ($p > 0.05$) (Table 2.2 and Figure 2.2). The *post hoc* Dunn test showed thata significant difference between CL and SIL ($p < 0.001$) and between FIL and SIL ($p < 0.001$) (see Appendix 1).

Principal Component Analysis (PCA) results revealed eight water chemistry variables *i.e.,* pH, DO, EC, water temperature, NO_3^-, NH_4^+, PO_4^{3-}, and Chl-*a* that showed a strong correlation (Pearson correlation*:* $r>0.6$) and were important in defining lake types and seasonality in physico-chemical variables patterns (Figure 2.3A). Three distinct clusters were recognized from the PCA and each cluster represented lake type and seasons, where CL (Lake Sibaya and Lake Mzingazi) were clustered together, and this was the same for FIL (Lake Tevrede Se Pan and Lake Banagher fresh) and the SIL (Lake Banagher salt and Lake Chrissiesmeer)during in summer and winter (Figure 2.3A and Figure 2.3B). During summer, SIL showed a strong and positive correlation to NO_3^-, NH_4^+, Chl-*a,* EC, and pH, while FIL showed a strongand positive correlation to DO and PO_4^{3-}, and CL was strongly correlated to water temperature (Figure 2.3A). In winter, Principal Component Analysis (PCA) also showed threedifferent clusters. Coastal Lakes were clustered together, and it was the same for both FIL and SIL. Dissolved Oxygen, water temperature, NH_4^+ and PO_4^{3-} showed a strong correlation to CL. While EC, pH, NO_3^-,and Chl-*a* showed a strong correlation to both FIL and SIL (Figure 2.3B).

Table 2. 2: Physico-chemical variables mean and (± standard deviation) recorded from three lake types (CL, FIL, and SIL) systemsin South Africa. The bold P-values represent significant differences (Kruskal-Wallis ANOVA, *p < 0.05*).

Physico-chemical variables	Coastal Lakes (CL)	Fresh Inland Lakes (FIL)	Salt Inland Lakes (SIL)	Seasons P-Value	Lake type P-Value
Water temperature (°C)	26.2 (±4)	16.8 (±2.1)	16.3 (±2.4)	**<0.05**	**<0.05**
DO (mg/L)	7.2 (±2.2)	6.3 (±3.3)	5.8 (±2.8)	**<0.05**	>0.05
pH	8.6 (±0.3)	9.3 (±0.8)	10.3 (±0.8)	**<0.05**	**<0.05**
EC (µS/cm)	1.1 (±2.1)	3.9 (±6.2)	5.3 (±3.9)	>0.05	**<0.05**
NO_{3^-} (mg/L)	2.3 (±5.5)	9.2 (±8.6)	17.9 (±33.6)	>0.05	**<0.05**
NH_4^+ (mg/L)	10.8 (±32.8)	1.8 (±3.1)	1.4 (±1.1)	**<0.05**	>0.05
PO_4^{3-}(mg/L)	5.8 (±5.7)	5.8 (±3.1)	4.3 (±3.1)	>0.05	>0.05
Chl-*a* (mg/m^3)	0.01 (±0.01)	0.06 (±0.1)	1.1 (±3.3)	>0.05	**<0.05**

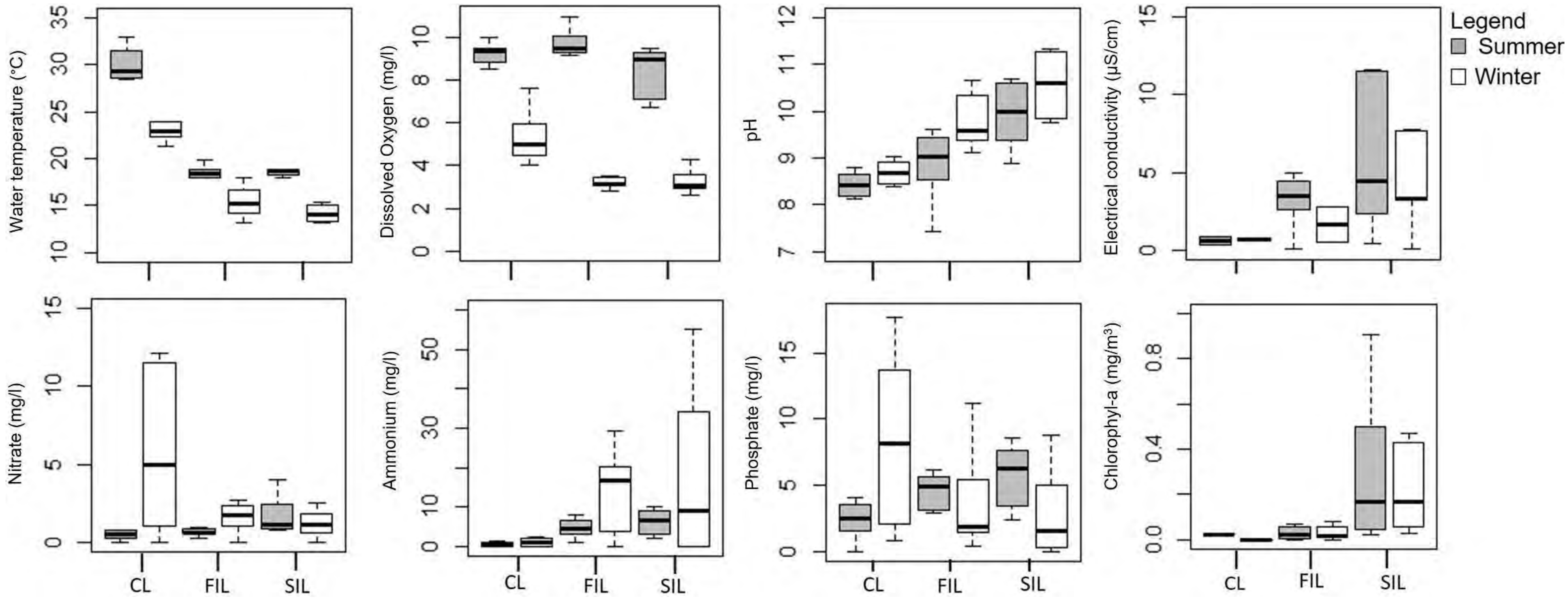

Figure 2.2: Boxplots indicating the concentration of physico-chemical variables measured from Coastal Lakes (CL), Fresh Inland Lakes (FIL), and Salt Inland Lakes (SIL). The thick line across the box, the box, the bottom whisker and top whisker indicate the median value, the interquartile range, minimum and maximum values, respectively.

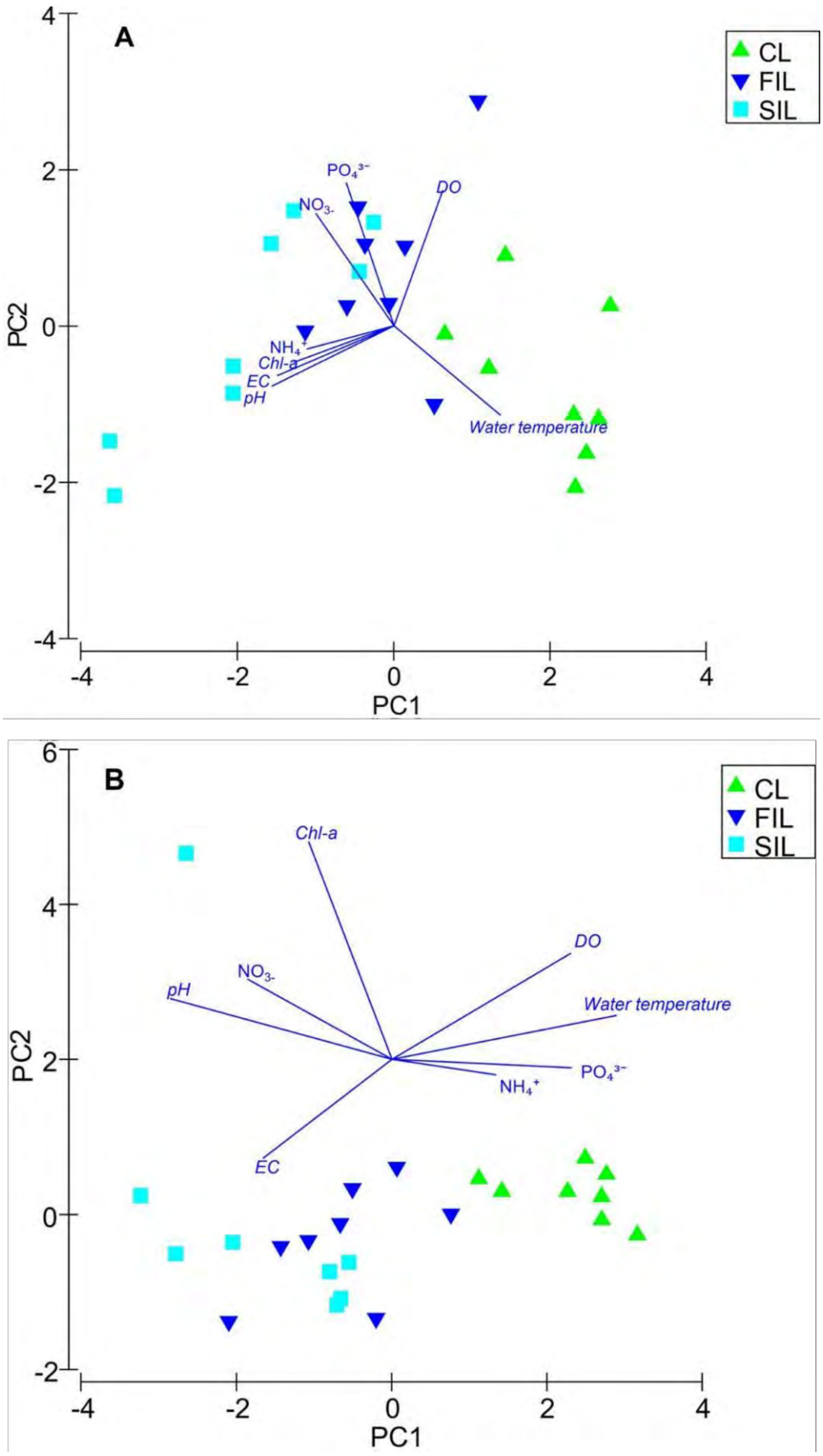

Figure 2.3: Principal Component Analysis (PCA) illustrating physico-chemical variables recorded from the six freshwater lakes during summer (A) and winter (B). Only significant variables with a strong Pearson correlation output of > 0.6 are represented on the plot.

2.3.2 Phytoplankton diversity and species composition

One hundred and twenty-two phytoplankton species were collected and identified during this study, belonging to seven Phyla which included Chlorophyta, Bacillariophyta, Cyanophyta, Chrysophyta, Dinophyta, Euglenophyta, and Cryptophyta groups respectively (see Appendix3 for full species list and abundance data). The most abundant species were *Microctinium* sp*, Spirulina mairor, Cyclotella meneghiniana, and Cocconeis placentula*, which belong to Chlorophyta and Bacillariophyta, whereas *Cytomonas* sp. *and Peridium enhrenberg w*ere the least abundant species. *Navicula* genus was the most represented with eight species followed by *Fragilaria* genus with five species (See Appendix 3).

Phytoplankton relative taxa abundance was not a significant difference ($p > 0.05$) between lake types for both seasons (Figure 2.4A). Taxa richness was also not significantly different in lake types in summer ($p > 0.05$) (Figure 2.4B). However, Pielou's evenness and biodiversity indices showed that CL and FIL were both significantly different from SIL ($p < 0.05$) in both seasons (Figure 2.4C). Shannon diversity index showed a significant difference between lake types for both seasons ($p < 0.05$) (Figure 2.4D). Phytoplankton relative taxa abundance was higher in SIL in summer (32 448 individuals) (Figure 2.4A). Furthermore, SIL recorded the highest taxa richness, whereas, CL was low in both seasons (Figure 2.4B).

In CL phytoplankton functional composition was dominated by Bacillariophyta with 59% in winter, followed by Chlorophyta with 27% of species dominating CL. While FIL was dominated by Chlorophyta at 49% and Bacillariophyta at 43%. Salt Inland Lakes was dominated by Bacillariophyta with 43% in winter (Figure 2.5A; Appendix 4). The least dominated phyla were Chrysophyta, Dinophyta, and Euglenophyta (See Appendix 4). In summer CL and FIL were largely dominated by Bacillariophyta with over 50% of species found dominating in CL and FIL (See Figure 2.5B; Appendix 5). While SIL were dominated by Chlorophyta with 38% of species found in the lakes (Figure 2.5B). Additionally, in summerthe least dominated species were Cyanophyta, Chrysophyta, Dinophyta, Euglenophyta and Cryptophyta with less than 10% of species in all lake types (See Appendix 5).

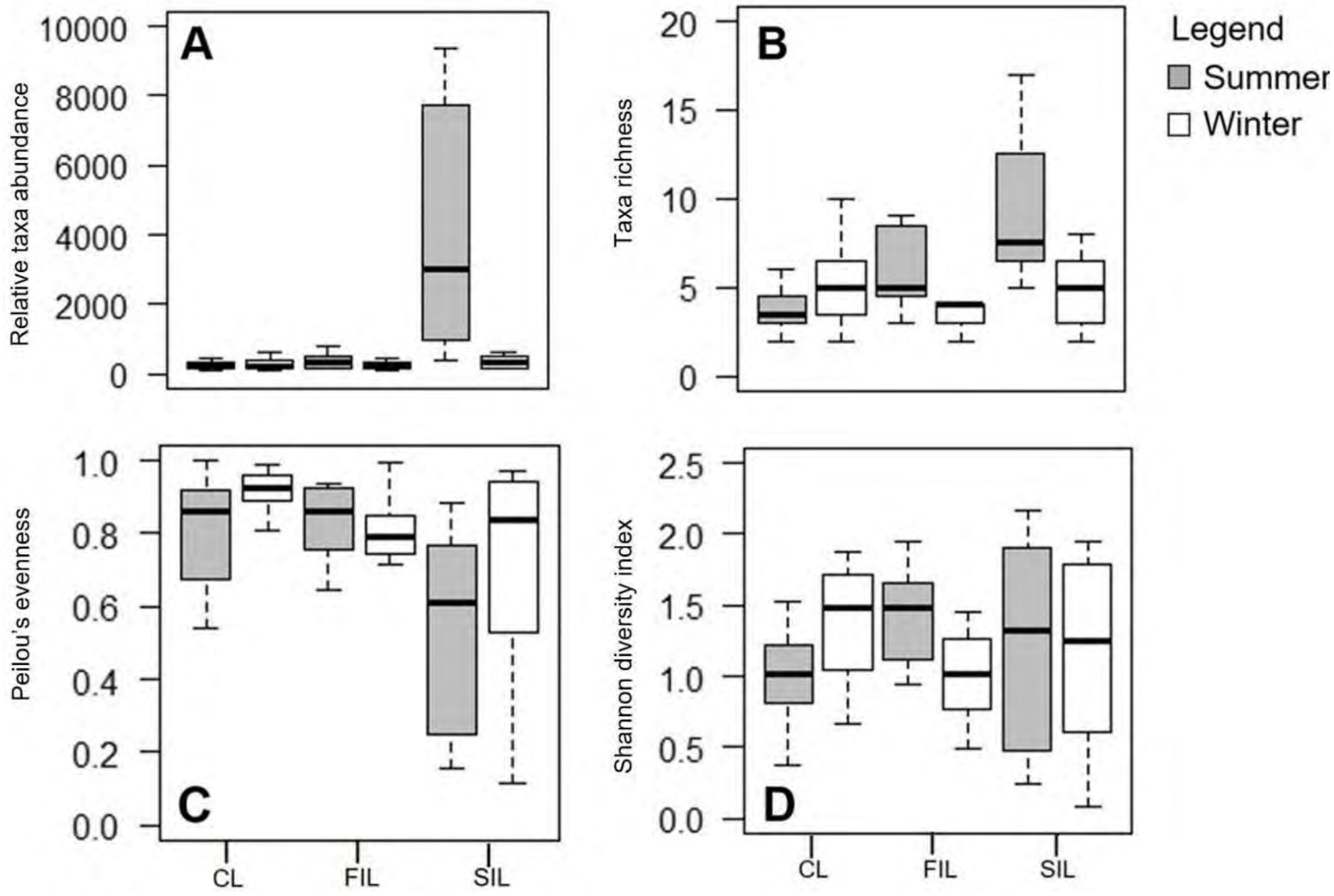

Figure 2.4: Phytoplankton biodiversity indices calculated for Coastal Lakes (CL), Fresh Inland Lakes (FIL) and Salt Inland Lakes (SIL): (A) Relative taxa abundance, (B) Taxa richness, (C) Peilou's evenness, and (D) Shannon's diversity index. The thick line across the box, the box, the bottom whisker and top whisker indicate the median value, the interquartile range, minimum and maximum values, respectively. The relative taxa abundance for summer in SIL was transformed by a factor of x10.

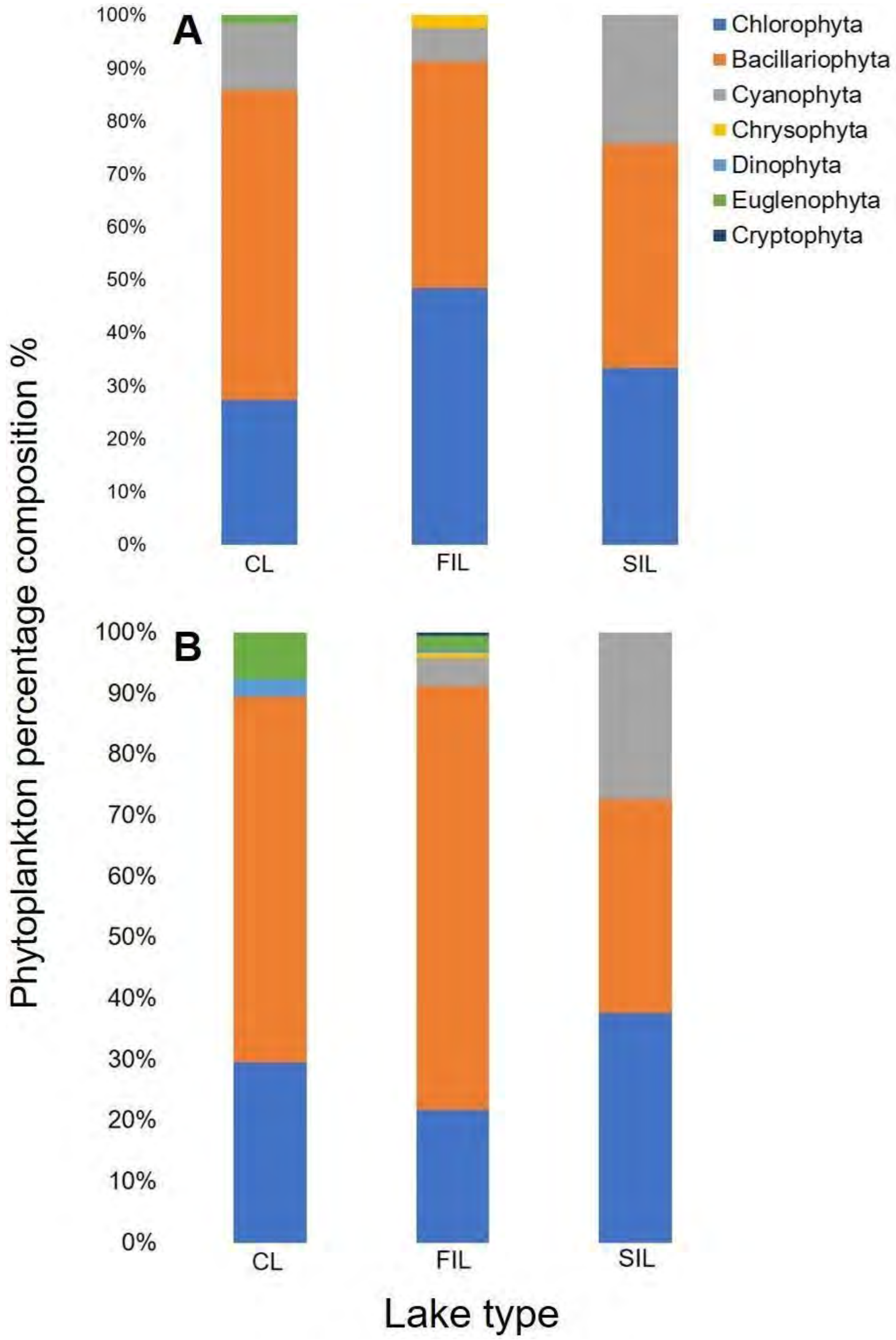

Figure 2.5: The percentage (%) composition of phytoplankton phyla in the six freshwater lakes from KwaZulu-Natal and Mpumalanga Provinces, South Africa. A = summer and B = winter.

Canonical analysis of principal coordinates (CAP) showed a significant difference in phytoplankton species composition between the lake types during summer seasons (Figure 2.6A). However, there was some overlap between FIL and SIL in species composition and *Spirulina maior* was positively and strongly associated with both lake types in summer. Whereas, *Schizothrix Mueller*, *Cyclotella ocellata*, *Lemnicola humgarica*, *Navicula capitatoradiata,* and *Scenedesmus bicadatus* were strongly correlated to SIL (Figure 2.6A). While for winter, CAP analysis showed three distinct clusters and each cluster represented lake type, indicating that phytoplankton species composition was different between lake types(Figure 2.6B). *Spirulina maior, Aphanocapsa grevillea, Surirella ovalis, Navicula veneta* and *Cyclotella meneghianian* were strongly correlated to SIL. While *Frustulia vulgaris, Anbaena* sp., *Epithemia adnate, Scendesmus* sp., and *Cocconeis pediculus* were strongly correlated to FIL. *Fragilaria tenera, Oocystis braun* and *Crucigenia tetrapedia* were strongly correlated to CL (Figure 2.6B). Additionally, CAP for summer showed a strong correlation and respectively indicative that phytoplankton species assemblage was indeed different between all three clusters, CAP1 $\delta=0.99$ and CAP2 $\delta=0.98$ (Figure 2.6A). This was the same with summer, CAP1 $\delta=0.99$ and CAP2 $\delta=0.98$ (Figure 2.6B).

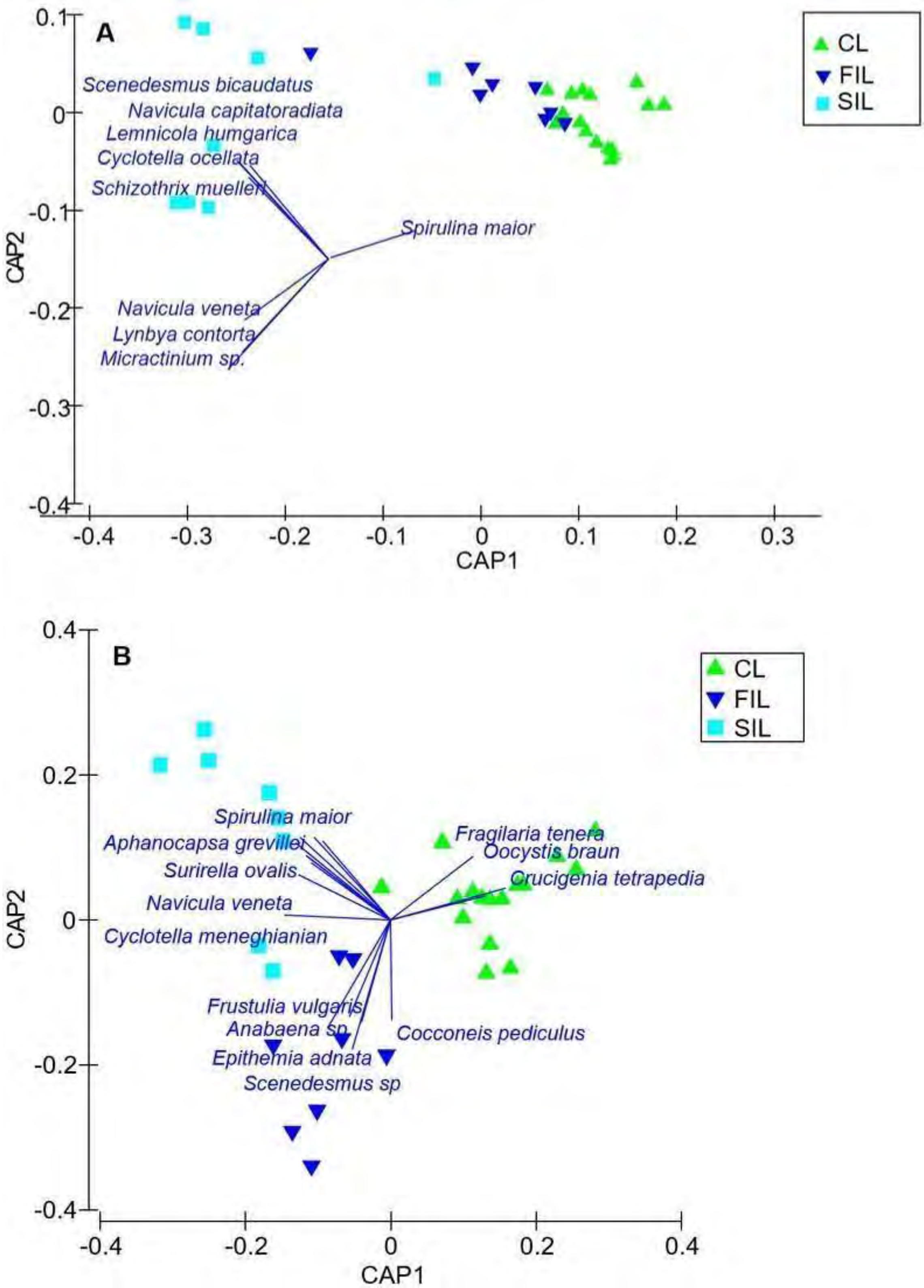

Figure 2.6: Canonical analysis of principal coordinates (CAP) ordination illustrating phytoplankton species composition from three lake types between two seasons, (A) summer and (B) winter. Only significant variables with a strong Pearson correlation output of > 0.6 are represented on the plot.

PERMANOVA revealed that phytoplankton species composition was a significant difference between lakes (PERMANOVA, pseudo-F = 3.91, P= 0.0001), and lakes and seasons (PERMANOVA, pseudo-F =3.91, P= 0.0001), thus in agreement with the CAP visual patterns (Table 2.3).

Table 2.3: PERMANOVA results comparing phytoplankton species composition patterns between lakes and seasons. Significant different P-values are highlighted in bold.

Factors	Degree of freedom	Pseudo-F	P-value
Sites	4	4.92	***P < 0.05***
Seasons	1	4.71	***P < 0.05***
Sites vs Seasons	4	3.91	***P < 0.05***

Multiple linear regression showed that pH, EC, DO and NH_4^+ explained 38% of phytoplankton relative abundance and this was significant. All the variables positively affected relative phytoplankton relative abundance and were all significant (Table 2.4). Phytoplankton species richness was influenced by EC, DO, NH_4^+, and PO_4^{3-} which explained a 15% variation in phytoplankton species richness with DO negatively affecting phytoplankton species richness, whereas EC, NH_4^+, and PO_4^{3-} showing a positive influence, but only PO_4^{3-} was significant (Table 2.4). Phytoplankton species evenness was negatively influenced by only pH and DO explaining 39% variation of species evenness and was both significant (Table 2.4). On the other hand, phytoplankton diversity was only negatively affected by pH which explained 17%variation of phytoplankton diversity and was significant (Table 2.4).

Table 2.4: Multiple Linear regression analysis showing t-statistics and P-value for regression coefficients, indicating if the value of the coefficient is significantly different from zero. Significant differences are in bold. S: taxa richness, N: relative taxa abundance, J': Pielou's evenness, and H': Shannon diversity index.

Factors	Prediction	Estimation	Standard Error	t-statistic	P-Value	Adjusted r^2	Degrees of freedom	F	P-Value
Relative abundance (N)	Intercept	-30717.4	9019.8	-3.41	***p* < 0.05**	0.38	4.43	8.06	***p* < 0.05**
	pH	10974.7	3566.2	3.18	***p* < 0.05**				
	EC	816.7	353.5	2.31	***p* < 0.05**				
	DO	2410.7	718.6	3.36	***p* < 0.05**				
	NH_4^+	501.5	293.0	1.71	***p* < 0.05**				
Species richness (S)	Intercept	0.37	2.42	0.15	*p > 0.05*	0.15	4.43	3.08	***p* < 0.05**
	EC	0.76	0.53	1.45	*p > 0.05*				
	DO	-0.73	0.53	-1.37	*p > 0.05*				
	NH_4^+	1.76	1.13	1.56	*p > 0.05*				
	PO_4^{3-}	1.17	0.63	1.86	***p* < 0.05**				
Pielou's evenness (J')	Intercept	5.34	0.82	6.54	***p* < 0.05**	0.39	2.45	16.02	*p > 0.05*
	pH	-1.79	0.32	-5.66	***p* < 0.05**				
	DO	-0.19	0.07	-2.67	***p* < 0.05**				
Shannon diversity (H')	Intercept	6.97	1.76	3.95	***p* < 0.05**	0.17	1.46	10.46	***p* < 0.01**
	pH	-2.44	0.75	-3.23	***p* < 0.05**				

2.4 DISCUSSION

The present study hypothesized that phytoplankton species composition will be different between lake types and seasons and that the geographical location and physico-chemical variable will be responsible for the difference. Results from the study agree with the hypothesis that lake types were different in terms of phytoplankton species composition, anddifferent physico-chemical variables influenced phytoplankton species composition distinction.

Phytoplankton species composition was structured by physico-chemical variables. It isgenerally recognized that changes in the physico-chemical characteristics have an influence on phytoplankton communities (Fathi *et al*., 2001; Liu & Shen, 2010). The physico-chemical variables in all the study lakes varied seasonally during the study period. As a result, Principal Component Analysis (PCA) showed three different clusters between lake types *i.e.,* CL, FIL, and SIL, and seasons, which demonstrated that each lake type was influenced by different physico-chemical variables.

As discussed before, phytoplankton structure may also be influenced by different drivers. Therefore, studies have reported that macrophyte communities influence phytoplankton structure (Pellegrini & Ferragut, 2018). In the present study, it was observed that all lake types had very low Chl-*a*, with the highest Chl-*a* concentration found in SIL (1.1 mg/m^3). The study by Dalu *et al*. (2014), on the Kowie Estuary mouth, found that high salinity levels might result in low Chl-*a* concentrations. However, in the current study low Chl-*a* concentrations were observed in high salinity lakes. According to Adams *et al*. (1999), freshwater systems that had more salt than others result in limited nutrient loading which in turn lower Chl-*a* concentration. The current study contradicts both studies by Dalu *et al*. (2014) and Adams *etal.* (1999). Furthermore, in the present study, FIL in which these lakes had both submerged and emerged vegetated showed relatively low phytoplankton species abundance, and this isbecause submerged aquatic plants compete with phytoplankton, hence low diversity.

Allanson (1979) also reported that there was no significant variation in the nutrient concentrations through the water column in the Lake Sibaya system. Increased nutrient concentrations have, however, been recorded in the lake over recent years, with Humphries& Benitez-Nelson (2013) reporting increased nutrient concentrations, especially in the western arm of the lake, and the DWS (2015) also reported increased nutrients in the sediment of the western arm. The present study also recorded an increase in nutrients in thelake. The increase in nutrient concentration is caused by an increase in human settlements and plantations around the lake. These factors are all contributing to increased nutrients in Lake Sibaya (Humphries & Benitez-Nelson, 2013; DWS, 2015).

Long-term research reported that light is also a crucial factor that affects the growth of phytoplankton in aquatic systems, however for our study this was not observed because wedid not measure water clarity as that would have explained low phytoplankton in coastal lakes. The present study showed that all lakes were significantly different from each other interms of phytoplankton species composition. The phytoplankton community was mainly composed of Bacillariophyta, Chlorophyta, Cyanophyta, and Cryptophyta in all sampled seasons. Peng *et al*. (2021) study on lakes in China, found that Bacillariophyta, Chlorophyta, Cyanophyta, and Cryptophyta dominated phytoplankton. In the present study, Bacillariophyta had a higher number of species compared to other groups. This was in accordance with the studies by Onyema *et al*. (2003), Onyema (2007), Nkwaji (2010), Fonge *et al.* (2013), were they observed that Bacillariophyta was the most common group, especially in summer. In addition, different taxa of phytoplankton respond differently to light conditions due to their different growth requirements. For example, phytoplankton such as Bacillariophyta usually grow under low light, this is because low light favours the growth of small cells (Sunda & Huntsman, 1997).

In the present study, phytoplankton relative abundance in all lakes was found to be maximum during the summer season as compared to winter, with about 39 833 individuals in summer and 12 856 individuals in winter. This was similar to the study by Hulya & Kaliwal (2009), where they were investigating phytoplankton dynamics in relation to physico-chemical factorsof Almatti reservoir of Bijapur District, Karnata State in India. In the study, it was observed that there was a high population of phytoplankton during the summer seasons as there was in winter. This was similar to the findings of Saify *et al*. (1986) investigating the biodiversity of wetlands with special reference to the physico-chemical factors. In their study, they also observed that phytoplankton was more dominant during summer than in winter. Even thoughthis is a wetland example, similar trends are observed in freshwater lakes, for example, in a study by Chaturvedi *et al.* (1999), which investigated the plankton community of polluted waters around Sanganer, Jaipur. In their study it was reported that summer is the more suitableseason for phytoplankton growth in freshwater systems than winter, this is because in summer there is a long duration of sunshine together with salinity increase, pH, and trophotropic activities. Literature shows that the blooming of phytoplankton in summer is mainly caused by high temperatures and light (Verma *et al*., 2001; Uz, 2007),and the present study showed the high temperature in summer ranging from (15 °C to 27 °C) which supports high phytoplankton development. In the present study relative taxa abundance for phytoplankton was transformed by a factor of x10 in summer for SIL, this was because there was a large number of *Micractinium* sp. (more than 5000 individuals) as compared to any identified species. In addition, the species was only observed to be present only in one SIL *i.e.*, Lake Banagher Salt. According to Venter *et al*. (2013), *Micractinium* spp. are the best-known eutrophic indicator species in standing waters and lakes.

Lakes that have high salinity are observed to have restricted fauna and flora composition (Talling & Talling, 1965; Hecky, 1971). This implies that the species richness of both plants and animals decreases with an increase in salinity (Oberholster *et al*., 2009a). The study donein Lake Tswaing highlighted that the absence of submerged or emerged aquatic macrophytesreduced the diversity of phytoplankton in the lake. This was also observed in the present study, in which in SIL there were no aquatic macrophytes observed, this then influenced the diversity of phytoplankton by reducing phytoplankton diversity in the lakes. According to Taylor *et al*. (2007a), phytoplankton species like *Cyclotella ocellata* occurs in mesotrophic to eutrophic waters with an elevated pH (optimum pH 8.4), this contradicts the findings by Fonge*et a*l. (2013) who observed that *Cyclotella* species mostly grow better in neutral to slightly alkaline environments. This was in accordance with results by Wetzel (1983) and Naz & Tukman (2004), who also reported that the abundance of *Cyclotella* species is related to oligotrophic waters. The present study agreed with both studies by Wetzel (1983) and Naz &Tukman (2004), that *Cyclotella* species were found dominating SIL with pH = 9.57 in Lake Chrissiesmeer and pH = 10.94 in Lake Banagher salt. In SIL, there was a decrease in the number of phytoplankton species as compared to CL and FIL, and according to Fonge *et al*. (2013), Parthasarathi *et al*. (2011) and Roubeix & Lancelot (2008), this was because most phytoplankton species do not tolerate high salinity.

According to Hadiyanto *et al*. (2021), the presence of salinity in a lake decreases the abundance of most phytoplankton species in the system *i.e., Spirulina sp*. However, this wasnot the case with our results because there was a high abundance of phytoplankton speciesparticularly *Spirulina sp*. in SIL *i.e.,* Lake Banagher salt. The results showed that there was low diversity of phytoplankton in Lake Sibaya in both winter and summer. The abundance of*Micractinium sp.* in Lake Banagher salt was remarkably high in summer as compared to its abundance in winter.

According to Venter *et al*. (2013) and Fonge *et al*. (2013), *Micractiniumsp.* is found in standing waters and is particularly common in eutrophic waters. This clearly explains why it was found to dominate Lake Banagher salt, this could be because Lake Banagher salt showed high levels of nutrient concentration (NO^- = 28.09 mg/L).

According to a study by Nankabirwa *et al*. (2019) seasonal sampling was used to assess the magnitude of local seasonal variation in phytoplankton composition and species abundancein freshwater systems. This was also the case with this study, where winter and summer sampling were used to track the phytoplankton species composition between lakes. As indicated before, temperature is one of the primary factors that determine the seasonal dynamics of species composition in phytoplankton (Falkowski & Raven, 1997). The study demonstrated that phytoplankton diversity was different across lake types and that SIL weresimilar in terms of phytoplankton assemblage composition. Salinity gradient is the main factor that drives phytoplankton diversity. Furthermore, physico-chemical variables attributed by the lake geographical position either coastal or inland influenced phytoplankton community structure. In addition, the study also demonstrated that anthropogenic activities *i.e.,* eutrophication negatively affect freshwater systems and that was reflected on the water quality, biodiversity and distribution of phytoplankton species.

CHAPTER 3: LITTORAL AQUATIC MACROINVERTEBRATE COMMUNITIES FROM COASTAL AND INLAND SOUTH AFRICAN LAKES

3.1 INTRODUCTION

Aquatic macroinvertebrates are regarded as one of the most sensitive aquatic organisms and they are widely used as biological indicators for assessing aquatic ecosystem health and integrity (Rosenberg, 1992; Ollis *et al*., 2006; Dalu *et al*., 2017; Van den Berg *et al.,* 2019). Aquatic ecosystem integrity can be negatively impacted by land-use developments, including agricultural activities, urban developments, and mining, which later affect water quality, quantity, and aquatic biodiversity (Nhiwatiwa *et al*., 2017). As a result, aquatic macroinvertebrates are involved in biogeochemical processes such as nitrogen and phosphorus biogeochemistry, carbon biosynthesis, and decomposition in aquatic environments (Dalu *et al*., 2021). Therefore, aquatic macroinvertebrate community dynamicscan be utilized for biomonitoring to demonstrate how both natural and human-caused environmental change and pollution affect aquatic ecosystem structure and functioning (Dalu*et al*., 2021).

Aquatic macroinvertebrates play an important role in the ecosystem by being consumers at the intermediate trophic level. They serve as channels by which bottom-up and top-down forces are transmitted (Wallace *et al*., 1999). Because of this, the classification of functional feeding groups helps in understanding our knowledge of trophic dynamics in aquatic systems(Gholizadeh & Heydarzadeh, 2020), and the balance of feeding strategies in aquatic macroinvertebrate communities (Gholizadeh & Heydarzadeh, 2020). In addition, feeding strategies are typical traits that reflect the adaptation of species to environmental conditions (Statzner *et al*., 2004).

In the last three decades, numerous bio-assessment techniques have been developed, and the South African Scoring System version 5 (SASS5), is the mostly used in South Africa (Mngandze *et al*., 2019). SASS5 was designed to measure riverine and stream water quality(Chutter, 1998; Dickens & Graham, 2002). For example, the presence of sensitive aquatic macroinvertebrates families *i.e.,* Plecoptera (stoneflies), Ephemeroptera (mayflies), andTrichoptera (caddisflies) is indicative of largely natural freshwater systems, whereas the proliferation of Oligochaeta (earthworms) and Chironomidae (midges), taxa generally known as severely tolerant to external disturbances (increase nutrients and reduced DO concentration), are indicative of a modified aquatic system (Bonada *et al*., 2005; Davies *et al*., 2010; Ratia *et al*., 2012; Olomukoro & Dirisu, 2014; Agboola *et al*., 2019). Biomonitoring using aquatic macroinvertebrate assemblages in lakes is limited and less developed compared to that of river systems (White & Irvine, 2003; Poikane *et al.*, 2016). However, in standing water bodies, like wetlands and lakes, the use of aquatic macroinvertebrates for biomonitoring has not been as successful (Burton *et al*., 1999; Awal & Svozil 2010; Gleason& Rooney 2017), including in South Africa (Bowd *et al*., 2006; Bird *et al*., 2013). As a result, Bird & Day (2010) and Bird *et al*. (2013) suggested that in South Africa there should be morestudies done on aquatic macroinvertebrates specifically in lakes, wetlands and pans.

The use of biological indicators is known to supplement conventional chemical and physical monitoring and improves the assessment of the ecological integrity of freshwater resources (Hussain, 2012). The distribution of aquatic macroinvertebrates in freshwater systems, suchas lakes, depends on biotype (Bird *et al*., 2014; Gleason *et al*., 2019), water quality (Hynes, 1970; Bass, 1995; Castella *et al*., 2001; Li *et al*., 2001; Sullivan *et al*., 2004; Miserendino & Masi, 2010; Clews *et al.,* 2014), landscape factors (Dutta & Malhotra, 1986; Engblom & Lingdell, 1999; Ometo *et al*., 2000; Miserendino & Pizzolon, 2003; Waite *et al*., 2004; Sporka *et al*., 2006; Joshi *et al*., 2007), vegetation and macrophytes (Declerck *et al.,* 2005; Subramanian et al., 2005; Schröder *et al.,* 2013). All these abiotic and biotic factors have been reported to influence aquatic macroinvertebrate community composition.

In South Africa research done on aquatic macroinvertebrates from natural permanent water bodies is limited. These studies include a few studies completed on the endorheic wetlands that occur in Mpumalanga province (Ferreira *et al*., 2009; De Klerk & Wepener, 2011; Victor, 3013; Foster *et al*., 2015; Burger *et al*., 2019). The earliest study done in the Mpumalanga province is that by Hutchinson *et al*. (1932) on Lake Chrissiesmeer and other depressions. The study revealed that the most common species in the area were *Lovenula excellens, Metadiaptomus transvaalensis* and *Daphnia gibba*. Furthermore, these species are increasingly being used as indicator species for water qualityin water bodies (Foster *et al*., 2015). In addition, more recent studies by Ferreira (2010) and Ferreira *et al*. (2012) also reported many aquatic macroinvertebrates in Mpumalanga pans. These included common crustaceans such as *Lovenula falcifera, Metadiaptomus* sp., *Daphnia carinata,* and various hemipterans and dytiscid predators. In the North West province, the study of aquaticmacroinvertebrates is even limited. These include a study by Hutchison *et al*. (1932), where they found that Barberspan a wetland in North West had similar physico-chemical variables as Mpumalanga systems, but there were differences in terms of aquatic macroinvertebrates that dominated the wetland. These species included, *Mesocyclops schuurmanae, Monia dubia, Ceriodaphnia riguadi* and *Daphnia barbata*. Therefore, maintaining and protecting unique and endemic aquatic macroinvertebrate biodiversity from different systems and studying how they respond to changes in the environment is critical forfreshwater conservation and management (Declerck *et al.,* 2005).

This chapter aims to provide baseline information on aquatic macroinvertebrate community structure for coastal and inland South African freshwater lakes which is currently lacking, by assessing whether different lake types support different aquatic macroinvertebrate species composition relative to their physical and chemical characteristics. It is expected that given the habitat structure and related physico-chemical variables, Fresh Inland Lakes (FIL) will have more diverse aquatic macroinvertebrates and different species composition as compared to Coastal Lakes (CL) and Salt Inland Lakes (SIL).

3.2 MATERIALS AND METHODS

3.2.1 Study sites

Details on the study sites, descriptions, and study design are described in Chapter 2 (page 16).

3.2.2 Aquatic macroinvertebrates data collection

Aquatic macroinvertebrate sampling was conducted following the procedure by Bird *et al* (2014). Where aquatic macroinvertebrates were collected using a 1-meter-long SASS net with a square frame (30 cm x 30 cm) and 1000 µm mesh size. Aquatic macroinvertebrates were dislodged by vigorously disturbing and sweeping submerged and emergent aquatic vegetation at four littoral zone sites (≤1-meter water depth) at each lake and season. A total of 27 sweeps (1 metre long) were done per littoral site to make up one sample of four sites per lake (27 sweeps x 4 sites per lake = 108 sweeps per lake), and this sampling effort was considered sufficient to represent aquatic macroinvertebrates at each site (Gabriels *et al*., 2010). Aquatic macroinvertebrates samples were transferred into 750 ml polyethylene containers and immediately preserved with a 70% ethanol solution. In the laboratory, samples were identifiedto the lowest possible taxon (either to genus or species level) using “Guides to Freshwater Aquatic invertebrates of Southern Africa" by Day *et al*. (1999), Day & de Moor (2002a, b) and de Moor *et al*. (2003a, b), Gerber & Gabriel (2002).

3.2.3 Data analysis

All data analyses were performed in R studio 4.2.0 (R Development Core Team 2022), and data visualization was completed in R studio and PRIMER version 6.1.16 (Clarke & Gorley, 2006).

To estimate aquatic macroinvertebrate biological diversity between lakes and seasons, relative macroinvertebrate abundance (N), taxa richness (S), Shannon’s diversity (H), H’= - $\Sigma^{s} i$ = 1 p_i ln P_i (where *pi* is the proportional abundance of taxa *i* in the sample given s taxa), and Pielou’s evenness index: $J' = \frac{H'}{In(s)}$ indices, were computed in PRIMER 6 version 6.1.16 and PERMANOVA+ version 1.0.6 using the DIVERSE function (PRIMER-E Ltd, Plymouth; Clarke & Gorley, 2006). Then to test for a significant difference in aquatic macroinvertebrate biodiversity indices, the Shapiro-Wilk test and Levene’s test were again employed to test for normality and homogeneity of variances in R Studio. The data were notnormally distributed (Shapiro-Wilk, $p < 0.05$) and the variances were not homogenous (Levene test, $p > 0.05$). Thus, the Kruskal-Wallis test was used to test for significant differences in aquatic macroinvertebrates biodiversity indices with lake types and seasons asa factor.

To compare aquatic macroinvertebrates species composition between lakes and seasons, aquatic macroinvertebrates species were categorized into taxa (Appendix 6), including Ephemeroptera, Odonata, Lepidoptera, Hemiptera, Coleoptera, Diptera, Decapoda, Gastropoda, Venerida, and Dermaptera. Additionally, to investigate aquatic macroinvertebrate functional diversity, aquatic macroinvertebrate taxa were grouped according to their functional feeding groups (FFGs): collector-filters, collector-gatherers, scrapers/herbivores, shredders, and predators, following Palmer *et al*. (1996), Tomanova *et al*. (2006), Zilli *et al*. (2008), Uwadiae, (2010), Masese *et al*. (2014), Ramírez & Gutiérrez- Fonseca, (2014), and Cummins, (2016).

Canonical Analysis of Principal Coordinates (CAP) was conducted to visualize aquatic macroinvertebrates species assemblage patterns between lake types *i.e.*, CL, FIL, and SIL, and seasons. Thereafter, to support the CAP results, PERMANOVA was used to test for significant differences in overall aquatic macroinvertebrates species composition between lake types and seasons. All the above analysis was performed using PRIMER version 6.1.16 and PERMANOVA+ version 1.0.6 (PRIMER-E Ltd, Plymouth; Clark & Gorley, 2005).

Multiple linear regression analysis was used to investigate which physico-chemical variables influenced aquatic macroinvertebrate biodiversity indices. Physico-chemical variables included pH, Dissolved Oxygen (DO), water temperature, Electrical conductivity (EC), ammonium (NH_4^+), phosphate (PO_4^{3-}), nitrate (NO_3^-), and Chlorophyll-*a* (Chl-*a*), which were predictor variables, and phytoplankton relative abundance, species richness, Shannon's diversity, and Pielous evenness were response variables. All statistical analyses wereconducted in R version 3.6.1 (R Core Team, 2019).

3.3 RESULTS

3.3.1 Aquatic macroinvertebrate diversity and species composition

A total of 87 aquatic macroinvertebrates species from 10 genus and 35 families were collected and identified during this study (Appendix 5). The most abundant group of aquatic macroinvertebrates were Coleoptera, Hemiptera, Odonata, and Gastropoda. Seven genera were recorded from the family Dytiscidae *i.e., Cybister* sp.*, Philodytes* sp.*, Hydatics* sp.*, Copelatus* sp.*, Darwinhychus* sp.*, Hydroglyphus* sp. *and Neptosternus* sp. The most commonly occurring family was Corixidae (Hemiptera) except in CL *i.e.,* Lake Mzingazi and Sibaya, and the family Baetidae was represented by the genus *Cloeon*, and the majority werefrom Lake Tevrede Se Pan and Lake Mzingazi. Physidae were found in all lakes except the SIL *i.e.,* Lake Chrissiesmeer and Banagher salt during all seasons, whereas the invasive Thiaridae (*Tarebia granifera*) was only recorded in CL (Appendix 5).

Aquatic macroinvertebrates relative taxa abundance was significantly different between all lake types ($p < 0.05$) for both seasons (Figure 3.1A). Taxa richness was not significantly different between CL and SIL for both seasons ($p > 0.05$) (Figure 3.1B). However, Pielou's evenness showed a significant difference between all lake types ($p < 0.05$) (Figure 3.1C). Whereas for Shannon diversity index showed that there was a significant difference between FIL and both CL and SIL in both seasons ($p > 0.05$) (Figure 3.1D). Aquatic macroinvertebraterelative taxa abundance was higher at FIL (5769 individuals), and lowest at SIL (1048 individuals) (Figure 3.1A). Similarly, taxa richness was high at FIL, and least at CL and SIL (Figure 3.3B). Peliou's evenness was higher in SIL, and low at CL, (Figure 3.1C). Shannon diversity index was high in FIL and low in CL (Figure 3.1D).

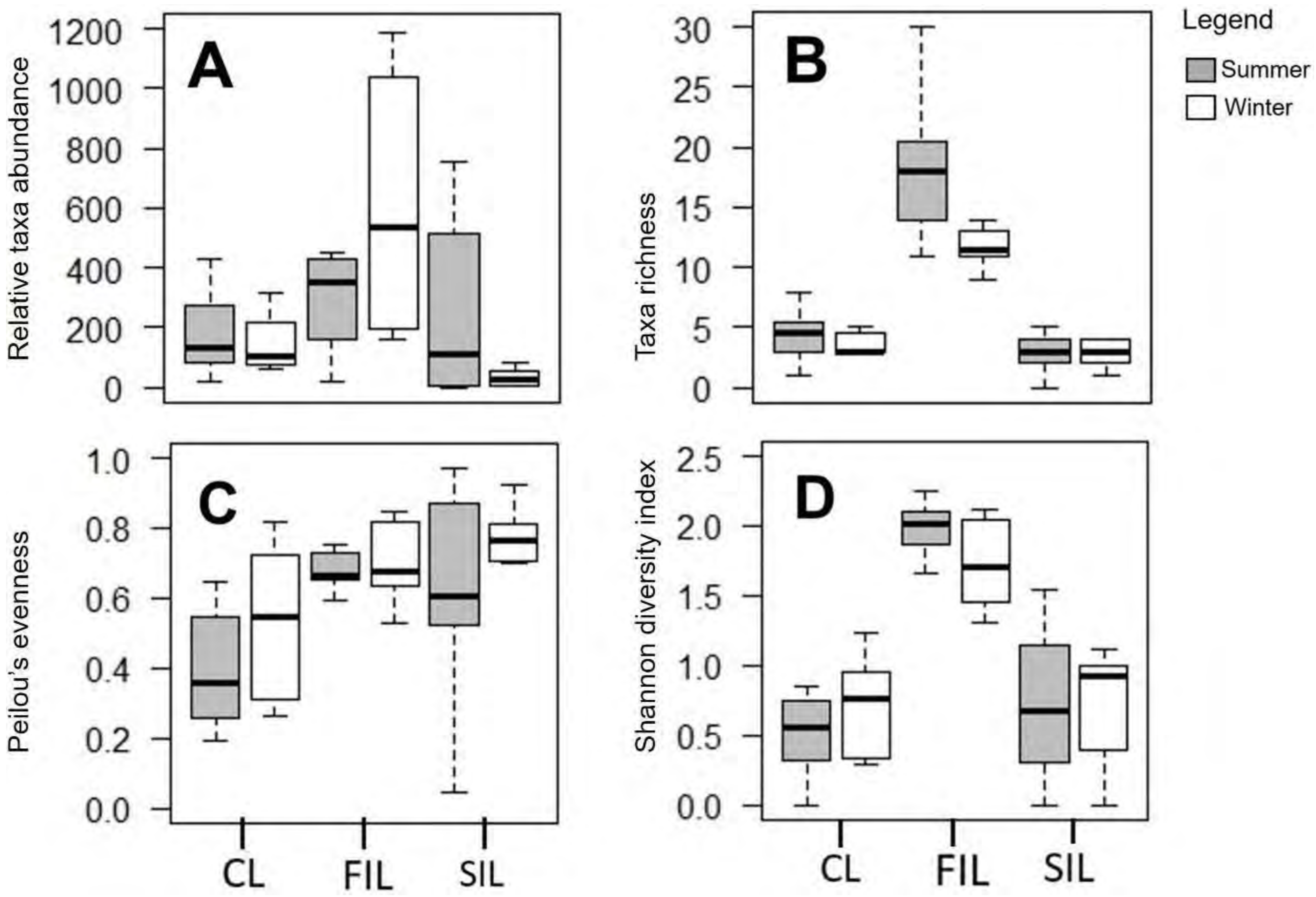

Figure 3.1: Aquatic macroinvertebrates biodiversity indices for Coastal Lakes (CL), Fresh Inland Lakes (FIL) and Salt Inland Lakes (SIL): (A) Relative taxa abundance, (B) Taxa richness, (C) Peilou's evenness, and (D) Shannon's diversity index from three lake types. The thick line across the box, the box, the bottom whisker and top whisker indicate the median value, the interquartile range, minimum and maximum values, respectively.

In CL aquatic macroinvertebrates were dominated by collector filters in winter with over 70% of species dominating the lakes, followed by grazers with 22% (See Figure 3.2A; Appendix 6). While FIL was dominated by predators 34%, collector gatherers with 30% and grazers 24%. The least dominating phyla were collector filters and shredders with a percentage of less than 20 (See Appendix 6). In addition, SIL was dominated by both predators and collectorgatherers, with predators with over 60% of species and collector gatherers with 32%. In summer CL was largely dominated by collector filters with over 90% of species dominating the lakes, followed by predators at 3% (See Figure 3.2B; Appendix 7). While FIL was mostly dominated by predators with 78% species, followed by collector filters at 9% and collector gatherers at 7%, and the least dominating phyla were grazers and shredders at less than 7%species dominating the lakes (See Appendix 7). In SIL predators dominated the lakes with 56% followed by collector gatherers at 41%. Collector filters, grazers, and shredders were the least dominant phyla with a percentage of less than 5% (See Figure 3.2B; Appendix 7).

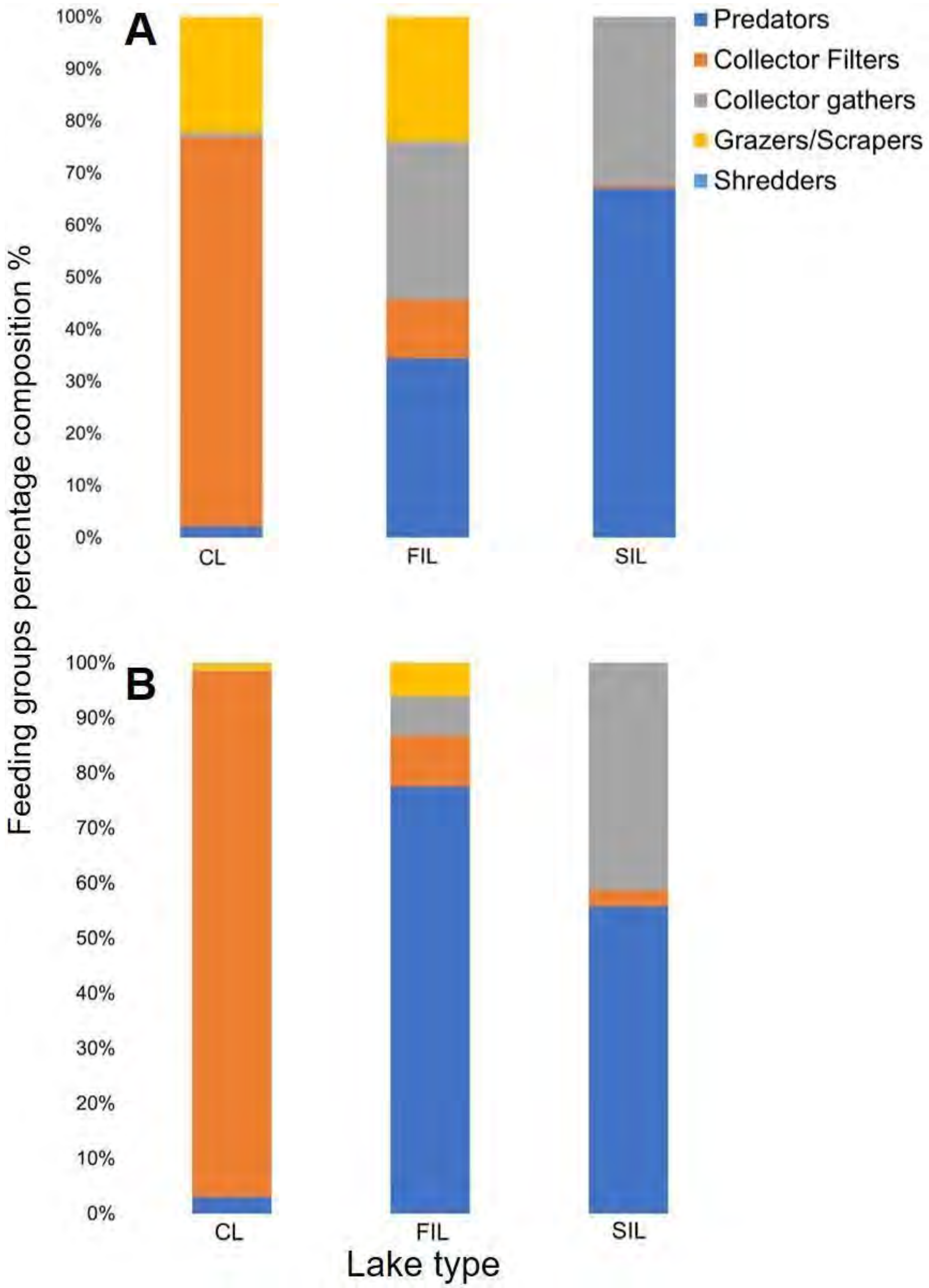

Figure 3.2: Aquatic macroinvertebrates feeding group percentage (%) composition from six freshwater lakes in KwaZulu-Natal and Mpumalanga Provinces, South Africa. A = Winter andB = Summer.

Canonical analysis of principal coordinates (CAP) ordination illustrated three distinct clusters representing aquatic macroinvertebrates species composition, where each cluster represented lake type (Figure 3.3A). Whereby CL (Lake Mzingazi and Lake Sibaya) were grouped forming a cluster, this was also the same with FIL (Lake Banagher fresh and Lake Tevrede Se Pan) and SIL (Lake Chrissiesmeer and Lake Banagher salt), they both formed separate clusters. Additionally, CAP showed that there was a significant difference betweenCL, FIL, and SIL in summer (Figure 3.3A). T*arebia granifera* for example showed a strong correlation towards CL during summer (Figure 3.3A). In contrast, *Enallagma glaucum, Anax*sp., *Ranatra* sp*., Appasus* sp., *Naucoris obseuratus, Helochares* sp., Chironominae, *Nychialimpida*, *Plea* sp. and *Bulinus africana* showed a very strong correlation towards FIL, whereas*Sigara* sp. and *Micronecta* sp. were positively associated with SIL (Figure 3.3A).

Aquatic macroinvertebrates also responded to habitat characteristics proxy for lake types and showed a distinct difference in species composition between lake types *i.e.,* CL, FIL, and SIL indicative by separate clusters (Figure 3.3B). Salt Inland Lakes clustered together indicating similarity in aquatic macroinvertebrates species composition, this was also true for FIL (Figure 3.4B). In addition, CL were also clustered together showing similarities in aquatic macroinvertebrates (Figure 3.3B). Furthermore, *Cloeon* sp., *Bulinus africanus*, *Nychia limpida*, *Anax* sp., *Naucoris obseur*atus, and *Tarebia granifera* were strongly correlated withCL. While *Micronecta* sp., *Aquarius distant*, *Helochares* sp., *Caenospella* sp. and *Bellamya capillata* were strongly correlated with FIL and SIL (Figure 3.3B).

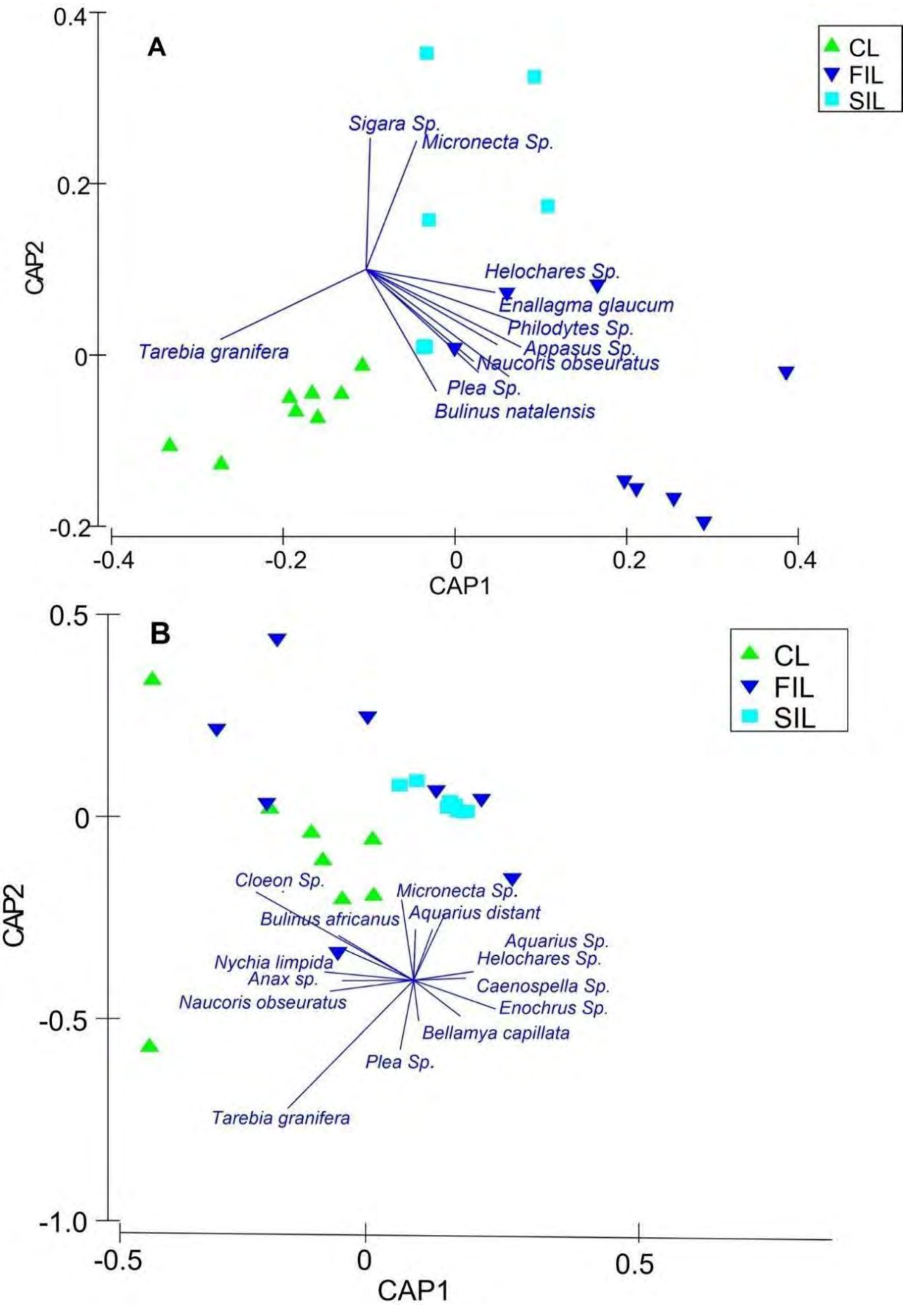

Figure 3.3: Canonical analysis of principal coordinates (CAP) ordination illustrating macroinvertebrate species composition in three lake types between summer (A) and winter (B). Only significant variables with a strong Pearson correlation output of r > 0.6 are represented on the plot.

PERMANOVA results supported the CAP patterns and showed that aquatic macroinvertebrates species composition was significantly different between lake types (PERMANOVA, pseudo-F=8.50, P= 0.0001), seasons (PERMANOVA, pseudo-F=5.35, P= 0.0001), and lakes x seasons (PERMANOVA, pseudo-F=2.58, P= 0.0001) (Table 3.1).

Table 3.1: PERMANOVA results comparing aquatic macroinvertebrate assemblagestructure between lakes and seasons. Significant differences are highlighted in bold.

Factors	Degree of freedom	Pseudo-F	P-value
Sites	5	8.50	***P < 0.05***
Seasons	1	5.35	***P < 0.05***
Site vs. Season	5	2.58	**P < 0.05**

Multiple linear regression analysis showed that predictor variables *i.e.,* pH, EC, and PO_4^{3-} explained <10% of aquatic macroinvertebrate relative abundance and that only pH and PO_4^{3-} positively influenced relative aquatic macroinvertebrate species abundance, while EC negatively affecting relative aquatic macroinvertebrate species abundance but was all variable was not significant (Table 3.2). Aquatic macroinvertebrate species richness was affected by pH, water temperature, and DO which explained the 17% variation in aquatic macroinvertebrate richness with pH and water temperature negatively affecting richness andDO positively affecting richness. Water temperature and DO were significant for species richness (Table 3.2). Aquatic macroinvertebrate Pielou's evenness was significantly and negatively affected by only water temperature, explaining <10% variation of aquatic macroinvertebrate species' evenness (Table 3.2). Aquatic macroinvertebrates Shannon diversity was affected by pH, water temperature, DO, and PO_4^{3-} which all explained 25% variation of aquatic macroinvertebrate diversity (Table 3.2). The pH, water temperature, and PO_4^{3-} negatively affect diversity whereas DO positively affects species diversity. The pH and water temperature were significant but DO and PO_4^{3-} were not significant (Table 3.2).

Table 3.2: Multiple Linear regression analysis between aquatic macroinvertebrate diversity indices and water chemistry. Significant differences in values are indicated in bold ($p < 0.05$).

Factors.	Prediction	Estimation	Standard Error	t-statistic	P-Value	Adjusted r^2	Degrees of freedom	F	P-Value
Relative abundance (N)	Intercept	-2312.34	1369.12	-1.69	$p > 0.05$	0.06	3.44	1.93	$p > 0.05$
	pH	1095.44	586.55	1.87	$p > 0.05$				
	EC	-109.83	64.12	-1.71	$p > 0.05$				
	PO_4^{3-}	97.51	62.98	1.55	$p > 0.05$				
Species richness (S)	Intercept	103.21	39.40	2.62	**$p < 0.05$**	0.17	3.44	4.25	**$p < 0.05$**
	pH	-24.96	12.61	-1.98	$p > 0.05$				
	Water	-16.32	4.96	-3.29	**$p < 0.05$**				
	Temperature	6.070	2.67	2.26	**$p < 0.05$**				
	DO								
Pielou's evenness (J)	Intercept	1.67	0.45	3.69	**$p < 0.05$**	0.09	1.46	5.97	**$p < 0.05$**
	Water	-0.37	0.15	-2.44	**$p < 0.05$**				
	Temperature								
Shannon diversity (H)	Intercept	16.28	4.17	3.91	**$p < 0.05$**	0.25	4.43	5	**$p < 0.05$**
	pH	-3.88	1.33	-2.93	**$p < 0.05$**				
	Water	-2.21	0.50	-4.41	**$p < 0.05$**				
	Temperature	0.39	0.27	1.48	$p > 0.05$				
	DO	-0.18	0.13	-1.39	$p > 0.05$				
	PO_4^{3-}								

3.4 DISCUSSION

The study provided an opportunity to understand the potential effects of physico-chemical variables on lake aquatic macroinvertebrate diversity and composition and produce aquatic macroinvertebrate inventory for natural lakes in South Africa. The results from this study highlighted that aquatic macroinvertebrates species composition was driven byphysico-chemical variables, thus in agreement with the hypothesis that given the habitatcomplexity and related physico-chemical variables, FIL was more diverse, and hadcompletely different aquatic macroinvertebrates communities as compared to the CL which was moderately diverse and SIL been the least diverse due to high salinity and absence of aquatic vegetation.

Lake types *i.e.,* CL, FIL, and SIL, and associated physico-chemical variables supported different types of aquatic macroinvertebrates for both seasons. Salinity acted as an environmental filter for aquatic macroinvertebrates preserving only species that have wide ecological tolerance more especially in SIL (Kefford *et al.,* 2004). In the present study and that of Reynolds *et al*. (2002) and Dalu *et al*. (2014), water quality variables were important andinfluenced aquatic macroinvertebrates assemblages. In SIL, it was observed that there was low diversity of aquatic macroinvertebrates species as compared to other lake types. In particular Lake Banagher salt where it was also observed that the lake had a huge flockof flamingos during both sampling seasons. According to literature by Geldenhuys (1982), Mepham (1987), and Seaman *et al*. (1991), a large number of bird species are found in most Southern African salt lakes. These bird species prefer mostly salty environments, this is because in salt lakes, there are few aquatic macroinvertebrates that survive in such environment except *i.e.,* brine fly larvae and brine shrimp. Therefore, these aquatic invertebrates are required by birds' nutrient needs (Conover & Bell, 2020). For example, Eared grebes (*Podiceps nigricollis*) for its survival need to consume 28,000 adult brine shrimp every day (Conover & Bell, 2020).

In addition, salt lakes accumulate and recycle nutrients far better than freshwater systems, producing large quantities of food for fish and birds (Wurtsbaugh *et al*., 2017; de Necker *et al*., 2021). However, in a study by Oberholster *et al*. (2009a) in LakeTswaing they observed very low numbers and taxa of aquatic macroinvertebrates throughout their study. The study concluded that this could be due to prevailing high salinity levels. The authors further suggested that the fine-grained inorganic sediment in Lake Tswaing influenced low numbers of aquatic macroinvertebratetaxonomic taxa. On the other hand, Etosha and Makgadikgadi pan have been known to be the only breeding pans of flamingos in Southern Africa (Berry, 1972; Childress *et al.*, 2008), however, observation of flamingos in the present study shows that Lake Banagher salt in the Mpumalanga region could be another breeding pan for these bird species, and accordingto the landowner (Malomane per. comms) this site has become a tourism attraction in the region..

Aquatic macroinvertebrates have adapted to certain environments and biotopes requirements, and changes to these environments may impact aquatic macroinvertebrate species diversity and abundance (Driver, 1977; Aiken, 1991; Dickens & Graham 2002; Hill, 2004; Van de Meutter *et al*., 2005). Our results showed that there was a significant difference in aquatic macroinvertebrate diversity between Coastal lakes and Inland lakes.In terms of diversity, it was found that inland lakes were more diverse as compared to coastal lakes, this was because each lake was different in terms of biotopes. For example, Lake Tevrede Se Pan and Lake Banagher fresh both had submerged and emergent aquatic plants, while Lake Sibaya, and Lake Mzingazi was dominated by emergent plants. Most studies (Glen *et al*., 1999; Bird *et al*., 2014; Mabidi *et al.,* 2017) conducted on the diversityof aquatic macroinvertebrates have shown that aquatic macroinvertebrate species mostly prefer vegetated biotopes. This is in line with a study by Cheruvelil *et al*. (2002) found a significantly higher density and biomass of aquatic macroinvertebrates in submerged vegetation than in freshwater systems with less vegetation. Additionally, Bird *et al*. (2014)

study also supported this concept, which investigated the influence of biotopes on invertebrate assemblage in alkaline wetlands in the Western Cape, South Africa. Their results showed that sites with submerged vegetation supported a high number of aquatic macroinvertebrates compared to open-water sites. This is because vegetation provides habitat complexity and therefore more habitat for aquatic macroinvertebrates, and food sources *i.e.,* periphyton grazing and better refuge from predators (Cheruvelil *et al*., 2002).

However, according to Mabidi *et al.* (2017), submerged vegetation supports low diversityof aquatic macroinvertebrates. This may be because, in this biotope, certain species cannot occupy the entire plant because it is submerged underneath the water (Glen *et al*., 1999). This was observed in the study by De Klerk & Wepener (2011), in which they were checking for the influence of biotope and sampling method on the assessment of the invertebrate community structure in endorheic reed pans in South Africa. In their study, they observed that pans were different in species abundance; this was contrary tothe difference in biotopes in these pans. For example, pans that had marginal and emergent vegetation supported a high number of aquatic macroinvertebrates compared to pans that were submerged, floating-leaved vegetation and had gravel, sand, and mud(GSM). This was in line with our finds where SIL that had sand and bedrock substrate, were not vegetated, thus had limited abundance of aquatic macroinvertebrates as compared to the CL and FIL which had submerged and emergent plants.

In the present study, two species of the family Lymnaeidae were collected, namely *Pseudosuccinea columella* and *Radix natalensis*. According to De Kock *et al.* (1989) and DeKock & Wolmarans (2008), *P. columella* populates several water bodies and is regarded as the most widespread alien species invading standing water bodies with muddy or soft substrate (De Kock *et al*., 1989). *Pseudosuccinea columella* was found in both CL and FIL, except in SIL *i.e.,* Lake Chrissiesmeer and Lake Banagher salt. This was also observed by Foster *et al*. (2015), who compared aquatic invertebrate communities between pans in North

West and Mpumalanga. Their study showed that *P. columella* invaded only the Mpumalanga pans. Due to their invasive ability to compete and overcome native species,these invasive species have led to a decrease in aquatic macroinvertebrates biodiversity(Miranda *et al*., 2010) to an extent that, these invasive snails are said to have displaced native snails.

This was also the case with the invasive *Tarebia granifera* snail, the species was found only in CL *i.e.,* Lake Sibaya and Lake Mzingazi, but there were no traces of this species in all inland lakes. According to Miranda *et al*. (2011), Miranda & Perissinotto (2014), Perissinotto *et al.* (2014), and Makherana *et al*. (2022), *T. granifera* prefers shallow and sandy habitats. Results from the present study agree that CL was more suitable habitat for the invasive Tarebia due to sandy substrate as compared to those found inland. Furthermore, *T. granifera* species are sensitive to low pH to an extent that it results in erosion of their shell, in addition, low EC reduces the growth and survival of snails. This makes sense because in the present study *T. granifera* was found dominating lakes withhigh pH, Lake Mzingazi (pH=8.33) and Lake Sibaya (pH=8.79) and low EC, Lake Mzingazi EC= 0.45 µS /cm and Lake Sibaya EC= 1.88 µS /cm.

Insufficient dissolved oxygen and high nutrient concentrations often indicate poor water quality, and this is characterized by the presence of pollution-tolerant chironomids (Cowardin, 1979; Brinkhurst, 1984; Thorp & Covich, 2009). In the present study, these pollution-tolerant species were seen in high abundance in Mpumalanga lakes, especiallyin FIL. This is usually associated with disturbed conditions either as a result of fluctuationsin nutrient levels or human landscape disturbances (Bird & Day, 2010), and in the presentstudy, this was caused by fluctuations in nutrients. Langdon *et al*. (2006) have classifiedlakes based on their trophic status, the diversity of *Chironomus* species, and their sensitivity to eutrophic conditions. Larvae of some Chironomidae *e.g.*, *Chironomus plumosus are* known for their ability to tolerate bad conditions, and they adapt to a wide range of environmental conditions such as sites that are polluted (Winner *et al*., 1980; Brinkhurst, 1984), but in the present study large abundance of chironomids was observedin non-polluted lake *i.e.,* Lake Tevrede Se Pan, this may have been caused by farming and cow grazing around the lake. However, according to their ecological preferences, these euryoid species can inhabit deep, muddy, or shallow substrates (Thorp & Covich, 2009). This is due to their abundance, and they are considered to be cosmopolitan species occurring throughout the world (Brinkhurst, 1984; Harrison, 2003; Griffiths *et al.*, 2015). They have haemoglobin as blood pigment, which enhances oxygen uptake, allowing them to survive in various environmental conditions (Griffiths *etal.*, 2015). According to Winberg (1978), the pollution index assumes that unpolluted waters are dominated by larvae of the subfamily Orthocladiinae and polluted water by larvae of the subfamily Tanypodinae, but in the present study, this was not the case; a large number of Orthocladiinae were found in both Lake Banagher salt and LakeBanagher fresh, and Tanypodinae were observed to be present in Lake Tevrede Se Pan.

Change in season also influence species abundance, richness, evenness, and diversity, different seasons may be dominated by different aquatic macroinvertebrates, for example, rainy seasons is long enough to allow all taxa not only those with a short life cycle, to complete their life cycles (Ferreira *et al*., 2012). For the present study, seasonalvariation played a crucial role in the structuring of aquatic macroinvertebrates, as illustrated by Appendix 6, high species abundance was observed in rainy seasons, this was greatly influenced by physico-chemical variables *i.e.,* D.O, pH, and NH_4^+. Seasonal differences in aquatic macroinvertebrates may be influenced by various factors such as water temperature, insect emergence, and nutrient inputs (Scheibler *et al*., 2020). Also, the life history of aquatic macroinvertebrates could explain why there was a significant difference in species diversity, evenness, abundance, and richness between seasons (Füreder *et al*., 2005).

In the present study aquatic macroinvertebrates were classified into functional feeding groups based on the indication by Barbour *et al*. (1999) and Merrit *et al*. (2002). Grouping aquatic macroinvertebrates into FFGs reveals shared or common feeding attributes, which can be valuable in understanding the complexity of aquatic macroinvertebrates communities (Gérino *et al*., 2003; Ramírez & Gutiérrez-Fonseca, 2014). In the present study, findings revealed that FIL lakes *i.e.,* Lake Banagher fresh and Lake Tevrede Se Pan, and Lake Sibaya were more heterogeneous as compared to other sites in both seasons. Predators were the most common functional category at most sites, followed by collector-filters, collector-gatherers, and scrapers being the least common. According to the study by De Necker (2015), the Pongola floodplain pans were also dominated by predators, followed by gathering collectors and filter collectors. Their results were also the same as the study by Ferreira (2010) in the Mpumalanga pans. Farrell *et a*l. (2015), suggested that variation in aquatic macroinvertebrates structure (FFGs) between different seasons may be due to alterations in food availability because of changes in seasons. Also, Hancock & Timms (2002), concluded structuring could be attributed to environmental conditions and the reproduction success of species.

This study provided baseline information on aquatic macroinvertebrates in South African lakes. The study gives a clear indication of habitat complexity and the presence of macrophytes provide spaces for many organisms to co-exist. It also reveals how salinity gradient influences aquatic macroinvertebrate's community structure. Furthermore, the study supports the growing literature that aquatic macroinvertebrates can be used reliable indicators for water quality assessment in lakes systems of South Africa.

CHAPTER 4: GENERAL DISCUSSION AND CONCLUSIONS

4.1 GENERAL DISCUSSION

Water is a vital resource in South Africa, and it plays a significant role in guaranteeing the country's economic growth and development (Basson, 2011; DWAF, 2002), as a result, the socio-economic well-being of South Africa depends on water resources such as lakes (Harding, 2015). A study by NWRS (2014) gives a summary of water resource management that South Africa faces. The document points out that South Africa lacks management strategies and training, that there are limited interventions done on South African lakes, and that they are not receiving the attention and status that they deserve. This has led to the sustainability of South Africa's freshwater resources reaching a criticalpoint.

Conservation of aquatic resources is crucial in South Africa (Taylor *et al*., 2007b), and urgent intervention is necessary, especially since there is an escalatingconcern for deteriorating water quality in freshwater systems in general (Richter *et al*., 1997; Matthiessen & Sumpter 1998; Lévêque 2001; Wood *et al*., 2003; Biggs *et al*., 2005; EPCN, 2008), which has significantly reduced theirability to function effectively (Brönmark & Hansson, 2002). As a result, poor water qualityposes a concern to human health and safety due to waterborne infections as well as having negative aesthetic (DWAF, 1996; Coetzee & Hill, 2012; Matthews & Bernard, 2015), because humans rely heavily upon lakes and ponds for domestic, industrial, and agricultural activities as well as for food supply (Amalu & Ajake, 2019). The limited freshwater surface waterways of South Africa are subject to environmental degradation, especially eutrophication. Sala *et al*. (2000) further argue that these disturbances seriously affect these freshwater systems and their biota. As shown in the present study, lakes are no exception, especially systems found in urban areas or developing catchments.

We know very little about phytoplankton and aquatic macroinvertebrates assemblages in South African large lakes, with only few studies done in the 1970s and 1980s (Appleton, 1977; Appleton, 1983), and the majority of the landscape has been influenced by global changes (NBA, 2018). Therefore, it is important to study, monitor and document the current aquatic organisms in this case phytoplankton and aquatic macroinvertebrate species assemblages to quantify changes over time (Brönmark & Hansson, 2002; Newton *et al*., 2011). Furthermore, with South Africa beinga water-scarce country (Dallas & Day, 2004), these lakes need local and international conservation status, and with good management and conservation plans, they can become a valuable ethno- and eco-tourist attraction (Van der Waal, 1997; Mantel *et al*., 2010).

This thesis was aimed to assess the species composition and diversity of phytoplanktonand aquatic macroinvertebrates from six South African freshwater lake systems, to produce comprehensive phytoplankton and aquatic macroinvertebrates species list and community assemblages structure. This was accomplished by pursuing specific objectives outlined in each chapter, which were geared at learning more about the natureof phytoplankton and aquatic macroinvertebrates biodiversity. Findings from this thesis supported the hypothesis and result from other studies including Bleich *et al*. (2011), Josefson & Hansen (2004), and Josefson & Göke (2013) with a conclusion that physico-chemical profiles are the main contributing factors in the structuring of phytoplankton species and aquatic macroinvertebrates. This was evident from the phytoplankton study(Chapter 2) and the aquatic macroinvertebrate study (Chapter 3).

Chapter 2 investigated phytoplankton diversity and species composition between Coastal lakes and Inland lakes, and how lake type and related physico-chemical characteristics influenced phytoplankton. There are limited studies on phytoplankton dynamics in South African lakes, making this project important and contributing significantly to the phytoplankton knowledge in Southern African lakes. It can be concluded that physico-chemical variables (salinity, pH, biotope structure and temperature) affect phytoplankton species diversity. This is because the concentration of water quality variables within an ecosystem influences aquatic system processes and biology. In the present study, most investigated physico-chemical variables had a concentration that exceeded the recommended limit of the South African Guidelines and World Health Organization tolerance limit for freshwater quality. The study by Allanson (1979) and a report by the Department of Water and Sanitation (DWS) on physico-chemical variables for over 20 years in the lakes, specifically Lake Sibaya, have indicated that this system is in a eutrophic state. These are in comparison with the present study indicating that these lakes are nutrient-enriched with high concentrations of nitrate, as a result, in the present study it was noticed that there was a decrease in water quality in these lakes, especially the SIL. As discussed before, the studied sites, despite their geographic disparities, had low Chl-*a*concentrations, which was surprising because an increase in nutrient levels increases Chl-a and lake productivity. All these lakes revealed similar levels of Chl-*a*, indicating that they are oligotrophic (Dunnink *et al*., 2016).

As discussed before, phytoplankton is a reliable indicator of lake water quality because they are constantly exposed to the aquatic environment; therefore, lake water quality andthe dominant diatom species could be used to represent changing environmental conditions over time (Chutter, 1998; Ndiritu *et al*., 2006; Martin & de los Reyes Fernández,2012). A total of 122 phytoplankton species that belong to seven Phyla were collected and identified during this study. As observed in the results (Appendix 1), Lake Banagher salt, Lake Sibaya, and Lake Chrissiesmeer, showed high cyanobacteria concentrations, and their presence was frequently linked to water quality issues. Agricultural activities arethe only factors that may have contributed to water pollution, which makes sense becausetwo of these lakes are located in Mpumalanga, and their surroundings are dominated mainly by farming.

According to Wetzel, (2001) and Ariyadej *et al*. (2004), phytoplankton of different speciescan endure a wide variety of temperatures, this includes having limitations by light and nutrients, therefore, and the dominance of species at different times and seasons is determined by these tolerance levels. Chapter 2 provided an excellent resolution and a better understanding because lakes with different temperatures and nutrients showed a significant difference in phytoplankton diversity and assemblages. The high dominance of phytoplankton in the study was employed as a water quality indicator. For example, allthe study lakes except Lake Banagher salt were dominated by *Cyclotella meneghiniana*,and according to Bronco & Senna (1995) and Ariyadej *et al.* (2004) this can be used as biomonitoring of oligo-mesotrophic waters.

Chapter 3 provided a baseline information on water quality and aquatic macroinvertebrate community structure in coastal and inland freshwater lakes. These results are critical forthe development of surrogates for biodiversity conservation and biomonitoring programs in freshwater lakes. Species assemblage was dominated by ten orders: Ephemeroptera, Odonata, Lepidoptera, Hemiptera, Coleoptera, Diptera, Decapoda, Gastropoda, Venerida, and Dermaptera (Appendix 3). The study hypothesis stated that each lake typewould be significantly different from the other, depending on salinity gradient and habitatcomplexity. The findings showed a significant difference in aquatic macroinvertebrates between lake types and seasons (see Chapter 3). For example, FIL *i.e.,* Lake Tevrede Se Pan and Lake Banagher fresh, had more aquatic macroinvertebrates than any other lake types, and this was be because these lakes had less salt, with their surroundings experiencing less anthropogenic activities, and were dominated by both submerged andemergent aquatic plants creating suitable habitat for diverse aquatic macroinvertebrates.This phenomenon is supported by several studies including Scheffer *et al.* (1984), Glen *et al*. (1999) Bird *et al*. (2014), Khudhair *et al*. (2019). Therefore, this gives a clear brief understanding that these lakes need to be protected. As a result, these freshwater lakesare endangered, and few are protected. As much as this is the problem, studies on lakesare needed to ensure that they are protected or conserved for future generations. Therefore, there is a growing interest in assessing these lake conditions, applyingrestoration strategies, and monitoring aquatic biota recovery.

4.2 GENERAL CONCLUSION

The study examined species assemblages and diversity of phytoplankton and aquatic macroinvertebrates in coastal and inland freshwater lakes to investigate biodiversity and species composition dynamics. The present study further provided baseline information on phytoplankton and aquatic macroinvertebrates species list for freshwater lakes in South Africa. This study added to our knowledge of phytoplankton and aquatic macroinvertebrate biodiversity patterns in South African freshwater lakes and provided insight into physio-chemical influence, and how salinity significantly influences andstructures phytoplankton and aquatic macroinvertebrate communities. The results also provide empirical evidence that will inform policy and the development of management strategies for freshwater lakes. With South Africa's population growing, and thus greaterdemands being made on its water resources, it is necessary to identify the major factorscontrolling the expression of the biota in the ecosystem, because an understanding of how the system functions is important for management. The study results will also contribute to the next National Biodiversity Assessment (2024) concerning biological datadeficiency noted in the previous NBA (2018) report, to understand how diverse these lakes are in terms of phytoplankton and aquatic macroinvertebrate species. The results will also contribute to a better knowledge of the richness and composition of phytoplankton and aquatic macroinvertebrates species in South African lakes. NBA (2018) identified 8 South African freshwater lakes, but we managed to sample 4, leavingfour lakes *i.e.,* Lake Fundudzi, Lake Barberspan, Lake De

Hoop, and Lake Groenvlei thatneeds to be investigated, so more studies are needed to assess and document the biology of these systems for their conservation. Further studies are necessary on these lakes to investigate more on how the phytoplankton and aquatic macroinvertebrates evolve overtime by using more techniques, for example, environmental DNA, that will helpidentify new species that might be found in the lakes.

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APPENDICES

Appendix 1: Dunn's *post hoc* test results showing the pairwise comparisons for Coastal Lakes (CL), Fresh Inland Lakes (FIL) and Salt Inland Lakes (SIL) for the different physico-chemical variables. NB: black and brown blocks represent significant differences at *p < 0.05* and *p < 0.001*, respectively.

	Water Temperature	DO	pH	EC	NO_3^-	NH_4^+	PO_4^{3-}	Chl-a
CL-FIL								
CL-SIL								
FIL-SIL								

Appendix 2: Spearman's rank order correlation (r_s) output for water quality variables. Correlation coefficients ≥ ±0.5 are highlighted in bold.

	Water Temperature	DO	pH	EC	NO_3^-	NH_4^+	PO_4^{3-}
Water Temperature (°C)							
DO (mg/L)	**0.57**						
pH	**-0.73**	-0.47					
EC (µS/cm)	-0.39	-0.10	**0.52**				
NO_3^- (mg/L)	-0.37	-0.09	0.35	0.12			
NH_4^+ (mg/L)	-0.15	-0.25	0.12	0.03	0.26		
PO_4^{3-} (mg/L)	0.16	0.25	-0.26	-0.16	-0.28	0.27	
Chl-*a* (mg/m^3)	-0.44	-0.04	**0.51**	0.35	0.36	-0.15	-0.27

Appendix 3: Phytoplankton species list and their relative abundances collected from six freshwater lakes in two seasons(winter and summer) between August 2020 and January 2021, South Africa.

Taxa	Lake salt	Banagher	Lake Fresh	Banagher	Lake Chrissiesmeer		Lake Mzingazi		Lake Sibaya		Lake Tevrede Se Pan	
	Winter	Summer	Winter	Summer	Winter	Summer	Winter	Summer	Winter	Summer	Winter	Summer
CYANOPHYTA												
Anabaena catenula												68
Anabaena sp.			145	27		44						
Anabaena bory											23	
Microcystis sp.							47		136			
Oscillatoria redekei						428						
Oscillatoria limnetica												44
Lyngbya contorta		105										
Schizothrix muelleri						316						
Spirulina maior	1403	9398										
Gloeocapsa alpina									187			
Merismopedia glauca									362			
CHRYSOPHYTA												
Mallomonas sp.			27	21								
Synura ehrenberg											23	
BACILLARIOPHYTA												
Achnanthidium crassum							359					
Achnanthidium saprophilum								23				

Achnanthidium minutissimum												145
Achnanthidium exiguum												
Synedra ulna												19
Staurosirella pinnata						21			100			
Tabellaria flocculosa									27			
Aulacoseira granulata							51	105				
Aulacoseira subarctica							47					
Aulacoseira muzzanensis									22			
Cocconeis pediculus				108		86	78	60		902	190	
Cocconeis engelbrechitii							95					
Cocconeis placentula		3666		54		54	90	21	51	78		89
Cyclotella meneghiniana	937		410	458	23	150		394	279		117	58
Cyclotella ocellata					140	770			131			442
Cymbella neocistula						21						
Cymbella tumida		23										
Diadesmis contenta	23	23										
Diadesmis confervacea											23	
Eunotia minor		23		22		42		246				40
Diatoma vulgaris					54		160					23
Fragilaria ungeriana							23					
Fragilaria nanana									27			
Fragilaria biceps							54			23		

Fragilaria tenera					23				279			21
Fragilaria ulna						64			22	111		
Gomphonema pumilum									25			
Gomphonema pseudoaugur										54		
Gomphonema affine												19
Gomphonema venusta		86										
Melosira varians					23				48		21	
Navicula bory						21	27					58
Navicula radiosa							118					
Navicula rhynchocephalia										27		
Navicula capitatoradiata						64						
Navicula rostellata						21						
Navicula veneta	73	66									21	
Navicula riediana		43										
Navicula tripunctata		23										
Nitzschia sp				54								
Pinnularia sp				21		21						
Rhopalodia gibba										82		
Diploneis elliptica				21								197
Diploneis subovalis										25		
Stephanodiscus hantzschii							105					

Stephanodiscus agassizensis				39	410			22			
Surirella ovalis				85							
Tabularia fasciculata				40						22	
Sellaphora stroemii	66										64
Staurosira elliptica										23	
Frustulia vulgaris		23									
Frustulia crassinervia			49		21						
Frustulia saxonica					43						
Eolimna minima				23					51		
Lemnicola hungarica					107						
Pseudostaurosira brevistriata	82			19							
Placoneis placentula											22
Pinnularia ehrenberg											39
Sellaphora pupula					43					23	
CRYPTOPHYTA											
Crytomonas sp			15								
DINOPHYTA											
Peridium enhrenberg											17
Peridinium sp.									93		
EUGLENOPHYTA											
Strombomonas sp.							320				67
Trachelomonas sp.								88			
Trachelomonas ehrenberg							23				
CHLOROPHYTA											

Ankyra sp.					21					
Chlamydomonas sp.			64					74		
Crucigenia tetrapedia								361	19	
Cosmarium sp.					21		47			
Dictyosphaerium nageli	47									
Euastrum sp.										
Micractinium fresenius							23			
Micractinium sp.	16014					28				
Monoraphidium sp.		27	108		21	98				
Monoraphidium contortum								27		
Oocysts braun								514		
Oocysts borgei					43					
Pandorina bory							864			
Pandorina sp.			21		43					
Pediastrum sp.									27	
Pediastrum tetras	23						47			
Pediastrum duplex				159	197					
Pediastrum samplex					108					
Scenedesmus sp.		822	193							
Scenedesmus bicaudatus				202	64					
Scenedesmus dispar										117
Staurastrum sp.									31	
Tetrastrum sp.					86			136		

Tetrastrum chodat		19										
Tetraedron minimum							23		54			
Epithemia sorex									21			
Epithemia adnata	47									31	141	22
Encyonema sp.						21		27				
Actinastrum sp.							50					
Achnanthes standeri					27	64						
Aphanocapsa litoralis	257											
Aphanocapsa grevillei	21											
Colostrum nageli				193		42						
Chlamydocapsa ampla					54							
Caloneis aequatorialis	128										47	
Discostella pseudostelligera									27			
Chlamydomonas sp.										92		
Encyonopsis leei								23				22
Encyonopsis subminuta					27							
Goniochloris fallax									23			
Gloecytis vesiculosa					19							
Peridiniopsis sp.								23	23			

Appendix 4: Phytoplankton percentage composition in three different lake types (Coastal Lakes, Fresh Inland Lakes and Salt Inland Lakes) in winter, KwaZulu-Nataland Mpumalanga, South Africa. Bolded values show aquatic macroinvertebrates compositions that were 20% and more ofthe total composition.

Phyla	Coastal Lakes (%)	Fresh Inland Lakes (%)	Salt Inland Lakes (%)
Chlorophyta	**27**	**49**	**33**
Bacillariophyta	**59**	**43**	**43**
Cyanophyta	13	6	**24**
Chrysophyta	0	2	0
Dinophyta	0	0	0
Euglenophyta	0	0	0
Cryptophyta	1	0	0

Appendix 5: Phytoplankton percentage composition in three different lake types (Coastal Lakes, Fresh Inland Lakes and Salt Inland Lakes) in summer, KwaZulu- Natal and Mpumalanga, South Africa. Bolded values show aquatic macroinvertebrates compositions that were 20% and more of the total composition.

Phyla	Coastal Lakes (%)	Fresh Inland Lakes (%)	Salt Inland Lakes (%)
Chlorophyta	**30**	**22**	**38**
Bacillariophyta	**60**	**68**	**35**
Cyanophyta	0	5	**27**
Chrysophyta	0	1	0
Dinophyta	3	1	0
Euglenophyta	7	2	0
Cryptophyta	0	1	0

Appendix 6: Aquatic macroinvertebrates species list and their relative abundances from six freshwater lakes found in SouthAfrica during two seasons (winter and summer) collected between August 2020 and January 2021. Feeding groups (Pr- Predators, S-Scrapers, CG-Collector gatherers, Sh-Shredders, Ft-Filter feeders).

Taxa		Lake Banagher salt		Lake Banagher Fresh		Lake Chrissiesmeer		Lake Mzingazi		Lake Sibaya		Lake Tevrede Se Pan	
	Feeding group	Winter	Summer	Winter	Summer	Winter	Summer	Winter	Summer	Winter	Summer	Winter	Summer
EPHEMEROPTERA													
Caenis sp.	CG/S											27	
Caenospella sp.	CG/S			1									
Cleon sp.	CG/S			704	13			401	9	35		248	108
ODONATA													
Parazyxomma sp.	Pr											1	6
Anax sp.	Pr			8	109					2			25
Enallagma glaucum	Pr			331	22							64	5
Trithemis werneris	Pr												2
Aeshna sp.	Pr										2		
Enallagna sp.	Pr											30	50
LEPIDOPTERA													9
Pyralidae	Ft						3					18	5
HEMIPTERA													

Micronecta sp.	Pr	4			31	12	732						28	190
Sigara sp.	CG	2	770		81	12	27				3		15	16
Naucoris sp	Pr													23
Naucoris obseuratus	Pr			6	111									86
Macrocoris sp.	Pr			2	8							22		96
Plea sp.	Pr			692	499							2		64
Aquarius distant	Pr				11								1	
Aquarius sp.	Pr				2								1	2
Eurymetra natalensis	Pr				4									1
Rhagadotarsus sp.	Pr				3									
Eurymetra sp.	Pr													1
Ranatra sp.	Pr			1	10									
Ochterus sp.	Pr													1
Appasus sp.	Pr				131							3		30
Nabis capsiformis	Pr				1									
Lethocerus sp.	Pr				1									4
Laccocoris sp.	Pr										10	10		
Anisops sp.	Pr	26	191	96		135	130						68	
Hebrus sp.	Pr										2			
Gerris swakopensis	Pr				4									
COLEOPTERA														

Neohydrocoptus sp.	Pr				1									
Chasmogenus sp.	CG				10									
Sternolophus sp.	Pr													5
Dytiscidae (Larvae)	Pr			268	70	3	14					1	99	64
Hydaticus sp.	Pr				8									3
Heterocerus sp.	Pr					5								
Philodytes sp.	Pr		10		10								2	
Amphiops sp.	CG			1										
Hydaticus sp.	Pr													4
Hydaticus latior	Pr				1									1
Hydaticus capicolla	Pr				6									5
Hydroglyphus sp.	Pr				1									
Neptosternus sp.	Pr				1									
Pseudobagous sp.	CG			2	2									
Rhyssemus sp.	Pr						3							
Nucleotops sp.	Pr						9							
Hydrophilidae	CG	2	2		19		6							3
Cybister sp.	Pr			1	7									
Neochetina sp.	Pr				1		4							1
Psuedobagous sp.	Pr				2									

Copelatus sp.	Pr				1									1
Allocotocerus sp.	CG				8									
Anacaena sp.	CG				3									
Helochares sp.	CG				50								2	
Berosus larvae	CG												20	
Berosus sp.	CG		3		12		1							
Darywinhychus sp.	Pr				2									3
Canthyporus sp.	Pr													20
Spertus sp.	Pr													2
Enochrus sp.	Pr		2	45	10	9	36							6
Hydochares sp.	Pr				1									
DIPTERA														
Chironomidae	CG/S/Pr			868	15	3			9	1			194	
Orthocladiinae	CG/S/Pr	36		312									20	3
Tanypodiinae	CG/S/Pr								1				10	1
Tabanidae	Pr					2	2							
Culicidae	CG												1	4
DECAPODA														
Potamonautes sidneyi	CG													3
Nychia limpid	CG			97										
GASTROPODA														

Bulinus africanus	Ft	519									18
Radix natalensis	Sh								1		
Tarebia granifera	F					761	286	226	934		
Pseudosuccinea columella	Sh				1				2		
Bulinus tropicus	Ft			1	28						
Bellamya capillata	Ft							4			
Bulinus natalensis	Ft		122			23	67	41	58	69	75
Afrogyrus sp.	Ft			1	30						8
VENERIDA											
Corbiculla fluminalis	Ft						4				
DERMAPTERA											
Labidura riparia	Pr				14						
Pseudobagous sp.	Pr									2	
Amphidoidae	Pr			15	2						
Afrocoenis sp.	Pr									1	
Profile adusta	Pr								24		
Agroptocorixa sp.	Pr		411								

Appendix 7: Aquatic macroinvertebrates percentage composition in three different lake types in winter, KwaZulu-Natal and Mpumalanga, South Africa. Bolded values show aquatic macroinvertebrates compositions that were 20% and more of the total composition.

Taxa	Coastal Lakes (%)	Fresh Inland Lakes (%)	Salt Inland Lakes (%)
Predators	2	**34**	**67**
Collector Filters	**75**	11	1
Collector Gatheres	1	**30**	**32**
Grazers/Scrapers	**22**	**24**	0
Shredders	0	0	0

Appendix 8: Aquatic macroinvertebrates percentage composition in three different lake types in summer, KwaZulu-Natal and Mpumalanga, South Africa. Bolded values show aquatic macroinvertebrates compositions that were 20% and more of the total composition.

Taxa	Coastal Lakes (%)	Fresh Inland Lakes (%)	Salt Inland Lakes (%)
Predators	3	**78**	**56**
Collector Filters	**95**	9	3
Collector Gatheres	<1	7	**41**
Grazers/Scrapers	1	6	0
Shredders	<1	0	<1