



THE MACROBENTHIC COMMUNITIES OF TWO ESTUARY TYPES WITH DIFFERENT ECOLOGICAL HEALTH STATUS

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A research thesis submitted in fulfilment of the academic requirements for the
degree of Masters in Marine Biology

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DECLARATION 2: CONFERENCE PRESENTATIONS

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Presentation 1

Nkosi, A.C.V.*, Radebe, T.P., Mackay, C.F. and Scharler, U.M. 2023. The ecology of macrozoobenthic communities and taxa of uMdloti Estuary during and after the El niño induced drought. 30 January – 2 February 2023, Bloemfontein, South Africa. Oral presentation.

Presentation 2

Nkosi, A.C.V.*, Scharler, U.M. and Mackay, C.F. 2022. The macrozoobenthos community structure and dynamics of uMdloti Estuary. 17th South African Marine Science Symposium. 20–24 June 2022, Durban, South Africa. Oral presentation.

Acknowledgements

I extend my sincere gratitude to the National Research Foundation (NRF) for their financial support, as well as the University of KwaZulu-Natal and Oceanographic Research Institute for making this study possible.

To my family, thank you for your unwavering prayers, support and encouragement, even though my field of study was unfamiliar to you. Your love and belief in me kept me standing strong, even during moments of doubt. A special thanks to my dad who sacrificed everything for my well-being and education from the very beginning and has continued to support me unconditionally ever since. To my sisters, Nda and Zinhle, thank you for always being my biggest cheerleaders and keeping my head above the water. To my late brother, I know you would have been proud of me, rest peacefully Ngwane.

To my friends, thank you for the encouragement and support throughout this journey. Fifi, Kajal and Njabulo, your late-night lab companionship filled with laughter, motivation and endless rants, will always hold a special place in my heart. To my Scharler Lab colleagues, thank you for creating a supportive and collaborative environment that made this journey worthwhile. I would like to thank Angel and Bianca for their GIS expertise and their assistance in creating maps.

To my supervisors, Prof. Scharler and Ms. MacKay, I am deeply appreciative of your guidance, mentorship and unwavering enthusiasm. Your passion for this field has been a source of inspiration and motivation. Thank you for believing in my potential, the opportunities you provided and for encouraging me to persevere through challenges. May you continue to be richly blessed.

Lastly, Isaiah 40:31 says, "But those who hope in the Lord will renew their strength. They will soar on wings like eagles, they will run and not grow weary, they will walk and not be faint." This verse perfectly captures my journey. I ran, walked and sometimes crawled, but I never gave up. Thank you Lord for being my constant source of strength and resilience.

Abstract

The subtropical estuaries of KwaZulu-Natal are among the most ecologically compromised, subjected to pressures from pollution, intensive agricultural practices and disruptions in freshwater flow regimes. Consequently, 44% of KZN estuaries are categorised as having a health status ranging from moderately to severely modified. Macrozoobenthic organisms are considered reliable biological indicators of estuarine health due to their sedentary nature and sensitivity to natural and human-induced changes. Monitoring community attributes such as composition, abundance and diversity provide valuable insights into ecological changes over time. This study investigates spatio-temporal changes in macrozoobenthic communities within two estuary types in KwaZulu-Natal, which have contrasting ecological health statuses. These include the heavily modified, temporarily closed (TCE) uMdloti Estuary and the near-natural, predominantly open (POE) uMlalazi Estuary. The analysis focuses on spatial and temporal variations in environmental parameters and community metrics such as species composition, abundance and diversity.

The uMdloti Estuary, characterised by frequent mouth closures exhibited relatively homogenous environmental conditions yet displayed spatial and temporal shifts in macrozoobenthic community structure. The observed temporal disruptions in community composition, abundance and diversity can be linked to changes in mouth state between seasons. The system was dominated by a combination of estuarine and freshwater taxa, with notable numerical dominance of opportunistic and invasive species. The numerical dominance of opportunistic and invasive taxa in the uMdloti Estuary highlighted compromised health due to chronic pressures in the system.

The uMlalazi Estuary, characterised by consistent freshwater inflow and a predominantly open mouth, exhibited well-defined salinity and sediment gradients. These gradients created diverse habitats that supported a wide range of taxa, with stable communities occupying distinct ecological niches along the estuarine gradient. A comparison of macrozoobenthic community attributes between the uMlalazi and uMdloti estuaries revealed differences linked to variations in morphological characteristics, pressure intensity and protection status.

Despite variations in community attributes between the systems, the community compositions shared similarities in key estuarine taxa, such as *Desdemona cf. ornata*, *Ceratonereis (Composetia) keiskama*, *Grandidierella lutosa* and *Grandidierella* spp., indicating a biogeographical species pool. These similarities overlook the estuary type and its health status to a certain degree. Therefore, these taxa appear not to be robust indicators and should be interpreted with caution. The study findings contribute to macrobenthos taxonomic inventories of TCEs and POEs in the subtropical estuaries of KZN. In addition, this study highlights the need to consider factors that influence the environmental variables such as the location of the system, the protection status and anthropogenic pressure in and around the system to better understand the prevailing community composition and distribution patterns.

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List of abbreviations

AMSL	Above Mean Sea Level
ANOVA	ANalysis of VAriance
CCA	Canonical Correspondence Analysis
DO	Dissolved Oxygen
DWA	Department of Water Affairs
DWS	Department of Water and Sanitation
EHl	Estuarine Health Index
ENA	Ecological Network Analysis
ICOLL	Intermittently Closed and Open Lakes and Lagoons
IPCC	Intergovernmental Panel on Climate Change
KZN	KwaZulu-Natal
LTCE	Large Temporarily Closed Estuary
MAR	Mean Annual Runoff
nMDS	non-metric Multi-Dimensional Scaling
MPB	Microphytobenthos
MSL	Mean Sea Level
NBA	National Biodiversity Assessment
PAST	PAleontological STatistics
PCA	Principal Components Analysis
PERMANOVA	PERMutational ANalysis of VAriance
PES	Present Ecological Status
POE	Predominantly Open Estuaries
POM	Particulate Organic Matter
PP	Phytoplankton
SA	South Africa
SIMPER	Similarity Percentages
SRZ	Summer Rainfall Zone
STCE	Small Temporarily Closed Estuary
STD	Standard Deviation
TCE	Temporarily Closed Estuary
TOC	Total Organic Content
TSS	Total Suspended Solids
USA	United State of America
WWTW	Waste Water Treatment Works

Chapter 1: Introduction

1.1 Study context

Estuarine ecosystems provide essential services, including food production, climate protection, nursery habitats, carbon sequestration and socio-economic benefits, supported by their complex ecological processes and diverse habitats (Barbier et al. 2011, Thrush et al. 2013, Comberti et al. 2015, Hope et al. 2020). These ecosystems are simultaneously affected by environmental, anthropogenic and climatic stressors (Kennish 2002, McLusky & Elliott 2004, van Niekerk et al. 2022). The intensification of anthropogenic impacts, driven by amplified demand for natural resources and increased coastal populations, has heightened these stressors in recent decades (van Niekerk et al. 2019, Mastrantonis & Dubininkas 2022). As a result, estuarine ecosystems are progressively losing their ability to continually change and adapt while continuing to provide their full range of essential services and sustain biodiversity within critical thresholds (Glibert et al. 2017, Kennish 2021). More than 50% of South African estuarine systems have undergone varying degrees of modification due to pressures such as pollution, agricultural cultivation and altered freshwater flows (van Niekerk et al. 2019). These pressures have been identified as major contributors to the degradation of estuarine ecosystem health, with 44% of KwaZulu-Natal (KZN) estuaries classified as moderate to severely modified health status (van Niekerk et al. 2019).

To understand the consequences and extent of these pressures on estuaries, macrobenthic fauna are typically used as biological indicators due to their sensitivity to both natural and human-induced changes in estuarine ecosystems (Pinto et al. 2009, Patrício et al. 2012, Drylie et al. 2020). The community assessment of macrobenthic fauna provides insight into the current condition and the long-term impacts of disturbances (Patrício et al. 2012, Veiga et al. 2016). This method is valuable for assessing estuarine ecosystem health and has been incorporated into international monitoring standards and monitoring indices (Borja et al. 2008, Puente & Diaz 2008, Pinto et al. 2009). This study aims to investigate the temporal and spatial variability of macrobenthos communities from two systems, a modified large temporarily closed estuary and a predominantly open estuary with good health status.

1.2 Definition of an estuary and estuary characteristics

At the interface between rivers and the sea are estuaries, uniquely characterised by the continuous mixing of riverine freshwater and oceanic saline water (Cooper 2001, Potter et al. 2010). Estuarine environments are shaped by climatic, topographical and geomorphological factors resulting in spatial and temporal variations in key parameters, including sediment, salinity, temperature and water levels (Cooper 2001, Harrison 2004, Ellero 2018). These variations play a crucial role in regulating critical ecosystem processes, such as salinity stratification, sediment transportation and nutrient cycling fostering diverse habitats (Attrill & Rundle 2002, McLusky & Elliott 2007, Perissinotto et al. 2010). As a result, estuaries are highly productive systems that sustain rich biodiversity, provide essential nursery grounds and offer sociological and economic benefits through tourism and fisheries (Lamberth & Turpie 2003, van Niekerk et al. 2019, Clark et al. 2022). These attributes collectively establish estuaries as among the most valuable ecosystems on Earth (van Niekerk et al. 2019).

Estuaries are complex ecosystems that vary widely depending on factors such as size, access to the sea and hydrodynamic conditions (Whitfield & Bate 2007). Consequently, this raises difficulties in constructing a universal definition of an estuary. Pritchard (1967) described estuaries as, *“a semi-enclosed coastal body of water which has a free connection with the open sea and within which seawater is measurably diluted with fresh water derived from land drainage”*. However, this definition excludes most estuaries that occur in southern Africa. In this regard, Day (1980) modified Pritchard’s (1967) definition of an estuary to *“a partially enclosed coastal body of water which is either permanently or periodically open to the sea and within which there is a measurable variation of salinity due to the mixture of sea water with fresh water derived from land drainage”*. In the most recent efforts to classify South African estuaries, van Niekerk et al. (2019) defined an estuary as *“A partially enclosed permanent waterbody that is either continuously or periodically open to the sea and that extends as far as the upper limit of tidal action, salinity penetration or back-flooding under closed mouth conditions”*.

1.3 Distribution and classification of South African estuaries

The South African coastline features approximately 290 estuaries, each with distinct biological compositions and classified into four biogeographical regions based on unique ecological features (van Niekerk et al. 2019). These regions span from tropical (Kosi Bay to uMgobezeleni) to subtropical (St Lucia system to the Mbashe River), warm temperate (Mendwana Estuary to Heuningnes Estuary) and cool temperate (Ratel Estuary to the Orange River) along the east coast (Harrison 2004, van Niekerk et al. 2019). Classification, however, relies on key estuarine processes and features, such as tidal prism, sediment stability, mixing processes and average salinity (van Niekerk et al. 2019).

Whitfield (1992) originally classified South African estuaries into five categories namely, permanently open, temporarily open/closed, estuarine bays, estuarine lakes and river mouths. However, this classification had limitations, as it primarily focused on functional estuary types and overlooked regional biogeography variations and a range of flora and fauna (van Niekerk et al. 2019). In response to these constraints, a revised classification system was introduced, incorporating interconnected components (van Niekerk et al. 2019). Firstly, it includes a biogeographical categorisation based on regional biotic responses. Secondly, it improves the categorisation of estuary types to highlight the differences between small and large estuaries. By considering these components, the new classification system provides a more comprehensive and appropriate way of categorising South African estuaries.

The new estuarine classification system introduces two additional types namely, estuarine lagoons and arid predominantly closed estuaries, while river mouth types are subdivided into small and large fluvially dominated estuaries based on size and sedimentology (van Niekerk et al. 2019). Temporarily open/closed estuaries were renamed and further divided into large and small temporarily closed estuaries (LTCEs and STCEs). Subsequently, the South African estuarine systems were divided into nine categories namely, estuarine lakes, estuarine bays, an estuarine lagoon, predominantly open, large and small temporarily closed, large and small fluvially dominated, small fluvially dominated and arid predominantly closed estuaries (van Niekerk et al. 2019). This study focused on predominantly open estuary (POEs) and large temporarily closed estuary (LTCEs).

1.3.1 Temporarily closed estuaries

There are 209 temporarily closed estuaries (TCEs) along the South African coast, with over 50% located in the subtropical region (van Niekerk et al. 2019). These estuaries are typically small, linear or funnel-shaped systems with an area of <15 ha in STCEs and >15 ha in LTCEs (van Niekerk et al. 2019). They are periodically disconnected from the sea by a sand bar that builds up across a mouth region during low to no freshwater inflow periods (Whitfield 1992, Perissinotto et al. 2010). Tidal ranges are minimal, varying from 25 – 50 cm in LTCEs to 15 – 30 cm in STCEs. Globally, estuaries with similar characteristics are found in Brazil (Netto et al. 2018), the south and west coast of the United States of America (USA) where they are known as seasonally open tidal inlets (Collins & Melack 2014) and in Australia where they are referred to as intermittently closed and open lakes and lagoons (ICOLLs) (McSweeney et al. 2017).

Mouth configuration in TCEs is influenced by wave conditions, currents, sediment characteristics and wind action (Perissinotto et al. 2010). On South Africa's east coast, high wave energy and large sediment particles contribute to a steep beach profile (Cooper 2001, Whitfield & Bate 2007). This profile includes a sandbar that rises between 0.5 and 5 m above mean sea level (AMSL) at the mouth, classifying these estuaries as perched systems. Perched systems are common along the subtropical region and are characterised by sandbars that significantly influence whether the mouth remains closed, opens, experiences over wash, or undergoes seepage (Cooper 2001, Whitfield & Bate 2007).

Depending on the interplay between the processes mentioned above, along with the size, beach profile and the level of mouth protection, TCEs can exist in one of five hydrodynamic states, namely the outflow phase, tidal phase, semi-closed phase and closed phase (Snow & Taljaard 2007). The closed mouth phase occurs during periods of low river inflow, characterised by the absence of tidal exchange and can persist for days to years (Cooper 2001, Perissinotto et al. 2010, McSweeney et al. 2017). External factors including climate change-related events such as droughts (IPCC 2023, van Niekerk et al. 2022, Scharler et al. 2023) and anthropogenic influences like freshwater modification can prolong this phase (Perissinotto et al. 2010, Brooker & Scharler 2020). During the closed mouth phase, saline intrusion may occur through marine overwash caused by high waves (Snow & Taljaard 2007).

In perched systems, the open phase is often short-lived compared to non-perched systems due to limited tidal prism which restricts marine intrusion (Cooper 2001). Conversely, marine-dominated estuaries without a berm are frequently open and can maintain a tidal regime for extended periods (Cooper 2001, Harrison 2004). In river-dominated estuaries, the open phase is highly seasonal, typically occurring during the wet season when substantial volumes of freshwater flow into the estuary, causing the mouth to breach (Perissinotto et al. 2010). This phase is characterised by low water levels and fluvial dominance. However, it ceases when sediment deposition establishes a sandbar at the mouth (Whitfield & Bate 2007).

1.3.2 Predominantly open estuaries

Predominantly open estuaries (POE) are the second most dominant estuary type along the South African coastline (Harrison 2002, van Niekerk et al. 2019) and mostly dominate the northern hemisphere along the North American and European coastlines (Potter et al. 2010). These systems are connected to the sea for more than 90% of the time and are characterised by a moderate tidal prism with amplitude ranges between 0.7 and 1.5 m, along with markedly varying channel sizes (10 to 7500 ha) (Whitfield 1992, van Niekerk et al. 2019). Such systems are supported by larger catchments (> 500 km²) that experience high runoffs throughout the year, in contrast to TCEs (Whitfield 1992, Whitfield & Bate 2007).

Generally, POEs have relatively wider mouth sizes, with freshwater inflow volumes being a primary factor influencing mouth dynamics (Cooper 2001). The primary drivers of mixing processes in POEs include both riverine and tidal inputs which create a marked vertical and horizontal salinity gradient along the estuary (Whitfield 2005). Predominantly open estuaries exhibit oligohaline conditions in the upper reaches, while middle reaches are characterised by brackish conditions and lower reaches are marine dominated (Whitfield 1992, Teske & Wooldridge 2001). Reduced freshwater inflow can shrink the oligohaline zone, causing the estuary to exhibit predominantly brackish to marine conditions (Scharler et al. 2023). During this time, some systems may develop a constricted mouth area and occasionally experience mouth closure (Kelbe & Taylor 2019, Scharler et al. 2023).

1.4 Estuarine health

Estuarine health provides a description of the extent to which an estuary has been modified from its original pristine state or established reference condition if the original state is unknown (Costanza 1992, Turpie et al. 2012). The assessment of estuarine health includes evaluating changes in the physical structure (e.g. morphology and habitats), as well as the ecosystem functions and processes, which are influenced by physical, chemical, biological, geographic and climatic factors (Costanza & Mageau 1999, Borja et al. 2012, Turpie et al. 2012). A healthy estuary is characterised by the absence of detrimental changes, the presence of biological communities reflective of its reference condition, functional redundancy (i.e. different species fulfilling similar ecological roles) and the persistence of high trophic level organisms (Elliott & Whitfield 2011, Thompson et al. 2012). In a healthy state, an estuarine ecosystem functions effectively, maintaining its natural variability and support stable and resilient communities (Turpie et al. 2012). The presence of resilient communities and the absence of destructive factors enable the estuary to sustain its ecological functions and processes, thereby enhancing its ability to recover from any potential stress (Elliott & Whitfield 2011, Turpie et al. 2012).

The Estuarine Health Index (EHI) is used for the quantitative assessment of estuarine health, integrating multiple ecological indicators into a comprehensive health score (Cooper et al. 1994, Turpie et al. 2012). The estuarine health score is determined through the evaluation of abiotic and biotic components, which include water quality parameters (e.g. salinity, temperature, dissolved oxygen), habitat diversity, biological communities and the presence of key indicator species (Cooper et al. 1994, Turpie et al. 2012). The determination of the EHI also involves the assessment of both historical and current data to capture temporal changes. This approach ensures that the assessment reflects the natural variability of the estuarine system and the impacts of anthropogenic influences, such as pollution and habitat modification (Turpie et al. 2002, van Niekerk et al. 2019). The scoring system employs a weighted index approach, assigning scores to each component based on its importance to the overall health of the estuary. For example, all abiotic and biotic components are each scored on a scale from 0 to 50, where a score of 50 indicates optimal conditions (i.e. high biodiversity and good water quality), while lower scores reflect degraded conditions (i.e. poor water

quality or a lack of key species) (Adams et al. 2002, Turpie et al. 2002). The final EHI is translated into present ecological status (PES) by summing the weighted scores of all indicators, providing a combined score that can range from ≤ 20 (F, critically modified) to ≥ 90 (A, unmodified) (Table 1.1) (Turpie et al. 2002, van Niekerk et al. 2019).

Table 1.1: Description of Estuarine Health Index (EHI) and Present Ecological Status (PES) estuary health categories (A to F), adopted from Turpie et al. (2002) and van Niekerk et al. (2019). A denotes an unmodified system and F denotes a critically modified system.

EHI Score	PES Category	Description
≥ 90	A	Unmodified. System remains in its natural state. No anthropogenic alterations to abiotic and biotic processes.
90 – 75	B	Near natural with modification. Slight alterations in natural habitats and biota, however, the fundamental functioning of the ecosystem remains intact.
75 – 61	C	Moderately modified. A minor transformation and loss in natural habitat and sensitive biota, ecosystem function is largely unaltered.
60 – 41	D	Heavily modified. Substantial loss in natural habitat and ecosystem function. Sensitive biota has been completely replaced by tolerant biota.
40 – 21	E	Severely modified. Ecosystem function is severely compromised. Extensive loss of natural habitat and biota
≤ 20	F	Critically modified. Modifications to the system have reached a critical threshold and basic ecosystem function been completely compromised. Natural abiotic processes and biota are lost. Such modifications are often irreversible.

The 2018 National Biodiversity Assessment revealed that approximately 63% of South African estuarine areas are classified as heavily modified (Category D), with 17% in a near-natural state (Category B) and 5% remaining completely natural (Category A) (van Niekerk et al. 2019). When considering the number of estuaries, however, 21% are still classified as pristine (Category A), while 41% are slightly modified (Category B), with the majority located in the warm temperate region, followed by subtropical estuaries. In KZN, most estuaries are classified as moderately modified (Category C) to critically modified (Category F), with only 16% in a near-natural state (Category B) (van Niekerk et al. 2019). The majority of heavily and severely/critically (Category E and F) modified estuaries are found in the cool temperate

region, particularly small systems like fluvially dominated, large and small temporarily closed and arid predominantly closed estuaries. These estuaries exhibit clear signs of degradation due to anthropogenic pressures (van Niekerk et al. 2019).

A high number of pristine estuaries are found in the warm temperate region. This can be attributed to the lack of urbanisation and industrial development in large coastal areas (van Niekerk et al. 2019). On the contrary, the subtropical estuaries show signs of degradation, primarily due to land-use related pressures such as pollution, sand mining and intensive sugar cane cultivation. (van Niekerk et al. 2019). However, the system degradation observed in subtropical estuaries is less compared to those in the cool temperate region (van Niekerk et al. 2019).

These anthropogenic pressures directly and indirectly impact estuarine health by altering water quality and biological communities. For example, studies conducted on heavily modified systems such as the uMdloti (Category D) and uMvoti Estuary (Category D) have shown that pollution, flow modification and sand mining disrupt hydrodynamics, degrade water quality and lead to habitat loss (Taljaard et al. 2017, Brooker & Scharler 2020, Mckelvey & Mackay 2025). Similarly, nutrient pollution in estuaries like the uMlalazi and uMdloti estuaries has been linked to shifts in biological communities, including the loss of key macrobenthic species and the occurrence of microalgal blooms (Mabaso 2002, Nozais et al. 2001). However, the overall health status of estuaries is not always determined by a single factor. For instance, Mckelvey & Mackay (2025) found that estuaries experiencing high sand mining pressure could still maintain relatively good health, while Mabaso (2002) suggested that despite shifts in biological communities and key species loss, uMlalazi Estuary remained in a near-natural state.

1.5 Current threats to estuaries

Estuaries are among the most degraded ecosystems globally due to a variety of natural and human-induced pressures (Gillanders & Kingsford 2002, Kennish 2002). There are three major pressures which are considered to rapidly deteriorate estuarine ecosystems namely, increasing urbanisation and industrialisation, increasing human population around the coast and impacts of climate change (Elliott et al. 2019, Defeo & Elliott 2021). Human population heavily relies on estuaries for different uses such as economic and social needs (Bocquier 2005, Swemmer 2011, van Niekerk et al. 2019, Lonsdale et al. 2022). Bianchi (2007) predicted that by 2025 about 75% of the human population will be living within coastal zones, while most of the population will dwell along the major rivers. Key pressures include pollution from agricultural runoff and urban development, habitat destruction due to coastal development and the effects of climate change such as droughts, floods and rising sea levels (van Niekerk et al. 2019, 2022). While both natural and anthropogenic factors impact estuarine environments, anthropogenic pressures are generally more detrimental (van Niekerk et al. 2019).

The synergistic effects of these pressures can lead to severe degradation of estuarine health, for example, nutrient loading from runoff can promote algal blooms, which deplete oxygen levels and harm aquatic life (Lemley et al. 2015, Nunes et al. 2022a, van Niekerk et al. 2022). Similarly, habitat loss from coastal development reduces the resilience of estuaries to climate change, making them more vulnerable to flooding and erosion (Kennish 2002, Dau et al. 2023, Roy et al. 2023). Over time, the cumulative impact of these interacting pressures can result in irreversible changes to estuarine ecosystems, significantly altering their structure and function (Elliott & Whitfield 2011). van Niekerk et al. (2019) identified six major threats to South African estuaries namely, freshwater inflow modification, overfishing and bait collection, pollution, habitat loss, climate change and biological invasion. For this study, freshwater modification, pollution and biological invasions are considered as the relevant and prominent pressures affecting various estuarine components of the investigated systems based on their direct and documented influence on the macrobenthic communities of the uMlalazi (Mabaso 2002) and uMdloti estuaries (Blaber et al. 1984, Scharler et al. 2020).

1.5.1 Flow modification

Flow modification refers to the alteration in the amount of freshwater entering the estuary, which could be an increase or decrease in water volumes received (Gudmundsson et al. 2019). Freshwater flow modifications may result from anthropogenic activities such as water abstraction through dams, urbanisation, agricultural and industrial developments and climate-induced pressures like droughts and floods (van Niekerk et al. 2012, Haddeland et al. 2014, Stein et al. 2021). Among these, upstream diversions and abstractions are the leading causes of reduced freshwater reaching estuaries (Gillanders & Kingsford 2002, Pollack et al. 2011a, Chilton et al. 2021, Clark et al. 2022). Additionally, human developments in and around estuarine systems, including wastewater treatment works (WWTW), agricultural runoff and industrial stormwater discharge, can elevate freshwater flow (Brooker & Scharler 2020, Nunes et al. 2022a). These discharges typically occur either directly into the estuary or upstream in the catchment areas, further impacting the hydrology and ecological balance of the estuarine environment (Gillanders & Kingsford 2002, Brooker & Scharler 2020, Nunes et al. 2022a).

Climate-related factors such as sea-level rise, frequent sea storm surges and floods elevate water levels and flow in coastal areas (Chust et al. 2011, Filho et al. 2022, van Niekerk et al. 2022, Roy et al. 2023). Rapid climate change alters tidal ranges in coastal areas, leading to more frequent flooding of marginal zones (Payne et al. 2016, van Niekerk et al. 2022). Rising sea levels and increased storm activity are reshaping estuarine geomorphology and physico-chemical characteristics through processes such as sediment erosion and seawater intrusion into upper reaches during low flow periods (Khojasteh et al. 2021, van Niekerk et al. 2022, Roy et al. 2023). These changes, including excessive inundation and elevated salinity directly affect community composition and distribution within the estuarine system (Khojasteh et al. 2021, Costa et al. 2022).

1.5.2 Pollution

Flows derived from anthropogenic activities have been recognised as a prominent source of pollution and a threat to the water quality of estuaries globally (Kennish 2002, Paerl et al. 2014, Lemley et al. 2016). In South Africa, like other countries, municipal and industrial wastewater, stormwater runoff and agricultural flows are major contributors to water quality degradation (Van Niekerk & Turpie 2011, Adams et al. 2020, Claassens et al. 2020). These flows introduce bacteria, harmful metals and pharmaceuticals while elevating nutrient concentrations, leading to eutrophication, hypoxia and anoxia, which in turn disrupt the ecological functions of the system (Cloern 2001, Lemley et al. 2017, Adams et al. 2019, Schmitz et al. 2024).

Agricultural runoff has significant impacts on estuaries, primarily through the introduction of excess nutrients and sediments (Lemley et al. 2014, Freeman et al. 2019, Olisah et al. 2021). The runoff is often rich in nitrogen and phosphorus from fertilisers and stimulates eutrophication which promotes the excessive growth of algae (Rabalais et al. 2010, Lemley et al. 2014, Glibert 2018, Adams et al. 2020, Ponnusamy et al. 2023). Algal blooms can reduce sunlight penetration and lead to oxygen depletion in the water, causing hypoxic or anoxic conditions that harm aquatic life (Coffin et al. 2017, 2018, Roman et al. 2019, Lemley & Adams 2020). Studies show that when agricultural runoff, particularly from fertilisers and pesticides, is the primary source of nutrient input, the water quality tends to be moderately degraded, with instances of severe modification occurring under intense agricultural practices (De Villiers & Thiar 2007, Lemley et al. 2014).

Discharge from WWTWs is considered a leading cause of deteriorating water quality in South African estuaries with 840 million litres of effluent discharged in estuaries daily (van Niekerk et al. 2019). The rapid increase in human population, along with demands for municipal waste disposal, deteriorating infrastructure, clogging of WWTWs pump stations and lack of skilled operators for WWTWs infrastructures, are the main causes of excessive volumes of WWTWs discharge (Paerl 2006, Van Der Merwe-Botha & Quilling 2012, Robb 2014). Over 50% of the impacted estuaries are located in the subtropical region, particularly in urbanised areas, with many of them being TCEs (van Niekerk et al. 2019). TCEs are especially vulnerable to excessive nutrient inputs due to their small tidal prism, extended water residence times and prolonged

periods of mouth closure. These factors allow nutrients from urban runoff and other discharges to accumulate, exacerbating the risks of eutrophication and other ecological impacts (Paerl 2006, Tweedley et al. 2016, Warwick et al. 2018).

1.5.3 Biological invasions

Biological invasions are a significant global threat to coastal ecosystems, driving biodiversity loss and altering ecosystem structure and function (Vitousek 1990, Mooney & Cleland 2001, van Wilgen et al. 2020, Whitfield et al. 2021). These are taxa that are accidentally or intentionally introduced through human intervention referred to as alien species. In some instances, these introduced species establish, thrive and spread from the initial point of introduction, eventually becoming invasive (Williams & Grosholz 2008, Pyšek et al. 2010). A number of invasive alien species have been reported in South African estuaries, including several macrobenthic invertebrates such as the polychaete *Ficopomatus enigmaticus*, the gastropod *Tarebia granifera*, and the bivalve *Corbicula fluminalis* (van Niekerk et al. 2019, Robinson et al. 2020, Whitfield et al. 2021). These organisms can spread passively, such as *T. granifera* being dispersed via bird droppings or water currents, or actively through human activities like recreational boating, which aids their movement to new areas (Appleton et al. 2009, Whitfield et al. 2021). Additionally, the development of hard structures, such as piers, facilitates the colonisation of *F. enigmaticus* (Schwindt et al. 2004).

KwaZulu-Natal estuaries, particularly TCEs, are highly susceptible to biological invasions due to degraded habitats and poor water quality (Paavola et al. 2005, Crooks et al. 2011, Frü et al. 2012, van Niekerk et al. 2019). Alien species often succeed in these environments because of their broad tolerance to various natural and human-induced stressors, such as pollution and extreme temperature or salinity fluctuations caused by climate-related changes (Miranda et al. 2010). These conditions place additional physiological stress on native species, leading to reduced population densities or, in severe cases, local extinctions (Miranda et al. 2010, Crooks et al. 2011). Invasive species possess traits that aid their successful establishment in new environments, such as rapid reproduction (r-strategy), phenotypic plasticity and high dispersal potential (Miranda et al. 2011, David et al. 2017, Peters et al. 2017).

Several concerns have been raised regarding the impact of invasive species, including their ability to outcompete native species for food and space, introduce pathogens and diseases (Mack et al. 2000), cause alterations in community structure (Perissinotto et al. 2014) and trophic networks (David et al. 2017, Gerber et al. 2024). For example, the native gastropod *Melanooides tuberculata* is known to co-occur with the invasive *T. granifera*, however, it is often outcompeted when unfavourable conditions occur. Similarly, *T. granifera* was reported to thrive and outcompete *Assiminea ovata* during a drought period that resulted in hypersaline conditions in the St Lucia Estuary (Miranda et al. 2011). *Tarebia granifera* is also known to modify habitats by changing the physical characteristics of the habitats they invade (McQuaid & Griffiths 2014, Whitfield et al. 2021) through forming dense populations in sediments, which alter sediment properties and disrupt local biodiversity (Appleton et al. 2009). Similarly, the polychaete *F. enigmaticus* builds extensive calcareous reefs on hard substrates which transform soft-sediment environments, altering species composition and ecosystem dynamics (Schwindt & Iribarne 2000, McQuaid & Griffiths 2014).

1.6 Macrozoobenthos communities as indicators of estuarine ecosystem health

Macrozoobenthic invertebrates are organisms larger than 0.5 mm in size and dwell at the bottom of water bodies (Perissinotto et al. 2004). Macrozoobenthos can be categorised into two groups namely, the infauna which reside within the sediment and epifauna which live on the sediment surface (Day 1981, Perissinotto et al. 2004, Cleto-Filho & Arcifa 2006). They are slow-moving creatures with a restricted physiological activity range and comprise diverse groups including polychaetes, oligochaetes, crustaceans and molluscs (Perissinotto et al. 2010). Their ecological characteristics such as relatively long life cycles, sedentary nature with limited mobility, habitation of a wide range of microhabitats and easy sampling effort, make them ideal indicators for assessing ecological changes and monitoring the health of estuarine environments (Wildsmith et al. 2009, Fitch & Crowe 2010, Veiga et al. 2016).

Macrozoobenthic organisms play a critical role in various ecological processes essential for the functioning of estuarine ecosystems (McLusky & Elliott 2004). They are significant contributors to the structure and dynamics of the estuarine food web (McLusky & Elliott 2004, Gray & Elliot 2009, Ortega-Cisneros 2013) and serve as ecological engineers (Sokołowski et al.

2012, Ortega-Cisneros 2013, Horn et al. 2017, Szczepanek et al. 2021). Additionally, macrofaunal organisms influence various biogeochemical processes necessary for the benthic environment and the overlying water column (Braeckman et al. 2010, Politi et al. 2019). These processes occur during the reworking and mixing of sediment particles through activities such as feeding, burrowing and irrigation (Gray & Elliot 2009, Hölker et al. 2015, Benelli et al. 2017, Martins & Barros 2022). For instance, tubificid worms enhance nutrients and organic matter processing by ingesting and excreting sediment (i.e. clay and silt), thereby redistributing them to various sediment layers (Mermillod-Blondin et al. 2001, Dafoe et al. 2011). Meanwhile, crustaceans such as sand prawns contribute to the oxygenation of benthic environments through their respiratory activities (Njozela 2012, Hölker et al. 2015). They achieve this by pumping and filtering sediment through the walls of their burrows, which enhances the exchange of gases and nutrients in the sediment (Adámek & Maršálek 2013, Venter et al. 2020).

Monitoring various community attributes such as abundance, richness, diversity, evenness and composition allows for the assessment of change over time corresponding to natural and anthropogenic changes (Dauer 1993, Elliott et al. 2007, Lohrer et al. 2012). A healthy environment remains within the fauna's physiological limits to withstand environmental stress and is capable of continually sustaining a stable and diverse community (Pearson & Rosenberg 1978, Lu & Wu 2007, Shivarudrappa & Briggs 2021). Diverse communities often support a range of life-history species, however, under stable environmental conditions, they tend to favour species with K-selected traits, such as slow growth, long lifespans, and active parental care (Pearson & Rosenberg 1978, Raymond 2022).

When stress is introduced, there is a decrease in diversity where sensitive species are eliminated first and are replaced by stress-tolerant or flexible r-selected strategists (Pearson & Rosenberg 1978, Raymond 2022). Opportunistic species are typically small-bodied and short-lived individuals which exploit disturbed environments through two successional stages (Pearson & Rosenberg 1978, Raymond 2022). The first-order opportunistic species serve as the primary pioneers of perturbed environments, establishing themselves shortly after a disturbance. They are succeeded by second-order opportunists that invade the environment at a later stage and compete with the first-order pioneers for resources (Pearson & Rosenberg 1978, Rosenberg et al. 2002, Raymond 2022).

1.6.1 Estuarine macrobenthos distribution and the response to environmental change

The distribution patterns and diversity of benthic communities in estuaries are determined by both abiotic and biotic components (McLusky & Elliott 2004). These components result in significant variation in faunal composition and abundance over spatial scales, from meters to kilometers and temporal scales, from days to years, due to their dynamic interaction (Bursey & Wooldridge 2003, McLusky & Elliott 2004, Cleto-Filho & Arcifa 2006). Key drivers of spatial distribution include gradients in salinity and sediment composition (Teske & Wooldridge 2003), which influence the structuring of benthic communities by selecting species adapted to specific physico-chemical conditions (Mannino & Montagna 1997, Allanson & Baird 1999, Teske & Wooldridge 2001, 2003, McLusky & Elliott 2004). Consequently, distinct communities occupy different regions of the estuary based on their tolerance to prevailing environmental conditions (Kraft et al. 2015).

In South African estuaries, the mouth region is inhabited by diverse communities of marine origin, the middle reaches support estuarine taxa and the upper regions are inhabited by taxa of freshwater origin (Mackay & Cyrus 1998, Teske & Wooldridge 2003). The estuarine group is eurytolerant and ubiquitous, comprising species such as polychaetes (*Ceratonereis (Composetia) keiskama* (hereafter *Ceratonereis keiskama*) and *Desdemona ornata*), amphipods (*Americorophium triaenonyx* and *Grandidierella lignorum*), tanaids (*Halmyrapseudes digitalis*) and the gastropod *Assiminea ovata* (Teske & Wooldridge 2004). These species are considered key estuarine taxa, particularly in the subtropical estuaries of KZN where they dominate majority of estuaries (Teske & Wooldridge 2003, Perissinotto et al. 2010).

Predominantly open estuaries exhibit greater diversity than TCEs attributed to a consistently open mouth facilitating continuous movement of marine groups and providing diverse niches along the salinity gradient (Teske & Wooldridge 2001). Mouth closure in TCEs prevents marine recruitment which may lead to a complete elimination of marine assemblages, where the estuarine taxa inhabit the mouth region, particularly in perched systems (Blaber et al. 1984, Vivier 2010, Stow 2011, Scharler et al. 2020). During the open-mouth phase, marine communities are introduced into the system, increasing biodiversity but can be associated

with reduced macrobenthos densities (Perissinotto et al. 2004, 2010, Stow 2011, Scharler et al. 2020). Upon mouth closure, the community transitions back to estuarine and freshwater assemblages. In river-dominated systems with a perched mouth region, extended periods of mouth closure result in the decline of estuarine assemblages and a predominance of freshwater communities (Scharler et al. 2020).

Sediment plays a crucial role in providing habitat and as a food source for macrobenthic organisms (Gray & Elliot 2009). Benthic species utilise sediment to construct protective structures such as tubes and burrows, providing shelter against predators and harsh environmental conditions (Kristensen & Kostka 2005, Gray & Elliot 2009). Burrow construction is influenced by factors such as feeding behaviour, activity levels, environmental conditions and sediment composition (Zoutendyk & Bickerton 1988, Gray & Elliot 2009, Venter et al. 2020).

Sandy substrates are considered unstable and low in organic matter, making them less hospitable to most organisms (Gray & Elliot 2009). These sediments are commonly inhabited by fast-burrowing, filter-feeding bivalves from the Verenidae family or deep-burrowing crustaceans like *Kraussillichirus kraussi* (Zoutendyk & Bickerton 1988, Gray & Elliot 2009). Sediments composed of medium to fine sand grains or heterogeneous particle sizes tend to support high macrobenthic diversity (Teske & Wooldridge 2003, Gray & Elliot 2009). These substrates provide a variety of microhabitats, such as burrows, crevices and spaces between particles, allowing different species to thrive (Edgar & Barrett 2002, Raymond 2022). The diversity of grain sizes creates niches for a range of feeding strategies, including filter-feeding, deposit-feeding and scavenging, promoting species coexistence (Hagerthey et al. 2002, Ysebaert et al. 2003, Gray & Elliot 2009).

In contrast, muddy substrates with tightly packed grain sizes promote extensive microbial activity, increasing oxygen demand and resulting in low oxygen levels which supports species tolerant of hypoxic conditions, such as oligochaetes. In addition, fine particle size and compact nature of muddy substrate pose challenges such as smothering of filtering mechanisms during locomotion, feeding and respiration (Gray & Elliot 2009).

Similarly, estuarine benthic organisms encounter a wide range of oxygen levels, from optimal concentrations ($> 5 \text{ mg}\cdot\text{l}^{-1}$) to hypoxic conditions ($< 3 \text{ mg}\cdot\text{l}^{-1}$) and anoxic levels ($\leq 1 \text{ mg}\cdot\text{l}^{-1}$)

(Whitfield & Bate 2007, DWS 2015). Under normoxicity, the benthic environment is characterised by thriving communities, inclusive of key taxa that dominate ecological processes such as sediment engineering, water filtration and nutrient cycling (Norkko et al. 2013, Gogina et al. 2014, Hale et al. 2016). As oxygen levels decline, the abundance and diversity of taxa may initially increase due to the infiltration of opportunistic species tolerant of oxygen-deficient habitats (Diaz & Rosenberg 1995, Hale et al. 2016, Tweedley et al. 2016). However, a significant decline in abundance and diversity occurs after prolonged hypoxic and anoxic conditions, affecting ecological processes and energy flow (Diaz & Rosenberg 1995, Taylor & Eggleston 2000, Gogina et al. 2014). Systems affected by hypoxic conditions support benthic communities dominated by small-bodied, r-selected taxa such as the polychaete *Capitella capitata* and oligochaetes, which are better adapted to prolonged hypoxia (Sturdivant 2011, Ahamefula 2012, Shivarudrappa & Briggs 2021). Sensitive species, such as brachyuran crustaceans, which can only tolerate mild, short-lived hypoxia, are the first to be eliminated before r-selected communities colonise (Llanos 1992, Levin et al. 2009, Ahamefula 2012, Tweedley et al. 2016, Collins et al. 2019).

1.7 Motivation and Problem statement

Over half of KwaZulu-Natal's estuarine systems are currently degraded due to a combination of human activities and climatic pressures, including freshwater abstraction, pollution, sand mining, intensive agriculture, fishing and droughts (van Niekerk et al. 2019). These stressors have led to habitat loss, frequent estuary mouth closures and nutrient enrichment, fundamentally altering the physico-chemical environments of these estuaries (Lemley et al. 2014, Scharler et al. 2020, van Niekerk et al. 2022). As a result, macrobenthic communities, which are essential for ecosystem functions such as sediment material cycling and food web energy flow, are under severe threat (Scharler et al. 2020, Ndhlovu et al. 2024). The effects of these pressures have been notable in the decline of endemic invertebrate species such as *Scylla serrata*, *Kraussillichirus kraussi*, *Upogebia capensis*, *Varuna litterata* and *Solen capensis* (van Niekerk et al. 2019). These taxa are prominent in performing ecosystem processes as keystone species and have been identified as threatened in South African estuaries (Wynberg 1991, Njozela 2012, Nel & Branch 2013).

The uMdloti Estuary is an example of a heavily modified system which has been significantly impacted by these pressures, leading to frequent mouth closure, hypoxic conditions and eutrophic events (Stretch & Zietsman 2004, Anandraj et al. 2008, Brooker & Scharler 2020, Scharler et al. 2023). Similarly, while the uMlalazi Estuary remains in relatively good ecological health, it has experienced mouth closures, hypoxia, high turbidity and algal blooms (Hill 1966, Mabaso 2002, Ortega-Cisneros 2013, Scharler et al. 2023). These ongoing challenges have led to a decline in the macrobenthos community diversity, abundance and assemblage, potentially affecting various ecological processes (Scharler et al. 2020, Gerber 2023). This raises concerns about the long-term health and sustainability of these systems, especially as these pressures are expected to intensify.

The contrasting conditions of the two estuaries offer a valuable opportunity for evaluation the macrobenthic community responses to varying degrees of key estuarine health threat namely freshwater modification, pollution, and biological invasions across different estuarine contexts. The findings will inform the ecological knowledge of macrobenthos communities in TCEs and POEs, providing valuable insight for effective conservation and management strategies within the biogeographical region. Additionally, the findings help bridge the gaps in macrobenthic data inventories for South African estuaries, as highlighted by the 2018 NBA.

1.8 Aim and objectives

This thesis aims to investigate the temporal and spatial variability in macrobenthos communities of a heavily modified large temporarily closed estuary (uMdloti Estuary) and a predominantly open estuary in a near-natural health state (uMlalazi Estuary).

This was achieved through the completion of the following objectives:

1. Analyse and quantify macrobenthic community from uMdloti and uMlalazi Estuary with regards to species composition, abundance and diversity.
2. Identify the spatial and temporal (seasonal) patterns of the macrobenthic community.
3. Explore possible relationships between macrobenthic communities and prevailing environmental conditions.

It was hypothesised that the macrobenthic communities in the uMdloti Estuary exhibit greater seasonal variability due to frequent mouth closure, whereas the uMlalazi Estuary show a more consistent pattern. Additionally, the spatial distribution of macrobenthic species are influenced by environmental gradients in both estuaries, with the uMlalazi Estuary showing greater spatial variability compared to the uMdloti Estuary.

1.9 Thesis layout

Chapter 1:

This chapter offers knowledge of estuaries along the coastline of South Africa with special attention to the estuaries in KwaZulu-Natal and the subtropical climatic region. This chapter also provides information on the distribution of macrozoobenthic communities in estuaries and their relationship with the estuarine environment.

Chapter 2:

Provides a description of the geographical area and location of the study sites. It also offers detailed outlines of field and laboratory protocols adopted for the current study. The section describes the data analysis methods applied to the environmental and biological data.

Chapters 3 and 4:

Focus on the spatial and temporal patterns of the macrozoobenthos of the uMdloti and uMlalazi Estuary, along with the related environmental variables. The investigation involves studying the community's composition and distribution patterns, which are linked to the environmental parameters measured.

Chapter 5:

General discussion, conclusion, and recommendation of potential research avenues from the findings in the previous chapters.

Chapter 2: General methods and materials

2.1 Study area

The study area is located in the subtropical region of KwaZulu-Natal (KZN) province on the east coast of South Africa. This coastal stretch comprises 16 POEs and 45 LTCEs and STCEs, shaped by the region's unique geomorphology and hydrology (van Niekerk et al. 2019). The climate and coastal waters are heavily influenced by the warm, south-flowing Agulhas Current, which affects local oceanography and biogeography (Harrison 2002). The KZN coast is classified as a summer rainfall zone (SRZ) and experiences highly seasonal rainfall, with annual precipitation ranging from 600 to 1200 mm, making it the wettest province in South Africa (Nash et al. 2016, Roffe et al. 2021).

The region is characterised as a microtidal coast, where semidiurnal tides dominate (Cooper 2001, McSweeney et al. 2014, 2017). Tidal fluctuations range from 0.8 m during neap tide to 1.8 m during spring tide, which plays a crucial role in the hydrodynamics and ecological functioning of the estuaries (Allanson & Baird 1999, Rautenbach et al. 2019). The study focuses on two estuary types, the large temporarily closed uMdloti Estuary and the permanently open uMlalazi Estuary (POE), located approximately 109 km apart (Figure 2.1). Both estuaries are influenced by various land use activities in and around their estuarine functional zones, particularly agricultural practices, urbanisation and industrialisation, tourism and recreation and mining and industrial waste (van Niekerk et al. 2019).

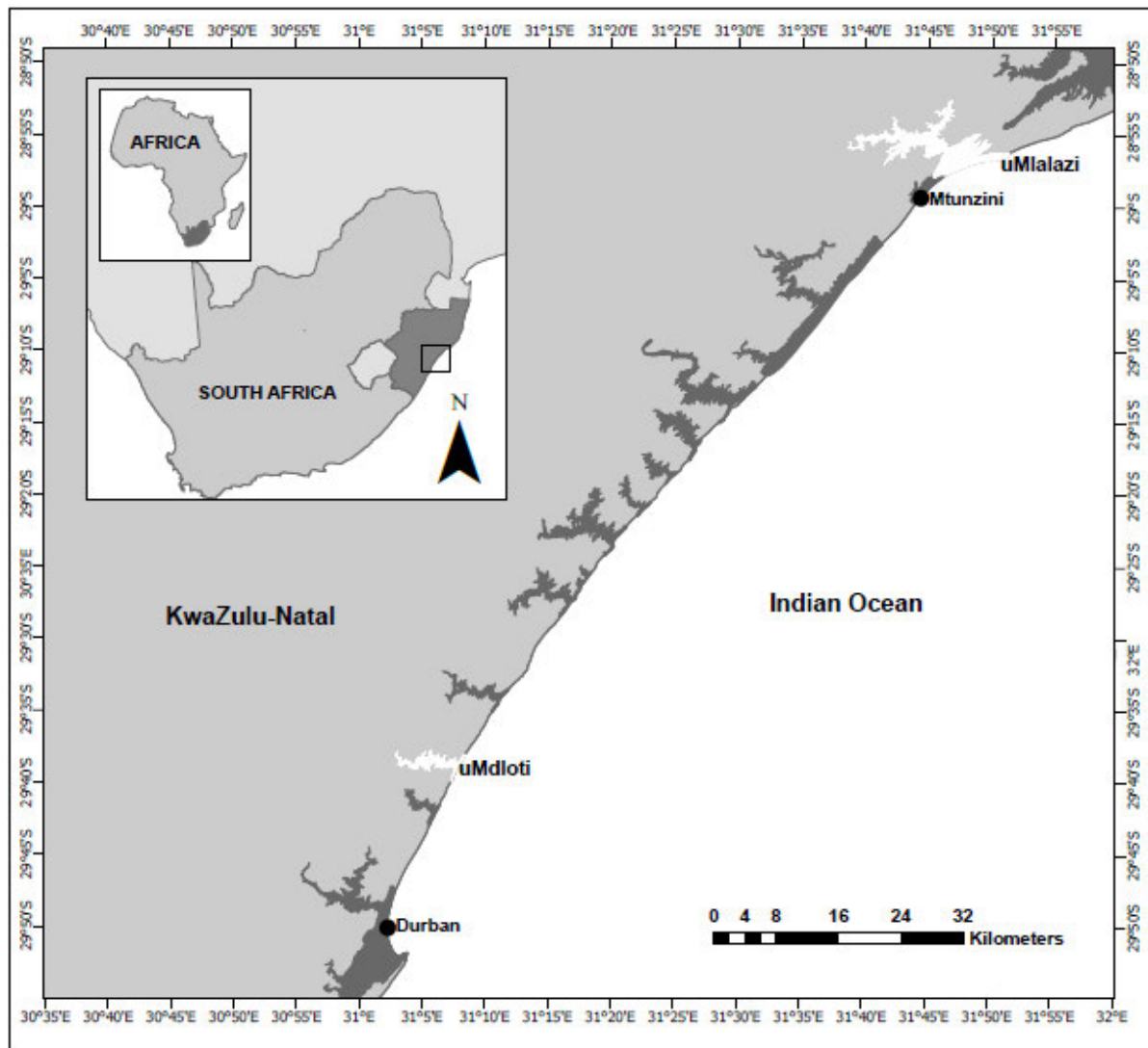


Figure 2.1: Map showing the location of the two study systems, uMlalazi and uMdloti Estuaries, on the east coast of South Africa.

2.3.1 Environmental variables- field sampling procedure

The uMdloti and uMlalazi Estuaries were sampled on a quarterly basis, with sampling conducted once a month during the dry season (June to August 2019) and the wet season (December 2019 to March 2020). To assess the spatial and temporal variability in environmental conditions across the estuaries, physico-chemical parameters were measured at the same time near the bottom of the water column at each site. A YSI multiprobe (model 6600) was employed to record environmental parameters such as temperature ($^{\circ}\text{C}$), pH and dissolved oxygen (DO) concentration ($\text{mg}\cdot\text{L}^{-1}$). Salinity (ppt) measurements were taken using

a refractometer. Phytoplankton chlorophyll-a concentration was quantified by collecting two replicates of subsurface water samples (~50 cm) from each site using 1 L bottles. Additionally, two replicates of 1 L water samples were taken for the analysis of suspended particulate organic matter (POM) and total suspended solids (TSS). All water samples were stored on ice and processed immediately upon arrival at the laboratory.

To estimate microphytobenthos (MPB) chlorophyll-a, duplicate sediment samples were collected from the top 1 cm of sediment using a twin corer and transferred into bottles containing 30 ml of 90% acetone. These samples were stored in a dark, cold environment for 96 hours to facilitate chlorophyll-a extraction (Nozais et al. 2001, Perissinotto et al. 2006). For the determination of sediment organic matter and grain size, two sub-samples were taken from the top 5 cm of the grab sample and preserved in 4% formalin to prevent decomposition and consumption by benthic invertebrates (Mackay 1996). All sediment samples were frozen before laboratory analysis.

2.4 Laboratory procedures

2.4.1 Total suspended solids (TSS) and suspended particulate organic matter (POM)

Whatman GF/F filter papers with a pore size of 1.2 µm were pre-combusted at 460 °C for 6 hours to remove any organic matter and then weighed. The filter papers were then cooled in a desiccator and weighed to record the initial mass. For the determination of TSS, 250 ml of each replicate water sample was filtered through the pre-weighed filter paper. The filter papers were then oven-dried at 60 °C for 6 hours to remove water content (Carrasco 2011). The dried filters were weighed again and the TSS, expressed in milligrams per litre (mg·l⁻¹), was calculated using the following formula:

$$TSS (mg \cdot l^{-1}) = \frac{TSS(g) \times 1000}{Volume \text{ filtered}}$$

In this formula, TSS (g) represents the difference between the pre- and post-dried weights of the filter paper. To determine POM, the same filter papers were further combusted at 460 °C

for 6 hours to burn off organic material (Carrasco 2011). The filter papers were weighed again and the weight loss due to combustion was used to calculate POM as follows:

$$POM (\%) = \frac{Initial\ dry\ weight - Ash\ weight}{Initial\ dry\ weight} \times 100$$

2.4.2 Phytoplankton (PP) chlorophyll-a and microphytobenthos (MPB)

A 250 ml water sample was filtered to measure total chlorophyll-a concentration using Whatman GF/F filters with a pore size of 1.2 µm. After filtration, the filter papers were placed in centrifuge tubes containing 8 ml of 90% acetone and stored in a dark, cold refrigerator (at approximately 10 °C) for 96 hours to allow for chlorophyll-a extraction. Following the extraction period, chlorophyll-a concentration was measured using a Turner 10-AU fluorometer, utilising the non-acidification procedure (Nozais et al. 2001). This method is highly sensitive to chlorophyll-a, reducing sensitivity to accessory pigments like chlorophyll-b and phaeopigments during chlorophyll-a measurements. It provides reliable chlorophyll-a values with only a 10% error and eliminates the need for acid correction steps required in traditional acidification method (Welschmeyer 1994).

Samples collected in muddy substrate or turbid stations would often clog filters due to high particulate matter. In such cases, less than 250 ml of water was filtered and the exact volume filtered was recorded and incorporated into the chlorophyll-a calculation. The chlorophyll-a concentration was then expressed as mg·m⁻³ using the following formula:

$$PP\ chlorophyll - a = displayed\ value \times \frac{AC(ml)}{Filtered\ Water\ (ml)}$$

Where:

- Displayed value is the reading on the fluorometer,
- AC (ml) is the volume of acetone used for extraction,
- Filtered Water (ml) is the actual volume of water filtered during the process.

To estimate Microphytobenthos (MPB) chlorophyll-a, the sediment sample containing 8 ml of acetone from each sample was centrifuged using the Eppendorf Centrifuge 5810 R at 4000 RPM for 30 minutes to allow sediment particles to settle (Nozais et al. 2001). The supernatant

was used for fluorometric analysis using a Turner Designs 10-AU fluorometer. The chlorophyll-*a* concentration was expressed as $\text{mg}\cdot\text{m}^{-2}$ using the following formula:

$$\text{Microphytobenthos chl} - a = \frac{\text{displayed value}}{1000} \times \text{dilution} \times \frac{AC(\text{ml})}{\text{Core Area}(\text{m}^{-2})}$$

Where:

- Displayed value is the reading on the fluorometer,
- Dilution is the dilution factor applied during sample preparation,
- AC (ml) is the volume of acetone used for extraction,
- Core Area (m^2) is the surface area of the core area used to collect sediment.

2.4.3 Sediment grain size

Sediment grain size analysis was conducted by Durban Environmental Mapping and Surveying Pty Ltd. The sediment was classified into seven categories based on the grain size classification systems of Wentworth (1922) (see Table 1).

Table 2.1: Sediment grain-size characterisation according to the (Wentworth 1922) grain scale:

Sediment type	Grain size (mm)
Gravel	>2 mm
very coarse sand	<2 mm >1 mm
Coarse sand	<1 mm >500 μm
Medium sand	<500 μm >250 μm
Fine sand	<250 μm >125 μm
Very fine sand	>125 μm >63 μm
Mud	< 63 μm

2.4.4 Total organic content

Sediment TOC was determined using the semi-quantitative loss on ignition method, a widely accepted approach in sediment analysis (Heiri et al. 2001, Schumacher 2002). This method determines organic matter content by measuring the weight loss of dried sediment samples after combustion (Schumacher 2002). The sediment was first homogenised and transferred into a pre-weighed crucible. The wet weight of the sediment with the crucible was recorded. The samples were then dried at 60 °C for 48 hours to remove moisture, after which they were allowed to cool in a desiccator to prevent moisture uptake before reweighing. After reweighing, the dried sediment was combusted at 450°C for 6 hours to remove organic matter, leaving behind ash. The organic matter content was calculated using the following formula:

$$TOC \% = \frac{Dry\ weight - Ash\ weight}{Dry\ weight} \times 100 \%$$

Where:

- Dry weight is the weight of the sediment after drying.
- Ash weight is the weight of the residue remaining after combustion.

The resulting TOC percentage was subsequently categorised as follows:

Table 2.2: The sediment organic matter percentage adopted from DWA (2010) as cited in Stow (2011).

TOC classification	Total organic content (%)
High	> 4
Moderate	2 – 4
Moderately low	1 – 2
Low	0.5 – 1
Very low	< 0.5

2.5 Macrobenthos field sampling procedure

Five replicates of macrobenthos samples were collected at each site using a van Veen grab with a sampling area of 0.024 m². Upon collection, the grab content was transferred into a bucket and approximately 10 litres of estuarine water was added to stir the sediment and release the organisms into suspension. The supernatant was then decanted through a 500 µm mesh cone sieve and this process was repeated five times to ensure thorough extraction of organisms (Cyrus & Martin 1988, Mackay 1996). For samples with muddy and coarse grain sizes, a few drops of 4% formalin were added to facilitate the release of benthic organisms from their burrows before the sieving process (Mackay 1996). The remaining sediment in the bucket was emptied into a 1000 µm mesh sieve to capture larger, heavier organisms such as gastropods and bivalves. All organisms retained in the sieves were transferred into a honey jar and preserved with 4% formalin stained with Rose Bengal (Blaber et al. 1984, Mackay 1996).

In the laboratory, the preserved samples were rinsed with tap water and then sieved through a 200 µm mesh sieve. The contents were placed in a petri dish for sorting. Each sample underwent a thorough sorting process, separating organisms into higher taxonomic groups. Following sorting, the organisms were identified to lower taxonomic levels using a Zeiss Discovery V12 stereo microscope and appropriate identification keys (Day 1967a, 1967b, Griffiths 1976, Kensley 1978, Kilburn & Rippey 1982, Day & de Moor 2002a, 2002b, Day et al. 2003, van Haaren & Soors 2009, Kakui & Angsupanich 2013). Macrobenthic organisms were identified to species level where possible, while those that could not be identified at the species level were classified to higher taxonomic levels such as genus, family or order.

2.6 Statistical Analysis

Descriptive statistics (range, mean and standard deviation) were calculated to observe general trends for abiotic and biotic data with respect to sites and seasons. Temporal and spatial changes in abiotic and biotic factors were examined using appropriate multivariate

analyses in PRIMER Version 6.1.13 (Clarke & Warwick 2001) and PAleontological STatistics (PAST) software program (Version 4.03) (Hammer et al. 2001).

Environmental data were log-transformed and normalised to meet the assumptions required for multivariate analysis. These data were then subjected to a Euclidean distance similarity matrix for ordination. This analysis tested the hypothesis that environmental variables exhibit significant variability among sites within each estuary and across seasons. To visualise these patterns and identify which variables contributed most to the observed variability, Principal Components Analysis (PCA) was performed (Clarke & Gorley 2006). Subsequently, a PERMutational ANalysis of VAriance (PERMANOVA) was conducted to test for any significant differences in environmental conditions that exist between sites and seasons.

Taxa abundance data were square-root transformed to reduce the weight of the contributing dominant taxa to achieve a balance in contribution between the rarer and more common species (Clarke & Warwick 2001). The subsequent analyses were then conducted using a Bray-Curtis similarity matrix on the square-root transformed abundance data. Hierarchical cluster analysis and non-metric multi-dimensional scaling (nMDS) ordination techniques were employed to visualise spatial variability in benthic communities. These analyses were used to test the hypothesis that the spatial distribution of benthic communities differs across sites based on species composition and abundance. Site similarities were assessed based on both species composition and abundance, with the expectation that sites with similar species composition and abundances would cluster closely together, while those with differing compositions and abundances would be positioned further apart (Clarke & Warwick 1994). The accuracy of the ordination was evaluated using the Kruskal stress value, where a low-stress value ($\text{stress} < 0.05$) suggests that the ordination plot accurately represents the relationships between samples. Stress values between 0.1 and 0.2 generally suggest a satisfactory fit (Clarke & Warwick 1994), while values between 0.2 and 0.3 are considered high, indicating a poor representation of the relationships among the samples. Higher stress values indicate reduced accuracy in the representation (Clarke & Warwick 2001). PERMANOVA was subsequently applied to test the hypothesis that significant differences in macrobenthic communities and densities exist across sites, seasons and their interactions.

The Similarity Percentages (SIMPER) routine in PRIMER was employed to assess the variability observed in benthic communities, as shown by the nMDS. This analysis was used to identify

which species contribute the most to the Bray-Curtis similarity and dissimilarity within and between different sites and seasons (Clarke & Warwick 2001). The analysis aimed to test the hypothesis that specific taxa drive the observed community patterns. This method identifies species that characterise a group (indicator species) and those that distinguish between two groups (discriminator species). To determine whether certain taxa contribute to the similarity or dissimilarity between groups, the Sim/SD ratio was used. The Sim/SD ratio is used to evaluate how consistently each taxon contributes to the average similarity within groups or dissimilarity between groups. An indicator or discriminator taxa will show a consistent contribution to dissimilarity/similarity contribution along with a high Sim/SD ratio. A taxon that is more abundant within a group tends to contribute more to the overall similarity among samples within that group. Conversely, a taxon that consistently contributes to the differences between groups serves as an effective indicator for distinguishing between those groups.

The DIVERSE program in PRIMER-E 6v statistical software (Clarke & Warwick 2001) was employed to analyse univariate community parameters at each site and season. The univariate measures included the Shannon-Wiener diversity index (H') and the Number of Species (S). These indices are widely used in ecological studies to quantify species diversity, incorporating both species richness (S) and number of species (E) (Clarke & Warwick 2001). The Shannon-Wiener diversity index (H') accounts for the proportion of each species relative to the total number of species and is calculated using the following formula:

$$H' = -\sum p_i \ln(p_i)$$

Where:

- H' denotes Shannon Weiner's diversity index,
- p_i is the proportion of the individual's contribution to the i^{th} population given by $p_i = \frac{n_i}{N}$,
- n_i is the density of the i^{th} species,
- N is the total number of individuals.

In addition, a two-way analysis of variance (ANOVA) was performed using PAST statistical software to test for significant differences ($\alpha < 0.05$) in species diversity (H') and the number of species (S) between sites and seasons. Where significant differences were detected, a

Tukey's Honestly Significant Difference (HSD) post-hoc test was applied to determine which specific sites or seasons differed significantly in their diversity or number of species.

To explore the relationship between the macrobenthic community structure and environmental variables, Pearson's correlations were initially performed on the environmental data to detect any significant correlations between variables. Where strong correlations ($r \geq 0.7$) were found, one of the correlated variables was removed to reduce redundancy and simplify further analysis. The BIOENV procedure within the BEST sub-program in PRIMER was subsequently used to identify a single or combination of environmental variables which best explained the community patterns. This approach identifies the combination of variables most strongly related to the biological data.

Finally, to visualise how different environmental gradients influenced species distributions, a Canonical Correspondence Analysis (CCA) was performed using PAST software (Hammer et al. 2001). This analysis relates species distribution to environmental factors, generating axes that represent these relationships. The strength of these relationships is reflected in the canonical eigenvalues (λ). Eigenvalues close to 1 indicate a strong relationship between community composition and environmental variables, while values close to 0 suggest weak or no relation.

Chapter 3: uMdloti Estuary

3.1 Introduction

The uMdloti Estuary is faced with various ecological challenges emanating from a combination of human-induced and climate related events such as droughts. These pressures have significantly affected the estuary's overall health, resulting in a health index score of 'D' which means it is largely modified (van Niekerk et al. 2019). Most of the prominent stressors are of anthropogenic origin such as flow modification, pollution from wastewater treatment works, agricultural and industrial return flows, artificial breaching and hypoxia (Blaber et al. 1984, Stretch & Zietsman 2004, DWA 2013, Taljaard et al. 2017).

A major contributing factor to the uMdloti Estuary's ecological decline is the Hazelmere Dam (constructed in 1976/7), situated 20 km upstream (Stretch & Zietsman 2004, DWA 2013). The dam, along with large-scale sugarcane farms and water extraction for urban and industrial purposes, has reduced the estuary's natural Mean Annual Runoff (MAR) by 20% (Stretch & Zietsman 2004, DWA 2013). This reduction in freshwater inflow has led to a dramatic increase in mouth closure frequency, shifting from a natural 5% to 69% of the time, with open-mouth periods lasting approximately seven days (Stretch & Zietsman 2004, Lawrie et al. 2010).

The uMdloti Estuary receives approximately 7.20 million cubic meters per year of treated sewage discharge from wastewater treatment works (WWTWs), which accounts for 4% of the total MAR (Stretch & Zietsman 2004, Brooker & Scharler 2020). During periods of low flow or closed-mouth phases, the discharge becomes a crucial freshwater source which drive the environmental conditions in the system (Brooker & Scharler 2020). However, this inflow, combined with agricultural runoff, has contributed to nutrient enrichment, fostering eutrophic conditions (Nozais et al. 2001, Thomas et al. 2005). This nutrient load has resulted in harmful algal blooms, the proliferation of invasive water hyacinth (*Pontederia crassipes*) and subsequent hypoxia, leading to fish kills and significant ecological disruptions (Thomas et al. 2005, Anandraj et al. 2008, Nunes et al. 2022b, Scharler et al. 2023).

The physico-chemical parameters of the uMdloti Estuary are influenced by an interplay of natural hydrological cycles and anthropogenic modifications and the mouth status (Thomas

et al. 2005, Brooker & Scharler 2020, Scharler et al. 2023). Natural hydrological cycles such as seasonal rainfall, govern the volume of freshwater inflows, which in turn influence the salinity, temperature, dissolved oxygen and nutrient concentrations within the estuary (Thomas et al. 2005, Anandraj et al. 2008, Scharler et al. 2023). Stronger inflows during the rainy season enhance tidal exchange and help remove accumulated pollutants, improving water clarity and oxygen levels. Conversely, during the dry season or when the estuary mouth is closed, the lack of flushing can result in stagnant water, which exacerbates hypoxic conditions and increases the accumulation of pollutants like nutrients and organic matter.

The salinity of uMdloti Estuary is influenced by the mouth state, duration of open mouth and the received stream flow volumes (Blaber et al. 1984, Thomas et al. 2005, Brooker & Scharler 2020, Scharler et al. 2023). During a closed mouth phase, the system becomes oligohaline and displays a well-mixed water column with no vertical and horizontal salinity variation (Lechman 2019, Scharler et al. 2023). Saltwater intrusion takes place during a closed mouth phase through overtopping (Lechman 2019) and during an open phase which is characterised by a defined horizontal gradient of marine conditions at the mouth and fresh at the head of the estuary (Blaber et al. 1984, Nozais et al. 2001, Deale et al. 2013). A vertical stratification has also been reported particularly in the mouth region and soon after the re-closure of the mouth, however, it does not persist (Blaber et al. 1984, Thomas et al. 2005, Deale et al. 2013). Due to its perched nature, the uMdloti Estuary may not experience full tidal exchange even when the mouth is open causing the estuary to retain predominantly freshwater conditions (Lechman 2019).

Dissolved oxygen concentrations vary spatially with lower levels at the upper reaches and higher towards the mouth (Blaber et al. 1984, Kibirige et al. 2006, Deale et al. 2013). The system experiences frequent hypoxic conditions which has been associated with biological factors (microalgae activity, bacterial load) and long inlet closure periods (Omarjee et al. 2020, Scharler et al. 2023). These conditions improve after a mouth breach when there is a tidal and riverine exchange.

The phytoplankton and microalgae of the uMdloti Estuary play a critical role in the ecological functioning, serving as primary producers at the base of the food web (Gerber et al. 2024). Chlorophyll-a levels in uMdloti Estuary are governed by the mouth state, nutrient availability, turbidity and light (Nozais et al. 2001, Perissinotto et al. 2004, Thomas et al. 2005). A closed

mouth creates stable conditions which allows proliferation of the suspended and benthic microalgae biomass leading to a high level compared to the open mouth phase. However, low microalgae biomass levels during the closed mouth phase have been recorded and were associated with low inorganic nutrients such as dissolved inorganic nitrogen and phosphorus (Nozais et al. 2001, Perissinotto et al. 2004, Anandraj et al. 2008). Upon a mouth breach, both pelagic and benthic microalgae are flushed into the ocean eliminating over 90% of microalgae in the system (Anandraj et al. 2008).

3.2.2 *Macrobenthic community*

The variability of macrobenthic communities in the uMdloti Estuary are closely linked to the estuary's unique hydrodynamic regime, which is influenced by the seasonal variations and state of the mouth (Scharler et al. 2020). The estuary is characterised by low community diversity and limited assemblages, largely due to its perched nature and frequent mouth closures, which restrict marine intrusion and hinder the establishment of diverse community structures (Forbes & Demetriades 2008, Stow 2011, Scharler et al. 2020).

Under frequent open-mouth conditions the community composition of the uMdloti Estuary comprises estuarine taxa, with common species including the polychaetes *Ceratonereis keiskama* and *Desdemona ornata*, along with amphipods such as *Grandidierella lutosa* and *Grandidierella lignorum* inhabiting the mouth region (Blaber et al. 1984, Forbes & Demetriades 2008). These species are typically associated with brackish to estuarine salinity ranges and well-oxygenated sandy habitats near the mouth. In contrast, the upper reaches of the estuary, which are strongly influenced by freshwater input and lower salinities, are typically dominated by Oligochaeta, Chironomidae and Baetidae insects, alien invasive species such as the freshwater bivalve (*Corbicula fluminalis*) and gastropod (*Tarebia granifera*) (Blaber et al. 1984, Forbes & Demetriades 2008, Scharler et al. 2020). Forbes & Demetriades (2008) noted that the absence of sensitive taxa, such as decapod crustaceans (prawns and crabs) and molluscs, is consistent with degraded ecological conditions and reflects the system's limited capacity to support more diverse and pollution-intolerant benthic communities.

During a closed-mouth phase, stable conditions often result in high macrobenthic densities (Perissinotto et al. 2004). However, if this phase is prolonged, diversity and composition are likely to be affected, with the system favouring opportunistic freshwater and invasive species over estuarine assemblages (Perissinotto et al. 2004, Scharler et al. 2020). Drought periods, reduced freshwater inflow and stagnant water promote organic matter accumulation, oxygen depletion and habitat homogenisation leading to the complete elimination of estuarine communities (Scharler et al. 2020). Breaching events provide opportunities for marine/estuarine species to recolonise, temporarily boosting diversity, but initial densities may remain low due to flushing effects (Scharler et al. 2020). Without regular tidal exchange the estuary remains dominated by limited brackish communities and freshwater species.

While previous studies have contributed to the understanding of macrobenthic communities in the uMdloti Estuary, much of the existing research either describes them based on broader TOCE contexts or focuses on the effects of mouth dynamics. Consequently, knowledge of how these communities respond to seasonal variability, particularly between wet and dry season conditions remains limited. To date, no study has compared macrobenthic community structures across different hydrological phases within the estuary. Therefore, there is a need to better understand how seasonal changes in environmental conditions influence the composition, diversity, and abundance of macrobenthic communities in the uMdloti Estuary.

The aim of this study is to investigate the short-term temporal and spatial variability of the uMdloti Estuary, with a focus on benthic communities. The specific objectives are to analyse the structure and composition of benthic communities across three stations during dry and wet seasons and to relate these patterns to environmental parameters, identifying the most influential factors shaping benthic communities. This approach will provide insights into the interactions between environmental conditions and biotic responses, offering a comprehensive understanding of the estuary macrobenthic invertebrate dynamics under variable hydrological conditions.

3.3 Methods and materials

3.3.1 Study area

The uMdloti Estuary (29°38' S, 31°08' E) is a large temporarily closed estuary (van Niekerk et al. 2019) located on the north coast, approximately 27 km north of Durban. The estuary drains 85 million cubic meters (MCM) of freshwater from the Mdloti River, with a catchment area of 550 – 597 km² and an overall river length of 88 km. The estuary spans a surface water area of 1.4 km (Forbes & Demetriades 2008), with depths ranging from 0.5 m to 2 m (Booyesen 2017, Scharler et al. 2023). The estuary is characterised by a 500 – 600 m wide sandbar across the mouth region, which rises 3 – 4 meters above mean sea level (MSL) and is often overtopped during spring high tides. (Stretch & Zietsman 2004). The sampling stations in this study were chosen to represent the different salinity zones encompassing the upper, middle and lower reaches. The lower reaches are located approximately 0.15 km from the estuary mouth and represent the marine-influenced zone. The middle reaches, associated with the euhaline conditions, are situated around 0.5 km from the mouth, while the upper reaches, which are freshwater dominated, are approximately 1 km upstream.

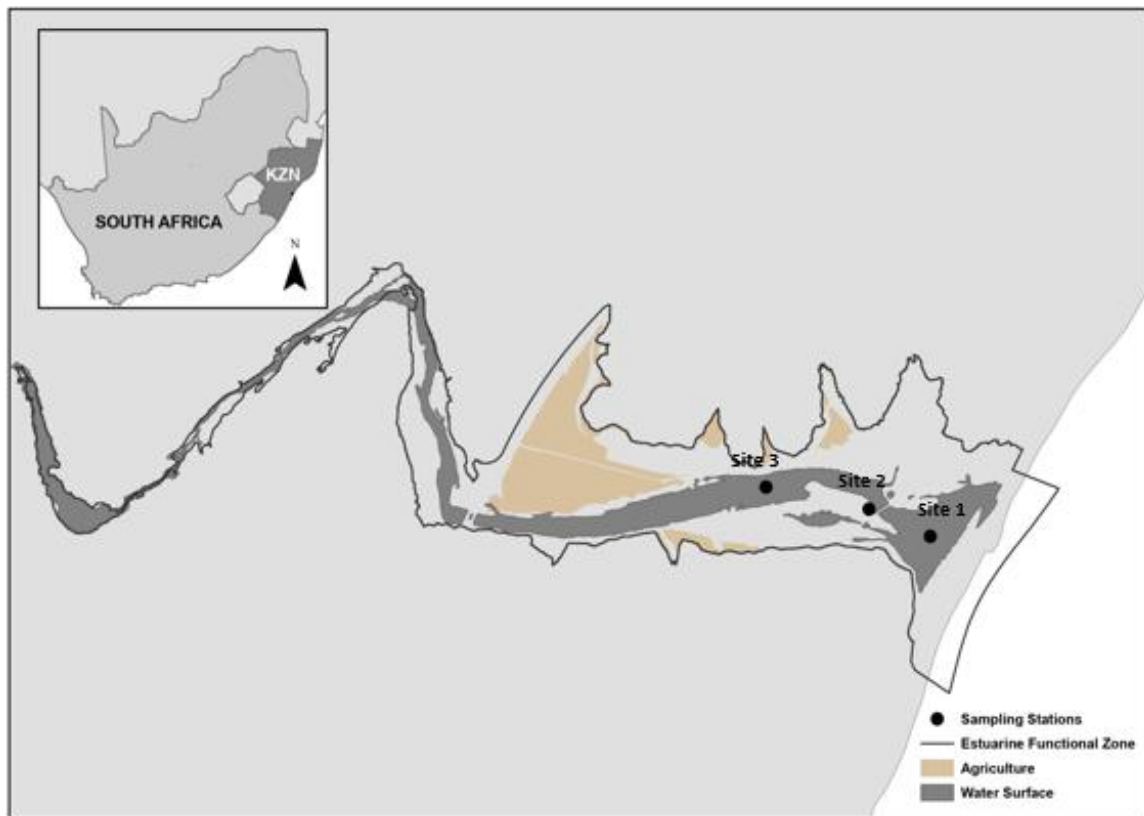


Figure 3.1: Map of South Africa and uMdloti Estuary (29°39'S; 31°07'E) with sampling sites, L: lower (site 1), M: middle (site 2) and U: upper reaches (site 3).

3.3.2 Methods and materials

Chapter 2 (General methods and materials) provides an in-depth overview of the standard procedures for collecting macrobenthos and environmental conditions (water and sediment), conducting laboratory processing and performing statistical analyses. Due to no POM data for Site 2 in July 2019 and Site 3 in January and March 2020, these months were excluded from the PCA, PERMANOVA, BEST and CCA analyses.

3.4 Results

3.4.1 Environmental variables

Water temperatures followed a typical South African subtropical region seasonal pattern across all sites, with temperature increasing from 15.00°C in the upper reaches during the dry (June – August 2019) season to 27.98°C during the wet season (December 2019 – January 2020 and March 2020) (Figure 3.2A).

In the uMdloti Estuary, DO concentration increased from the upper to lower reaches during dry periods, with an overall average of $4.47 \pm 0.68 \text{ mg}\cdot\text{l}^{-1}$. Minimum ($3.53 \text{ mg}\cdot\text{l}^{-1}$) and maximum ($8.62 \text{ mg}\cdot\text{l}^{-1}$) DO concentrations were recorded during the dry season in the upper and lower reaches, respectively (Figure 3.3B). DO concentrations below $5 \text{ mg}\cdot\text{l}^{-1}$ were consistently observed in the upper reaches, except for $5.15 \text{ mg}\cdot\text{l}^{-1}$ in December 2019. The middle and lower reaches were generally well oxygenated ($> 5 \text{ mg}\cdot\text{l}^{-1}$), although slight declines were observed in January 2019, with DO concentrations of $4.28 \text{ mg}\cdot\text{l}^{-1}$ and $4.60 \text{ mg}\cdot\text{l}^{-1}$, respectively.

The pH levels ranged between acidic (6.12) to basic conditions (8.24) and were both recorded in the wet season (December 2019 – January 2020 and March 2020). The overall average pH in the dry season (June – August 2019) was 7.11 ± 0.39 and 7.60 ± 0.66 in the wet season. The uMdloti Estuary was generally characterised by oligohaline (<5) conditions from the lower to the upper reaches (Figure 3.2D). Salinities ranged between 0 – 2 ppt during the dry season with elevations observed in the wet season ranging from 2 – 12 ppt. Minimum salinities of 0.00 were recorded in the upper and middle reaches in June 2019 and these sites remained oligohaline even during the subsequent open phase in January and March 2020. The highest salinity level (12.47 ppt) was recorded in the lower reaches during an open mouth phase in January 2020. This phase allowed marine water to enter the estuary, creating a salinity gradient that increased progressively from the upper to the lower reaches.

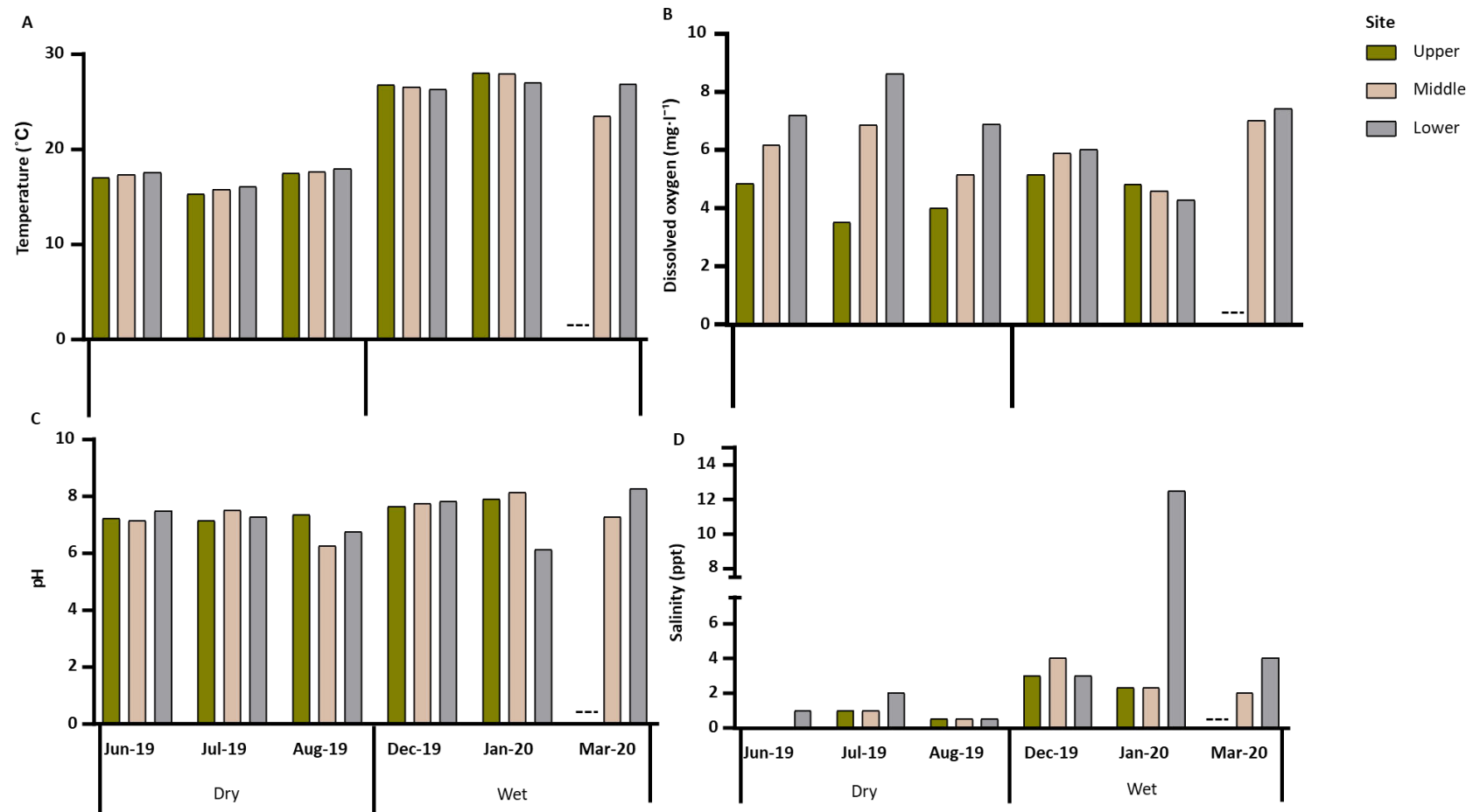


Figure 3.2: Temperature (A), Dissolved oxygen B), pH (C) and Salinity (D) measured at the bottom of the water column in uMdloti Estuary during a dry season represented by June, July and August 2019 and wet season represented by December 2019, January and March 2020. Dotted line represents missing data.

Although there were no spatial and temporal significant patterns in phytoplankton (pp) chlorophyll-a, low concentrations were more prevalent during the dry and cooler season across all stations (Figure 3.3A). Exceptions were observed in July 2019, with peak concentrations recorded in the middle and upper reaches at $13.98 \pm 0.16 \text{ mg}\cdot\text{m}^{-3}$ and $12.72 \pm 0.19 \text{ mg}\cdot\text{m}^{-3}$, respectively. Elevated pp chlorophyll-a prevailed during the wet season with an average concentration of $8.13 \pm 5.50 \text{ mg}\cdot\text{m}^{-3}$. There was a notable decrease in March 2020 due to flushing that occurred after the mouth breach event.

Wide ranges of suspended particulate organic matter percentages (Figure 3.3B) were evident throughout the study period with an average of $39.80 \pm 24.61\%$. The lowest value (3%) was recorded in March 2020 at the lower reaches. The TSS was stable across all sites during the study period except the observed peaks in August 2019, reaching the highest value overall (Figure 3.3C). In the wet season, TSS levels increase in January 2020, marking the second-highest peak before declining slightly by March. The dry season generally shows more fluctuation, while the wet season stabilises with moderate TSS levels.

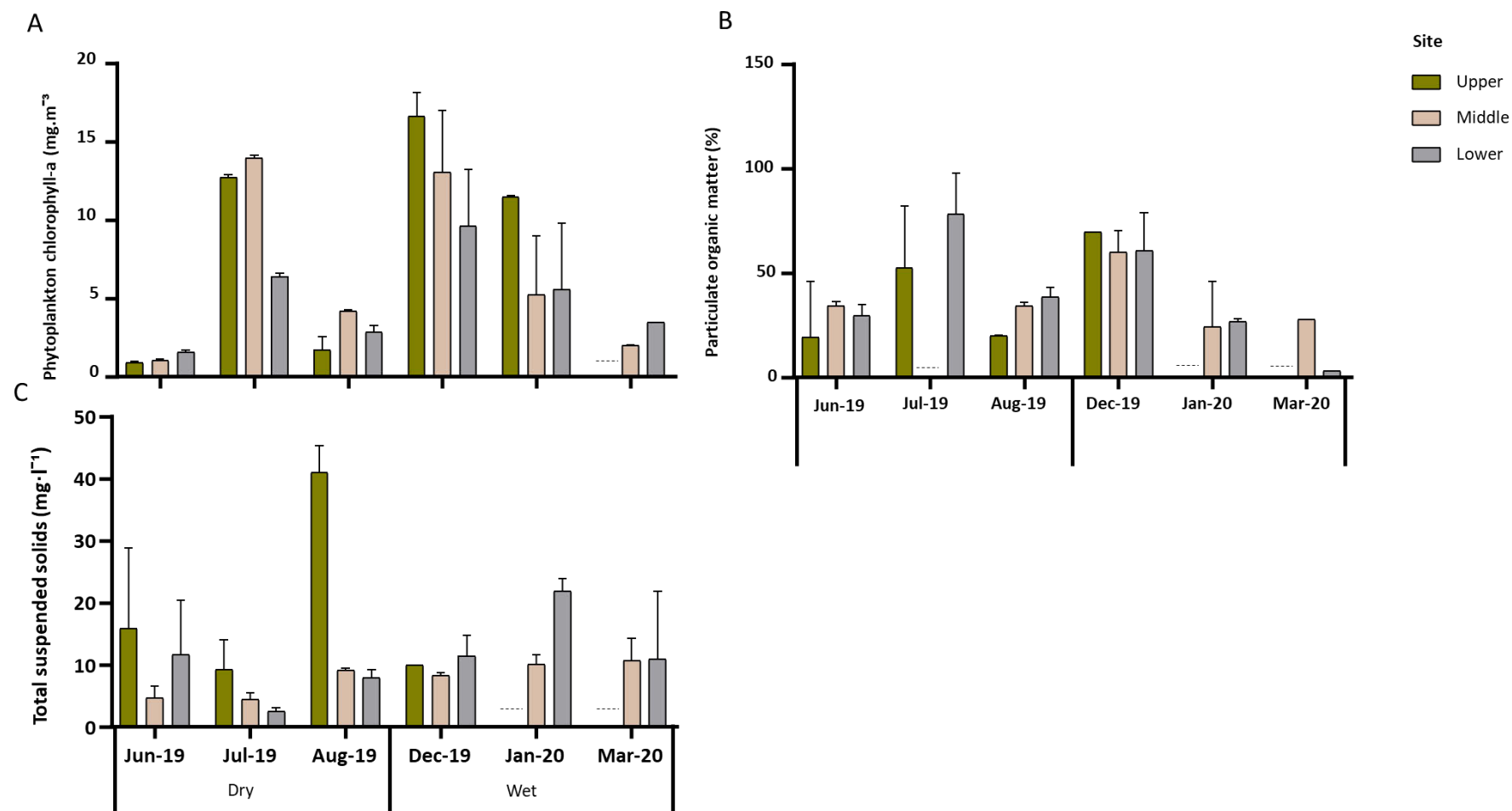


Figure 3.3: Mean ($\pm$ STD) suspended particulate organic matter (A), phytoplankton chlorophyll-a (B) recorded during the wet and dry season in uMdloti Estuary. Dotted line represents missing data.

The PERMANOVA results identified a significant temporal difference in environmental parameters across stations (Pseudo-F=3.03, df=1, p-perm=0.008) (Table 3.1). This variability was further visualised through the PCA (Figure 3.4) where, PCA 1 and PCA 2 explained 31.8% and 24.6% variability of the data respectively, accounting for 58.8% of the total variability. PC1 separates samples primarily based on POM and DO, which are associated with dry season. The second component reflects differences in TSS with higher values noted during this period as well. High salinity, pH and chlorophyll-a were linked to the wet season, with phytoplankton chlorophyll-a strongly correlating with pH, suggesting both responded similarly to increased freshwater input, changes in mouth state and temperature. POM And DO were closely aligned, suggesting these factors increased together, particularly noticeable in August 2019. TSS on the other hand, was inversely related to DO and POM. Although the PERMANOVA results were not statistically significant, visual patterns highlight that sites vary in their characteristics, with the upper site tending towards more turbid and lower in DO environments.

Table 3.1: Permutational Multivariate Analysis of Variance (PERMANOVA) results of temperature, dissolved oxygen, pH, salinity, suspended particulate organic matter and water column pp chlorophyll-a concentrations.

Factor	Pseudo-F	df	Unique perms	P (perms)
Site	1.31	2	999	0.285
Season	3.03	1	999	0.008

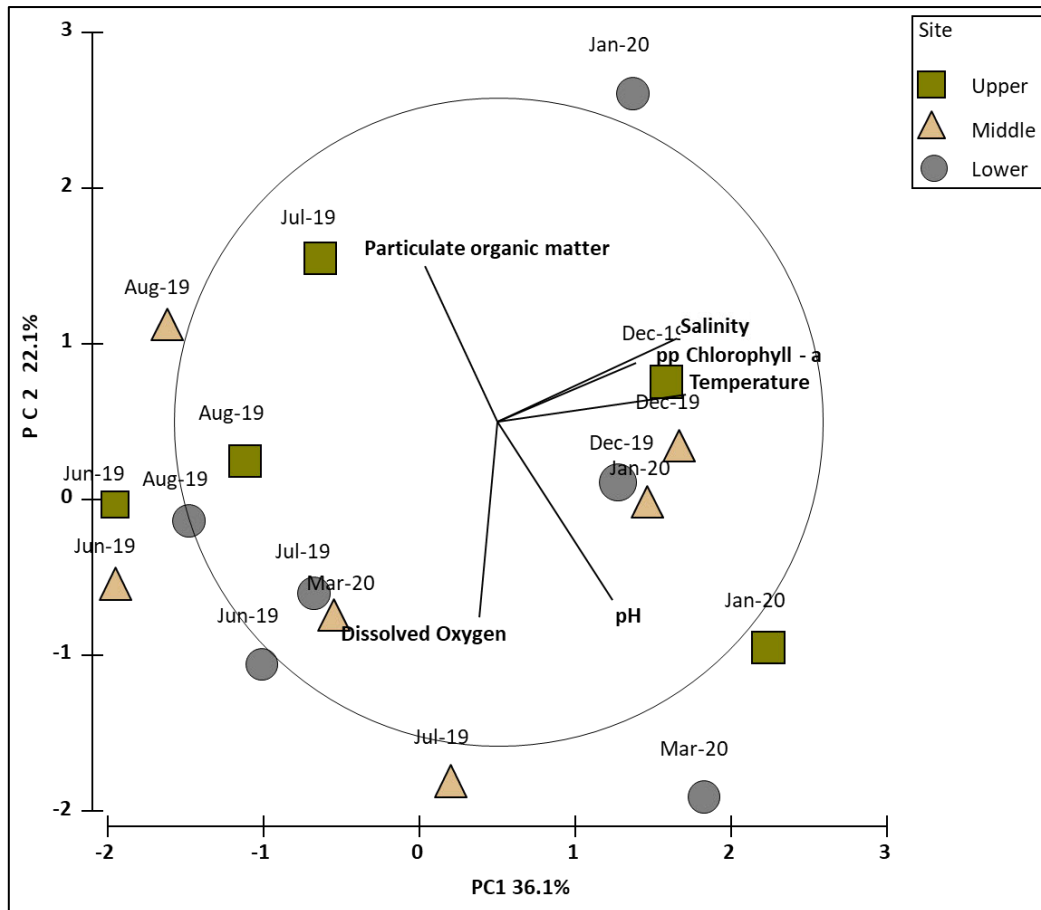


Figure 3.4: Principal Components Analysis (PCA) ordination plot showing sites based on water column parameters at three sampling sites of uMdloti Estuary for the dry (June – August 2019) and wet (December 2019 – January 2020 and March 2020) seasons.

3.4.2 Sediment characteristics

Sediment grain sizes displayed a consistent contribution to the overall sediment composition and distribution patterns amongst sites with no marked seasonal changes (Figure 3.5). Sediment grain sizes varied from fine sand to gravel with medium and coarse sand across at all sites and seasons. The uMdloti Estuary was characterised by notably low total sediment organic content (TOC), with values ranging from a low 0.12 % in the lower reaches in January 2019 to a moderately low 1.25 % in the middle reaches in July 2019. (Figure 3.6). Total organic content was highest in the middle reaches during the dry season, while lower TOC levels were observed in the lower and upper reaches.

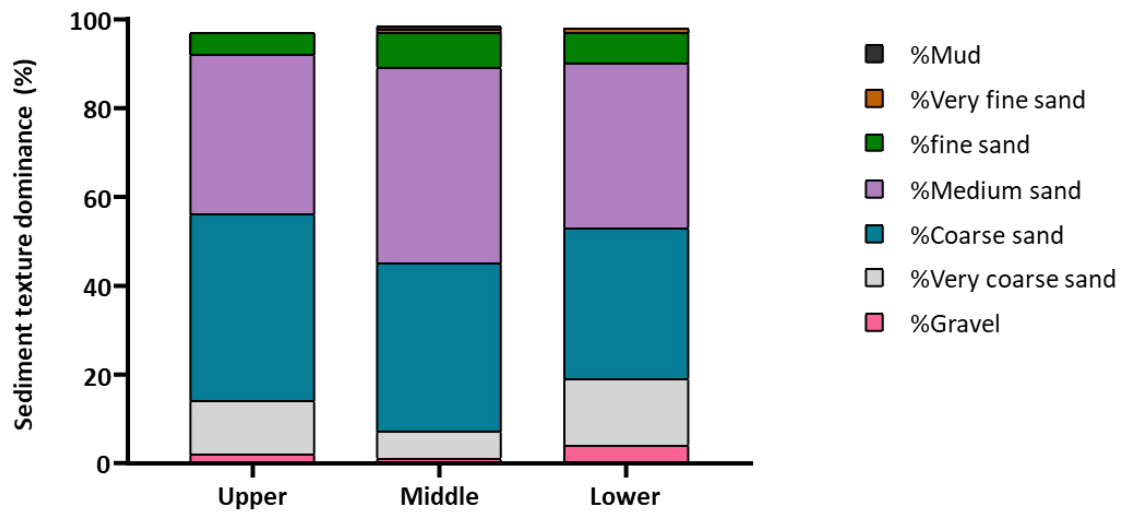


Figure 3.5: Distribution of sediment particle sizes in the three reaches of the uMdloti Estuary, averaged across wet and dry seasons.

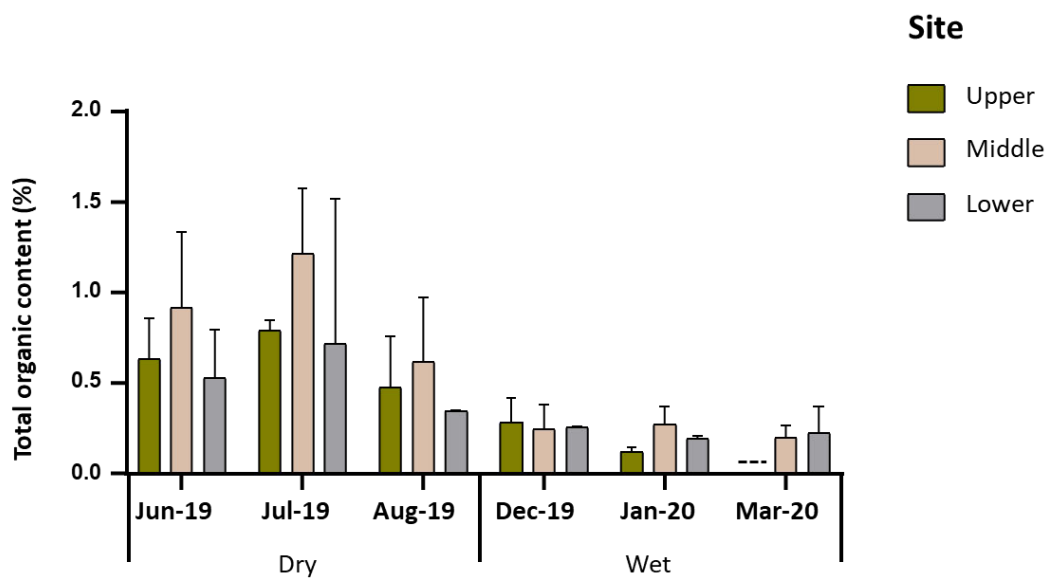


Figure 3.6: Mean ($\pm$ STD) sediment total organic content measured across three stations (upper, middle, lower) of uMdloti Estuary. Dotted line represents missing data.

The PCA did not reveal distinct groupings, however, stations clustered based on dominant sediment grain sizes (Figure 3.7). The first component axis, explaining 53.11% of sediment variability, was characterised by medium sand and total organic content. The second component axis, accounting for 28.3% of variability, featured gravel, very coarse sand and coarse sand.

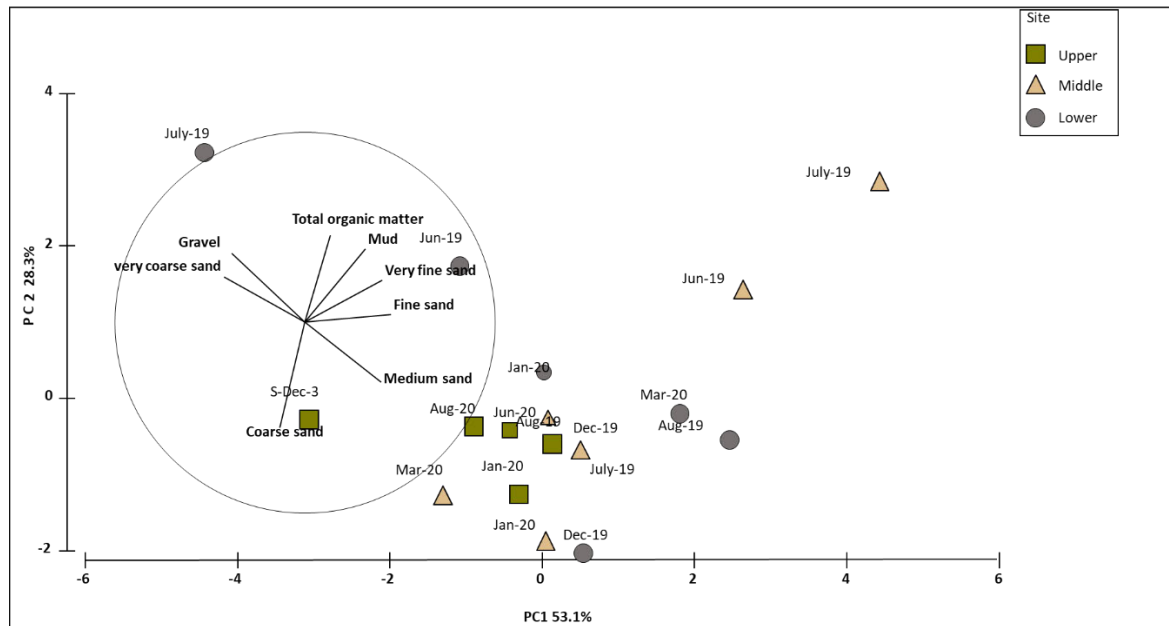


Figure 3.7: Principal Component Analysis (PCA) ordination plot of the sediment characteristics of uMdloti Estuary over the wet and dry season.

3.5 Macrobenthos community analysis

3.5.1 Taxonomic composition

The uMdloti Estuary was characterised by the abundance of the polychaetes *D. cf. ornata* and *C. keiskama*, the oligochaete Naididae sp1, the bivalve *C. fluminalis*, the gastropod *T. granifera* and the amphipods *G. lutosa* and *Grandidierella* spp. (Table 3.2). The upper reaches were dominated by *C. fluminalis* (>2000 ind·m⁻²) and *T. granifera* (>2000 ind·m⁻²) in both

seasons. Together these taxa contributed to more than 50% of the total composition in terms of abundance (Figure 3.8).

During a closed mouth phase, the middle reaches were characterised by a combination of freshwater (gastropods, oligochaetes) and estuarine (polychaetes, amphipods and bivalve) taxa with seasonal influence playing a role in the total percentage contribution of individual taxa (Table 3.2 and Figure 3.3). A mouth breach event in January 2020 and March 2020 resulted in drastic change in species composition and abundance due to flushing and scouring. In January 2020, the middle reaches were dominated by freshwater tolerant species such as *T. granifera* (66%), *C. fluminalis* (10%) and Naididae sp1 (10%). In contrast, ten previously recorded taxa together contributed only 7% to the total species composition. In March 2020, only 4 taxa were recorded (Table 3.2) again *T. granifera* together and *C. fluminalis* being numerically dominant together accounting for 87% of the infauna abundance. During this period, the freshwater gastropod *Melanoides tuberculata* (1%) which is native to South African systems, was also observed.

The lower reaches were characterised by estuarine taxa such as *D. cf. ornata*, *C. keiskama*, *G. lutosa*, *G. lignorum* and *Grandidierella* spp., which are typical residents in subtropical temporarily closed estuaries (Figure 3.8). *Desdemona cf. ornata* particularly displayed higher proportions from June to August 2019 and December 2019 contributing between 41 % and 71 % (Figure 3.8). However, the open phase in January 2020 resulted in marked decline of *D. cf. ornata* and an introduction of marine/estuarine taxa such as *Prionospio* spp. (20 %) which together with *G. lutosa* (21 %) and *G. lignorum* (20 %) became the important contributors to benthos abundance. An increase in density of *C. keiskama* (1928 ± 517) was evident in March 2020 where it made up more than 60 % of total abundance, followed by *Hiatula lunilata* (16 %) and *T. granifera* (11 %).

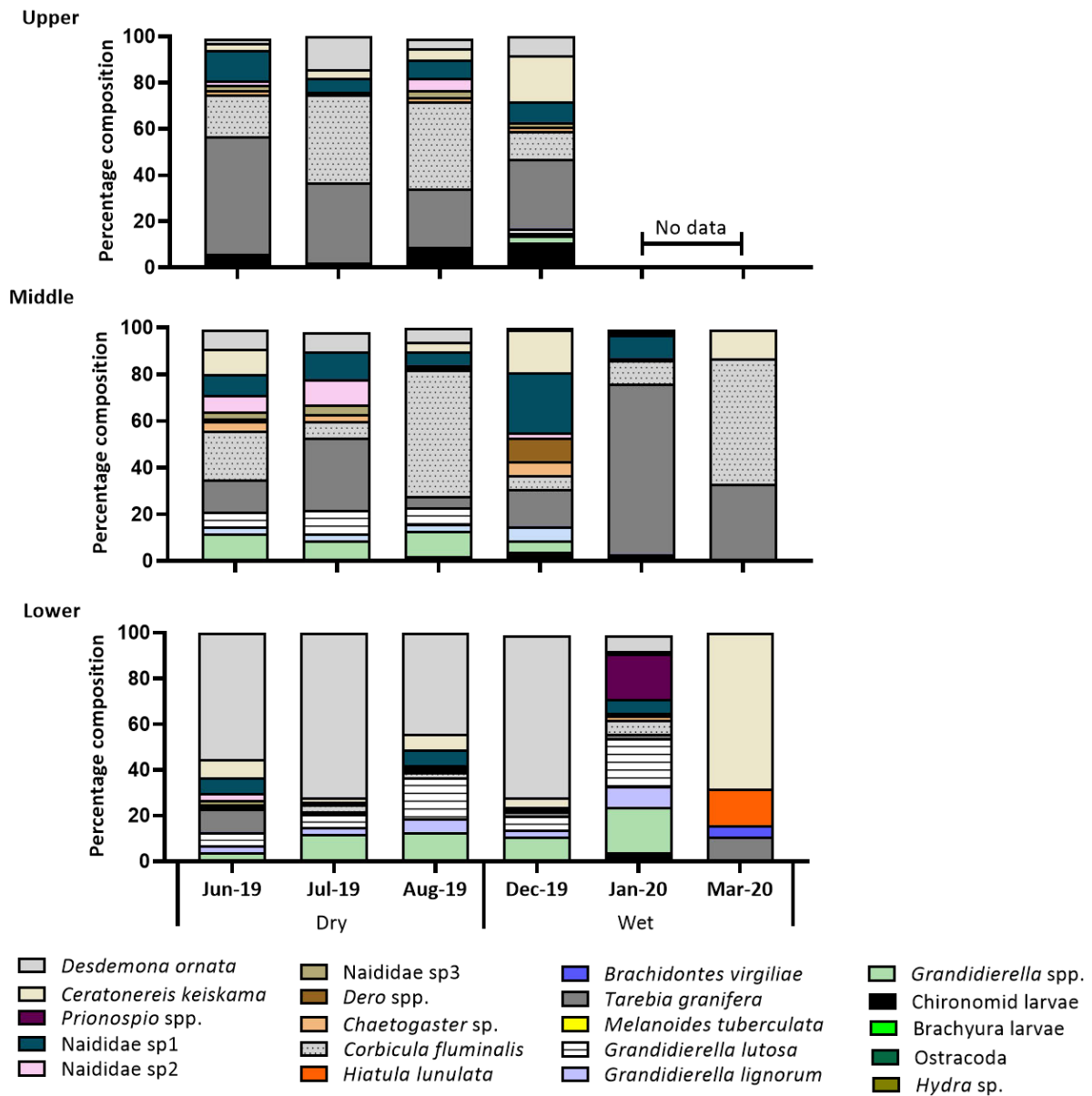


Figure 3.8: Composition percentage of macrobenthos densities recorded at the three sampling sites of the uMdloti Estuary for the period June 2019 – March 2020 in the upper, middle and lower reaches.

Table 3.2: Average macrobenthos taxa abundance recorded in uMdloti Estuary during the season (June 2019 – August 2019) and wet season (December 2019 – March 2020) season from upper (U), middle (M) and lower (L) reaches. – denote missing data.

	Jun-19			Jul-19			Aug-19			Dec-19			Jan-20			Mar-20		
Taxa	U	M	L	U	M	L	U	M	L	U	M	L	U	M	L	U	M	L
Polychaeta																		
<i>Desdemona cf. ornata</i>													-			-		
<i>Ceratonereis (Composetia) keiskama</i>													-			-		
<i>Prionospio</i> spp.													-			-		
Clitellata													-			-		
Naididae sp.1													-			-		
Naididae sp.2													-			-		
Naididae sp.3													-			-		
<i>Chaetogaster</i> sp.													-			-		
<i>Dero</i> sp.													-			-		
Bivalvia													-			-		
<i>Corbicula fluminalis</i>													-			-		
<i>Brachidontes virgiliae</i>													-			-		
<i>Hiatula lunulata</i>													-			-		
Gastropoda													-			-		
<i>Tarebia granifera</i>													-			-		
<i>Melanoides tuberculata</i>													-			-		
Malacostraca													-			-		
Brachyura larvae													-			-		
<i>Grandidierella lutosa</i>													-			-		
<i>Grandidierella lignorum</i>													-			-		
<i>Grandidierella</i> spp.													-			-		
Ostracoda													-			-		
Insecta													-			-		
Chironomid larvae													-			-		
Hydrozoa													-			-		
<i>Hydra</i> sp.1													-			-		
Density (ind·m ⁻²)	0		≤ 10		11 - 100		101 - 500		501 - 1000		1001 - 2000		>2000					
Density key																		

Macrobenthos densities exhibited distinct spatial and temporal distribution patterns (Figure 3.9). During the dry season, the average density was $15877.33 \pm 3308.42 \text{ ind}\cdot\text{m}^{-2}$, calculated across all three sampling stations and months, which was more than double the average density observed during the wet season ($7621.33 \pm 5696.06 \text{ ind}\cdot\text{m}^{-2}$). The peak macrobenthic densities during the study were observed in the lower reaches, with $11.120 \text{ ind}\cdot\text{m}^{-2}$ recorded in the dry season (July 2019) and $11.984 \text{ ind}\cdot\text{m}^{-2}$ in the wet season (December 2019). The latter represented the highest faunal density recorded throughout the study, exceeding those at other sites. Despite this, overall macrobenthic densities were considerably reduced during the wet season, likely because of system flushing during an open mouth phase.

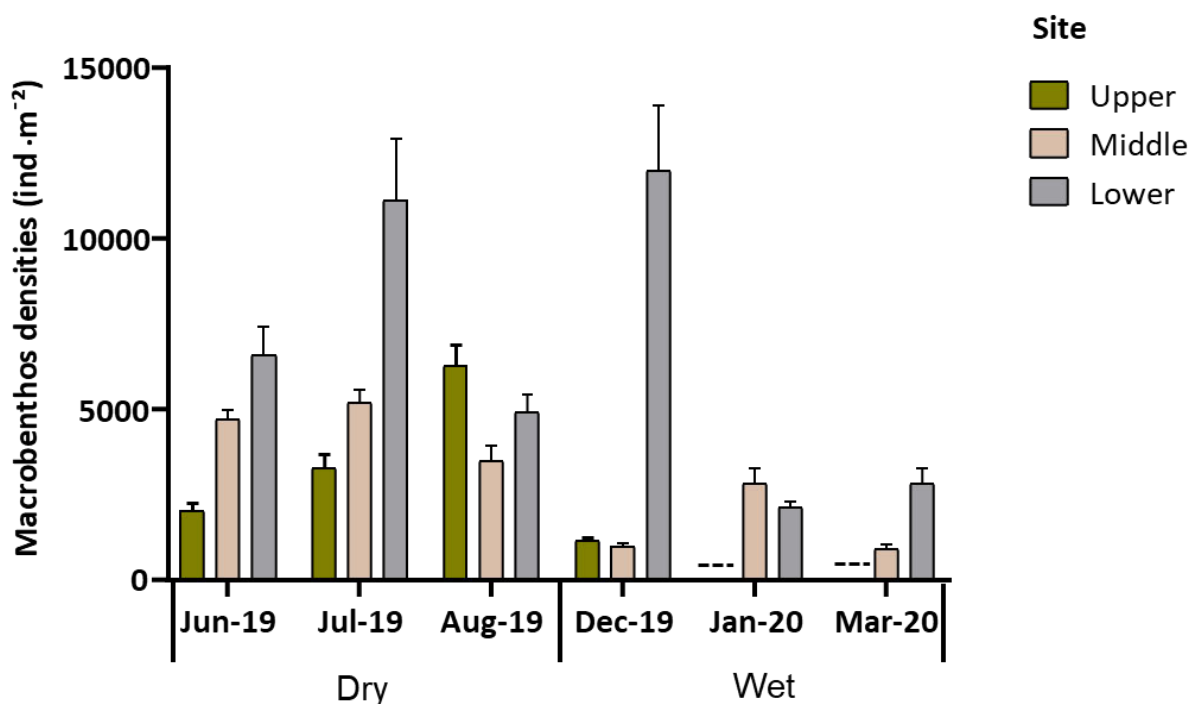


Figure 3.9: Mean macrobenthos density ($\text{ind}\cdot\text{m}^{-2} \pm \text{STD}$) from the upper, middle and lower reaches of uMdloti Estuary sampled in the wet and dry season. Dotted line represents missing data.

The Shannon-Wiener diversity index (H') and number of species (S) fluctuated throughout the study period, displaying no consistent trend across sites or seasons (Figure 3.10). The highest S ($2.27 \pm 0.21 \text{ STD}$) and H' ($10.8 \pm 2.5 \text{ STD}$) were recorded in the middle reaches in June 2019, and the lowest values ($S = 0.58 \pm 0.56 \text{ STD}$; $H' = 2.00 \pm 0.40 \text{ STD}$) also occurred in the middle reaches during the wet season (March 2020). However, these differences were not statistically significant across sites (S , $df = 2$, $F = 0.22$, $p = 0.81$; H' , $df = 2$, $F = 0.08$, $p = 0.9$) or seasons (S ,

df = 1, F = 1.16, p = 0.30; H', df = 1, F = 1.11, p = 0.24). A slight increase in diversity and species abundance was observed in the upper reaches in August 2019, driven by the numerical proliferation of *C. keiskama* and the appearance of *G. lutosa*, *G. lignorum* and *Grandidierella* spp. Similarly, an increase was noted in the lower reaches in January and March 2020, coinciding with the rise in numerical abundance of these species alongside the introduction of *Prionospio* spp. and *H. lunulata*.

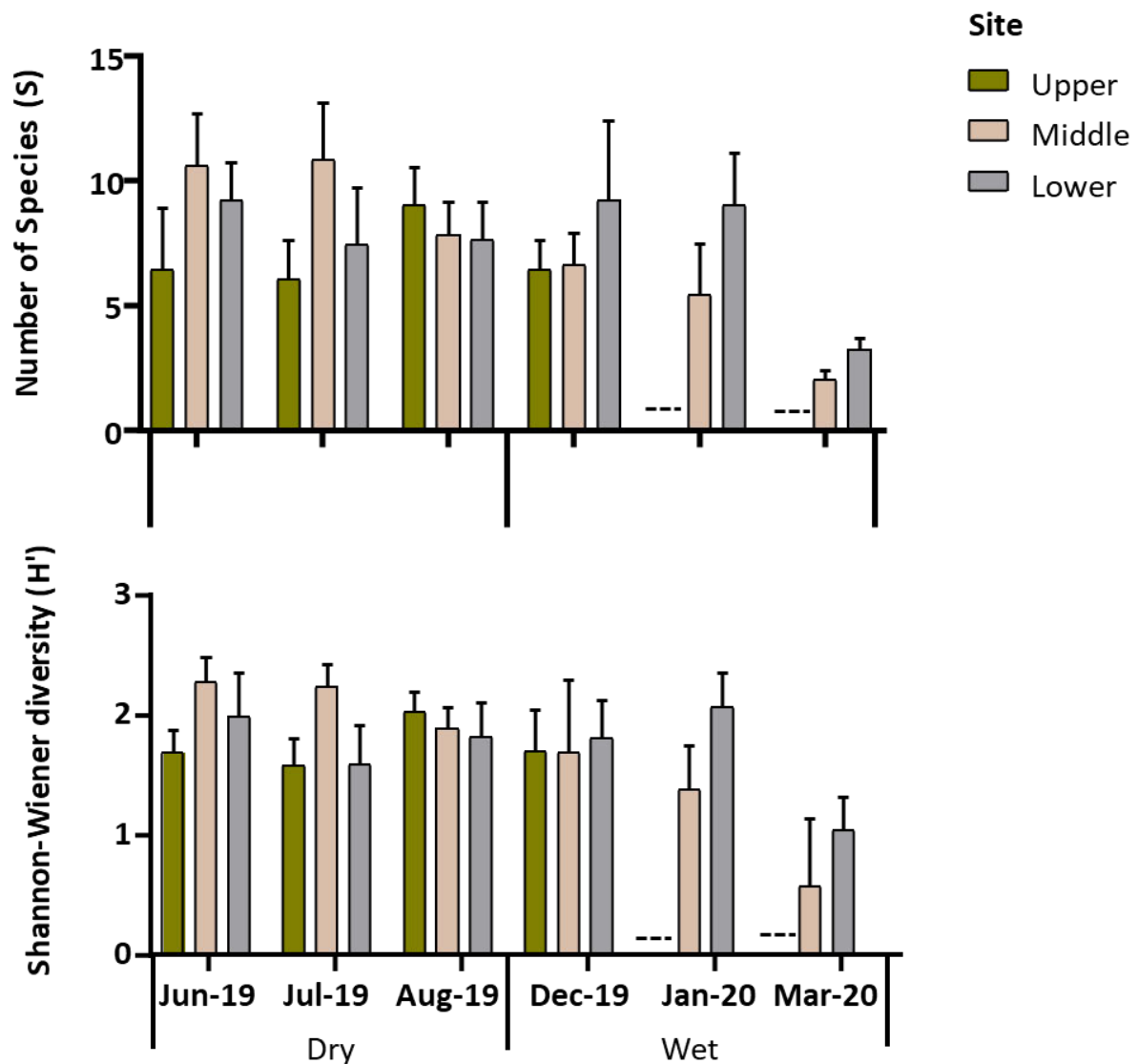


Figure 3.10: Mean number of species ($S \pm \text{STD}$) and Shannon-Wiener diversity index ($H' \pm \text{STD}$) for macrofaunal community sampled during the wet and dry of uMdloti at three sampling sites. Dotted line represents missing data.

There were statistical differences in spatial (Pseudo-F=2.27, df=2, p-perm=0.03) and temporal (Pseudo-F=11.16, df=1, p-perm=0.001) variation (Table 3.4). To visualise these variations, the

MDS was overlaid with a hierarchical cluster at 60% similarity and revealed 3 major groups with 2 outliers (Figure 3.11). The MDS ordination also showed that the sampling sites displayed a distribution gradient from the lower reaches (group 1) to the upper reaches (group 3). Group one was formed by the samples collected from the lower reaches and the main distinguisher of this group was the numerical contribution of *D. cf. ornata*. The second group was characterised by a unique community representing a transition between communities recorded from the lower and upper reaches during the dry season. Group three was shaped by the freshwater taxa (oligochaetes, chironomid insects and *T. granifera*) from the middle and upper reaches across all seasons. The outliers separated distinctly from the three groups owing to the numerical increase of taxa such as *D. cf. ornata*, Naididae sp1, *C. fluminalis*, *T. granifera* in January 2020 and the presence of marine/estuarine taxa (*Prionospio* spp. and *H. lunulata*) during March 2020 of the wet sampling season.

Table 3.3: Permutational Multivariate Analysis of Variance conducted on macrobenthos community densities in the uMdloti Estuary for the period of June – August 2019 and December 2019, January and March 2020.

Factor	df	Pseudo-F	Unique perms	P (perms)
Site	2	2.27	999	0.031
Season	1	2.57	999	0.001
Site x Season	2	0.73	997	0.060

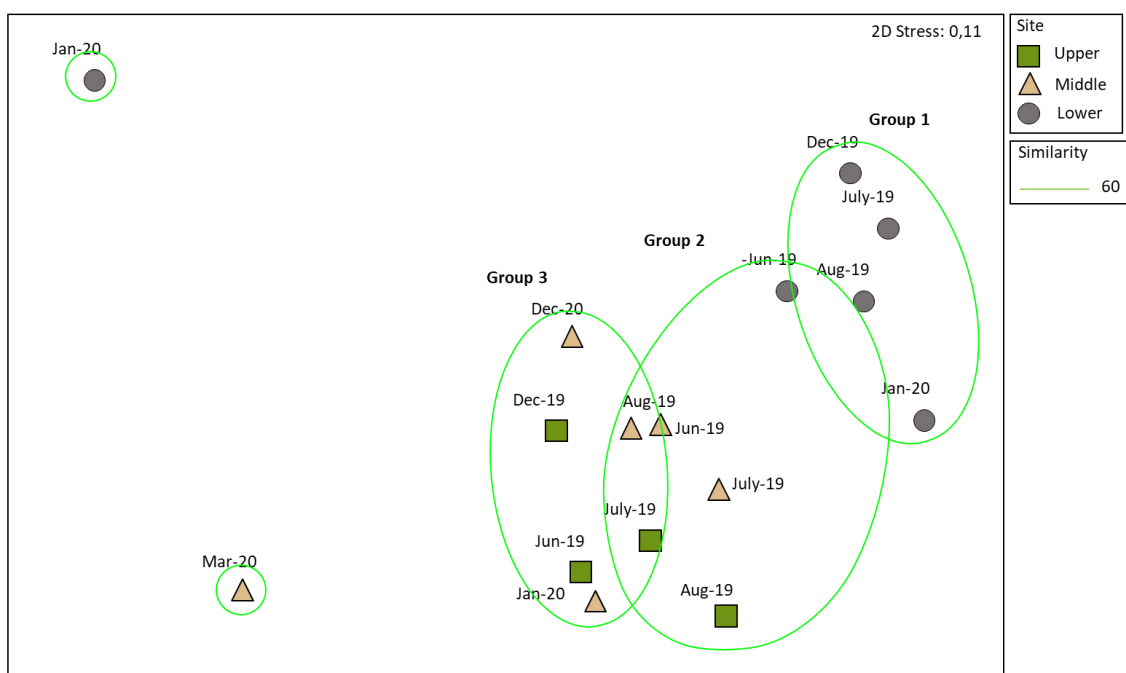


Figure 3.11: nMDS of uMdloti Estuary square-root transformed macrobenthos abundance data from three sites (upper, middle and lower) recorded during dry (June to August 2019) and wet (December 2019, January and March 2020).

The SIMPER analysis identified 12 taxa that were responsible for similarities between all three sites (Table 3.5). The lower reaches exhibited the lowest similarity to other stations (39.75 %), with eight taxa contributing significantly to the observed similarity within this site. Among these, *D. cf. ornata* accounted for the largest contribution (23.95 %), followed by *C. keiskama* (13.37 %), the amphipod species *G. lutosa* (12.56 %) and *Grandidierella* spp. (11.69 %) (Table 3.5). This site also recorded the highest dissimilarity compared to the upper reaches (57.04 %) and the middle reaches (53.44 %), with *G. lutosa*, *Grandidierella* spp., *T. granifera*, *D. cf. ornata* and *C. fluminalis* identified as prominent discriminating taxa (Table 3.6). The middle reaches showed a similarity of 46.10 % with both the lower and upper reaches (Table 3.5). The top five indicator taxa contributing to this similarity were *T. granifera* (19.46 %), *C. fluminea* (19.35 %), *Naididae* sp1 (10.30 %), *Grandidierella* spp. (9.11 %) and *C. keiskama* (9.05 %). The lowest dissimilarity (40.30 %) was recorded between the middle and upper reaches, with *C. fluminalis*, *T. granifera* and *Grandidierella* spp. identified as the main distinguishing

taxa. The top four most dominant taxa of the middle reaches along with *D. cf. ornata* (8.99) *C. keiskama* (8.50) chironomid larvae (6.77%) cumulatively contributed 90.37 % of the similarity in the upper reaches (Table 3.5).

Table 3.4: The taxa accounting for 90% of the cumulative within-group similarity, as determined by a one-way SIMPER analysis of square-root transformed macrobenthic abundance data, listed in descending order of their average similarity contribution.

Sites	Species	Average similarity	Contribution%
Lower	<i>Desdemona cf. ornata</i>	12.61	23.95
	<i>Ceratonereis keiskama</i>	7.04	13.37
	<i>Grandidierella lutosa</i>	6.61	12.56
	<i>Grandidierella</i> spp.	6.15	11.69
	<i>Tarebia granifera</i>	4.54	8.63
	<i>Grandidierella lignorum</i>	4.47	8.49
	Naididae sp1	3.43	6.51
	<i>Corbicula fluminea</i>	2.97	5.65
Middle	<i>Tarebia granifera</i>	11.95	19.46
	<i>Corbicula fluminea</i>	11.89	19.35
	Naididae sp1	6.33	10.3
	<i>Grandidierella</i> spp.	5.59	9.11
	<i>Ceratonereis keiskama</i>	5.56	9.05
	<i>Desdemona cf. ornata</i>	4.59	7.47
	<i>Grandidierella lutosa</i>	3.85	6.27
	<i>Grandidierella lignorum</i>	3.19	5.19
	Chaetogaster sp	2.26	3.68
	Naididae sp2	2.19	3.56
Upper	<i>Tarebia granifera</i>	21.26	30.39
	<i>Corbicula fluminea</i>	15.45	22.09
	Naididae sp1	9.53	13.63
	<i>Desdemona cf. ornata</i>	6.29	8.99
	<i>Ceratonereis keiskama</i>	5.95	8.5
	Chironomid larvae	4.74	6.77

Table 3.5: Top five primary discriminating taxa contributing more than 2% to the average dissimilarity between sampling stations, with Dissimilarity/Standard Deviation in parentheses. The dissimilarity percentage (DS) is highlighted in bold.

	Lower	Middle	Upper
Lower			
Middle	DS = 53.44 <i>Desdemona cf. ornata</i> 12.23(1.43) <i>Grandidierella</i> spp. 4.68(1.3) <i>Corbicula fluminalis</i> 4.66(1.9) <i>Ceratonereis</i> (Composetia) keiskama 4.52(0.87) <i>Grandidierella lutosa</i> 4.46 (1.32)		
Upper	DS = 57.04 <i>Desdemona cf. ornata</i> 11.72(1.59) <i>Corbicula fluminalis</i> 5.80(1.30) <i>Grandidierella lutosa</i> 5.63(2.12) <i>Grandidierella</i> spp. 5.55(2.02) <i>Tarebia granifera</i> 5.41(1.92)	DS = 40.30 <i>Corbicula fluminalis</i> 5.36(1.42) <i>Tarebia granifera</i> 5.10(1.54) <i>Desdemona cf. ornata</i> 3.23(1.24) Chironomid larvae 3.20(1.45) <i>Grandidierella</i> spp. 3.18(1.47)	

3.5.2 Macrobenthos drivers

A combination of salinity, pH, particulate organic matter and coarse sand ($\rho=0.526$, sig level = 0.1) were the environmental variables that best explained the distribution patterns of macrobenthic taxa. The first axis, with an Eigenvalue of 0.43, accounted for 57.88% of the variation, while the second axis, with an Eigenvalue of 0.19, explained 25.73%. Together, these two axes captured 83.6% of the total variation in macrobenthos abundance data. The CCA further provided a visual insight into the relationship between biological communities and abiotic factors (Figure 3.12).

The results of the Canonical Correspondence Analysis (CCA) revealed that pH, dissolved oxygen (DO%) and coarse sand percentage were the most significant drivers of variability in macrobenthic community composition and density in the uMdloti Estuary. In contrast, salinity and total organic content (TOC) were found to be the least influential factors. The CCA plot demonstrated a clear separation between samples from the lower reaches and those from the middle and upper reaches. However, samples from the middle and upper reaches clustered together, indicating minimal variability in the taxa composition between these two sites. Estuarine taxa such as *G. lutosa*, *G. lignorum* and *D. cf. ornata* were closely associated with well-oxygenated, saline conditions in the lower reaches, with *Grandidierella* spp., displaying a strong association with POM. In contrast, *C. keiskama* showed a strong correlation with elevated pH levels, which coincided with its higher abundance during the sampling periods of January and March 2020. Stress-tolerant taxa, including oligochaetes from the family Naididae, *C. fluminalis* and chironomid larvae, were predominantly associated with coarse sand sediments and high organic matter content in the upper and middle reaches. Additionally, *T. granifera* and *Dero* sp. were positioned near the origin of the CCA plot, suggesting that their distribution was moderately influenced by a mix of the environmental variables, rather than any single dominant variable.

Table 3.6: Summary of BIOENV results showing the correlation values of macrobenthos communities and environmental variables measured at three stations of uMdloti Estuary.

No. of variables	Correlation	Environmental variables
5	0.508	Dissolved oxygen, salinity, pH, particulate organic matter, coarse sand
4	0.513	Dissolved oxygen, salinity, pH, particulate organic matter
4	0.526	Salinity, pH, particulate organic matter, coarse sand

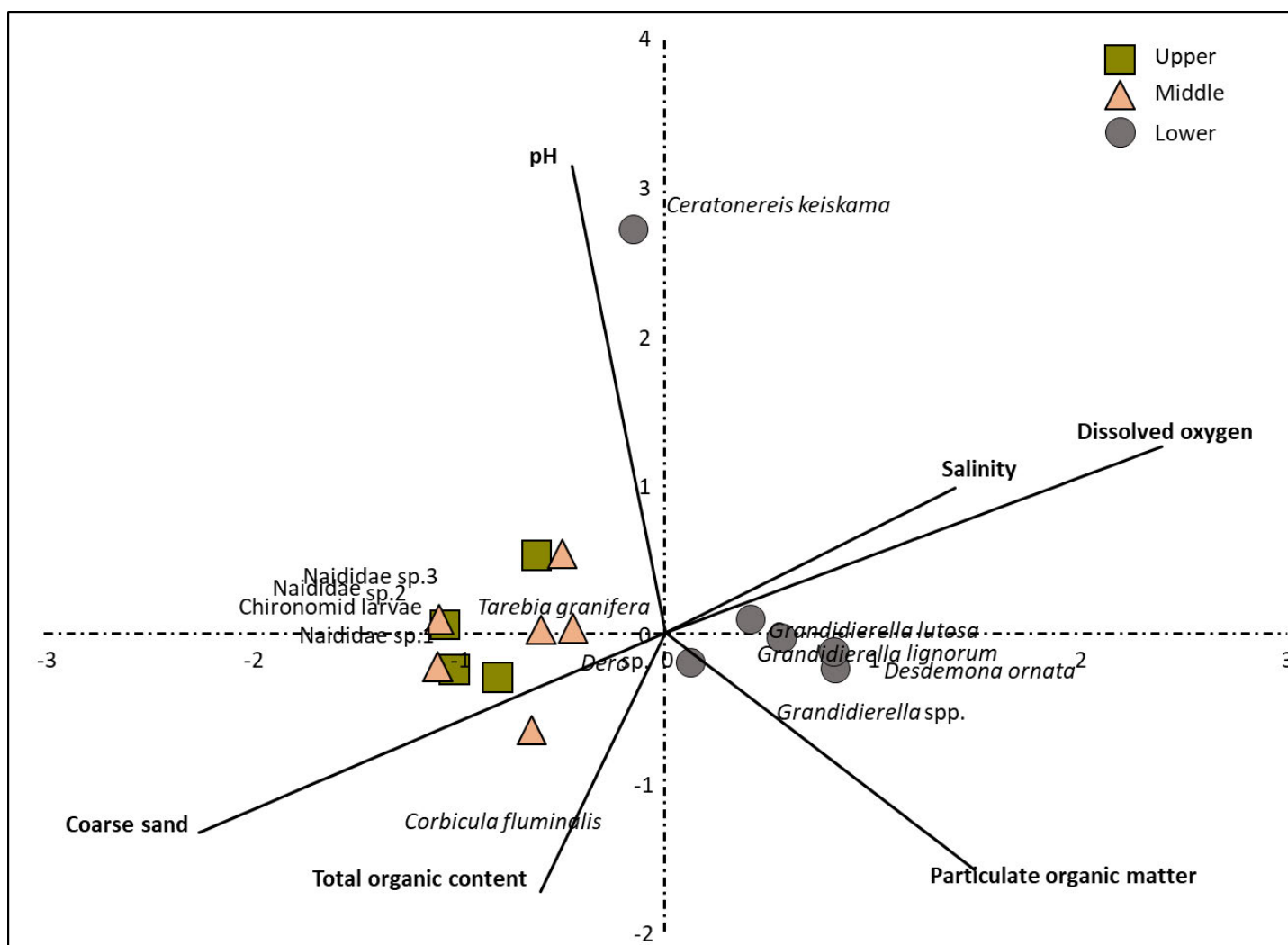


Figure 3.12: Canonical Correspondence Analysis (CCA) of benthic invertebrate density for the three sites sampled in the uMdloti Estuary.

3.7 Discussion

3.7.1 Physico-chemical parameters

The environmental parameters of the uMdloti Estuary were strongly influenced by seasonal variability, a common characteristic of TCEs in subtropical regions. During the dry season, reduced rainfall significantly limited freshwater inflow, causing the estuary mouth to close. Under these conditions, the estuary relies on wastewater as the primary freshwater source (Brooker & Scharler 2020), which results in reduced salinity, elevated nutrient concentrations and the potential for stagnation (Cloern 2001, Kennish 2002, Lemley et al. 2014, Scharler et al. 2023). In contrast, increased rainfall during the wet season generates sufficient freshwater inflow to breach the estuary mouth and temporarily open it. This opening facilitates tidal exchange, which restores the estuarine environment by resetting salinity and nutrient levels (Skinner et al. 2006, Human et al. 2016, Slinger 2024).

The uMdloti Estuary retained its oligohaline state (< 2 ppt) during the period of low flow/dry season and only transitioned to mesohaline (> 5 ppt) conditions during the wet season following the mouth breach events in January and March 2025. In January 2020, high salinity was recorded at the estuary mouth, with marked variability compared to the more uniform salinity levels observed in the middle and upper reaches. This variability emanates from the perched characteristic of the estuary which limits tidal intrusion into the upper regions.

During the current study, particularly in the dry season, the DO and pH levels in the uMdloti Estuary displayed a stable and consistent spatial trend, with DO increasing from the upper to the lower reaches. In contrast, phytoplankton chlorophyll-a, exhibited wide fluctuations, peaking in July 2019 but remaining low throughout most of the dry season. During the wet season, as temperatures increased, there was an elevation in photosynthetic productivity. Although the PCA indicated a positive association between chlorophyll-a and DO concentrations (Figure 3.4), the data showed a decline in DO levels from December 2019 to January 2020, despite an increase in chlorophyll-a concentration (Figure 3.2B and 3.3A). This suggests that other factors may have influenced DO levels during this period, possibly overshadowing the expected positive effect of higher chlorophyll-a on oxygen production.

The decline in DO is usually associated with elevated respiratory processes, likely driven by the decomposition of organic matter and increased bacterial activity (Lemley et al. 2015, Lemley & Adams 2020, Schmidt & Diallo 2024, Shen & Qin 2024). However, in the uMdloti Estuary, Omarjee et al. (2020) and Scharler et al. (2023) reported that phytoplankton chlorophyll-a has little to no correlation with DO variability. Instead, microphytobenthos activity and bacterial respiration are more likely to influence DO concentrations. pH displayed no consistent pattern during the study period. There were minimal fluctuations with a slight increase in pH during the wet season and a strong correlation with phytoplankton productivity (Figure 3.4). Increased photosynthetic activity tends to elevate pH by reducing dissolved CO₂, while a reduction in phytoplankton and the dominance of respiratory processes can lower pH (Carstensen & Duarte 2019).

Naturally, when an estuary breaches, there is an inverse reaction where the accumulated phytoplankton is flushed out, leading to a decrease in phytoplankton chlorophyll-a levels (Perissinotto et al. 2002, Thomas et al. 2005, Murrell et al. 2007, Navas-Parejo et al. 2020). This process is often accompanied by an increase in pH and DO levels due to an exchange with oxygen-rich oceanic water which also provides buffering in estuarine water (Perissinotto et al. 2010, Lemley & Adams 2020, Omarjee et al. 2020, Shen & Qin 2024). However, in perched TCEs with high nutrient and organic influx, the opposite effect can occur during an open phase. Due to limited flushing, biological processes (i.e. respiration and microbial decomposition) become the primary drivers influencing chlorophyll-a, DO and pH variability. In the current study, these claims were possibly supported by the elevated chlorophyll-a during the open phase, a decline in pH level (6.1) particularly in lower reaches and low dissolved oxygen during the open phase (January 2020). The reopening of the estuary mouth in March 2020 seems to have influenced DO concentrations and pH levels, with DO levels recovering. This improvement was likely due to the exchange with oxygen-rich ocean water, which enhanced the oxygenation of the estuarine water (Shen & Qin 2024). Meanwhile, the oceanic water exchanges with estuarine waters provided a buffering effect on pH, promoting an increase in pH, particularly in the mouth.

3.7.2 Macrobenthos community structure

The uMdloti Estuary was characterised by the abundance of the polychaetes such as *D. cf. ornata* and *C. keiskama*, the oligochaete Naididae sp1, the bivalve *C. fluminalis*, the *T. granifera* and the amphipods *G. lutosa* and *Grandidierella* spp. These are a combination of freshwater and estuarine taxa that are common in the east coast TCEs (Teske et al. 2006, Perissinotto et al. 2004). *Tarebia granifera* was first documented in the uMdloti Estuary just over a decade ago by Stow (2011) and subsequently reported by (Scharler et al. 2020). However, there are no prior records according to Blaber et al. (1984), Forbes & Demetriades (2008) and Perissinotto et al. (2004).

In the current study, the uMdloti Estuary macrofaunal densities, number of species and diversity were higher during the dry and closed mouth phase and significantly lower in the open mouth phase. This pattern mirrors observations in other TCEs, such as the aManzimtoti Estuary (Stow 2011), iMpenjani Estuary (Ortega-Cisneros 2013) and in different parts of the world such as Camacho Lagoon in South Brazil (Netto et al. 2012) and Rio Grande in the USA (Montagna et al. 2022). Reduced densities during the open-mouth phase result from scouring and flushing, eliminating biota from the estuary. The taxa assemblage and composition showed significant spatial and temporal variability along the estuarine gradient. True estuarine taxa were recorded in the lower reaches, while the middle reaches displayed a mosaic of estuarine and freshwater communities and the upper reaches a residence to freshwater groups. However, estuarine taxa such as *C. keiskama*, *D. cf. ornata* and *Grandidierella* spp. were able to proliferate across all estuary regions, as previously noted by Forbes & Demetriades (2008) and Perissinotto et al. (2004). This pattern is common in TCEs that frequently attain an open mouth state such as iMpenjani Estuary, uTongati Estuary and iLovu Estuary (Forbes & Demetriades 2008).

Mouth-breaching events influenced community structure and composition, with notable variations among sites, especially impacting the lower reaches (closer to the mouth). The open mouth conditions in TCEs establish a link to the marine environment, promoting the movement of marine groups and enhancing community diversity (Montagna et al. 2022, Perissinotto et al. 2004, Scharler et al. 2020). In the uMdloti Estuary, this led to the introduction of marine/brackish taxa, including *Prionospio* spp. polychaetes and the *H.*

lunulate gastropod. Mouth breaching caused abrupt environmental changes, favouring a few species. This was evident in December 2020 (middle and upper reaches), January 2020 and March 2020, where there were exceptionally low densities and an introduction of additional marine/estuarine taxon in the lower reaches. Peak densities observed at the mouth in December 2020 could be attributed to the brief opening that took place in late November 2020, before our sampling date. This mouth breach event may have facilitated an increase in estuarine taxa densities, particularly *D. cf. ornata* and *Grandidierella* spp.

Following a disturbance, communities are usually dominated by opportunistic species which are small individuals tolerant of a disturbance (Elliott & Quintino 2007). This response was evident in the lower reaches of the uMdloti Estuary where the community was dominated by *Prionospio* spp. which are small-bodied opportunistic polychaetes that rapidly pioneer disturbed environments (Pearson & Rosenberg 1978, Chollett & Bone 2007). Succeeding a stressful period is a recovery stage characterised by the abundance of large-bodied individuals, which are usually common before the disturbance (Elliott & Quintino 2007). During the current study, the recovery stage was marked by the invasion of taxa such as *C. keiskama*, *H. lunilata* and *G. lignorum*. A similar recovery trend was observed by Vivier (2010) in the iNhlabane Estuary after a flood event, where a steady increase in these species replaced opportunistic taxa. Conversely, in the Siyaya Estuary, Mackay (1996) noted that these taxa acted as pioneers in disturbed areas. Similarly, this study noted that *Grandidierella* amphipods and *C. keiskama* persisted within the system and subsequently assumed a key role in recolonising areas that were depleted of species.

3.7.3 Macrobenthos drivers

Important parameters that explained macrobenthos community patterns included DO, coarse sand, POM, pH, salinity and TOC. Salinity, DO and POM showed a strong influence on the abundance of the amphipod species, *G. lutosa*, *G. lignorum*, *Grandidierella* spp. and the polychaete *D. cf. ornata*. Various studies have recorded the occurrence of the *Grandidierella* amphipod species in estuaries with mesohaline to marine conditions (Schlacher & Wooldridge 1996, Vivier 2010, Masikane et al. 2014) as well as in estuaries with oligohaline conditions (Stow 2011). These amphipods exhibit a range of physiological and morphological adaptations

which enable them to cope with a wide range of salinity and abrupt environmental salinity changes (Masikane et al. 2014). They employ active osmoregulation strategies, using specialised ion-transporting epithelial cells in the coxal gills to regulate electrolyte balance, allowing the amphipods to maintain constant osmotic pressure even in the highly variable salinities of estuarine environments (Kikuchi & Matsumasa 1993).

Grandidierella amphipods exhibit suspension and deposit feeding strategies, which involve surfacing from their burrows to collect detritus (McLusky & Elliott 2004), which may explain their association with POM. During the early developmental stages, they employ filter-feeding behaviour, allowing them to filter food material in response to high river inflows (Read & Whitfield 1989). Similarly, *D. cf. ornata* demonstrates versatile feeding strategies based on the prevailing environmental conditions. While they can typically function as suspension feeders in organic-rich muddy environments (Schlacher & Wooldridge 1996, Rodil et al. 2008) they have been observed thriving in organically deprived sandy substrates evident in the iNhlabane Estuary (Vivier 2010) and uMdloti Estuary (this study). *Desdemona cf. ornata* showed a positive association with POM, particularly during months with elevated POM concentrations such as July and December 2019. However, its persistence even during periods of low POM (March 2020) suggest a wider tolerance to fluctuating food availability. While *Grandidierella* amphipods and *D. cf. ornata* were negatively associated with coarse sand and organic matter, the uMdloti Estuary still provided optimal habitat for these taxa, as the sediment particles fell within their preferred size range (0.15 mm – 1.0 mm) (Cyrus & Martin 1988).

Ceratonereis keiskama was associated with higher pH levels in the lower reaches of the estuary, which is indicative of healthy dissolved oxygen levels and high primary productivity. *Ceratonereis keiskama* it is a surface deposit feeder, consuming a mix of detritus, microalgae, and organic particles in the sediment, particularly in areas of high primary productivity (Ortega-Cisneros 2013). Although a negative association with POM and TOC was observed, *C. keiskama* has been previously linked to areas of high sediment organic matter, suggesting that its occurrence may be influenced by localised organic enrichment. Its flexible diet (Ortega-Cisneros & Scharler 2015, Rishworth et al. 2018) likely enables it to exploit a range of food sources under varying conditions.

The freshwater taxa such as Oligochaetes (Naididae sp1, Naididae sp2, Naididae sp3 and *Dero* sp.), Chironomid larvae and *T. granifera* associated with oligohaline environment, with coarse sand and organically enriched substrate. However, *T. granifera* was positioned closer to the central region of the nMDS plot suggesting that it is adaptable to intermediate environmental conditions and may tolerate a range of factors. These taxa are key components of macrobenthic communities in the headwaters of most estuaries (Perissinotto et al. 2004, Forbes & Demetriades 2008, Ngqulana et al. 2010, Vivier 2010, Stow 2011). In addition, they have been reported as indicators of compromised system health and community resilience. For example, these taxa have dominated macrobenthos communities of heavily modified systems health due to pollution (particularly nutrient enrichment) with associated frequent hypoxic and anoxic conditions such as uMvoti Estuary (Swemmer 2011, Vezi et al. 2020).

The uMdloti Estuary faces ecological pressure from two invasive species of concern, namely, *T. granifera* and *C. fluminalis*. Elevated densities of *T. granifera* can lead to reduced species diversity and shifts in community composition, making it a potential bioindicator of environmental degradation (Appleton et al. 2009, Miranda et al. 2011). While *T. granifera* showed no specific association with any environmental in the CCA, previous studies have shown that its abundance often correlates with organic enrichment, poor water quality and reduced ecological integrity (Miranda et al. 2011, Scharler et al. 2020). With available knowledge on the water quality of uMdloti Estuary including elevated nutrient loads, periodic low dissolved oxygen events, and episodic freshwater inflows, the observed proliferation of *T. granifera* may reflect ongoing environmental stress and the system's reduced capacity to support diverse native benthic assemblages.

Corbicula fluminalis, like *T. granifera*, is an invasive species that thrives in freshwater and limnetic estuarine environments (Hakenkamp & Palmer 1999, Sousa et al. 2008). To date, no published research is available detailing the ecology of these bivalves in South African estuaries. However, they have been documented as an invasive species in other parts of the world such as Spain (Minho Estuary) (Sousa et al. 2008). Sousa et al. (2008) further identified coarse sand and organic matter as key abiotic factors contributing to its abundance, particularly in organically polluted sites. These findings align with the results of this study, as *C. fluminalis* dominated the middle and upper reaches of the uMdloti Estuary. These sites are characterised by elevated nutrient and organic matter levels due to discharge from

wastewater treatment plants and agricultural runoff (Nozais et al. 2001, Tagliarolo & Scharler 2018). The observed dominance of *C. fluminalis* suggests its adaptability to nutrient-enriched habitat, however, further research is needed to fully understand its ecological impact and adaptive mechanisms. *Corbicula fluminalis* is a filter feeder that can also adopt a deposit-feeding strategy depending on the availability of food sources (Vaughn & Hakenkamp 2001, Sousa et al. 2008). They have a high metabolic rate, supported by a dual-feeding mode that allows them to filter-feed on organic matter in the water column and pedal-feed on organic matter in the sediment (Hakenkamp & Palmer 1999). Such adaptability enables *C. fluminalis* to exploit a broad range of resources, potentially consuming more food in invaded environments than native fauna (Hakenkamp & Palmer 1999). This competitive advantage poses a significant threat to native species, as it may lead to resource depletion and displacement of local populations.

In conclusion, this study revealed that the macrofaunal community structure in the uMdloti Estuary responds significantly to seasonal variations, particularly influenced by the open and closed states of the estuary mouth. Higher densities, diversity and number of species were observed during dry and closed-mouth phases, while open-mouth phases led to substantial declines due to flushing effects. Environmental variables, such as DO levels, salinity gradients and organic content, emerged as key drivers of macrobenthic community structure, exhibiting pronounced seasonal shifts tied to the estuarine mouth state. The short-term seasonal responses of macrobenthic communities to changes in salinity, dissolved oxygen and phytoplankton levels provided insight into how these communities may respond to long-term climatic and anthropogenic changes.

The presence of estuarine taxa such as the *Grandidierella* amphipods and *C. keiskama* reflect relatively healthy estuarine environmental conditions. These taxa have been associated with improved ecosystem function, increased diversity and resilience in response to drought-induced stress, as these taxa contribute to cycling and food web stability (Gerber et al. 2024). Furthermore, these taxa have also been observed to be sensitive to prolonged unfavourable environmental conditions such as oligohaline and hypoxic environments (Scharler et al. 2020, Ndhlovu et al. 2024), making them good indicators of environmental change. While the presence of estuarine taxa is typically indicative of functional and resilient estuarine conditions, the concurrent presence of invasive species may reflect localised or underlying

stressors that compromise ecological balance. Despite the observed improved environmental conditions and benthic communities, the estuary remains under considerable anthropogenic stress, reflected in its ecological health score of D. This contrast highlights the complexity of estuarine systems, where signs of recovery or resilience in native communities may coexist with pressures that threaten long-term ecological integrity. Future research should focus on monitoring additional global change impacts, such as nutrient enrichment, flow modification and biological invasions, to better understand their effects on macrobenthic communities and to guide effective management strategies aimed at enhancing the resilience and long-term health of the uMdloti Estuary.

Chapter 4: uMlalazi Estuary

4.1 Introduction

The uMlalazi Estuary is located within the uMlalazi Nature Reserve, which is protected under the National Environmental Act (No. 57 of 2003) and managed by Ezemvelo KwaZulu-Natal Wildlife (uMlalazi Nature Reserve: Integrated Management Plan: 2009–2013, 2009). The uMlalazi Estuary exhibits a near-natural (Category B) ecological health status, with minimal alterations from its original pristine condition (van Niekerk et al. 2019). The system has a high biodiversity priority, is an important nursery area for fish and supports significant bird life (van Niekerk et al. 2019). The uMlalazi is associated with important ecological habitats namely, swamp forest (100 ha), intertidal (42 ha) and supratidal (34.4 ha) saltmarsh, reeds and sedges (43 ha), mangrove (45 ha) and open water (140 ha) area (Van Niekerk et al. 2019, Taylor 2020). Despite this, it is not exempted from anthropogenic pressures with a documented history of pressures such as flow modification, pollution, poor land use and concerns related to recreational activities (Mabaso 2002, Kelbe & Taylor 2019, Schmitz et al. 2024).

4.1 Morphodynamics and physico-chemical characteristics

Even though the uMlalazi Estuary is a predominantly open system, it occasionally experiences temporal mouth closures during periods of extremely low river flow, often associated with droughts (Kelbe & Taylor 2019, Scharler et al. 2023). These closures are further aggravated by reduced freshwater inflows due to upstream anthropogenic activities such as water abstraction and land-use changes in the catchment (Kelbe & Taylor 2019, Scharler et al. 2023). The uMlalazi Estuary historically experienced frequent mouth closures in the 1800s and early 1900s (Hill 1966). During these years, the system was artificially breached to prevent extended mouth closures which led to flooding of the surrounding area and significant mangrove die-offs post-colonisation (Hill 1966). Hill (1966) reported that the uMlalazi Estuary mouth closed five times between 1945 and 1952, while Kelbe & Taylor (2019) reported that it closed five times between 1993 and 2013 and later closed two times between 2013 and 2017.

The physico-chemical parameters of the uMlalazi Estuary display a strong spatial and temporal variability, primarily driven by freshwater inflow volumes, tidal intrusion and the estuary size. This variability is further influenced by short-term climatic events such as floods (Mabaso 2002) and droughts (Scharler et al. 2023) as well as human activities such as freshwater abstraction and pollution (Mabaso 2002). Salinity within the estuary is primarily determined by river runoff, which governs dilution processes (Mabaso 2002, Scharler et al. 2023). Typically, salinity forms a gradient from marine conditions near the mouth to brackish in the middle reaches and freshwater upstream (Ortega-Cisneros 2013, DWS 2015). Reduced river flow disrupts this gradient, causing salinity to fluctuate between brackish and marine conditions (Scharler et al. 2023), while floods result in predominantly fresh conditions, with marine influence limited to the mouth (Mabaso 2002).

The uMlalazi Estuary is typically well-oxygenated but periodically experiences hypoxic conditions, particularly in the middle and upper reaches (Mabaso 2002, Scharler et al. 2023). The primary drivers of these low-oxygen events are attributed to the characteristics of the sediment, especially in areas with muddy substrates, along with increased turbidity, elevated nutrient concentrations and biological factors (Mabaso 2002, Scharler et al. 2023). These hypoxic conditions tend to intensify during periods of low freshwater water flow or drought, while improved hydrological flushing during wet periods mitigates these conditions (Scharler et al. 2023). The DWS (2015) noted that dissolved oxygen (DO) levels in the estuary largely reflect natural conditions, assigning an 80% score on the water quality health index.

Particulate organic matter (POM), microphytobenthos (MPB) and phytoplankton chlorophyll-a exhibit significant seasonal variability in response to shifts in the hydrological regimes of the uMlalazi Estuary (Ortega-Cisneros 2013, Scharler et al. 2023). These components play a critical role in sustaining ecosystem function by supporting primary production, nutrient cycling and food web dynamics (Ortega-Cisneros 2013). The seasonal fluctuations in MPB and phytoplankton chlorophyll-a concentrations serve as key indicators of phytoplankton responses to variations in nutrient availability, light conditions and water residence times (Ortega-Cisneros 2013). Higher chlorophyll-a levels are typically observed during periods of low flow and limited tidal flushing (Ortega-Cisneros 2013, Scharler et al. 2023). During these conditions, the system tends to stabilise, enabling biomass accumulation in both pelagic and benthic environments, provided that nutrient availability remains sufficient.

4.2. Macrobenthos of uMlalazi Estuary

Predominantly open estuaries provide highly dynamic environmental conditions due to the consistent water exchange between the sea and the river compared to the temporarily open or closed counterparts (Teske & Wooldridge 2001, Ortega-Cisneros 2013). This connectivity influences the structure of the macrobenthic assemblages, which include diverse macrobenthic taxa and lower densities compared to TCEs (Teske & Wooldridge 2001, McLusky & Elliott 2004). The variation in benthic macrobenthos distribution is driven by water quality, substrate influences and natural factors (i.e. seasons), including seasonal population shifts (Blaber et al. 1984, Lu & Wu 2007, MacKay et al. 2010, Scharler et al. 2020, Szczepanek et al. 2021).

The macrobenthic communities of the uMlalazi Estuary are an essential component of the ecological stability and function of the system, particularly in relation to nutrient cycling and the transfer of energy within the estuarine food web. (Ortega-Cisneros & Scharler 2014, Gerber et al. 2024). Studies have shown that groups such as polychaetes, bivalves and amphipods play a vital role in the bioturbation and bioirrigation of sediment and the recycling of nutrients (i.e. carbon and nitrogen) (Ortega-Cisneros 2013, Gerber et al. 2024). In addition, these organisms play a critical role in sustaining the food web by contributing to the detritus-based energy flow, particularly during unfavourable climatic conditions like drought (Gerber et al. 2024).

The first study on macrobenthic communities in the uMlalazi Estuary was conducted by Hill (1966), identifying a diverse array of 74 taxa. Dominant macrobenthic groups included Brachyura, gastropods, bivalves, and polychaetes. Among these, ecologically significant arthropod species such as *Varuna litterata*, *Paratylodiplax blephariskios*, *Scylla serrata*, five *Sesarma* species, four *Uca* species, and three *Ocypode* species were particularly abundant within the system. Hill (1966) also recorded other important species like *Upogebia africana* and three species of *Penaeus* decapods. However, subsequent research by Mabaso (2002) reported a shift in the dominant group from Brachyura to various species of Polychaeta. Mabaso (2002) attributed this shift to alterations in substrate composition and deteriorating water quality. Further studies on the macrobenthos of uMlalazi Estuary reported a slight similarity in community composition and also reported relatively healthy macrobenthic

communities with a health index score of 80 (DWS 2015). Despite the reported environmental changes, studies by Ortega-Cisneros (2013) and DWS (2015) reported that the macrobenthic community of the uMlalazi Estuary remained dominated by taxa such as polychaetes *Dendronereis arborifera* and *Prionospio* spp., the tanaid *Halmyrapseudes cooperi*, the amphipod *Americorophium triaenonyx*, the brachyuran *P. blephariskios* and the mollusc *Brachidontes virgiliae*.

Despite the uMlalazi Estuary being a protected system of high ecological value, it faces multiple anthropogenic threats (van Niekerk et al. 2019). These include reduced freshwater inflows due to upstream water abstraction, human settlement, nutrient pollution from wastewater treatment plant discharge and aquaculture and agricultural runoff (Mabaso 2002, Kelbe & Taylor 2019, Scharler et al. 2023, Schmitz et al. 2024). The projected increases in seasonal precipitation variability (IPCC 2023), coupled with increasing anthropogenic pressures are expected to degrade ecologically significant estuarine habitats (van Niekerk et al. 2022). This degradation threatens the quality of environmental conditions and structure of the macrobenthic community, risking the loss of species essential for nutrient cycling, sediment stabilisation and food web dynamics (Savage et al. 2002, Baeta et al. 2011, Donázar-Aramendía et al. 2019, van Niekerk et al. 2019, Drylie et al. 2020, Lécuyer et al. 2024, Gerber et al. 2024). A decline in these key species could severely weaken the resilience and capacity to provide essential services (Watson et al. 2019, Gerber et al. 2024).

Given the critical ecological roles of macrobenthic species, the notable loss of critical benthic species and rising pressures on estuarine environments, it is essential to understand the extent and drivers of these changes. Therefore, this study aimed to investigate the variability of the macrobenthic community of the uMlalazi Estuary. To achieve this, the community was assessed in terms of species density and community structure over temporal (seasonal) and spatial scales for the period June 2019 – August 2019 and December 2019, January and February 2020. In addition, the study examined the relationships between environmental parameters and the macrobenthos community to identify key factors influencing these variabilities.

4.3 Methods and materials

4.3.1 Study area

The uMlalazi Estuary (28° 56' 42" S, 31° 48' 58" E) is a POE located on the northeastern coast of South Africa (Figure 4.1). It is fed by the Mlalazi River, with a catchment drainage area of 397 km² and receives a mean annual runoff (MAR) of 124.6×10^6 m³ (Van Niekerk & Turpie 2011, van Niekerk et al. 2019). This estuarine system is characterised by its elongated and narrow shape, with a width ranging from 50 to 200 m, flowing towards the sea between sand dunes (Hill 1966, DWS 2015, Kelbe & Taylor 2019). The mouth is oriented in the north-east direction, extending 4 km towards the sea with a width varying from 14 to 88 m and a length of 4.4 km (Kelbe & Taylor 2019). The system experiences tidal influence for about 15 km (DWS 2015).

For the current study, uMlalazi Estuary was divided into three regions based on the salinity gradient along the estuary (Hill 1966, Mabaso 2002, DWS 2015). Sites 1 and 2 are situated in the lower reaches and display a strong tidal influence while sites 3 and 4 represent the middle reaches and site 5 is the upper reaches with a predominant fluvial influence.

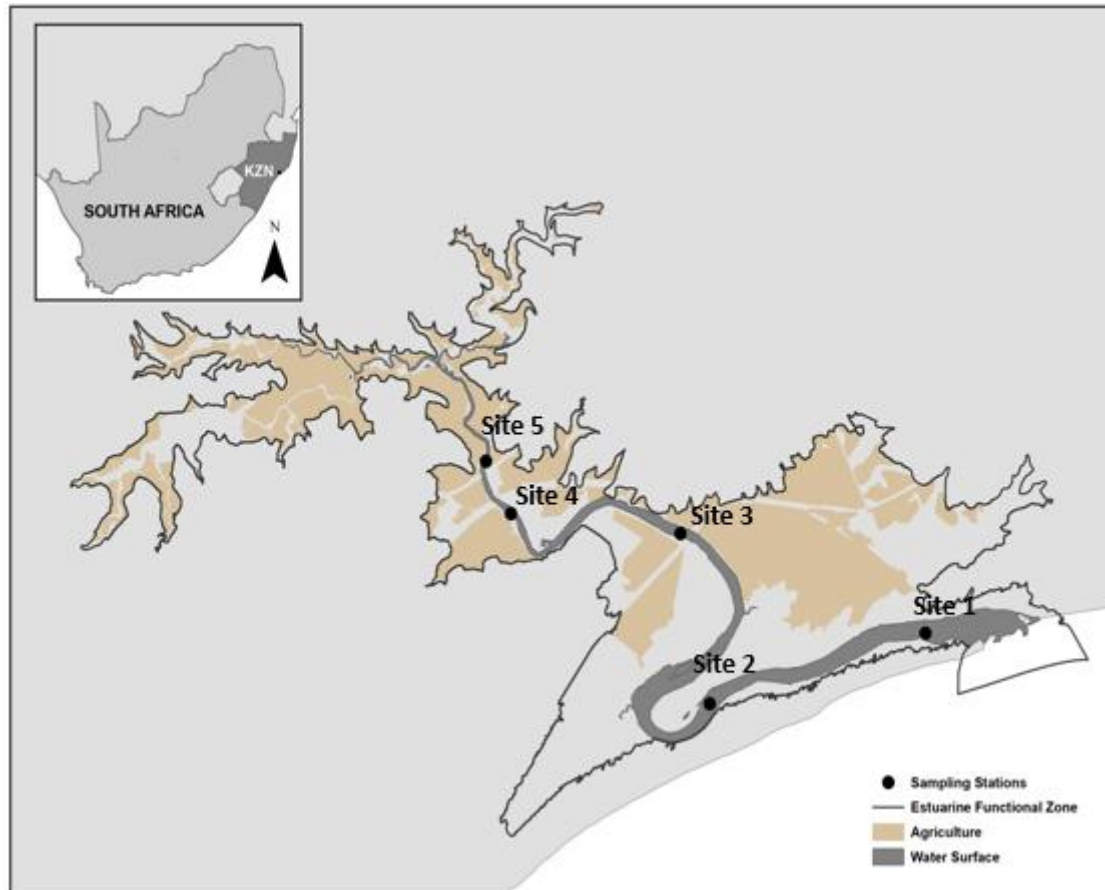


Figure 4.1: Location and map of uMlalazi Estuary showing the distribution of study sites. Sites 1 and 2 represent the lower reaches, sites 3 and 4 the middle reaches and site 5 the upper reaches.

4.3.2 Methods and materials

Chapter 2 (General Materials and Methods) provides an in-depth overview of the standard procedures for collecting macrobenthos and environmental conditions (water and sediment), conducting laboratory processing and performing statistical analyses. Data from site 3 in December 2019 and site 5 in January 2020 were excluded from the PCA, PERMANOVA, BEST and CCA analyses due to missing YSI and/or MPB chlorophyll-a data for these months.

4.4 Results

4.4.1 Environmental variables

An expected seasonal difference was observed between the dry and wet seasons (Figure 4.2A) with mean dry and wet temperatures of 20.47 ± 0.89 and 26.86 ± 3.61 °C respectively. Overall, difference between the dry and wet seasons were marked by a large temperature range between 18.81°C in July 2019 at site 3 and 31.22°C in January 2020 at site 4.

A strong spatial and temporal salinity gradient prevailed varying from marine to freshwater (Figure 4.2B). As a predominantly open estuary, the uMlalazi Estuary retained high salinities at sites 1 and 2 (closer to the mouth) during the dry (range, 33 – 35 ppt) and wet (range, 26 – 35 ppt) seasons, while other sites fluctuated greatly between the seasons ranging between 1 and 30 ppt. At the beginning of the wet season, a significant decline in salinity was observed in the upper reaches, where salinities reached 3 and 1 ppt, indicating an increase in freshwater influence. During the dry season, the system varied from brackish to marine (9 – 35 ppt) and fresh to marine (1 – 35 ppt) during the wet season.

The pH in uMlalazi Estuary consistently ranged between 7 and 8.37 except in July 2019 where a slightly lower (6.65) pH was recorded at site 5 (Figure 4.2C). The highest pH value was recorded at the lower reaches in June 2019.

The uMlalazi Estuary was well oxygenated all through the study period and across all sites with DO concentrations ranging from $4.96 \text{ mg}\cdot\text{l}^{-1}$ – $8.78 \text{ mg}\cdot\text{l}^{-1}$ (Figure 4.2D). Congruent with the existing estuarine salinity gradient in the system, DO concentrations increased from the upper reaches to the lower regions with the lowest DO concentrations observed in site 4. DO concentrations were slightly lower during the wet season, ranging from $4.96 \text{ mg}\cdot\text{l}^{-1}$ to $8.63 \text{ mg}\cdot\text{l}^{-1}$, compared to the dry season when DO concentrations ranged between $5.73 \text{ mg}\cdot\text{l}^{-1}$ and $8.78 \text{ mg}\cdot\text{l}^{-1}$.

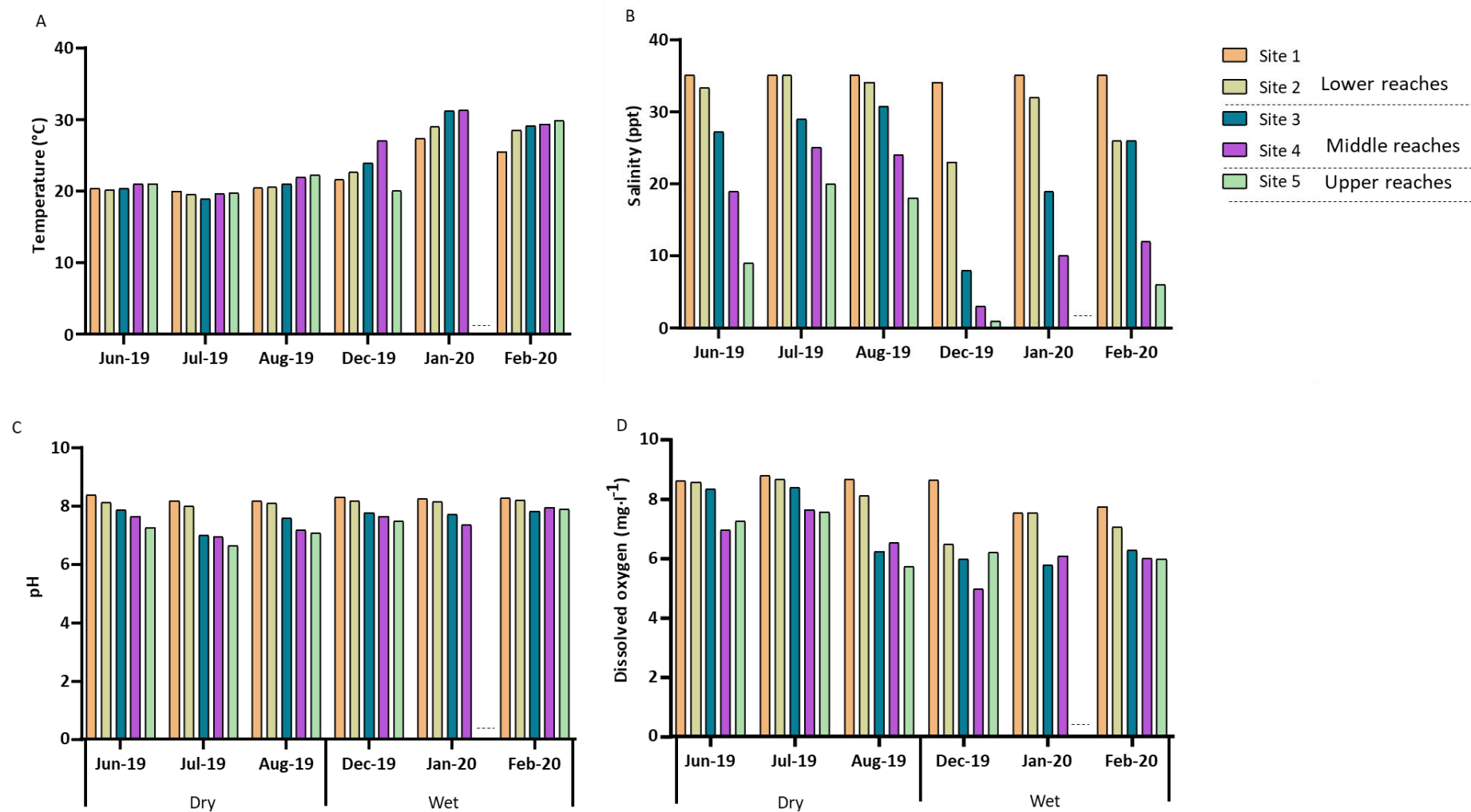


Figure 4.2: Measurement of seasonal (wet and dry) environmental variables at five sites (1 – 5) at the uMlalazi Estuary: temperature (A), salinity (B), pH (C) and dissolved oxygen (D). Dotted line represents missing data.

The suspended POM of uMlalazi Estuary displayed no obvious spatial pattern (Figure 4.3A). High POM concentrations were generally noted at site 5, ranging from $25 \pm 70 - 43 \pm 10.60\%$. Site 5 was also associated with the lowest POM ($9.50 \pm 1.20\%$) in December 2019. A decline in POM was observed during the wet season across all sites, particularly in December, where it ranged between 9.50 ± 1.20 to $19.9 \pm 0.30\%$, with values increasing from site 5 to site 1.

Total suspended solids (TSS) concentrations were highly variable throughout the study period and across all sites (Figure 4.3B). Slightly elevated concentrations were observed during the dry season (range, $21.48 \pm 10 \text{ mg}\cdot\text{l}^{-1} - 113.82 \pm 2.12 \text{ mg}\cdot\text{l}^{-1}$) compared to the wet season (range, $17.00 \pm 5.37 \text{ mg}\cdot\text{l}^{-1} - 101.86 \pm 41.10 \text{ mg}\cdot\text{l}^{-1}$) and it was a common trend across all sites. Site 3 generally had high suspended solids concentration, particularly during the dry season ranging from $104.10 \pm 11.68 \text{ mg}\cdot\text{l}^{-1} - 194.14 \pm 15 \text{ mg}\cdot\text{l}^{-1}$ which was followed by sites 2 and 1 with ranges from $56.60 \pm 10 \text{ mg}\cdot\text{l}^{-1} - 113.80 \pm 2.12 \text{ mg}\cdot\text{l}^{-1}$ and $69.62 \pm 0.42 \text{ mg}\cdot\text{l}^{-1} - 112.70 \pm 7.21 \text{ mg}\cdot\text{l}^{-1}$ respectively. Sites 4 (range, $20.20 \pm 9.90 \text{ mg}\cdot\text{l}^{-1} - 69.30 \pm 6.31 \text{ mg}\cdot\text{l}^{-1}$) and 5 (range, $17.00 \pm 5.37 \text{ mg}\cdot\text{l}^{-1} - 58.68 \pm 0.85 \text{ mg}\cdot\text{l}^{-1}$) had relatively lower TSS compared to the sites at the lower regions of the estuary.

The MPB in uMlalazi Estuary displayed a wide range in concentrations ($32.48 \pm 2.00 \text{ mg}\cdot\text{m}^{-2} - 680.06 \pm 113.52 \text{ mg}\cdot\text{m}^{-2}$) but no temporal trend across all sampling sites (Figure 4.3C). MPB values $> 50 \text{ mg}\cdot\text{m}^{-2}$ were generally recorded at sites 1 to 3 except for $34 \pm 1.90 \text{ mg}\cdot\text{m}^{-2}$ recorded at site 1 in August 2019. Sites 4 and 5 were generally MPB deprived with ranges between 17 and $69.30 \text{ mg}\cdot\text{m}^{-2}$. The highest MPB concentration ($194 \pm 1.44 \text{ mg}\cdot\text{m}^{-2}$) was recorded at site 3. Phytoplankton concentration notably varied between the dry and wet seasons across sites 1 to 5 (Figure 4.3D). During the dry season, the concentration remained relatively stable, ranging from 1.36 ± 0.17 to $11.39 \pm 0.72 \text{ mg}\cdot\text{m}^{-3}$. In contrast, the wet season showed much greater variability, with a maximum concentration of $41.24 \pm 4.77 \text{ mg}\cdot\text{m}^{-3}$ and a minimum of $3.43 \pm 0.06 \text{ mg}\cdot\text{m}^{-3}$. In contrast, the wet season showed much greater variability, with a maximum concentration of $41.24 \pm 4.77 \text{ mg}\cdot\text{m}^{-3}$ and a minimum of $3.43 \pm 0.06 \text{ mg}\cdot\text{m}^{-3}$ (Figure 4.3D).

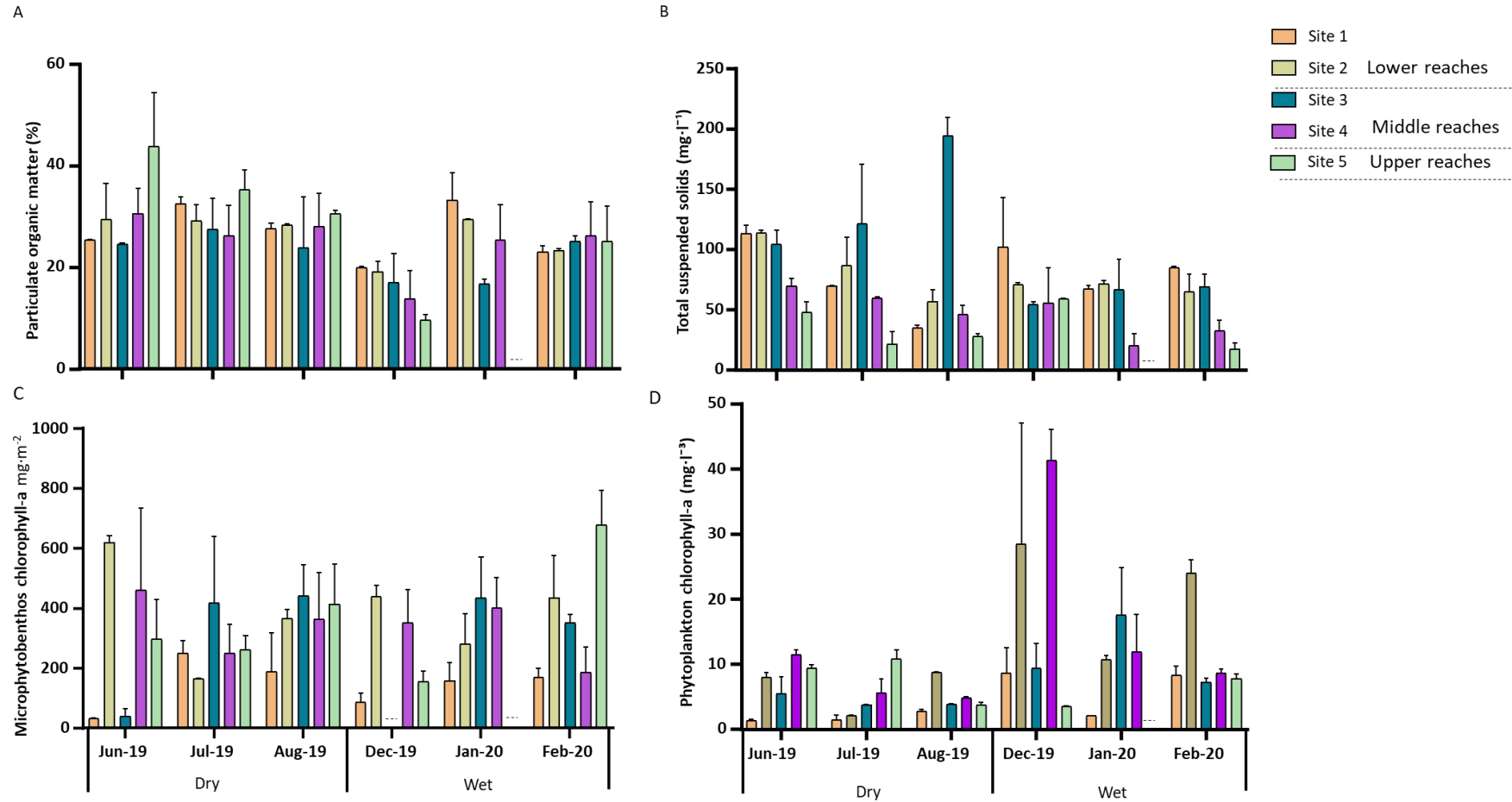


Figure 4.3: Spatial variations in average ($\pm$ STD) of (A) suspended particulate organic matter, (B) total suspended solids, (C) microphytobenthos chlorophyll-a and (D) phytoplankton chlorophyll-a in the uMlalazi Estuary during the dry season (June – July 2019) and wet season (December 2019 – February 2020). The dotted line indicates missing data.

The sediment grain sizes ranged from mud to gravel, with the median phi values ranging between -0.54Φ and 3.8Φ (Table 4.1). Coarse and medium sand dominated the sediment grain-size classes, contributing proportions ranging from 16 % – 55.97% and 14.34% – 43.69% respectively. Very coarse sand and gravel dominated site 3 to site 5 with the highest contribution of 27.88 % and 17.37% at site 4. The contribution of mud was relatively high at site 1 (12.97 %), site 3 (9.92 %) and site 5 (5.14 %). The presence of mud at site 1 (mouth region) is unusual for estuaries on the subtropical coast and was only observed in June and July 2019.

Table 4.1: Overview of average ($\pm$ STD) site-specific (averaged across all seasons per site) sediment particle sizes (%) measured from five sites in the uMlalazi Estuary.

Variable	Site 1	Site 2	Site 3	Site 4	Site 5
%Gravel	0.14 $\pm$ 0.10	0.37 $\pm$ 0.19	6.42 $\pm$ 7.62	17.37 $\pm$ 15.83	4.57 $\pm$ 3.16
%Very coarse sand	0.75 $\pm$ 0.77	7.12 $\pm$ 3.25	24.69 $\pm$ 17.31	27.88 $\pm$ 10.69	16.53 $\pm$ 11.26
%Coarse sand	16.60 $\pm$ 12.28	44.11 $\pm$ 7.33	32.58 $\pm$ 15.89	34.02 $\pm$ 13.52	55.97 $\pm$ 8.38
%Medium sand	33.14 $\pm$ 28.38	43.69 $\pm$ 10.21	14.34 $\pm$ 19.56	11.30 $\pm$ 8.59	20.66 $\pm$ 5.94
%Fine sand	27.23 $\pm$ 17.50	2.76 $\pm$ 1.16	9.80 $\pm$ 11.57	2.64 $\pm$ 1.81	0.93 $\pm$ 0.24
%Very fine sand	9.16 $\pm$ 9.66	0.19 $\pm$ 0.09	2.27 $\pm$ 1.49	1.66 $\pm$ 0.96	0.34 $\pm$ 0.24
%Mud	12.97 $\pm$ 17.22	1.75 $\pm$ 0.91	9.92 $\pm$ 9.52	5.14 $\pm$ 3.82	0.99 $\pm$ 0.822

Sediment TOC at uMlalazi Estuary varied from very low to medium across sites and seasons (Table 4.2). The dry season was associated with higher organic matter ranging between 0.01% and 3.5% than the wet season which varied between 0.3% and 1.7% across all sites. The sediment at sites 1 and 3 had very low to moderate total organic content (0.4% to 3.5%), while site 2 recorded the least organic content varying between very low and low (0.01% to 0.8%). The organic content recorded at site 4 was comparable to site 5 ranging from low to moderately low.

Table 4.2: Quantity and distribution of sediment total organic content between seasons at five sites of uMlalazi Estuary.

	Total organic Content %				
	Site 1	Site 2	Site 3	Site 4	Site 5
Jun-19	3.23	0.01	0.25	0.82	0.15
Jul-19	3.56	0.83	1.30	1.36	0.52
Aug-19	0.45	0.54	3.72	0.82	0.36
Dec-19	0.42	0.62	1.64	0.91	1.35
Jan-20	0.34	0.41	0.90	0.71	-
Feb-20	0.93	0.45	1.70	1.42	0.8

The PERMANOVA analysis on environmental parameters revealed temporal ($p(\text{perm}) = 0.001$, pseudo-F = 9.45, df = 1) and spatial ($p(\text{perm}) = 0.001$, pseudo f = 4.27, df = 4) differences (Table 4.3). The PCA biplot illustrates the spatial and temporal variation in environmental variables across five sites (1 to 5) during dry (June – August 2019) and wet (December 2019 – February 2020) seasons (Figure 4.4). Principal components 1 and 2 (PC1 and PC2) explain a combined 55% of the total variance, with PC1 accounting for 37.7% and PC2 for 17.3%. Phytoplankton chlorophyll-a, MPB, temperature and DO were the primary drivers of environmental variation during the wet season, while TSS and salinity were more prominent during the dry season. The most influential environmental variables along PC1 include phytoplankton chlorophyll-a, MPB chlorophyll-a and temperature, which positively correlated with each other and strongly contributed to the differentiation of sites, particularly in the wet season. Total suspended solids and salinity were negatively correlated with chlorophyll-a and temperature. These variables were more closely associated with the dry

season, particularly for sites 1 and 2. pH and TOC were less influential, but showed some relationship with the dry season, particularly at sites 1 and 2 in July 2019.

Table 4.3: PERMANOVA output for the two-way (site $\times$ season) analysis conducted on environmental parameters of uMlalazi Estuary df=degree of freedom, F=critical values of PERMANOVA and p=level of statistically significant difference. Significant effects ($P \leq 0.05$).

Factor	df	Pseudo-F	Unique perms	P(perm)
Site	1	9.45	999	0.001
Season	4	4.27	999	0.001

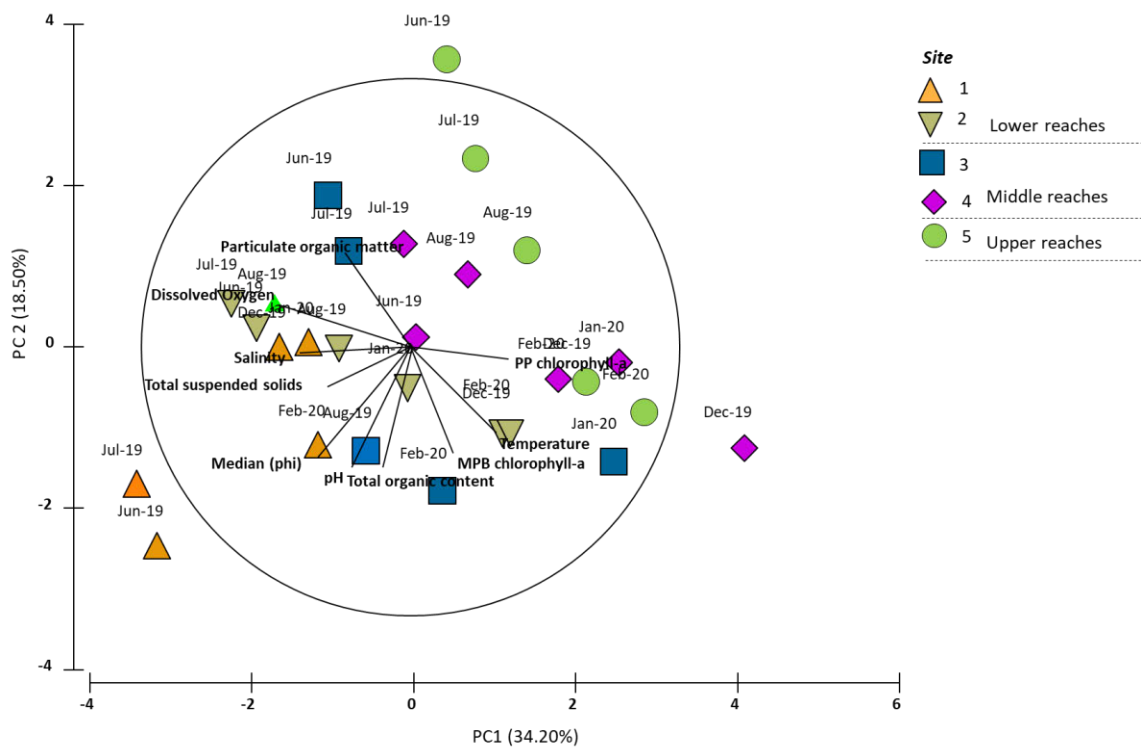


Figure 4.4: Principal component analysis (PCA) ordination diagram for physico-chemical parameters and sediment characteristics of uMlalazi Estuary during the dry (June - August) and wet (December to February) season of 2019 to 2020.

4.4.2 Macrobenthos taxa

A total of 76 taxa (Appendix 4.1) were documented from the five sampling sites during the dry and wet study periods (Table 4.4). The macrofaunal composition encompassed a diverse array of taxa, with polychaetes (32 taxa) being the most diverse group (Appendix 4.1). Notably, *D. arborifera*, *P. sexoculata*, Naididae oligochaetes, *B. virgiliae* and *H. cooperi* consistently attained densities greater than 1000 ind·m⁻² across sites throughout the study duration (Tables 4.4). Among the 76 taxa recorded, 66 taxa were recorded during the dry season, while 59 were recorded during the wet season. Seven taxa exhibited high densities (> 1000 ind·m⁻²) during the dry season, including, *Desdemona cf. ornata*, *Prionospio cf. sexoculata*, the native and invasive gastropods *Assiminea ovata* and *T. granifera*, as well as common amphipods typical of the subtropical coast of KZN (*Grandidierella* spp and *A. triaeonyx*) (Table 4.4). Estuarine polychaetes such as *C. keiskamma* and *Prionospio cf. bocki* were among the three taxa that occur in the highest densities (>1000 ind·m⁻²) during the wet season.

Taxa such as *Desdemona cf. ornata*, *D. arborifera*, *Prionospio* species, *H. cooperi*, *B. virgiliae*, *C. keiskamma* and *A. triaeonyx* were consistently present at all sampling stations, although their densities varied temporally (Figure 4.5). Site 1 constituted few organisms characterised by the presence and numerical abundance of *A. ovata*, *Iphinoe truncata* and Apseudidae sp1. The middle (site 3) and upper (sites 4 and 5) reaches were characterised by a combination of estuarine and freshwater taxa. *Dendronereis arborifera* and *H. cooperi* were numerically important taxa in terms of abundance, collectively contributing over 50% of the total taxa density at site 3 (Figure 4.5). There were notable similarities in taxa composition between sites 4 and 5, with *B. virgiliae*, Naididae sp.1, *D. cf. ornata* and *Grandidierella* spp. identified as key taxa. However, these sites were distinguished by variations in taxa densities and the presence of certain exclusive species. For instance, while *T. granifera* was unique to site 5 and was absent from the other sites (Figure 4.5).

Table 4.4: Spatio-temporal macrobenthos density (ind·m⁻²) data of the uMlalazi Estuary during the dry season (June 2019 – August 2019) and wet season (December 2019 – March 2020). 1 – 5 denotes sites.

	Jun-19					Jul-19					Aug-19					Dec-19					Jan-20					Feb-20				
Taxa	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5
Polychaeta																														
<i>Desdemona cf. ornata</i>																														
<i>Ceratonereis keiskama</i>																														
<i>Dendronereis arborifera</i>																														
<i>Glycera natalensis</i>																														
<i>Prionospio</i> sp1																														
<i>Prionospio</i> sp2																														
<i>Prionospio</i> cf. sexoculata																														
<i>Prionospio saldanha</i>																														
<i>Prionospio</i> cf. bocki																														
<i>Prionospio sexoculata</i>																														
Clitellata																														
Naididae sp1																														
Naididae sp2																														
Gastropoda:																														
<i>Assiminea ovata</i>																														
<i>Tarebia granifera</i>																														
Bivalvia:																														
<i>Brachidontes virgiliae</i>																														
Amphipoda:																														
<i>Grandidierella</i> spp																														
<i>Grandidierella lutosa</i>																														
<i>Americorophium triaenonyx</i>																														
Tanaidacea																														
<i>Apseudidae</i> sp1																														
<i>Halmyrapseudes cooperi</i>																														
<i>Sinelobus stanfordi</i>																														
Isopoda																														
<i>Cyathura estuaria</i>																														
Brachyura																														
<i>Paratyloidiplax blephariskios</i>																														
Cumaceans																														
<i>Iphinoe truncata</i>																														
Platyhelminthes																														
<i>Platyhelminthes</i> sp1																														
Density (ind·m ⁻²)	≤ 10					11 - 100					101 - 500					501 - 1000					1001 - 2000					2001 - 3000				
Density key																														

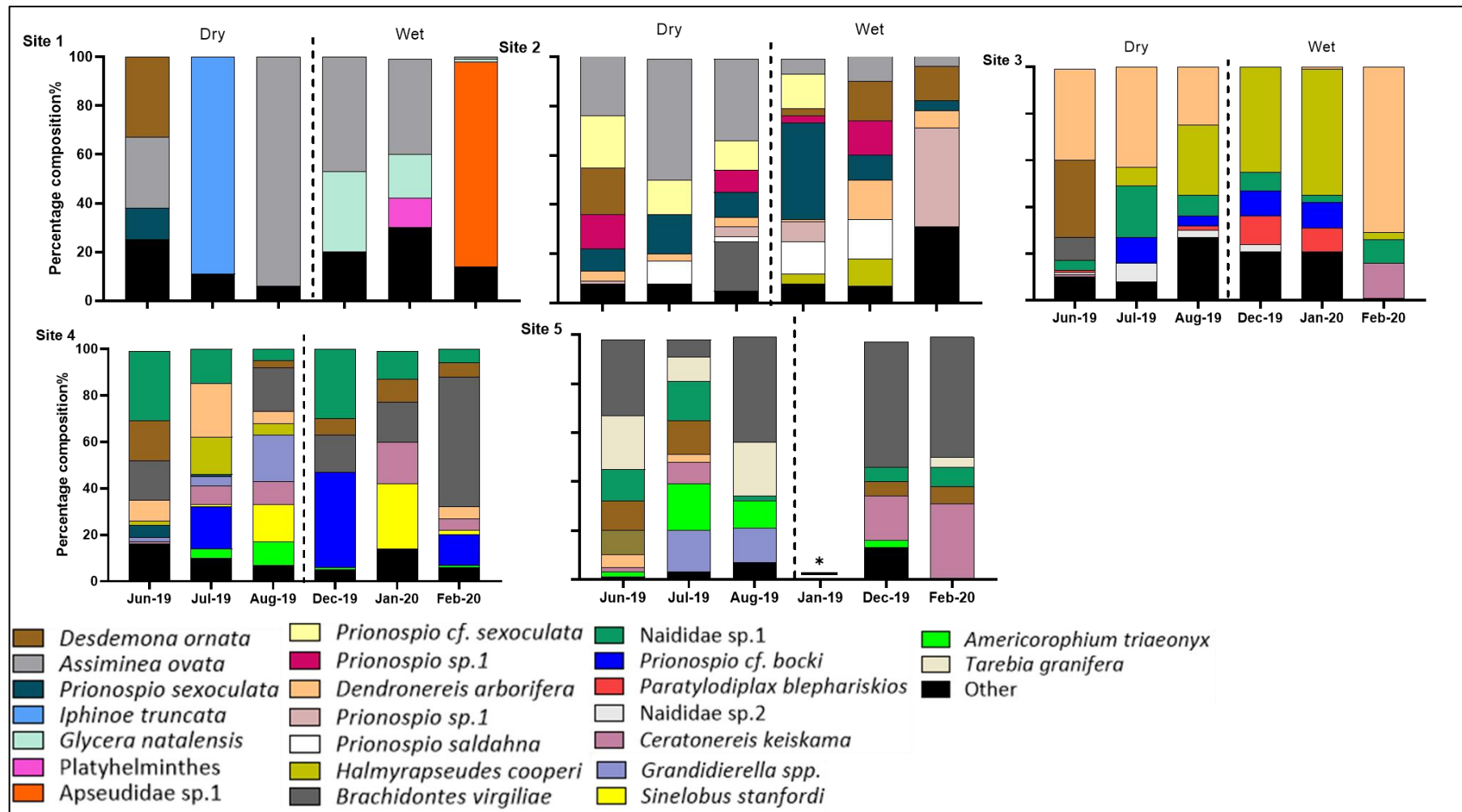


Figure 4.5: The distribution of numerically dominant (> 90% of community density) taxa across all five sites of uMlalazi estuary. Taxa which contributed less than 2 % of the total abundance were grouped together as "Other". Sites 1 and 2 represent the lower reaches, Sites 3 and 4 are in the middle reaches and Site 5 represents the upper reaches.

4.4.3 *Macrobenthos community structure*

There was limited seasonal variation in benthic densities observed between the dry and wet seasons across all sites (Figure 4.6A). Generally, the dry season exhibited higher densities, ranging from $224 \pm 24 - 8472 \pm 508 \text{ ind}\cdot\text{m}^{-2}$ compared to the wet season, which ranged between $120 \pm 8 - 6640 \pm 460 \text{ ind}\cdot\text{m}^{-2}$. The highest densities ($8470 \pm 502 \text{ ind}\cdot\text{m}^{-2}$) were recorded during the dry season at site 5, while the lowest ($120 \pm 8 \text{ ind}\cdot\text{m}^{-2}$) densities were noted at site 1 during the wet season. Overall, site 1 consistently showed lower total densities (range, $120 \pm 8 - 1120 \pm 125 \text{ ind}\cdot\text{m}^{-2}$), except for February 2020 ($4448 \pm 443 \text{ ind}\cdot\text{m}^{-2}$). Macrobenthos densities increased from site 1 to 3 and decreased at site 4, this pattern was observed in June and July 2019, December 2019 and January 2022.

The number of species followed a similar seasonal pattern, with higher species counts observed during the dry season for most sites, particularly Sites 2, 3 and 5 (Figure 4.6B), but there were no significant seasonal differences. Site 1, however, consistently showed low species richness across all periods ranging from $1 \pm 0.08 - 10 \pm 1$. Sites 3 and 4 generally maintained high species richness across both seasons, with the highest average number of species recorded at 14 ± 4 and 14 ± 2 , respectively. A two-way ANOVA revealed no significant seasonal difference in species richness ($df = 1$, $F = 0.868$, $p = 0.363$), while richness differed significantly among sites ($df = 4$, $F = 5.238$, $p = 0.005$) (Table 4.5). Tukey's post-hoc further revealed that species richness at site 1 significantly differed from sites 3, 4 and 5.

The Shannon-Wiener diversity index (H') remained relatively stable across sites and seasons (Figure 4.6C). Site 4 consistently showed the highest diversity, with values ranging from 1.36 ± 0.37 to 2.22 ± 0.21 . Site 1, however, exhibited lower diversity values due to fewer species and higher dominance of certain species, except for February 2019, when it recorded the highest diversity (2.26 ± 0.42). The stability in diversity reflects the consistent number of species richness and counts across seasons, indicating that the overall diversity was unaffected by seasonal changes. This conclusion aligns with ANOVA results, which, like species richness, showed no significant seasonal variation in diversity ($df = 1$, $F = 0.06$, $p = 0.80$), but significant spatial differences ($df = 4$, $F = 5.183$, $p < 0.005$) driven by site 1's lower diversity compared to Sites 3, 4 and 5 (Table 4.5).

Table 4.5: ANOVA showing post-hoc comparison for density and diversity of macrobenthos communities between sites. Values in bold indicate significant differences.

No. of Species (S) (df = 4. F = 5.238. p = 0.005)					
	Site 1	Site 2	Site 3	Site 4	Site 5
Site 1					
Site 2	0.097				
Site 3	0.005	0.65			
Site 4	0.008	0.75	0.99		
Site 5	0.014	1,00	0.64	0.74	
Shannon-Diversity (df = 4. F = 5.183. p = 0.05)					
	Site 1	Site 2	Site 3	Site 4	Site 5
Site 1					
Site 2	0.073				
Site 3	0.033	0.994			
Site 4	0.004	0.676	0.879		
Site 5	0.019	0.933	0.994	0.987	

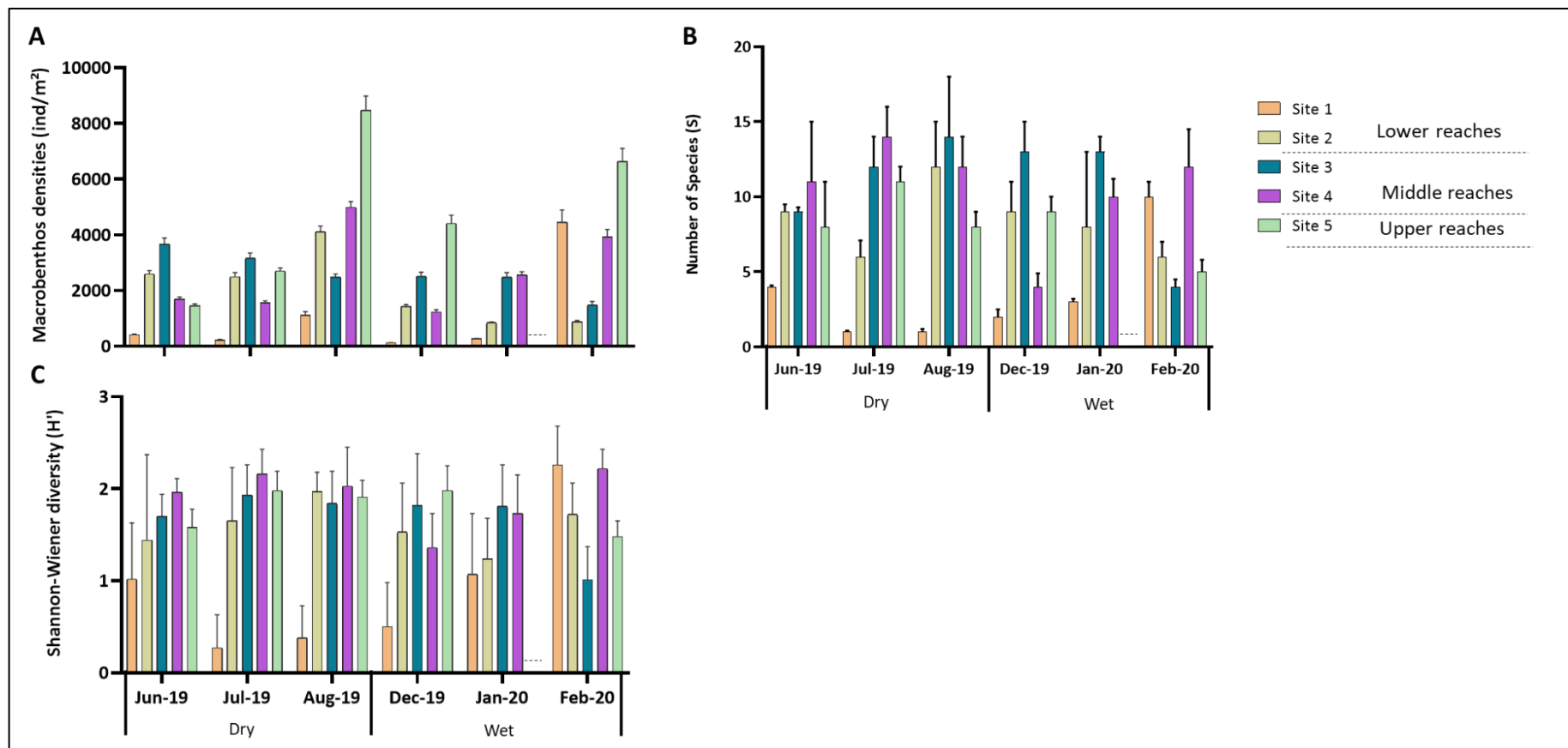


Figure 4.6: Macrobenthic community diversity indices showing averages ($\pm$ STD) for (A) density ($\text{ind} \cdot \text{m}^{-2}$), (B) Number of species (S), (C) Shannon-Wiener diversity (H') across five sites during dry and wet seasons at uMlalazi Estuary. Dotted lines represents missing data.

The MDS plot illustrated the spatial and temporal distribution of community composition across five sites sampled over the wet and dry seasons (Figure 4.7). Samples clustered into five distinct groups, suggesting varying degrees of similarity in community structure based on both sites and seasons. These groups displayed a conspicuous spatial distribution overriding seasonal variation that could be attributed to a gradual shift along the salinity gradient. Group 2 included samples from sites 3, 4 and 5, indicating that these sites shared similar community structures over this period, likely due to common environmental conditions that shaped similar species compositions. Unlike the other sites, Site 1 exhibited high variation in community composition. Consequently, its samples did not cluster together like those from other sites but were instead distributed across four groups. This showed that site 1, consists of unique compositions, reflecting the influence of site-specific conditions and temporal changes on communities. Group 4 combines samples from sites 1 and 2 and site 1 from June 2019, indicating that these samples had similar compositions, possibly due to shared ecological influences such as similar water quality parameters, sediment characteristics, or seasonal biological processes. The stress value of 0.11 indicates a reliable two-dimensional representation, meaning that the plot effectively captures the dissimilarities among samples.

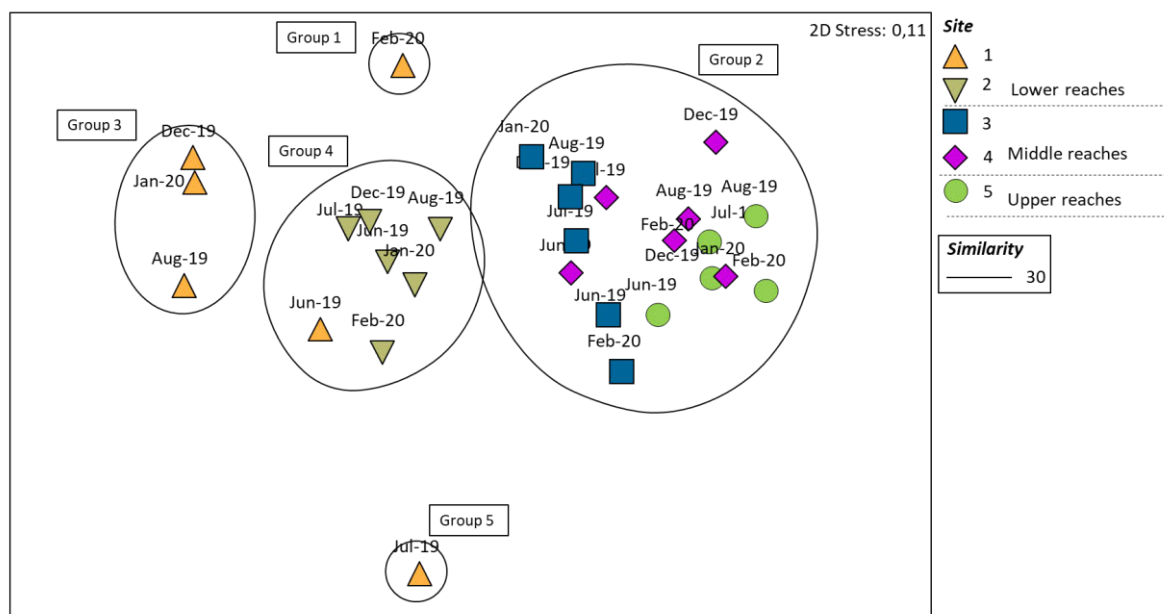


Figure 4.7: non-metric Multidimensional ordination (nMDS) ordination of square-root transformed macrobenthos density data from uMlalazi Estuary during the wet (June – August 2019) and dry season (December 2019 – February 2020).

SIMPER analysis revealed that the sample groupings as observed in the MDS were influenced by a specific subset of taxa, with their distributions indicating gradual spatial and temporal shifts instead of distinct separations between groups. Overall, 17 taxa were responsible for similarities (Table 4.6) and dissimilarity (Table 4.7) between sample sites. Site 1 consisted of only 3 taxa contributing > 90% to overall site average similarity. Seven taxa contributed >2% to within group similarity (58.87 %) at site 2. The gastropod *A. ovata* and polychaetes *P. sexoculata*, *Prionospio cf. sexoculata*, *D. cf. ornata*, *Prionospio* sp2 and *D. arborifera* were the important contributors to within group similarity. The polychaetes, *Prionospio* and *D. cf. ornata* also contributed > 2% to dissimilarity (average dissimilarity 76.59%) between sites 1 and 2 (Table 4.7). *Halmyrapseudes cooperi*, *D. arborifera* and Naididae sp1 were typifying taxa at site 3 and contributed >2% to within-group similarity. *Halmyrapseudes cooperi* and Naididae sp1 were amongst the important discriminators between sites 3 and 1 and sites 3 and 2 (Table 4.7). The oligochaeta and *B. virgiliae* were amongst the common taxa contributing to similarities at sites 4 (average similarity, 48.87%) and 5 (average similarity, 57.66%). *Brachidontes virgiliae* together with *T. granifera*, *C. keiskama*, *Prionospio cf. bocki* and *A. triaenonyx* contributed > 2% to the dissimilarity between sites 4 and 5 (average dissimilarity, 44.40%).

Table 4.6: SIMPER results of square-root transformed macrobenthic density data. Listed taxa account for 90% cumulative contribution percentage of the within group similarity.

Site	Av. similarity	Species	Av. similarity	Sim/SD	% Contrib.	% Cum.
1	28.99	<i>Assimineia ovata</i>	16.2	1.01	55.86	55.86
		<i>Glycera natalensis</i>	8.61	0.7	29.7	85.56
		<i>Desdemona ornata</i>	3.5	0.41	12.07	97.63
2	58.87	<i>Assimineia ovata</i>	14.81	4.06	25.16	25.16
		<i>Prionospio sexoculata</i>	10.69	7.71	18.16	43.32
		<i>Prionospio cf. sexoculata</i>	7.44	0.91	12.63	55.95
		<i>Desdemona ornata</i>	5.46	1.22	9.28	65.22
		<i>Dendronereis arborifera</i>	5.28	1.25	8.98	74.2
		<i>Prionospio</i> sp2	5.2	0.87	8.83	83.03
		<i>Prionospio cf. saldanha</i>	3.38	1.22	5.74	88.77
		<i>Prionospio</i> sp1	2.87	0.64	4.88	93.65
3	46.36	<i>Halmyrapseudes cooperi</i>	12.12	4.8	26.14	26.14
		<i>Dendronereis arborifera</i>	12.04	2.63	25.97	52.11
		Naididae sp1	8.83	6.56	19.04	71.16
		<i>Prionospio cf. bocki</i>	2.55	1.24	5.5	76.66
		Naididae sp2	2.42	1.27	5.23	81.89
		<i>Prionospio sexoculata</i>	1.71	0.6	3.7	85.59
		<i>Sinelobus stanfordi</i>	1.44	0.41	3.12	88.7
4	48.87	Naididae sp1	12.2	3.7	24.96	24.96
		<i>Brachidontes virgiliae</i>	8.4	2.74	17.19	42.15
		Naididae sp2	7.14	1.24	14.62	56.76
		<i>Dendronereis arborifera</i>	4.49	1.18	9.19	65.95
		<i>Prionospio cf. bocki</i>	4.18	0.91	8.56	74.51
		<i>Ceratonereis keiskamma</i>	3.78	1.2	7.74	82.25
		<i>Halmyrapseudes cooperi</i>	3.29	0.58	6.73	88.98
		<i>Grandidierella</i> spp	2.07	0.91	4.24	93.22
5	57.66	<i>Brachidontes virgiliae</i>	15.72	2.49	27.26	27.26
		Naididae sp1	9.6	7.69	16.64	43.9
		<i>Tarebia granifera</i>	8.41	3.13	14.58	58.48
		Naididae sp2	7.03	1.48	12.19	70.68
		<i>Ceratonereis keiskama</i>	5.75	1.28	9.97	80.64
		<i>Americorophium triaenonyx</i>	4.79	0.75	8.31	88.95
		<i>Grandidierella</i> spp	2.9	0.5	5.02	93.97

Table 4.7: SIMPER analysis results revealing the top 5 discriminating taxa from taxon abundance data showing the main discriminating taxa, contributing over 2% to between-site dissimilarity from sites 1 to 5. Sim/SD ratio is presented in parentheses, DS represents the dissimilarity percentage (highlighted in bold).

	Site 1	Site 2	Site 3	Site 4	Site 5
Site 1					
Site 2	DS =76.59% <i>Prionospio sexoculata</i> 9.9(1.8) <i>Prionospio cf. sexoculata</i> 8.9(1.4) <i>Desdemona ornata</i> 7.6(1.46) <i>Assiminea ovata</i> 7.5(0.82) <i>Prionospio</i> sp2 7.06(1.58)				
Site 3	DS=91.74% <i>Dendronereis arborifera</i> 16.1(1.35) <i>Halmyrapseudes cooperi</i> 13.82(2.3) <i>Naididae</i> sp1 9.32(2.3) <i>Assiminea ovata</i> 6.83(1.3) <i>Apseudidae</i> sp1 5.24(0.8)		DS=80.33 <i>Halmyrapseudes cooperi</i> 8.8(1.8) <i>Dendronereis arborifera</i> 7.9(1.3) <i>Assiminea ovata</i> 7.26(1.96) <i>Naididae</i> sp1 6.4(2.8) <i>Prionospio sexoculata</i> 4.93(1.5)		
Site 4	DS= 95.13 <i>Brachidontes virgiliae</i> 12.3(1.6) <i>Naididae</i> sp1 11.6(2.2) <i>Prionospio cf. bocki</i> 8.05(0.94) <i>Naididae</i> sp2 7.8(2.3) <i>Assiminea ovata</i> 7.06(1.12)	DS=90.80 <i>Brachidontes virgiliae</i> 9.3(1.5) <i>Naididae</i> sp1 8.1(2.5) <i>Assiminea ovata</i> 7.9(1.9) <i>Prionospio cf. bocki</i> 5.6(1) <i>Prionospio sexoculata</i> 5.7(1.9)	DS=57.06 <i>Brachidontes virgiliae</i> 7.9(1.5) <i>Dendronereis arborifera</i> 6.6(1.1) <i>Halmyrapseudes cooperi</i> 6.3(1.1) <i>Ceratonereis (Composetia) keiskama</i> 4.1(1.3) <i>Prionospio cf. bocki</i> 4(0.1)		
Site 5	DS=96.78 <i>Brachidontes virgiliae</i> 20.8(2.5) <i>Tarebia granifera</i> 10.2(1.5) <i>Naididae</i> sp1 10(3.6) <i>Ceratonereis (Composetia) keiskama</i> 9.9(1.2) <i>Naididae</i> sp2 8.7(2.4)	DS=93.48 <i>Brachidontes virgiliae</i> 14.6(1.8) <i>Assiminea ovata</i> 8.2(1.8) <i>Ceratonereis (Composetia) keiskama</i> 7.9(1.1) <i>Naididae</i> sp1 7(3.3) <i>Tarebia granifera</i> 6.6(1.6)	DS= 70.92 <i>Brachidontes virgiliae</i> 12.4(1.6) <i>Dendronereis arborifera</i> 7.9(1.7) <i>Halmyrapseudes cooperi</i> 6.5(1.5) <i>Ceratonereis (Composetia) keiskama</i> 6.1(1.1) <i>Tarebia granifera</i> 5.9(1.5)	DS=44.40 <i>Brachidontes virgiliae</i> 8.2(1.3) <i>Tarebia granifera</i> 6.2(1.6) <i>Ceratonereis (Composetia) keiskama</i> 5.6(1.1) <i>Prionospio cf. bocki</i> 4.1(1.1) <i>Americorophium triaenonyx</i> 3.6(1.2)	

4.4.4 Macrobenthos drivers

According to the BIOENV results, macrofaunal communities were influenced by a combination of environmental factors including salinity, gravel, mud and TSS (Table 4.8). The first two principal components accounted for 65.47% of the variance and axes 1 and 2 explained 44.80 % and 20.67%, respectively. The distribution of macrobenthos communities in the uMlalazi Estuary reflects environmental heterogeneity within the system, with community composition shifting gradually from marine in the lower reaches to brackish and freshwater communities in the middle and upper reaches. The CCA showed that salinity was the most important environmental variable in structuring the benthic communities, followed by sediment characteristics (percentage mud and gravel) (Figure 4.8). *Assiminea ovata*, the opportunistic polychaetes from the genus *Prionospio* and Apseudidae sp.1 were associated with increasing salinity and TSS concentration. The taxa at sites 4 and 5 were mostly associated with increasing grain size, temperature and increasing MPB Chl-a concentration influencing the abundance and distribution of *S. stanfordi*, oligochaetes and *C. keiskama*. The mud crab *P. blephariskios*, *H. cooperi*, *Prionospio cf. bocki* and *D. arborifera* were structured by muddy substrates, while the *A. triaenonyx* and *Grandidierella* spp., *B. virgilae* and gastropod *T. granifera* were closely associated with coarse substratum and high POM concentrations.

Table 4.8: BIOENV results from the BEST analysis showing the weighted Spearman rank correlation between environmental variables and macrobenthos taxa. The best combination of environmental variables is highlighted in bold.

Number of variables	Correlation	Environmental variables
2	0.3	Gravel, Mud
3	0.4	Salinity, Gravel, Mud
4	0.3	Salinity, Total suspended solids, Gravel, Mud

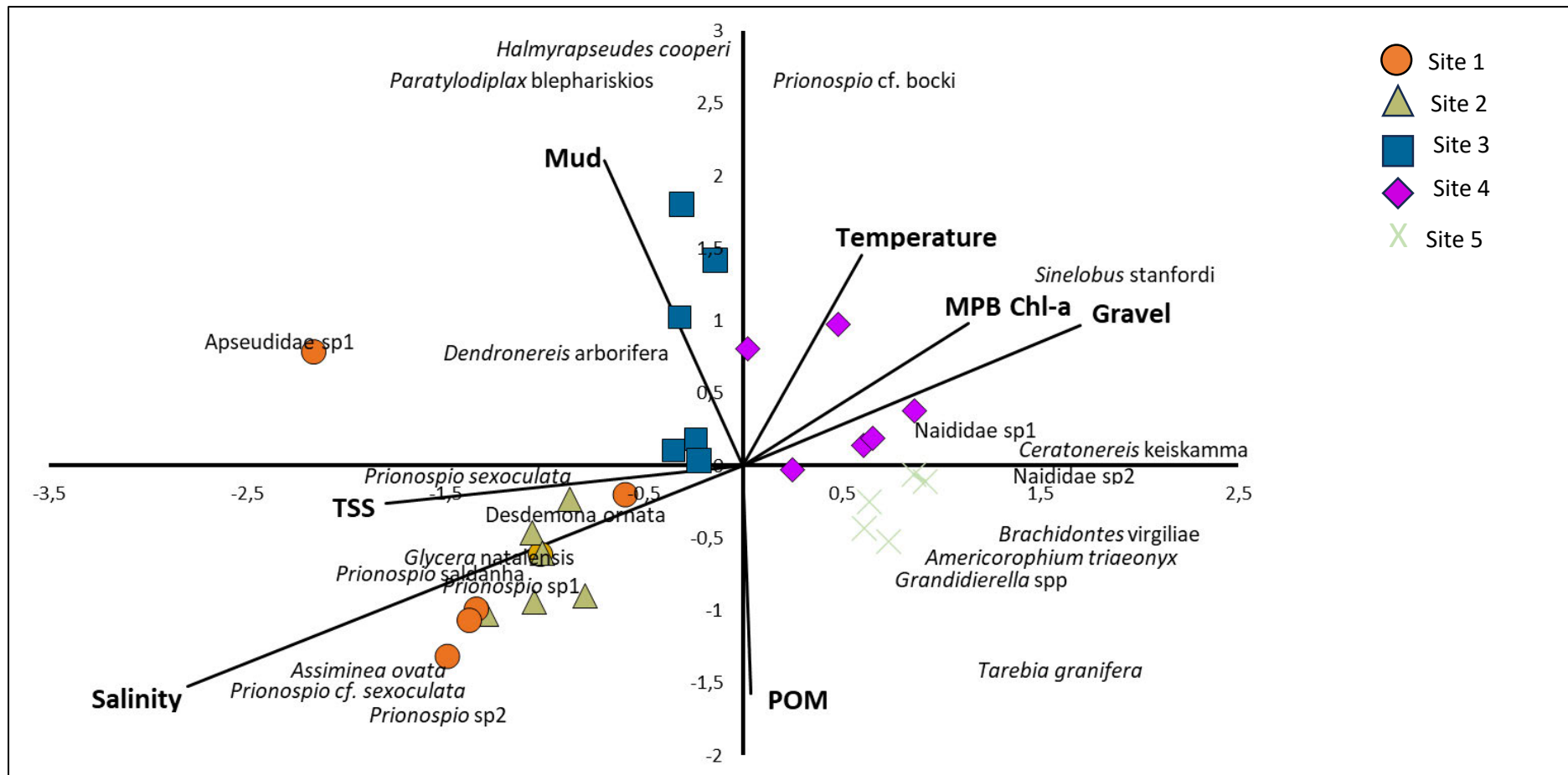


Figure 4.8: Canonical Correspondence Analysis (CCA) illustrating the relationship between environmental parameters and macrobenthic taxa that contribute 90% to the total macrobenthos density. Sites 1 and 2 represent the lower reaches, Sites 3 and 4 are in the middle reaches and Site 5 represents the upper reaches.

4.6 Discussion

4.6.1 Environmental variables

The salinities in POEs are regulated by the interplay between two key factors namely, freshwater inflow from the surrounding catchment and the influx of seawater prompted by tidal currents (Cooper 2001, Whitfield & Bate 2007). During the wet season, freshwater inflow increases, which directly impacts the salinity concentrations in the estuary, resulting in a noticeable decrease in salinity (van Niekerk et al. 2022). The extent of this decrease is dependent on the volume of freshwater received. This pattern was observed in the uMlalazi Estuary, where salinity decreased during the wet season. The estuary also displayed a strong horizontal gradient, with salinities decreasing towards the headwaters. These temporal and spatial patterns have been documented in previous studies of the uMlalazi Estuary (Mabaso 2002, Vezi 2017, Nche-Fambo 2021, Scharler et al. 2023).

In the current study, phytoplankton exhibited strong seasonal variation, with chlorophyll-a concentrations increasing during the wet season, potentially due to rising temperatures. Higher temperatures tend to enhance photosynthetic activity by increasing metabolic rates in phytoplankton, which leads to higher chlorophyll-a production (Fernandes et al. 2012, Navas-Parejo et al. 2020). While temperature appears to be a primary driver, pp chlorophyll-a is also influenced by other factors, such as nutrient availability, light availability and water residence time (Froneman 2002, Thomas et al. 2005, Wang & Zhang 2020, Hall et al. 2023). Benthic chlorophyll-a (MPB) concentrations were generally higher than pelagic chlorophyll-a, which may be linked to their adaptability to a wide range of environmental conditions (Mundree et al. 2003, Sanilkumar et al. 2009, Lemley et al. 2016). This trend is common in South African estuaries, where it commonly exceeds phytoplankton by 1 to 3 orders of magnitude (Nozais et al. 2001, Perissinotto et al. 2002). Benthic microalgae are better adapted to low light penetration, salinity fluctuations, rapid nutrient utilisation and a broader range of habitat conditions (Perissinotto et al. 2002, Sanilkumar et al. 2009, Underwood 2010).

Dissolved oxygen and POM displayed an inverse relationship with increased temperature and chlorophyll-a concentrations. In estuarine ecosystems, studies indicate that a relationship between DO and POM is linked to chlorophyll-a production, temperature, decomposition rates and hydrodynamic influences (Countway et al. 2007, Liang et al. 2023). Rising temperatures accelerate microbial activity, increasing the decomposition rate of organic matter, which can reduce POM concentrations when phytoplankton blooms (Cloern 2001, Iriarte et al. 2010, Guo et al. 2015, Ye et al. 2017, Onabule et al. 2020, Wang & Zhang 2020, Li et al. 2023). As algal blooms decay, they contribute to oxygen consumption, decreasing oxygen levels (Li et al. 2023). This effect was noticeable in the DO concentrations which decreased with increasing temperature, even though it remained well oxygenated ($>5 \text{ mg}\cdot\text{l}^{-1}$), with a slightly lower DO concentration recorded at site 4 ($4.96 \text{ mg}\cdot\text{l}^{-1}$) in the wet season. In contrast, POM did not show any obvious seasonal variation except for a slight decrease at the onset of the wet season. Freshwater inflow is a significant source of suspended organic matter, with a tendency for a substantial portion settling in areas closer to the upper reaches rather than the mouth region (Bartl et al. 2019, van Niekerk et al. 2022). This is reflected in generally high POM concentrations in the upper reaches.

The uMlalazi Estuary was characterised by fine sand at the mouth, a muddy substrate in the middle reaches and coarser sand grains in the upper reaches. However, the substratum heterogeneity was observed at each site throughout the study period. Hill (1966) reported that the mouth region was composed of a mixture of mud with medium and fine sand. Similar results were observed in the current study which is an unusual observation in KZN estuaries. The middle reaches were the most heterogeneous sites in terms of particle size. The upper regions (sites 4 and 5) of the estuary were characterised by comparatively coarser sediment textures relative to the other sites. This aligns with the principle that larger sediment particles carried by river currents tend to accumulate and deposit in the upper estuarine reaches (McLusky & Elliott 2004). The observed spatial distribution of sediment grain sizes and the distinctive characteristics of sediment texture specific to each site are like findings presented in Hill (1966).

4.6.2 Macrobenthos community structure

The macrobenthos communities consisted of a diverse range of marine, estuarine and freshwater taxa, with polychaetes being the most dominant group, represented by 32 species. They were followed by amphipods (11 species), gastropods (seven species) and bivalves (five species). Other groups, such as Oligochaeta (four species), Brachyura (four species) and Tanaidacea (three species), were less represented in terms of diversity. The uMlalazi Estuary was comprised of Southern African endemic *Prionospio* species, cosmopolitan (e.g. *Heteromastus cf. filiformis*) and Indo-Pacific (*Phyllodoce capensis* and *Sigambra parva*) polychaetes (Mackay & Cyrus 1998). While the study reported a high diversity of polychaete species, it is important to note that many identifications based on historical literature such as Day (1967a, 1967b), require re-evaluation (Simon et al. 2022). Simon et al. (2022) highlighted that numerous species such as *Heteromastus filiformis*, *Capitella capitata* and members of the Phyllodocidae family are likely unresolved cosmopolitan or categorised as pseudocosmopolitan species. These taxa may represent complexes of cryptic species rather than a single widely distributed species. As molecular techniques advance, many of these identifications are expected to be revised, leading to a better understanding of their true diversity and distribution.

The *Prionospio* genus was the most diverse polychaete group with seven species distributed across all five sites, each occupying its preferred niche. Two out of the seven species (*P. sexoculata* and *P. cirrifera*) have been reported in numerous studies in South African estuarine systems such as the Lake St. Lucia (Owen & Forbes 1997, Pillay & Perissinotto 2008), Nhlabane Estuary (Vivier 2010), uMhlathuze Estuary (Mackay & Cyrus 1998, Izegaegbe 2019), Keiskamma Estuary (Teske & Wooldridge 2001) and Groot Berg (Wooldridge & Deyzel 2009). In the current study, *Prionospio sexoculata* was the most common and numerically abundant species in the uMlalazi Estuary. Although *Prionospio* polychaetes are common in South African estuarine systems, their phenotypic plasticity complicates taxonomic identification, often resulting in classification at higher taxonomic levels. For example, *Prionospio* spp. has been recorded as *Prionospio* spp. by Mackay & Cyrus (1998) and Mabaso (2002), while others are identified only to the family level as Spionidae (Ortega-Cisneros 2013, Vezi 2017). This suggests that this group may include various cryptic species that are overlooked during

benthic inventories in South African estuaries. To address this, molecular and experimental studies are critical for resolving taxonomic uncertainties, identifying cryptic diversity and investigating their behaviour, ecological interactions and environmental adaptations.

The benthic communities in the uMlalazi Estuary were also characterised by a high numerical abundance of polychaetes (*D. arborifera* and *Prionospio* species), the tanaid (*H. cooperi*), the bivalve (*B. virgiliae*) and the gastropod (*A. ovata*). These taxa are also frequently observed in different estuarine types along the KwaZulu-Natal coast (Teske et al. 2006, Perissinotto et al. 2010), including river mouths (Forbes & Demetriades 2008, Ngqulana et al. 2010, Vezi et al. 2020), estuarine lakes (MacKay et al. 2010, Vivier 2010, Cyrus et al. 2011) and estuarine bays (Vivier & Cyrus 2014, Izegaegbe et al. 2020). The ubiquity and dominance of these taxa across multiple systems highlight their broad environmental tolerance, ecological plasticity and resilience. As generalist species, they thrive in diverse conditions, with their distribution within the uMlalazi Estuary additionally reflecting variations in their ecological roles across different habitats.

Seasonality had no significant effect on macrofaunal density, species diversity, or composition. However, Site 1 consistently exhibited lower macrofaunal density and species richness compared to other sites, with the nMDS analysis revealing high variability in community structure. This variability and reduced diversity were likely driven by harsh site-specific conditions, including constant exchange with the sea and sediment instability caused by scouring (Owen & Forbes 1997, Perissinotto et al. 2010, Ortega-Cisneros & Scharler 2014). Consequently, Site 1 was characterised by low taxonomic richness and frequent occurrences of singletons, aligning with the principle that elevated stress or disturbance typically reduces diversity (Clarke & Warwick 2001). The effects of these harsh conditions were most evident in the high dissimilarity observed between Site 1 and both Sites 4 and 5, with the community structure at Site 1 being highly distinct from those at the other sites. In contrast, the middle and upper reaches, with their coarse and heterogeneous substrate, supported a more diverse community by providing numerous microhabitats. This aligns with the principle that substrate heterogeneity promotes greater faunal diversity (Gray 1977, Gray & Elliot 2009). These findings highlight the strong influence of site-specific environmental factors in shaping spatial macroinvertebrate communities, with temporal fluctuations at Site 1 likely reinforcing its unique composition, even as seasonal effects remained minimal.

4.6.3 Macrobenthos drivers

Macrobenthic communities are spatially distributed in response to an interplay between various chemical (Buss et al. 2002) and physical (Harrison et al. 2007, Hope 2016) factors which prevail in the estuary at different temporal scales (Cloern et al. 2017, Mestdagh et al. 2020). Consequently, their distribution is largely shaped by each organism's tolerance range to a specific or combination of variables (Dallas & Day 2004). Macrobenthic invertebrates that exist in environments falling within their tolerance range demonstrate maximum growth rates and reproduction, whereas deviation from this range leads to physiological stress, diseases, or the displacement of organisms from the area (Dallas & Day 2004).

The CCA revealed the most influential environmental variables in structuring the benthos of uMlalazi Estuary were salinity and sediment grain sizes. Taxa strongly associated with high salinity levels were predominantly found in sites 1 and 2, which mainly comprised euryhaline marine/estuarine species like *G. natalensis*, *A. ovata* and *Prionospio* polychaetes. These taxa are well-documented in several South African estuaries and are regarded as true estuarine species, displaying a remarkable capacity to thrive across a wide range of environmental conditions (Dix et al. 2004, Henninger & Froneman 2011, Perissinotto et al. 2014, Gupta et al. 2018). *A. ovata* and *Prionospio* polychaetes have been reported to coexist in areas with fine-grained sediments, high organic matter and high salinities (Mabaso 2002, Stow 2011, MacKay et al. 2010, Izegaegbe 2019, Cronin-O'Reilly 2021).

Assiminea ovata displayed a positive association with increasing salinity concentrations. This gastropod is recognised as a marine/brackish species, commonly dominating habitats with higher salinity levels compared to brackish environments (Vivier 2010, Stow 2011, Ortega-Cisneros 2013) and even thriving under hypersaline conditions (Miranda et al. 2010). However, a molecular study by Perissinotto et al. (2014) identified cryptic species within the *Assiminea* group, with one lineage predominantly marine and *A. ovata* primarily associated with brackish habitats. These findings echo the importance of conducting further molecular studies to accurately discern all species occurring in estuarine systems and better understand their ecology. In addition, Miranda & Perissinotto (2012) and Pillay & Perissinotto (2008) identified MPB as the primary dietary source for *A. ovata*. However, in the current study, a

negative correlation was observed between *A. ovata* and MPB, while a positive association was observed with POM, although to a lesser extent. This phenomenon could be attributed to the gastropod's versatile dietary preferences, which include the capacity to shift its diet towards particulate matter, filamentous algae and selected meiofauna species (Pillay & Perissinotto 2008, Miranda & Perissinotto 2012).

Prionospio polychaetes species tolerate a wide range of environmental conditions, including disturbed, hypoxic and organically enriched habitats (Mackay & Cyrus 1998, Dix et al. 2004). Their adaptability is facilitated by flexible feeding strategies, euryhalinity, respiratory pathways and their ability to construct burrows in various sediment types (Dix et al. 2004). *Prionospio* polychaetes such as *P. sexoculata*, *Prionospio* sp.1 and sp.2 were affiliated with polyhaline conditions and fine to coarse sediments, whereas *P. cf. bocki* was associated with brackish environments rich in mud. These polychaetes are tubicolous and can construct mucous-lined burrows or tubes in varying types of sediments but most preferably fine to coarse sand (Dix et al. 2004) which explains their diversity and ubiquitous trend in the uMlalazi Estuary. *Prionospio* burrows allow them to position branchiae anteriorly and remain in their burrows while feeding, keeping their respiratory pathway independent of sediment interactions, which reduces predation risk and enhances their tolerance to challenging estuarine conditions (Dauer 1985, Wilson 1990). In hypoxic conditions, these organisms enlarge their branchiae which enhances their oxygen uptake (Dauer 1985, Ellis et al. 2006, Qu et al. 2015).

Prionospio polychaetes are widely recognised as indicators of environmental disturbance or pollution (Dix et al. 2004). They have been recorded in many disturbed estuarine regions worldwide. For example, Vivier (2010) observed high *Prionospio* populations in heavily polluted areas of the iNhlabane Estuary and Keeley et al. (2014) reported a high numerical abundance in Forsyth Bay, New Zealand, where salmon farming led to organic enrichment (TOC levels of 15% - 20%). Similarly, hypoxic conditions in the Swan-Canning POE led to large polychaete mortalities, followed by high *Prionospio* abundance (Tweedley et al. 2016). In the uMlalazi Estuary, *Prionospio* species were abundant at Site 2, an area receiving wastewater treatment discharge. However, the discharge levels remain within ecologically acceptable thresholds and do not compromise water quality (Nche-Fambo 2021). This observation suggests that the presence of *Prionospio* in undisturbed systems may indicate localised

natural environmental stress, but it does not necessarily signify significant pollution or ecosystem degradation. Moreover, during periods of severe ecological deterioration, these organisms exhibit adaptability and may even increase in abundance. Consequently, Keeley et al. (2014) emphasise that in systems such as the uMlalazi Estuary, where *Prionospio* persists even under good conditions, these polychaetes cannot be solely relied upon as pollution or disturbance indicators.

Halmyrapseudes cooperi, *P. blephariskios* and *D. arborifera* displayed a strong affinity for muddy substrates. Studies by Owen & Forbes (2002), Bate et al. (2011) and Izegaegbe et al. (2020) have documented the species in euryhaline environments with sandy sediment. In the uMlalazi Estuary, however, the distribution of *P. blephariskios* appears to be primarily driven by substrate type, explaining its sole occurrence at site 3. Owen et al. (2000) found that its presence in sandy substrates depends on the proportion of mud contained, which influences sediment organic matter content and burrow construction. *Paratyloidiplax blephariskios* plays a crucial role in muddy estuarine substrates, functioning as a keystone species (Smith et al. 1991). This role is exhibited during burrowing and feeding activities, which oxygenate the mud, promote vertical nutrient transport and alter sediment topography (Warren & Underwood 1986, Smith et al. 1991, De Villiers et al. 2009).

In contrast to *P. blephariskios*, *H. cooperi* and *D. arborifera* were also found in sandy and polyhaline environments but showed a strong preference for muddy areas, where they were more abundant. *Halmyrapseudes cooperi* displayed a negative relationship with suspended POM. Although suspended and sediment POM are regarded as key food sources for this species, *H. cooperi* can also feed on bacteria and MPB (Ortega-Cisneros & Scharler 2015). The observed negative association between this species and suspended POM suggests a potential reliance on alternative food sources, such as MPB and detrital material, especially in systems where these resources are abundant. This observation is consistent with the conclusions of studies by Blaber et al. (1983) and MacKay et al. (2010) which linked the abundance of *H. cooperi* to the presence of elevated detrital material.

Sinelobus stanfordi, *A. triaenonyx* and *B. virgiliae* are deposit and suspension/filter feeders that occupy a wide range of habitats with semi-permanent or permanent hard substrates such as stones, rocks and macrophytes (Allanson & Baird 1999, van Haaren & Soors 2009, Montagna & Palmer 2011, Perissinotto et al. 2014, Ferreira et al. 2015, Nel et al. 2016). These taxa have

been found to dominate areas with submerged macrophytes in various estuaries. However, in the uMlalazi Estuary, where submerged macrophytes are absent (DWS 2015), these taxa were highly abundant in sites surrounded by reeds and coarse to gravel-textured substrates.

Mussels in the Mytilidae family secure themselves to hard surfaces using a collagenous fibre known as byssus (Sees & Suchanek 1992). Byssus production is affected by several environmental factors including temperature, salinity and flow velocity (Sees & Suchanek 1992, Dolmer & Svane 1994). In the uMlalazi Estuary, *B. virgiliae* mussels were commonly observed forming colonies on stones, as well as on gastropod and bivalve shells, primarily at sites 4 and 5. In contrast, at sites 2 and 3, these mussels were found individually and in lower numbers, unattached to any hard object. In an experimental study conducted by Bell & Gosline (1996), it was found that mussels exposed to conditions beyond their tolerance range responded by closing their shells and halting byssus production. A similar response was observed by Nel et al. (2014) in St Lucia Estuary when salinity levels exceeded 26, which is beyond their optimal salinity range. The spatial distribution of *B. virgiliae* in the current study aligns with these findings, as salinity levels at sites 2 and 3 consistently remained above 25, while those in sites 4 and 5 were below this threshold. However, attributing *B. virgiliae* spatial distribution to this observation may be inaccurate, as these findings are based on counts and factors like turbidity, temperature and flow rate were not assessed.

Sinelobus stanfordi and *A. triaenonyx* play an essential role in sediment dynamics as deposit feeders, primarily consuming detrital material (Cyrus & Martin 1988, van Haaren & Soors 2009). *Americorophium triaenonyx* exhibits versatile feeding strategies based on available food resources, including suspension and grazing feeding (Gerdol & Hughes 1994a, 1994b, Ford & Honeywill 2002). Research has shown that amphipods in the Corophiid family (i.e. *A. triaenonyx*) exhibit a strong preference for MPB as a primary food source, which they consume by grinding with their well-developed mandibles (Zajac & Whitlatch 1982, Gerdol & Hughes 1994a, Backer et al. 2010). This preference was demonstrated through correlations between reduced MPB concentrations and the presence of these amphipods in estuarine environments, confirmed by both *in situ* and experimental studies (Gerdol & Hughes 1994a, Hagerthey et al. 2002, Ysebaert et al. 2005).

In conclusion, the uMlalazi Estuary highlights the vital role of consistent freshwater inflows and low pollution levels in supporting healthy and diverse estuarine ecosystems. These

conditions support diverse niches that sustain sensitive and key estuarine taxa, which serve as indicators of ecosystem health and contribute essential traits for maintaining ecological functions and overall resilience. However, maintaining these favourable conditions requires regular monitoring and strict management of freshwater inflows and pollution control. This is particularly important in light of the evident impacts of key threats to estuarine systems, as outlined in Chapter 1, including nutrient enrichment (Schmitz et al. 2024), reduced habitat variability (Scharler et al. 2023), and the presence of invasive species such as *T. granifera* and *Ficopomatus enigmaticus* (Appendix 4.1)(current study).

Chapter 5: General discussion and conclusions

Estuaries undergo constant changes due to natural and human-induced pressures (Defeo & Elliott 2021). Estuarine size, morphology and hydrodynamics determine how these systems respond to pressures, often resulting in shifts in biological communities (van Niekerk et al. 2022). Consequently, an ecosystem under stress is anticipated to demonstrate changes in its species assemblage and functional roles (Lohrer et al. 2012, Ellis et al. 2015, Raymond 2022). Macrobenthic communities are widely recognised as reliable indicators of estuarine health over time, offering insight into ecological conditions. This study focused on short-term (seasonal) changes in macrobenthos communities of the uMdloti and uMlalazi Estuaries, with the aim of understanding the temporal and spatial variability of macrobenthic communities in these systems. Specifically, to answer the question, how do environmental gradients influence macrobenthic diversity, density and composition across space and time? The characteristics of the macrobenthic community in the uMdloti Estuary differed both spatially and temporally, primarily structured by hydrology-related factors such as mouth state and salinity, sediment and DO concentration. In contrast, macrobenthic communities in the uMlalazi Estuary only displayed spatial differences driven by pronounced salinity and sediment gradients, consistent with the estuary's predominantly open mouth. The contrasting results of the current study highlight the impact of short-term changes in community structure driven by localised seasonal environmental variations. These changes are influenced by broader-scale factors, including morphological characteristics, the intensity of pressures on the system and the protection status of the area.

5.1 Estuarine type, state and response to pressures

The current study investigated a TCE and POE subjected to similar pressures (i.e. freshwater modification, nutrient pollution) but different levels of protection status and locations (urbanised and non-urban areas). Despite the similarities in pressures, the estuaries demonstrated distinct spatial and temporal responses due to differing environmental contexts and protection measures.

While the uMdloti Estuary showed no significant spatial gradient in terms of environmental variables, the uMlalazi Estuary varied in both time and space. The uMdloti Estuary showed a lack of spatial variation, particularly in salinity levels and sediment characteristics. This lack of variation has been linked to significantly reduced freshwater inflows reaching the system, which have resulted in oligohaline conditions (Brooker & Scharler 2020) and insufficient sediment transportation. Although the current study recorded optimal environmental conditions in terms of stable DO levels and chlorophyll-a concentrations, occasional episodes of hypoxia and acidic conditions were also observed. While these conditions may result from natural processes, in the uMdloti Estuary and other systems with compromised health, they have been associated with elevated pollution of water and sediment from urban contaminants and modifications in hydrological regimes by upstream impoundments and water abstraction (Adams et al. 2002, Govender 2009, Taljaard et al. 2009, Hale et al. 2016, Brooker & Scharler 2020, Bate et al. 2023). Additionally, these conditions have been associated with destruction in riparian areas, poor agricultural practices including subsistence crop cultivation and animal grazing and commercial agriculture where catchment banks are encroached by sugarcane (Govender 2009, Van Niekerk & Turpie 2011, Lemley et al. 2014, van Niekerk et al. 2019).

The uMlalazi Estuary benefits from several natural buffering factors such as its predominantly open mouth, protection status, rural location, protected riparian areas and steady freshwater inflows help maintain critical salinity and sediment gradients, ensure optimal oxygen levels and reduce the frequency of harmful algal blooms. These factors collectively promote a more stable and resilient ecological environment. This comparison highlights the importance of protecting and improving the management of estuaries to maintain their natural function, which guarantees continued ecosystem services.

5.2 Macrobenthic responses to estuary type, state and pressures

The macrobenthic communities of uMdloti Estuary suggested the possibility of localised habitat disturbance. This was evident in the reduced macrobenthos diversity and extreme seasonal shifts in community structure observed during open-mouth periods. Generally, the estuary was dominated by stress-tolerant, opportunistic and invasive species, including

Chironomidae, Oligochaeta, *Tarebia granifera* and *Corbicula fluminalis*. In continuously disturbed estuaries such as uMdloti Estuary, these communities remain resilient and persist over time even when conditions improve (Dias et al. 2022). For example, they dominated the estuary during the drought period (Scharler et al. 2020) despite frequent hypoxic and eutrophic conditions (Scharler et al. 2023) and they continue to thrive under varying environmental conditions (current study). Although this was the case, the communities displayed a spatial gradient, where these taxa were predominantly found in the upper reaches, indicating signs of environmental degradation, while the lower reaches supported typical key estuarine taxa. This suggests that spatial distribution patterns are strongly influenced by localised conditions that alter the physico-chemical environment in specific areas.

In contrast, the stable and optimum environmental conditions, together with distinct estuarine gradients in the uMlalazi Estuary, create diverse ecological niches, fostering diverse and stable communities inhabiting distinct spatial zonation patterns. These habitats permitted greater diversity, with a higher numerical abundance of key estuarine taxa such as *Dendronereis arborifera*, *Prionospio sexoculata*, *Americorophium triaenonyx*, *Halmyrapsuedes cooperi* and *Sinelobus stanfordi*. Additionally, the presence of keystone taxa such as *Paratyrodiplox blephariskios* and *I. truncata* serves as indicators of a healthy environment. Taxa such as *Ceratonereis keiskama*, *D. arborifera* and *P. blephariskios* occupy critical functional roles, serving as the primary link for transfer of energy to higher trophic levels and facilitating nutrient cycling (Ortega-Cisneros & Scharler 2015).

Despite these differences, both estuaries supported relatively similar key estuarine macrobenthic taxa comprising *C. keiskama*, *D. cf. ornata*, *Grandidierella* spp. amphipods and the *Brachidontes virgiliae* bivalve. The presence of these taxa highlights the biogeographic species pool which disregards the estuary type and its health status to a certain degree. These key estuarine taxa generally thrive under favourable environmental conditions, with their distribution constrained under adverse conditions. Natural stress such as oligohalinity (current study) and hypersalinity (Pillay & Perissinotto 2008, MacKay et al. 2010, Cyrus et al. 2011) does not have deleterious effects on these taxa due to their broad environmental tolerance (Elliott & Quintino 2007). However, it has been noted that these taxa are sensitive to poor water quality, which is associated with severe hypoxia, anoxia and eutrophic

conditions (Diaz & Rosenberg 1995, Ndhlovu et al. 2024) making them reliable indicators of environmental health. While there were some similarities between these estuaries, their differences were highly pronounced. Moreover, the observed patterns are not directly comparable, as they result not only from differences in estuary type but also variations in the degree of exposure to environmental pressures and protection statuses. This variability makes it impossible to generalise these trends or the responses of macrobenthic communities to broader patterns typical of subtropical estuaries.

The presence of key estuarine taxa, alongside a notable prevalence of stress-tolerant species, suggests that benthic macroinvertebrate communities in both estuaries may be influenced by a combination of natural stressors and anthropogenic impacts. Stress-tolerant taxa are common in estuarine environments due to the inherently challenging conditions of these habitats (Elliott & Quintino 2007, Dauvin & Ruellet 2009). However, their numerical dominance alone does not necessarily indicate poor ecosystem health (Simboura & Zenetos 2002, Borja et al. 2012). Of greater concern to ecologists is the increasing number of invasive species such as *T. granifera*, *C. fluminalis*, *Polydora* spp. and *Ficopomatus enigmaticus* (Appendix 4.1). Their presence in both estuaries highlights growing environmental pressures on the health of these systems. Given the ecological impact of invasive species and their potential to reshape estuarine dynamics, there is a critical need for regular monitoring. This effort should focus on tracking invasive taxa across systems to better understand their spread, assess their impacts and develop strategies to mitigate their influence.

5.3 Limitations and recommendations for future research

The current study did not capture the full estuarine gradient of the uMlalazi Estuary, particularly in the upper reaches, beyond the brackish regions. This excluded area extends approximately 3 km upstream to the estuarine-river interface, where salinity gradually declines to below one (Mackay, F. unpublished data). This region is expected to support distinct brackish and freshwater species. Given the inherent variability in salinity across estuarine systems, it is essential to expand monitoring efforts to cover a wider range of sites, including those situated further upstream. Salinity gradients within estuaries play a crucial role in structuring community composition and influencing ecosystem function (Teske &

Wooldridge 2004, Dias et al. 2022). Excluding the upper reaches from analysis provides an incomplete understanding of these communities, their ecological functions and their responses to the environmental conditions unique to these regions. Additionally, Schmitz et al. (2024) argued that the upper reaches of the uMlalazi Estuary are experiencing severe environmental degradation due to organic contamination and heavy metals. While the study acknowledged the localised impacts of various pressures across different regions of the estuary, it emphasised the urgent need for mitigation strategies guided by a catchment-to-coast management approach.

The study assessed the TCE in a semi-urbanised area and the protected POE, which has clear ecological and environmental differences. While the results provide valuable insights into macrobenthos dynamics in these contrasting estuary types, the variation in anthropogenic pressures and natural processes between the two makes it challenging to make generalised drivers of observed patterns. A comparative study focusing on estuaries of similar types (e.g. TCE vs. TCE or POE vs. POE), subject to similar pressures would offer a more controlled understanding of macrobenthos responses and ecosystem dynamics. Using similar estuary types with comparable pressures offers several advantages for ecological studies such as minimising confounding variables and ensuring that observed differences are more likely due to the specific pressures under investigation rather than inherent differences in estuary characteristics. These focused comparisons also provide clearer management implications, enabling the establishment of targeted conservation and restoration strategies for estuaries facing similar challenges.

To date, studies on macrobenthos in the uMdloti and uMlalazi estuaries have primarily focused on taxonomic characteristics and distribution in relation to environmental variables, overlooking aspects such as functional traits. Taxonomic studies provide insights into biodiversity inventories by identifying and classifying species, allowing researchers to assess species richness, detect biogeographic patterns and monitor how they change over time (Alves et al. 2014, Drylie et al. 2020). However, solely utilising the taxonomic method may not fully explain how changes in benthic biodiversity affect the functioning of the system (Alves et al. 2014, Dias et al. 2022). Studying functional traits is beneficial for understanding critical ecological processes like nutrient cycling, bioturbation and oxygen transport, which directly impact ecosystem functioning (Pearson & Rosenberg 1978, Drylie et al. 2020, Dias et al. 2022).

Including functional traits in these studies would have provided an opportunity to identify the roles specific species play in supporting various ecosystem functions. In the uMdloti Estuary, where species composition is less variable, functional trait studies could have highlighted which functions are potentially not being fulfilled and how their absence affects the overall system. Temporally, examining functional traits could reveal how seasonal changes (wet and dry periods) influence these processes, offering insights into how the system operates under different climatic conditions and enabling predictions of its behaviour during extreme climatic events. Spatially, this approach could uncover how species adapt to local environmental conditions and the functional roles they adopt under different scenarios.

The study revealed that, although these systems may be under pressure, spatial responses can vary depending on external factors such as land use surrounding the sampling area, the intensity of activities and proximity to pollution sources. For instance, in the uMdloti Estuary, the upper reaches were comprised of macrobenthos communities that are indicative of compromised health, whereas the lower reaches displayed healthier conditions. Similarly, in the uMlalazi Estuary, Schmitz et al. (2024) demonstrated that metal and organic pollution effects are pronounced in areas near human settlements and regions with reduced water flow. This shows the effects of the localised response of environmental conditions to pressures, which have been reported as critical in shaping macrobenthos patterns in estuaries (Harmer 2014). Therefore, further research to investigate the direct effects of anthropogenic stressors on these communities is necessary. Specifically, studies should focus on establishing correlations between macrobenthos and pollution from nutrients and metals, which have emerged as major pollutants compromising quality in these estuarine systems (Nozais et al. 2001, Thomas et al. 2005, van Niekerk et al. 2019, Schmitz et al. 2024). Additionally, there is a knowledge gap regarding the concentrations and effects of organic pollutants in these systems, as well as their potential influence on macrobenthic communities, highlighting the need for further research on this subject.

The study further highlighted the influence of mouth dynamics on benthic communities. Both estuaries experience periodic mouth closures, though the uMlalazi Estuary is less frequently affected. Reports of critical ecological disasters such as mangrove die-offs (Taylor 2020) highlight the need for ongoing seasonal monitoring to assess how these closures impact hydrological functions and the implications of seasonal variability in both estuaries.

Understanding how mouth closures and hydrological variability impact estuarine functions is crucial for informing efforts to restore freshwater inflows, a critical component of estuarine health and ensure adherence to environmental water requirements outlined in the National Water Act (Act 36 of 1998). Restored freshwater inflows encourage natural mouth breaches, reducing reliance on artificial interventions (Claassens et al. 2022). Additionally, improved flows may enhance estuarine functional zones and restore riparian buffer zones, which are vital for filtering runoff and managing nutrient loads entering these systems (Stromberg et al. 2013, van Niekerk et al. 2019, Taylor 2020).

Moreover, restoration efforts must be tailored to the unique characteristics of each estuary, considering differences in estuary type, the intensity of pressures, geomorphological features and their current ecological states. Finally, Long-term monitoring is essential to address the escalating pressures on these systems and the associated loss of biodiversity. Such studies will help identify resident species, track species loss and evaluate the functional roles currently fulfilled, those at risk of decline and the broader implications for the estuarine ecosystem.

5.4 Final conclusions

In conclusion, the spatio-temporal changes of macrobenthic communities in the uMdloti and uMlalazi Estuary were structured by responses to a combination of hydrological regimes, morphological features and anthropogenic pressures in and around the systems. The protected status of the uMlalazi Estuary is a critical factor influencing the ecological outcomes observed in this study. Protection measures such as restricted access and regulated land use within the estuarine functional zone (EFZ), likely play an important role in maintaining good water and sediment quality and preserving high macrobenthos diversity.

The benthic communities of the uMlalazi Estuary highlight the importance of diverse habitats in supporting varied taxa composition that sustain essential ecosystem processes. For example, the estuary has been reported to have an efficient nutrient cycling capacity and robust trophic interactions (Ortega-Cisneros 2013, Gerber et al. 2024). This suggests resilience against extreme conditions such as eutrophic, hypoxic and anoxic conditions, as well as reduced primary production. Evidence from previous studies highlights that the system

remains well-oxygenated, with low nutrient enrichment and minimal algal blooms (DWS 2015) even during adverse consequences of low flows during droughts (Scharler et al. 2023). These conditions may also be attributed to the efficiently performed functions by various taxa such as *P. blephariskios*, *C. keiskama* and *D. arborifera* which contribute to sediment oxygenation, sediment reworking and nutrient recycling in the sediment, thereby improving the overall quality of the habitat.

In contrast, the imbalance in hydrological dynamics caused by a significant reduction in freshwater inflow combined with accumulating nutrients has led to a marked decrease in biodiversity. This reduction in species diversity has resulted in restricted critical ecosystem functions (i.e. nutrient cycling and bioturbation), potentially contributing to hypoxia, acidity and occasional eutrophication, particularly during the closed-mouth phase when there is no exchange with the sea. Although mouth breaches temporarily reset environmental conditions and facilitate the recruitment of estuarine and marine taxa, the system remains under chronic environmental stress. As a result, it struggles to fully restore key taxa essential for maintaining critical ecosystem functions. This makes the estuary increasingly vulnerable to functional losses in the face of future species loss and extreme hydrological shifts, thus reducing its buffering capacity to environmental stress. This is particularly concerning given the predicted extreme hydrological variation between the dry and wet seasons (IPCC 2023). Such changes may rapidly accelerate the loss of both taxonomic and functional diversity during dry seasons, potentially exceeding the rates of biodiversity loss documented during the 2014 – 2016 drought period.

Both systems urgently require effective measures to control anthropogenic pressures. While the impacts on the uMlalazi Estuary may appear minimal at present, the gradual loss of keystone species over the years suggests potentially severe long-term consequences for the ecosystem (outlined in Chapter 4). In contrast, the uMdloti Estuary, despite being classified as highly modified, has demonstrated resilience in its benthic communities and retained its biodiversity significance (van Niekerk et al. 2019). Addressing these challenges through targeted conservation and management efforts will be crucial to safeguarding the health and functionality of both estuaries, ensuring they continue to provide vital ecosystem services and support resilient communities.

This study revealed the composition of estuarine communities along the KZN coast and contrasted benthic communities in a semi-urbanised TCE and a near-natural POE. Based on macrobenthic communities, the current study revealed that the uMlalazi Estuary exhibited better ecological condition compared to the uMdloti Estuary, with higher taxonomic richness, diversity, and density in the macrobenthic assemblage. The uMlalazi Estuary also supported more sensitive and ecologically significant taxa. While common estuarine species found in both systems serve as indicators of a well-functioning benthic environment, their presence alone cannot fully determine the health of an estuarine system. Utilisation of these taxa as indicators of ecological health should be approached with caution. Additional factors such as nutrient levels and land use, need to be considered to establish stronger links between community structure and ecosystem health.

6. References

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Appendix

Table 4.1: List of taxa recorded in the uMlalazi Estuary across five sites (1 – 5) during the dry (Jun – Aug 19) and wet (Dec 19 – Feb 20). – denotes missing data.

	Jun-19					Jul-19					Aug-19					Dec-19					Jan-20					Feb-20				
Taxa	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5
Polychaeta:																														
<i>Capitella cf. capitata</i>																														
<i>Heteromastus cf. filiformis</i>																														
<i>Desdemona cf. ornata</i>																														
<i>Ceratonereis (Composetia) keiskama</i>																														
<i>Dendronereis arborifera</i>																														
<i>Dendronereis sp1</i>																														
<i>Potamilla sp1</i>																														
<i>Ficopomatus enigmaticus</i>																														
<i>Glycera natalensis</i>																														
<i>Glycera sp1</i>																														
<i>Phyllodoctidae capensis</i>																														
<i>Goniada sp1</i>																														
<i>Prionospio sp1</i>																														
<i>Prionospio cf. sexoculata</i>																														
<i>Prionospio sp2</i>																														
<i>Prionospio saldahna</i>																														
<i>Prionospio cf. bocki</i>																														
<i>Prionospio sexoculata</i>																														
<i>Prionospio cirrifer</i>																														
<i>Sigambra parva</i>																														
<i>Aphelocheata filibranchia</i>																														
<i>Aphelocheata sp1</i>																														
<i>Aphelocheata sp2</i>																														
<i>Aonides sp1</i>																														
<i>Polydora sp1</i>																														
<i>Notomastus aberans</i>																														
<i>Polydora sp2</i>																														
<i>Scolecopsis (Scolecopsis) squamata</i>																														
<i>Sphaerosyllis sp1</i>																														
<i>Scalibregmatidae sp1</i>																														
<i>Sphaerosyllis sp1</i>																														
Unknown 1																														
Density (ind·m ⁻²)	≤ 10					11 - 100					101 - 500					501 - 1000					1001 - 2000					2001 - 3000				
Density key																														

Table 4.1: Continued.

	Jun-19					Jul-19					Aug-19					Dec-19					Jan-20					Feb-20				
Taxa	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5
Oligochaeta:																														
<i>Chaetogaster</i> sp1																														
Naididae sp1																														
Naididae sp2																														
Naididae sp3																														
Gastropoda:																														
<i>Assiminea ovata</i>																														
<i>Nassarius kraussianus</i>																														
<i>Nassarius</i> sp1																														
<i>Neverita didyma</i>																														
<i>Tarebia granifera</i>																														
<i>Haminoea natalensis</i>																														
Unknown 2																														
Bivalvia:																														
<i>Dosinia hepatica</i>																														
<i>Tellina</i> sp1																														
<i>Eumarcia paupercula</i>																														
<i>Brachidontes virgiliae</i>																														
<i>Solen cylindraceus</i>																														
Mysida:																														
<i>Mesodopsis africana</i>																														
Amphipoda:																														
<i>Afrochiltonia capensis</i>																														
<i>Grandidierella</i> spp																														
<i>Grandidierella lutosa</i>																														
<i>Americorophium triaenonyx</i>																														
<i>Hourstonius pusilla</i>																														
<i>Urothoe pinnata</i>																														
<i>Victorapsia chilensis</i>																														
<i>Melita zeylanica</i>																														
<i>Melita orgasmos</i>																														
<i>Boltzia minuta</i>																														
<i>Caprellidae</i> sp1																														
Density (ind·m ⁻²)	≤ 10					11 - 100					101 - 500					501 - 1000					1001 - 2000					2001 - 3000				
Density key																														

Table 4.1: Continued.

	Jun-19					Jul-19					Aug-19					Dec-19					Jan-20					Feb-20				
Taxa	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5
Tanaidacea:																														
<i>Apseudidae sp1</i>																														
<i>Halmyrapseudes cooperi</i>																														
<i>Sinelobus stanfordi</i>																														
Isopoda:																														
<i>Uromunna sheltoni</i>																														
<i>Cyathura estuaria</i>																														
<i>Leptanthura laevigata</i>																														
Decapoda:																														
<i>Penaeus monodon</i>																														
<i>Brachyuran larvae</i>																														
<i>Paratylodiplax blephariskios</i>																														
<i>Hymenosoma sp1</i>																														
<i>Neorhynchoplax bovis</i>																														
Cumacea																														
<i>Iphinoe truncata</i>																														
Hydra:																														
<i>Hydra sp1</i>																														
Hirudinea:																														
Nemertea sp1																														
Platyhelminthes:																														
Platyhelminthes sp1																														
Unkown 3																														
Density (ind·m ⁻²)	≤ 10					11 - 100					101 - 500					501 - 1000					1001 - 2000					2001 - 3000				
Density key																														