MICROPLANKTON COMMUNITY STRUCTURE IN THE SUBTROPICAL MLALAZI ESTUARY

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As the candidate’s supervisor I have approved this thesis/dissertation for submission.

Signed: _____________ Name: _____________ Date: ___________

ABSTRACT

In a country like South Africa that is prone to drought, achieving good quality of water is necessary to ensure the nation's water resource sustainability in the interest of all water users (National Water Act (No. 36 of 1998). River flow and rainfall influence the estuarine environment and biological indicators (phytoplankton (chl *a*)), system variables (salinity, turbidity, dissolved oxygen, and temperature), and nutrients (phosphate, nitrate, and nitrite) are some of the core water quality variables considered in ecological reserve assessments. Globally, microplankton (phytoplankton and ciliate) community structure (composition, abundance, and biomass) varies with physico-chemical parameters, making them good bioindicators of water quality. Phytoplankton are one of the main primary producers in estuaries, forming the basis of trophic webs. Ciliates are a valuable tool for indicating hydrological conditions and trophic status and can be used as an additional bioindicator to phytoplankton. Characteristics that make ciliates potential bioindicators include very high metabolic rates, short generation times, broad geographic distribution, and their rapid response to changing environmental factors. Ciliates dominate the microzooplankton abundance in coastal water bodies and graze on phytoplankton directly, making ciliates ecologically crucial in transferring energy to higher trophic levels, and controllers of phytoplankton community structure, through top-down pressure on phytoplankton. The main attributes of ciliates used for water quality monitoring are feeding habits (bactivores, carnivores, omnivores, and algivores), species composition, abundance, and biomass.

For each water quality variable, a concentration range (minimal ('excellent') to severely modified ('poor') within the water resource should be determined. This emphasises the importance of studying these variables in near-pristine water resources to determine the minimally modified concentrations. There are few microplankton community composition studies in South African oligotrophic or non-eutrophic systems that are important in determining reference conditions and community structures for possible future changes in

water quality. In this study, microplankton (phytoplankton and ciliates) and the physico-chemical parameters in the subtropical Mlalazi Estuary of good to fair water quality status were evaluated bi-weekly for one year. This evaluation aimed to assess the spatio-temporal variability of the microplankton community structure and to understand the potential drivers of these changes in an estuary. During this study period, 17 ciliate genera including 12 species in the subclass Choreotrichia, five in the subclass Oligotrichia, five in the subclass Haptoria, one Hypotrichia and one Scuticociliata. In terms of abundance and biomass choreotrichs and oligotrichs were dominant and 65% of the ciliate species were reported at all reaches. With respect to phytoplankton, 97 genera were recorded. This included 68 diatoms (Bacillariophyta), 21 dinoflagellates (Dinophyta), three chlorophytes (Chlorophyta), three cyanobacteria (Cyanophyta), one cryptophyte (Cryptophyta) and one euglenophyte (Euglenophyta). Diatoms were the most diverse with 182 species followed by dinoflagellates with 40 species. Overall, cryptophyte was the most numerically dominant ($1.97 \pm 2.82 \times 10^5$ Cells/L) and cyanobacteria were the least ($0.75 \pm 2.2 \times 10^1$ Cells/L). Biweekly sampling showed patterns and details that would otherwise not be revealed with the commonly used seasonal sampling schedule. The biweekly sampling showed that there was a very high variability in microplankton community structure (abundance, biomass, species composition) within a season and that sampling once per season was not indicative of the community in that season. No variable was highlighted as a driver for phytoplankton taxon richness. The microplankton community structure varied spatially and temporally, and the estuarine water quality status ranged from oligotrophic and mesotrophic. The study showed that salinity, temperature, turbidity, total oxidised nitrogen (TON) concentration and dissolved inorganic phosphorus (DIP) concentration explained most of the variability in the microplankton community abundance and biomass.

Furthermore, spatial characterisation indicated that diatoms and dinoflagellates dominated at the lower reaches where salinity was higher while chlorophytes and cryptophytes dominated at the upper reaches. Higher nutrient concentrations in summer led to diatoms' dominance while dinoflagellates dominated in winter when nutrient concentrations were low at the lower reaches. Higher temperatures in summer favoured the growth of chlorophytes over cryptophytes at the upper reaches. A shift in diatom type associated with salinity and turbidity was identified. Chain-forming pelagic diatoms associated with a marine environment were most abundant at the lower reaches while benthic taxa dominated at the upper reaches where turbidity was high, and salinity was lower. 25% of the dinoflagellates recorded were potentially harmful (PH) species. These were *Gonyaulax spinifera*, *Karlodinium veneficum* (original name: *Gyrodinium estuariale*), *Prorocentrum cordatum*, *P. micans*, *P. triestinum* and *Scrippsiella trochoidea*. However, low nutrient concentrations recorded in the estuary did not encourage bloom initiation. Given that these PH species are present in the Mlalazi and are known harmful species in systems with poor water quality, their presence is not be used as an indicator of poor water quality. However, water quality investigations should focus on the abundance of these species (rather than presence or absence) and toxicity. A narrow range in temperature and water quality from oligotrophic to mesotrophic accounted for near consistency in the ciliate community composition along the salinity gradient of the estuary and dominance of choreotrichs and oligotrichs throughout the study period at all sites. These ciliates, *Tintinnopsis* spp., *Rimostrombidium* sp., *Strobilidium* sp., *Spirotontonia* sp., *Stenosemella* sp., and *Strombidium* spp. can be used as a guide of what to expect in an oligo– to mesotrophic predominantly open subtropical estuaries with limited anthropogenic influences Except for the Scuticcociatids, *Uromena* sp. which can be used as an indicator of declining water quality (TON concentration of 90 µM).

During this study, the estuary's water quality was good to fair, however, given the presence of potentially harmful dinoflagellates in the Mlalazi Estuary, a potential increase in nutrient concentration could initiate blooms leading to poor water quality. The estuary has low potential of becoming eutrophic and river flow is a main source of nutrients. However, during droughts and low river flow, primary productivity leading to bloom conditions is possible suggesting other sources of nutrients. The practice of water abstraction in South African estuaries, agriculture along the estuary and the extreme nature of seasonal events such as extended droughts and floods demand continuous monitoring of water quality in this estuary despite the current good ecological status. This study contributes to understanding the variability in biological indicators (microplankton communities) associated with nutrients and system variables which are the main variables in ecological reserve assessment (Skowno et al., 2019; Van Niekerk et al., 2019; DWS, 2015; Palmer et al., 2005). The study also provides a baseline of the microplankton community of an estuarine ecosystem in near pristine condition and key species to focus on during monitoring rather than the whole microplankton community.

PREFACE

The work described in this PhD thesis was carried out in the School of Life Sciences, University of KwaZulu-Natal, Westville, from February 2014 to November 2020, under the supervision of Prof. Ursula M. Scharler.

These studies represent original work by the author and have not otherwise been submitted in any form for any degree or diploma to any other tertiary institution. Where use has been made of the work of others it is duly acknowledged in the text.

Signed [redacted]…………………………

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DECLARATION 1 - PLAGIARISM

I, Nche-Fambo Fru Azinwi, declare that

1. The research reported in this thesis, except where otherwise indicated, is my original research.

2. This thesis has not been submitted for any degree or examination at any other university.

3. This thesis does not contain other persons' data, pictures, graphs or other information, unless specifically acknowledged as being sourced from other persons.

4. This thesis does not contain other persons' writing, unless specifically acknowledged as being sourced from other researchers. Where other written sources have been quoted, then:

a. Their words have been re-written but the general information attributed to them has been referenced. Where their exact words have been used, then their writing has been placed in italics and inside quotation marks, and referenced.

5. This thesis does not contain text, graphics or tables copied and pasted from the Internet, unless specifically acknowledged, and the source being detailed in the thesis and in the References sections.

Signed [redacted]…………………………

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DECLARATION 2 - PUBLICATION

Details of contribution to publications that form part and/or include research presented in this thesis are as follows:

Publication 1

Fru Azinwi Nche-Fambo and Ursula M. Scharler. Submitted. Spatial variability can shape seasonal responses of phytoplankton communities in subtropical estuaries (Marine Ecology Progress Series).

Publication 2

Fru Azinwi Nche-Fambo and Ursula M. Scharler. In Preparation. Spatio-temporal pattern of the diatom community along a salinity gradient in a subtropical estuary.

Publication 3

Fru Azinwi Nche-Fambo and Ursula M. Scharler. In Preparation. The spatio-temporal variability of dinoflagellate species in a subtropical South African estuary, with a focus on potential harmful species.

Publication 4

Fru Azinwi Nche-Fambo and Ursula M. Scharler. In Preparation. Planktonic ciliates in the oligo- to mesotrophic subtropical Mlalazi Estuary.

Conference poster

Nche-Fambo, F.A., Scharler, U.M. 2017. Dinoflagellate diversity in a subtropical permanently open estuary: emphasis on potentially harmful species. Phycological Society of America, California.

Nche-Fambo, F.A., Scharler, U.M. 2017. Dinoflagellate diversity in a subtropical permanently open estuary: emphasis on potentially harmful species. Phycological Society of South Africa, Cape Town.

Nche-Fambo, F.A., Scharler U.M. 2016.Variability of microalgae biomass and community structure in a permanently open KwaZulu-Natal estuary. School of Life Science Research Day, Durban.

Other Publications

Wang, H., **Nche-Fambo, F.A.**, Yu, Z. and Chen, F. 2018. Using microalgal communities for high CO2-tolerant strain selection. Algal Research, 35: 253-261.

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Dedication

I dedicate this Thesis to my adorable mom **Mangie Fambo Phoebe Akwa** and dad **Tangie Fambo Wilfred Nche.**

Table of Content

Abbreviations and Notations.

% = Percent

°C = degrees Celsius

CCA = Canonical correspondence analysis

CV = Coefficient of variation

Cells/L = Cells per litre

Chl *a* = Chlorophyll a

df = degrees of freedom

TON = total oxidised nitrogen

DIP = Dissolved inorganic phosphorus

DISTLM = Distance based linear modelling

DO = Dissolved oxygen

GF/F = Glass Fibre Filter

KZN = KwaZulu-Natal

µm = micrometre

µM = micro molar

mg/L = milligrams per litre

m = metre

pg C/L = picogram Carbon per litre

n = Sample size

nMDS = Non-metric multidimensional scaling

NTU = Nephelometric Turbidity Units

NO_2^+ = Nitrite

NO_3^- = Nitrate

PO_4^{3-} = Phosphate

SD = Standard Deviation

sp. = Species (singular)

spp. = Species (plural)

Chapter 1. GENERAL INTRODUCTION

Background

Estuaries

An estuary is defined by Potter et al. (2010) as “a partially enclosed coastal body of water that is either permanently or periodically open to the sea and which receives at least periodic discharge from a river(s), and thus, while its salinity is typically less than that of natural seawater and varies temporally and along its length, it can become hypersaline in regions when evaporative water loss is high and freshwater and tidal inputs are negligible”. Estuaries mainly receive nutrients from inflowing rivers (Nunes et al., 2019; Yamaguchi et al., 2019) and they have a higher nutrient concentration than the surrounding ecosystems increasing their potential for primary production (Freeman et al., 2019; Elliot and McLusky, 2002). Vertical and horizontal salinity gradients are characteristics of these systems (Adams et al., 2020; Adams, 2014; Louw, 2007). Local topography, tidal mixing, and freshwater inflow are the drivers of the degree of salinity gradients (Van Nierkerk et al., 2019; Louw, 2007). As a transition between land and sea, estuaries are very complex and dynamic ecosystems with constant changes in the environmental conditions (Van Niekerk et al., 2019; Acreman et al., 2014; Jennerjahn and Mitchell, 2013; Schumann et al., 1999;). The dynamic nature of the estuarine environment and the high potential for primary production provides estuaries with a wide variety of habitats and resources to support a diverse group of fauna and flora (Elliot and McLusky, 2002). The high turbidity and availability of food in estuaries compared to the surrounding aquatic systems also enable estuaries to serve as a shelter and nursery ground for organisms of high economic value such as fish and prawns (Abrantes et al., 2019; Rezende et al., 2019; Forbes and Demetriades, 2005).

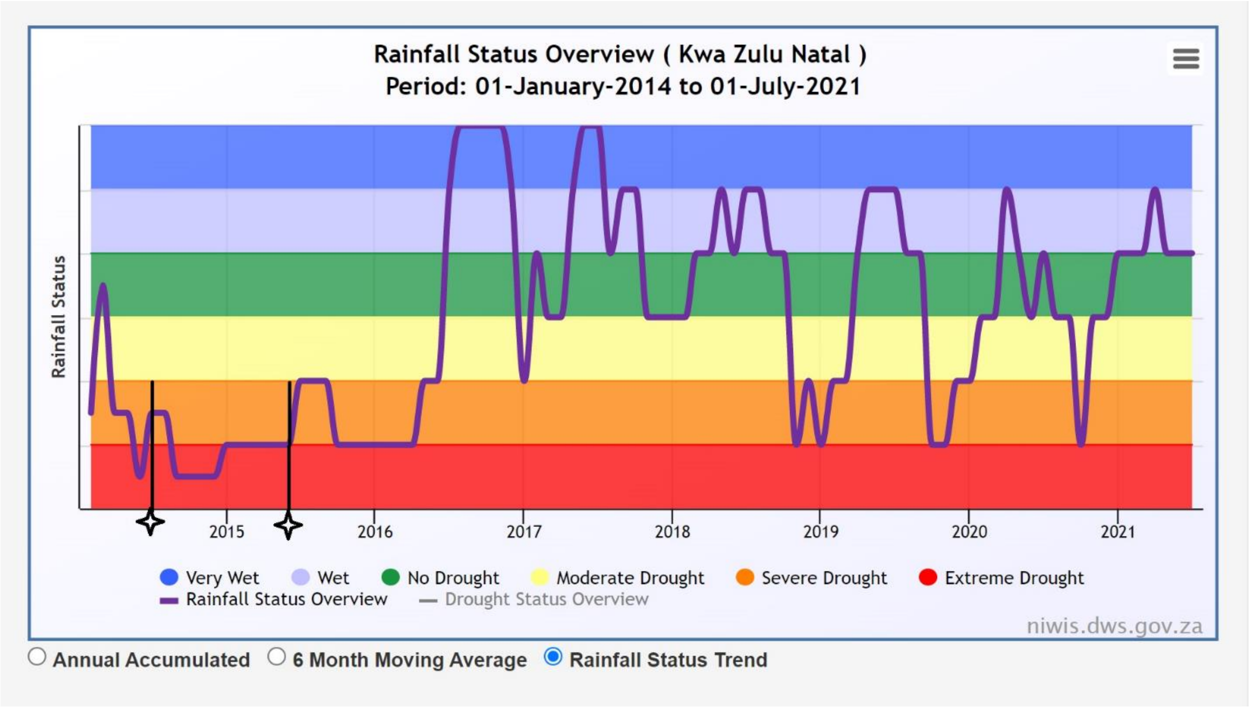
Estuaries are therefore a source of subsistence for people and attract their settlement (Abrantes et al., 2019; Jennerjahn and Mitchell, 2013), making them systems of high socio-economic importance. Globally, other common activities along estuarine environments include mining, agriculture, and aquaculture all of which promote industrialisation and urbanisation along the estuary.

The continuous growth of the human population around estuaries and their watersheds acts as a source of anthropogenic pressure on estuaries. This may cause alterations in the ecological functioning of these systems and their ability to function as nursery grounds (Nicolas et al., 2007). Effluent discharge from waste water treatment plants, nutrient enrichment from agricultural runoff, water abstraction for use in agriculture, aquaculture, municipal use are examples of anthropogenic pressures (Allanson and Read, 1995). With the increase of these anthropogenic pressures on estuaries, water quality has degraded globally accompanied by features such as eutrophication and harmful algae blooms (Paerl and Barnard, 2020; Adams et al., 2016; Bricker et al., 2008; Paerl and Scott, 2010).

Along the South African coastline, there are 290 estuaries, catergorised into nine groups, estuarine lake, estuarine bay, estuarine lagoon, predominantly open, large temporarily closed, small temporarily close, large fluvially dominated, small fluvially dominated, and arid predominantly closed estuaries (Van Niekerk et al., 2020). 45 of which are predominantly open estuaries, and 17 are in the subtropical biogeographic region of South Africa. Mostly referred to as Permanently open estuaries (POE) (Whitfield, 1992), until revised in 2020 by Van Niekerk et al. (2020), predominantly open estuaries are defined as estuaries open to the sea for more than 90% of the time (Van Niekerk et al., 2020). Predominantly open estuaries serve as substantial nursery grounds for anadromous fish and marine organisms. Withing South Africa, prolonged mouth closure due to droughts and human manipulation are some of the main threats to the functioning of estuaries. In particular, dams upstream and the water abstraction present a threat to predominantly open estuaries. For example, the Uilkraals Estuary, which was predominantly open, is now seemingly permanently closed since 2018 and only breached artificially, changing it to a large, temporarily closed estuary. Prolonged mouth closure hinders the recruitment of fish from the sea and inhibits estuarine nursery function. Also, prolonged mouth closure may lead to accumulation of nutrients and higher residence time, leading to eutrophication.

The above threats, including the redirection of freshwater from the estuary for agricultural purposes and input from wastewater treatment plants into the estuary, have led to devastating effects in South African estuaries, including those in the KwaZulu-Natal province (KZN) located in the subtropical biogeographic region. Examples of such devastating effects include prolonged hypersalinity and cyanobacteria blooms in the St. Lucia estuarine lake system (Muir and Perissinotto, 2011) and eutrophication and fish kill in the temporarily open Mdloti estuary (personal observation). However, near pristine estuaries, with limited human interference still exist in South Africa and KZN.

A recurrent and regular feature of the South African climate is drought. A drought is defined as a shortage of precipitation over an extended period of less than 70% the normal precipitation. Hydrological drought is associated with precipitation shortage on a longer time scale (12 months to 2 years or more) and its effect on subsurface or surface water supply (Rouault and Richards, 2003). There are eight rainfall regions in South Africa and according to the Weather Service of South Africa, it is not unusual for droughts to last over three years in each region. For KwaZulu-Natal province, in summer months, maximum rainfall is predominantly reported. Droughts that persist for several years also referred to as extreme droughts are sometimes triggered by El Niño Southern Oscillation (ENSO) event. This happens when warm sea surface water intrudes into eastern and central tropical Pacific Ocean and returns at least once per decade (Rouault et al., 2003). In 2014-2016, South Africa experienced the combined effects of a strong El Niño event and a severe drought (Baudoin et al., 2017). This study was conducted during a period of extreme drought (Fig. 1.1).



○ Annual Accumulated ○ 6 Month Moving Average ● Rainfall Status Trend

Figure 1.1: Rainfall status overview in KwaZulu-Natal province from January 2014 to July 2021 showing extreme drought conditions during this study period (highlighted by the star) from July 2014 to June 2015 (https://www.dws.gov.za/niwis2/DroughtStatusManagement/RainfallStatusOverview).

Changes in flood patterns and freshwater input can have significant effects on the functioning of estuaries (Hitchcock and Mitrovic, 2015). All estuaries are sensitive to variations and reductions in freshwater input. Also, due to South Africa's arid nature, runoff is not necessarily directly linked to catchment size (Van Nierkerk et al., 2019; Whitfield and Bate, 2007). There is generally significant river flow and large catchment for Predominantly Open estuaries throughout the year (Whitfield, 1992). Freshwater is an important variable controlling biological productivity coastal areas and estuaries globally including South Africa (Lemley and Adams, 2020). In South Africa, the recognition of water as an important natural resource and government asset led to the development of the National Water Act (No. 36 of 1998) (Adams, 2014; Department of Water Affairs (DWA), 2009). In response to the Act, DWA outlined resource-directed measures to provide frameworks that ensured the protection of water resources for the basic needs of humans and ecological reserve such as water in rivers to ensure the sustainability of aquatic ecosystems and related aquatic ecosystem services in a long term (DWA, 2009). The act recognises that achieving good quality of water is necessary (National Water Act (No. 36 of 1998). The main water quality variables considered in ecological reserve assessments are system variables (salinity, turbidity, dissolved oxygen, and temperature), nutrients (phosphates, nitrates, and nitrites), toxic substances, and biological indicators (chlorophyll a (phytoplankton and periphyton) (Malan and Day, 2012; Palmer et al., 2005). For each variable, a concentration range (minimal (‘excellent’) to severely modified (‘poor’) within the water resource should be determined (Palmer et al., 2005). This highlights the importance of studying these variables in near-pristine water resources to determine the minimally modified concentrations.

Estuaries and coastal management

Despite the importance of estuaries, in South Africa, estuaries are the most threatened and least protected ecosystems (Whitfield et al., 2020; Skowno et al., 2019: Van Niekerk et al., 2019). 86% of estuary types are threatened. In terms of total surface area of estuaries, 99.9% of the total estuarine surface area is threatened. Estuarine ecosystems are the most threatened ecosystem in South Africa. With the wide range of pressures affected estuaries and their catchments, estuaries are also the most challenging ecosystem to protect (Skowno et al., 2019; Waltham et al., 2019). This mirrors the poor ecological state of estuaries, of which the most concerning aspects include water quality degradation and changes in the hydrological regimes. Freshwater flow reduction has impacted 30% of South African estuaries, especially large predominantly open estuaries (e.g., Orange and Groot Berg) (Van Niekerk et al., 2020; Skowno et al., 2019).

Around areas such as estuaries, characterized by high productivity, accessibility and rich in natural resources, human activities tend to concentrate. Ecosystems in good ecological conditions are better able to cope with climate change impacts which in turn help people adapt. Estuarine ecosystems need attention such as direct management and prudent spatial planning (Skowno et al., 2019). Data is needed to inform such management. On a national scale, carrying out monitoring programs for estuaries are important for management and future assessment. However, there is limited long term monitoring data leading to difficulties in in establishing decisions with a high level of confidence (Skowno et al., 2019; Van Niekerk et al., 2019; DWA, 2015). Due to the lack of high confidence decisions or poor decisions, the maximum benefits of estuaries are achieved. Surveys on estuarine abiotic and biotic components are urgently needed in South Africa to ensure the best resource protection, use and allocation especially along the KwaZulu-Natal and Cape West coast where the estuaries are most threatened (Skowno et al., 2019). Also, 63% of the estuarine area are heavily modified leading to a reduction in productivity, fisheries livelihood, food security, and recreational enjoyment (Van Niekerk et al., 2019). To address the threatened state of these estuaries, a 9-point Strategic Management Framework which focuses on implementation and compliance across key sectors including water quality management and freshwater allocation as well as conservation interventions such as the expansion of protected areas, increased fisheries management and compliance, and restoration to base flows and water quality should be developed (Skowno et al., 2019; Van Niekerk et al., 2019). However, such restoration is possible when the base flow and water quality of the estuarine system at pristine or near-pristine condition is known.

In defining the ecological health status (‘excellent’ to ‘poor’) of South African estuaries, a scoring of biotic health requires information on macrophytes, invertebrates, fish, birds, and phytoplankton (DWS, 2015). Of the 290 estuaries in South Africa, the conditions of only 10% are classified as ‘excellent’ (Whitfield and Baliwe, 2013). Here, an estuary with an ‘excellent’ ecological status is defined as one that appears to be in near-pristine condition, i.e., is subjected to negligible human impact (Whitfield and Baliwe, 2013). Along the subtropical coastline of South Africa (Fig. 1. 2), only 20% of estuaries are in their natural state or classified as estuaries of ‘excellent’ ecological status. 36% of the estuaries, including the Mlalazi Estuary, are in a near-natural state or classified as ‘good’ (Skowno et al., 2019; Whitfield and Baliwe, 2013). Estuaries with a ‘good’ ecological status are those with no major anthropogenic influences (Whitfield and Baliwe, 2013). However, 17% of the predominantly open estuaries in South Africa’s subtropical region are classified as highly vulnerable, suggesting a possible degradation in the future, and 18% of all the estuaries in the subtropical region are already classified as ‘poor’ or heavily to critically modified (Skowno et al., 2019). Estuaries with a poor ecological status are those with major ecological degradation arising from a combination of several anthropogenic influences (Whitfield and Baliwe, 2013). To address this issue, estuarine management authorities are striving to achieve and maintain excellent and good ecological health status. Studies of the estuaries with a ‘good’ ecological status will provide a baseline for monitoring future changes in estuaries and the ability to sustain them.

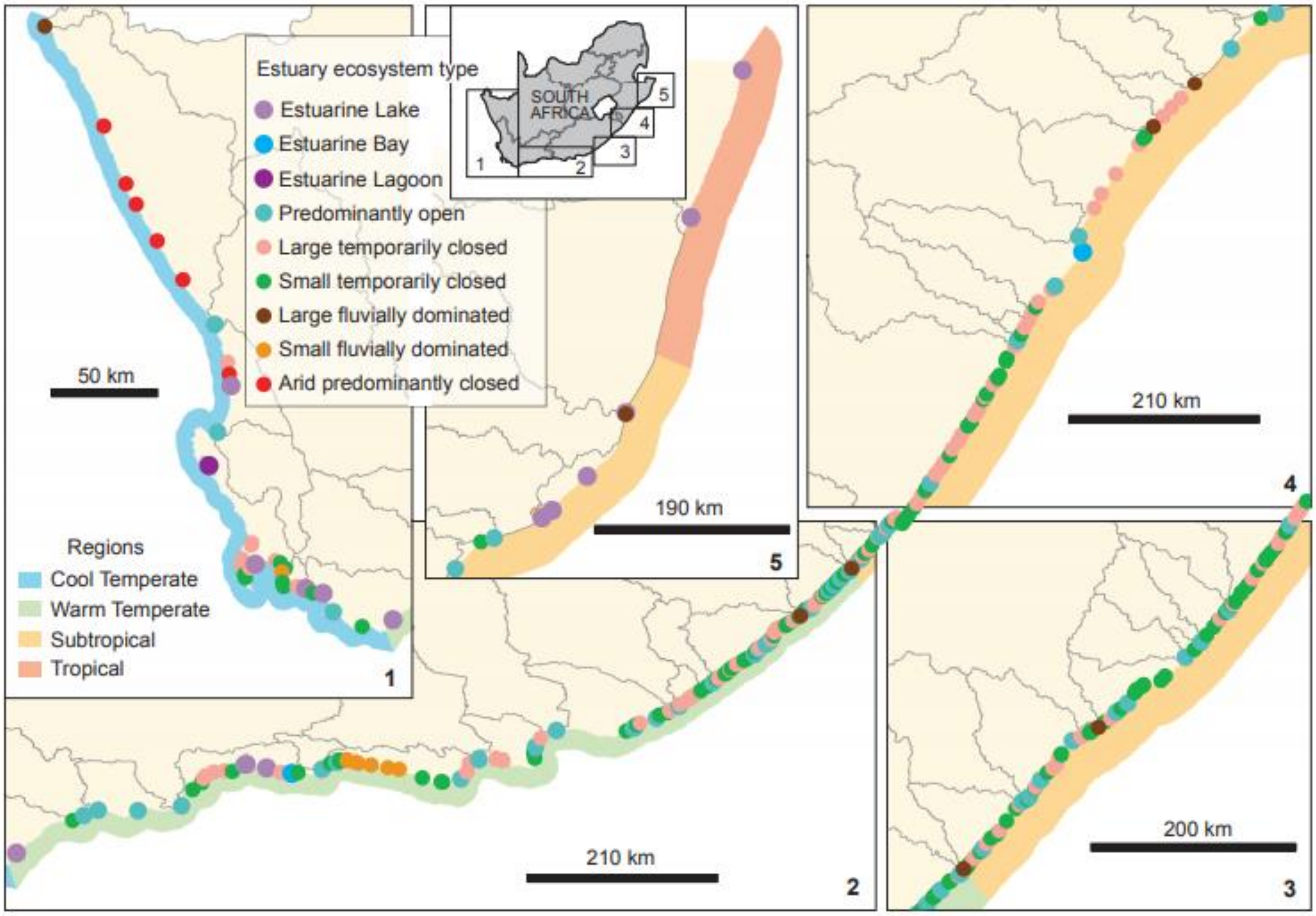


Figure 1.2: South Africa’s estuary types and four biogeographical regions; Cool Temperate (1), Warm Temperate (2), Subtropical (3 and 4) and Tropical (5) (van Niekerk et al., 2020).

The Mlalazi Estuary

According to the resource-directed measures, a water catchment could be used to meet human needs, where the catchment is highly developed with natural habitat loss, or conserved for ecological importance. The

Mlalazi catchment is minimally used and deserves a high conservation status. The Mlalazi Estuary is a subtropical, predominantly open estuary located on the east coast of South Africa (van Niekerk et al., 2020) in KZN. The mouth of this estuary was open throughout the study period. The Mlalazi Estuary mouth closes for about 4% of the time during major droughts (Taylor, 2020; Kelbe and Taylor, 2019; DWS, 2015). This estuary is under the protection of the provincial conservation agency, Ezemvelo KwaZulu-Natal (EKZN) Wildlife within the Umlalazi Nature Reserve. In addition, the presence of mangroves in the Mlalazi Estuary makes the estuary a priority system for protection (Skowno et al., 2019). The Mlalazi Estuary has a longitudinal salinity gradient that increases from the upper to the lower reaches and ranges from 0.1 to 32.8 (DWS, 2015; Cisneros, 2013; Vezi, 2013). The estuary is relatively shallow with a mean depth of 1-2.5 m (DWS, 2015) and experiences higher rainfall during the spring and summer months (Harrison, 2004). Chlorophyll *a* concentration, a proxy for phytoplankton biomass, is generally low, ranging from 0.3 to 11.25 µg/L (DWS, 2015; Ortega-Cisneros, 2013; Vezi, 2013), 1.1µg/L in May (dry season) and 0.3µg/L in November (wet) season, characteristic of oligotrophic to mesotrophic estuaries based on the National Coastal Condition Assessment (NCCA) thresholds for chl *a* concentration (U.S. EPA, 2001) and Lemley et al. (2015).

Though the estuary is situated within a nature reserve, human activities associated with the estuary are poaching, water abstraction for aquaculture and sugarcane farming, and recreational activities (DWS, 2015). Anthropogenic sources of nutrients that this estuary receives are effluent discharges from the Mtunzini municipal Waste Water Treatment Works and the Zini Fish Farm (DWS, 2015). Despite the anthropogenic activities associated with the estuary, the Mlalazi Estuary was classified as having a good ecological health status and an estuarine health score of 80% (DWS, 2015). This implies that the estuary is minimally affected by anthropogenic factors, therefore, of good ecological status. However, the confidence level assigned to the biological health score of the ecological assessment of this estuary was low due to limited information on certain biological groups such as the phytoplankton, where data from a single sampling date was used for the evaluation (DWS, 2015). There is, therefore, a lack of comprehensive information on the phytoplankton assemblage of this estuary. Given that the water quality of the Mlalazi

Estuary is minimally influenced by anthropogenic factors, knowledge on the microplankton community structure of an estuary, classified as having a good ecological status can be used as a reference point for possible future changes in the estuary. The study is particularly important as it establishes the microplankton community an estuary of good ecological status, during a period of drought which is a usual occurrence in South Africa. This study focuses on the water quality aspect of the ecological health status in relation to microplankton (phytoplankton and ciliates).

Phytoplankton

Phytoplankton are microscopic organisms ranging from <2 to 200 µm in size, found in the water column with their spatial distribution, horizontal and vertical position being controlled mainly by water motion (Wirtz and Smith, 2020; Haris, 1986). They are photosynthetic and one of the main primary synthesizers of organic matter (carbon) in aquatic ecosystems forming the bases of the trophic webs in the ecosystem (Peña et al., 2019; Konoplya and Soares, 2011; Ramdani et al., 2009; Fielding et al., 1991). They, therefore, provide a link between inorganic dissolved nutrients available, higher trophic levels, and ultimately to the top predators (Wilken et al., 2017; Miller et al., 1996). An understanding of the variation in phytoplankton abundance, biomass, and species composition gives a comprehension of the primary energy stock of the system. A change in the microalgae community structure therefore has the potential to eventually influence the food web structure of the estuary (Peña et al., 2019; Mitra et al., 2004).

Phytoplankton and water quality

Phytoplankton communities are a combination of a variety of very different species constantly changing in response to changes in the environment and are often good indicators of ecosystem health (López Abbate et al., 2017; Paerl et al., 2010; Blanco et al., 2008; Snow, 2006). Some species produce toxins and blooms causing a nuisance and poor water quality conditions and are good indicators of water quality (Paerl and Barnard, 2020; Ramdani et al., 2009; Lemoalle and Talling, 1998). Therefore, the phytoplankton community composition has been used as an important biological factor in monitoring aquatic systems. According to DWS (2015), a ‘high ecological status is attained when the composition and abundance of phytoplankton taxa are consistent with undisturbed (pristine) conditions', highlighting the importance of examining the phytoplankton community structure in a pristine or near-pristine environment. Phytoplankton community structure (composition, abundance, and biomass) remains a reliable indicator of water quality conditions (such as an influx of nutrients and a reduction in freshwater leading to changes in salinity) and estuarine health (Snow, 2016; Paerl et al., 2010; Blanco et al., 2008).

The use of phytoplankton as bioindicators of water quality has been successful in estuarine systems. In the United States of America, an example is the Virginia Chesapeake Bay monitoring program which monitors Chesapeake Estuary, the largest estuary in the country (Zhang et al., 2021; Wolny et al., 2020; Mulholland et al., 2018; Marshall et al., 2008). In Australia, an example is the Ecosystem Health Monitoring Program (EHMP) monitoring, Moreton Bay, the largest estuarine bay in Australia (Saeck et al., 2019a, Saeck et al., 2019b). In Europe, an example is the Central de Tratamento de Resíduos Sólidos Urbanos, monitoring the Tagus Estuary, one of the largest estuaries in Europe (Brito et al., 2014). For estuaries with decades of data, such as the Chesapeake Bay, phytoplankton monitoring programs have helped the region develop nutrient thresholds for surface waters, leading to an overall improvement of water quality and a decrease in HAB events (Zhang et al., 2021; Wolny et al., 2020; Saeck et al., 2019a; Mulholland et al., 2018; Brito et al., 2014; Marshall et al., 2008). In South Africa, studies such as Lemley and Adams (2020) and Lemley et al. (2017) have also established thresholds for nutrient concentrations and relationship between freshwater input and HAB events in some estuaries.

Phytoplankton diversity

Diversity, defined as early as 1943 as the number of items and their relative frequencies (Fisher et al., 1943), is most often represented in phytoplankton ecology as the number of species in a taxonomic unit (taxonomic richness) (Borics et al., 2020). Phytoplankton diversity is a characteristic that affects the functioning of ecosystems such as resilience and resource use efficiency across considerable environmental gradients from freshwater, to brackish and marine environments (Borics et al., 2020; Ptacnik et al., 2008). Selection or environmental filtering amongst other factors influence taxon richness. Phytoplankton composition and richness are controlled by locally acting environmental filters such as nutrients and abiotic interactions such as competition (Borics et al., 2020; Chalar, 2009). Species of similar preference and tolerance to environmental factors therefore coexist. Maximum diversity is often noted at intermediate levels of phytoplankton biomass and lowest during blooms (Irigoien et al., 2004). Factors reducing the phytoplankton diversity may have a direct negative effect on the predictability and amount of aquatic primary production (Ptacnik et al., 2008). Despite the importance of phytoplankton productivity and the effects of phytoplankton diversity on ecosystem functioning, globally, studies on phytoplankton diversity have not increased due to the dominance of eutrophication studies (Borics et al., 2020), and the patterns of phytoplankton diversity in South African estuaries remains poorly characterised.

Phytoplankton are a very diverse group of plankton. The most common phytoplankton groups are the bacillariophytes, chrysophytes, chlorophytes, cryptophytes, dinophytes, cyanophytes, and xanthophytes (Haris, 1986). Knowledge of the dynamics of diatoms and dinoflagellates is of utmost importance because diatoms and dinoflagellates are the taxonomic groups most often associated with phytoplankton blooms in estuaries (Lemley and Adams, 2020; Lemley et al., 2018; Carstensen and Heiskanen, 2007; Sarthou et al., 2005). Moreover, phytoplankton in estuarine systems are mostly dominated by bacillariophytes (Muñiz et al., 2018; Dalu et al., 2017; Ramdani et al., 2009; Dijkman and Kromkamp, 2006; Huang et al., 2004; Johnson, 1977). In estuaries, diatoms are the most diverse phytoplankton group (Muñiz et al., 2018; Malviya et al., 2016; Armbrust, 2009; Bates and Trainer, 2006), with possibly over 100,000 extant species (Mann and Droop, 1996). They are ubiquitous, very distinctive due to the presence of siliceous cell walls called frustules (Korsunsky et al., 2019; Ramdani et al., 2009; Round et al., 1990) and readily absorb nutrients compared to other phytoplankton groups (Reynolds, 2006; Lomas and Glibert, 2000; Lomas et al., 1999) such as dinoflagellates.

Photosynthetic or autotrophic forms of dinoflagellates are important members of the phytoplankton community, and heterotrophic forms for the zooplankton community (Jeong, 1999). Dinoflagellates are biflagellate and well-known causative agents of red tides and harmful algae blooms (Lemley and Adams, 2020; Ndhlovu et al., 2017; Tango et al., 2005). They are one of the most ecological and economically important phytoplankton groups in aquatic systems (Carty and Parrow, 2015).

Spatial variability and temporal evolution of phytoplankton community composition

Phytoplankton are found in varying aquatic ecosystems from oligohaline (<4) to hypersaline estuaries (>35) with species adapted to different environmental conditions. Species-specific adaptations can be seen in the changes in community composition and species abundance with time and space. An increase in phytoplankton biomass is usually linked to the availability of nutrients (Adams et al., 2020; Lemley et al., 2019; Reynolds, 2006). The availability of nutrients in an estuary depends greatly, but not exclusively, on water inflow from rivers, associated with rainfall, catchment, and land-use (Adams et al., 2020; Nunes et al., 2019; Adams and Bate, 1999). High amounts of nutrients, especially phosphorus and nitrogen in an estuarine system in addition to long water residence time and river flow rate, often lead to blooms and eutrophication (Adams et al., 2020; Lemley and Adams, 2020; Howarth and Marino, 2006; Bricker et al., 2003). Other factors affecting the community structure (taxa composition, abundance, and biomass) of phytoplankton in estuaries include light, turbidity, salinity, temperature (Wang et al., 2019; O'Boyle and Silke, 2010; Reynolds, 2006; Haris, 1986) and grazing (Lürling, 2020; Kibirige and Perissinotto, 2003). These conditions vary on spatial and temporal scales, leading to the existence of spatio-temporal variations in phytoplankton diversity and community structure in estuaries.

Phytoplankton being photosynthetic needs solar energy in the form of light for photosynthesis (Charalampous et al., 2018; Mitra et al., 2004). Turbidity strongly influences light availability to phytoplankton cells by interfering with the penetration of light through the water column. Flocculation when freshwater meets saline water can cause additional estuarine turbidity (Horemans et al., 2020). Also, the inflow of water from rivers causes turbulence, bringing in new sediment and particulate matter by the re-suspension of already existing particulate matter, thus increasing turbidity.

With an increase in the salinity gradient, a higher plankton diversity is accommodated because different phytoplankton and protozoan species are adapted to different salinity regimes (Adams and Bate, 1999; Olli et al., 2019). Certain phytoplankton species are euryhaline, from marine (salinity 35) to freshwater conditions (salinity <4). However, some are adapted only to marine conditions, some exclusively brackish (salinity of 5-20) or adapted only to freshwater conditions (Reynolds, 2006). This amongst other factors causes spatial variations in the phytoplankton community structure in estuaries.

Temporally, the occurrence of seasonal events may further complicate the spatial ecosystem structure and variability of phytoplankton communities, as in tropical and subtropical estuarine systems where temporal changes are highly influenced by precipitation [e.g., Port Basin Estuary, Brazil (Santiago et al., 2010), Bay of Bengal (Sahu et al., 2014) and St. Lucia, South Africa (Nche-Fambo et al., 2015)]. Temporal patterns have been associated with factors such as temperature, salinity, nutrient concentration, and turbidity. For example, in predominantly open estuaries along the subtropical coastline of South Africa, salinity is lower in summer while nutrient concentration and turbidity increase in summer with an increase in rainfall (Harrison, 2004). Spatio-temporal changes in the phytoplankton community are therefore expected as communities adapt to the changing environment. Temporal variability always exceeds overall spatial variability in plankton community structure (composition and abundance) in estuaries (Klais et al., 2015). These temporal variations in the occurrence of plankton communities in South Africa are usually expressed as monthly (e.g., Nche-Fambo et al., 2015; Gama et al., 2005) or seasonal (e.g., Dalu et al., 2018; Dalu et al., 2014; Bate and Smailes, 2008) variations. However, phytoplankton growth rates vary from a few doublings per day to one doubling per week or ten days (Haris, 1986) and their life span varies on a time scale of hours to a few days (Karl and Marra, 2012; Marra, 2002). Due to this fast growth rate and the temporal changes in the estuarine environment, in situ field studies of phytoplankton community structure (diversity, abundance, and biomass) requires more frequent sampling (Lemley et al., 2016) rather than the conventional seasonal or monthly sampling applied in most studies investigating the phytoplankton in South African estuaries.

Temporal and spatial patterns in community structure provide a means of evaluating ecological changes in ecosystems in response to pressures that are detrimental to the ecosystem such as nutrient loading (Nunes et al., 2019; Liu, 2008). However, the determination of patterns in phytoplankton community structure even at the group level (e.g., diatoms, dinoflagellates) can only be possible with knowledge of the species present (taxonomy), their distribution, and abundance. Knowledge of the species present, their distribution, and abundance is limited in South African estuaries, especially estuaries in the subtropical region.

In other biogeographic regions where more studies on the phytoplankton community structure in estuaries have been reported, the severity of degradation of the estuary identified, indicators of eutrophic water quality conditions noted, and strategies have taken to ameliorate the situation (Harding et al., 2019, 2016). Such strategies include mouth breaching (Lemley and Adams, 2020; Harding et al., 2019, 2016). In the subtropical region of South Africa, key phytoplankton species have been associated with hypersalinity (Carrasco and Perissinotto, 2012; Muir and Perissinotto, 2011). It is important to determine reference conditions (phytoplankton community) and identify key indicator species.

Ciliates

Ciliates, commonly characterised by the presence of cilia (Lynn, 2010), are protozoans that occur in aquatic systems (Agatha, 2011) and dominate the microzooplankton abundance in coastal water bodies (Kanayama et al., 2020; Wasserman et al., 2013; Ojaveer et al., 2010; Kibirige et al., 2002). Ciliates graze directly on bacteria, phytoplankton such as diatoms and dinoflagellates, and other large zooplankton. Hence, ciliates play an important role in the pelagic carbon flow and are ecologically crucial in transferring energy to higher trophic levels (Kanayama et al., 2020; Jiang et al., 2013). As primary consumers of phytoplankton, ciliates control phytoplankton community structure in aquatic systems through top-down pressure on phytoplankton. Thus, ciliates can have a significant positive relationship with phytoplankton biomass (Noman et al., 2019; Nche-Fambo et al., 2016).

Different sites in an estuarine system may have unique species defined by environmental conditions (Munawar et al., 2020; Xu and Soininen, 2019; Pfister et al., 2002). Along the estuary, the highest diversity and abundance of ciliates usually occur in the mesohaline regions where there is a mixture of marine and freshwater species (Pfister et al., 2002). The least diverse and least abundant ciliate community is found towards high salinities of >24 as few species such as *Euplotes* sp. are adapted to osmotic stressors (Li et al., 2019; Urrutxurtu et al., 2003; Pfister et al., 2002).

Ciliates and water quality

Characteristics of ciliates include very high metabolic rates, short generation times, broad geographic distribution, and rapid response to changing environmental factors. Because of these characteristics, ciliates are a valuable tool for indicating hydrological conditions and trophic status, and some species are used as water quality bioindicators (Basuri et al., 2020; Polat et al., 2019; Sivasankar et al., 2018; Grinienė et al., 2011; Pfister et al., 2002). For example, in oligotrophic regions, tintinnids are dominant and are the main consumers of primary production, greatly influencing the planktonic assemblage (Noman et al., 2019). The main attributes of ciliates used for water quality monitoring are feeding habits (bactivore, carnivores, omnivores, and algivores), species composition, and abundance (Dias et al., 2021; Segovia et al., 2016). Increase in eutrophication leads to increase in bacterial density which has a direct effect on ciliates (Dias et al., 2021; Sivasankar et al., 2018; Segovia et al., 2016). Communities dominated by bacterivorous ciliates is an indication of poor water quality while communities dominated by algivorous ciliates are indicators of good water quality (Dias et al., 2021; Sivasankar et al., 2018; Segovia et al., 2016). Loricate ciliates are dominant in areas with good water quality, however, some *Tintinnopsis* species such as, *T. beroidea*, *T. brasiliensis*, and *T. tubulosoides*. can be used as indicators for poor water quality due to their significant correlation with nutrients (Feng et al., 2015). In South Africa, the dominance of *Fabrea Salina*, in the St. Lucia estuarine lake system was an indicator of its hypersaline nature (Nche-Fambo et al., 2016). However, other ciliate studies in South Africa have not reported ciliate compositions in relation to abundance or biomass, making it difficult to suggest what ciliate taxa can expect to be used as bioindicators in South African estuaries.

Generally, high densities of ciliates are found in areas with high organic content due to the presence of high bacteria concentration and areas of high chl *a*, due to a high concentration of phytoplankton. Ciliate abundance is highest in eutrophic parts of a system than non-eutrophic parts. The ciliate abundance representing poor water quality is therefore depending on the system in question. For example, in the Kochi estuary, ciliate abundance of 1.25×10^2 Cells/L was associated to good water quality while an abundance of 9.46×10^2 Cells/L was associated to eutrophic condition (Sivasankar et al., 2018). In the Pirapó River Bazin, abundance representing eutrophic conditions ranged from 1.28×10^2 Cells/L to 4.67×10^3 Cells/L (Sergovia et al., 2016). Ciliate attributes can therefore be used as an alternative bioindicator to phytoplankton and to describe a healthy trophic structure. Because bacteria and phytoplankton are the main food sources for ciliates, it is important that in addition to phytoplankton, ciliates are included in the preliminary study of an aquatic system to understand the community structure, reference conditions and indicator species in relation to water quality. The use of ciliates as bioindicators of water quality has been successful in lotic systems (Dias et al., 2021; Segovia et al., 2016), marine systems (Guo and Xu, 2019; Pereira et al., 2018; Wu et al., 2016) and estuarine systems (Sivasankar et al., 2018; Kim et al., 2012; Jiang et al., 2011). For example, *Tintinnopsis beroidea* and *Mesodinium pupula* are a potential bioindicators of eutrophic conditions or poor water quality, dominating in areas with high nutrient concentration (DIN of 22 µM and DIP of 2.64) associated with large amounts of anthropogenic inputs (Sivasankar et al., 2018; Jiang et al., 2011). Furthermore, the association of these species to high chl *a* concentration of eutrophic levels indicates that they can be used in place of phytoplankton as bioindicators.

Phytoplankton and ciliate community structure in South African estuaries

A synthesis of microalgae studies in South African estuaries shows a lack of thorough assessments of microalgae communities and adequate sampling periods or intensity (Lemley et al., 2016). Currently, of the nine comprehensive studies (to phytoplankton species level) that have investigated the effects of environmental variables on the phytoplankton in South Africa, three have been limited to estuarine systems already experiencing pressures from anthropogenic factors (See Table 1.1). These anthropogenic factors are hypersalinity in St. Lucia Lake (Nche-Fambo et al., 2015; Bate and Smailes, 2008) and eutrophication in the Sundays Estuary (Lemley et al., 2018, 2016; Kotsedi et al., 2012) and Gamtoos Estuary (Lemley and Adams, 2020). Three of such studies exist for estuaries of good water quality conducted in the Kwesani, Cunge, Mtwendwe, Mtendwe, Kowie, and Maitland estuaries in the warm temperate region (Dalu et al., 2018, 2014; Gama et al., 2005). There exists very little knowledge on estuaries in the subtropical region with good ecological health status such as the Mlalazi Estuary.

Studies on the estuarine ciliates in South Africa are limited to the St. Lucia Estuary (Nche-Fambo et al., 2016; Carrasco and Perissinotto, 2012), Mpenjati Estuary (Kibirige et al., 2002), Kasouga Estuary (Wasserman et al., 2013) and the Kariega Estuary (Froneman and McQuaid, 1997). There is, therefore, limited knowledge on the ciliate community structure and its variability in South African estuaries and the question of whether ciliates can be used as an alternative bioindicator to changing water quality has not been addressed.

Purpose of the research

Despite the importance of phytoplankton and ciliates and the onward degradation and vulnerability of estuaries on the South African subtropical coast due to human influences, very little is known of the microplankton community structure and variability in the estuaries of good ecological status. Such knowledge can be used as a comparison for future conditions and as a tool in projects aimed at reducing the severity of harmful effects of anthropogenic pressures on estuaries.

Research aim and objectives

The overall aim is to assess the variability of the microplankton community structure in subtropical estuaries of good ecological status through the biweekly investigation of the phytoplankton and pelagic ciliates to develop a baseline data set, outline the thresholds of potential concern and identify key water quality bioindicator species.

Specific Objectives

In this study, different microplankton attributes (phytoplankton species, phytoplankton abundance, phytoplankton biomass (chlorophyll a), ciliate abundance, ciliate biomass and ciliate feeding habit) were studied in relation to some core water quality parameters (total oxidized nitrogen, dissolved inorganic phosphorus, dissolved oxygen, turbidity, salinity, and temperature) to achieve the above aim. The following objectives will be used:

1 Characterise the diversity and spatio-temporal phytoplankton community structure (composition, abundance, and biomass) in predominantly open estuaries of good to fair water quality and evaluate the influence of the physico-chemical environment on the phytoplankton community.

2 Determine the diatom community structure in estuaries with a good to fair water quality, assess the biweekly-seasonal patterns and their driving factors.

3 Examine the temporal evolution of the dinoflagellate community composition and the factors influencing the occurrence of potentially harmful dinoflagellate species in the Mlalazi Estuary.

4 Investigate the temporal succession of the ciliate assemblage, functional groups, and the physico-chemical variables influencing the ciliate community structure in the Mlalazi Estuary.

Thesis structure.

The thesis consists of six chapters. Chapter 1 introduces the microplankton (phytoplankton and ciliate) research, past phytoplankton and ciliate community structure studies in South African estuaries and highlights its importance. Chapter 2 describes the overall phytoplankton species composition, examines the spatial and temporal variability of the phytoplankton groups, and discusses their ecological characteristics and relationship between the phytoplankton assemblage and environmental factors in an estuary of good to fair water quality status. Chapters 3 and chapter 4 focus on the diversity of the diatoms and dinoflagellates respectively in an estuary with a good to fair water quality, highlighting in detail the spatio-temporal dynamics of these two important phytoplankton groups. Chapter 5 reports on the temporal evolution of the ciliate community structure and functional groups in an estuary of good ecological health status and discusses the driving physico-chemical factors. Chapter 6 summarises the main findings of the research and suggestions for future research.

Chapter 2. SPATIAL VARIABILITY SHAPES SEASONAL RESPONSES OF PHYTOPLANKTON COMMUNITIES IN SUBTROPICAL ESTUARIES

Abstract

Phytoplankton community structure studies are of great importance in the determination of ecological and reference conditions for possible future changes in aquatic environments. This research examined the variable responses of the phytoplankton community structure (species composition, abundance, and biomass) in space and time of an oligo– to mesotrophic predominantly open, subtropical estuary over a one-year period. Ninety-seven phytoplankton genera (68 diatoms, 21 dinoflagellates, three chlorophytes, three cyanobacteria, one cryptophyte, and one euglenophyte) were identified. The total abundance per site which ranged from 9.81 x 10^3 to 1.93 x 10^6 Cells/L, increased from the lower to the upper reaches and was highest in winter and lowest in spring. Salinity influenced the spatial distribution of taxa while temperature and nutrient concentrations influenced the temporal variability. Diatoms and dinoflagellates dominated in the saline lower reaches, with the former more abundant in summer under higher dissolved inorganic phosphorus (DIP) concentration conditions and the latter dominating in winter when nutrient concentrations were low. Chlorophytes and cryptophytes dominated in the less saline upper reaches. Chlorophytes dominated in summer under high temperatures, and cryptophytes were the most abundant group in the estuary, especially in spring. The period of dominance by a phytoplankton group ranged from two weeks to three months (a season). Given the low phytoplankton abundance and biomass, high richness, low nutrient concentrations, and lack of substantial anthropogenic pressures on the estuary, the phytoplankton community structure of this estuary provide a reference point for possible future changes in the water quality of this system as well as similar estuaries globally.

Key Words: Cryptophytes, salinity, water quality, Mlalazi Estuary, South Africa.

2.1. Introduction

Phytoplankton play a central role in ecosystems as a link between inorganic dissolved nutrients and upper trophic levels (Kanayama et al., 2020; Wirtz and Smith, 2020; Miller et al., 1996). They are important in understanding biogeochemical processes, ecosystem metabolism and production systems in food webs supporting fisheries (Cloern and Jassby, 2010). The seasonal changes in variables such as temperature, freshwater input, salinity and nutrient concentration influence the dynamics of phytoplankton communities within estuarine environments (Jendyk et al., 2014; Cloern and Jassby, 2010). Seasonal patterns in phytoplankton composition are more common in temperate estuaries that experience marked seasonal differences of cold, dark winters and warm summers with longer daylight hours (Adolf et al., 2006; D'Alcala et al., 2004; Turner et al., 1983). Although tropical regions have defined wet and dry seasons characterised by heavy rainfall and little to no rainfall respectively, they do not experience extreme seasonal temperature variability (Harrison, 2004; Taylor and Tulloch, 1985). Several studies have found no obvious seasonal trend in phytoplankton community composition in tropical estuaries (Fonge et al., 2013; Álvarez-Góngora and Herrera-Silveira, 2006). For example, in the coast of the Yucatan Peninsula within the Gulf of Mexico (Álvarez-Góngora and Herrera-Silveira, 2006) and in the Douala Estuary in Cameroon, (Fonge et al., 2013), a shift in the dominant phytoplankton group with change in seasons from wet to dry could not be detected.

However, the physical and hydrographic properties of estuaries lead to complex and contrasting phytoplankton dynamics amongst these systems (Cloern and Jassby, 2010; Cloern, 1996) and the presence of a global seasonal trend in phytoplankton community composition has been questioned (Duarte et al., 2009; Cebrián and Valiela, 1999). Moreover, site-specific factors can play an important role in driving phytoplankton dynamics in estuaries (Cloern and Jassby, 2010).

The South African coastline has nearly 300 estuaries ranging from sub-tropical to temperate and the few published estuarine phytoplankton community ecology studies have focused on anthropogenically highly modified estuaries, some of which are facing extreme conditions such as eutrophication (e.g., the Sundays

Estuary [Lemley et al., 2018; Kotsedi et al., 2012] and Gamtoos Estuary (Lemley and Adams, 2020)), or hypersalinity (St. Lucia Estuary [Nche-Fambo et al., 2015]). Studies conducted in estuaries with a typical salinity gradient (0.5 to 35) and low anthropogenic pressure (e.g., Kwesani, Cunge, Mtwendwe and Mtendwe [Dalu et al., 2018]; Kowie river-estuarine system [Dalu et al., 2014] and Van Stadens and Maitland estuaries [Gama et al., 2005]) have been limited to warm temperate estuaries in South Africa's Eastern Cape Province.

Phytoplankton community ecology studies in oligotrophic estuaries are scarce (Muniz et al., 2018) despite the fact that these systems are suitable to serve as reference conditions of community structure and their ecology under near natural conditions. This investigation was conducted on the Mlalazi Estuary, South Africa, located within the uMlalazi Nature Reserve area and under protection by the Ezemvelo KwaZulu-Natal (EKZN) Wildlife conservation authority. This estuary has a good water quality status (Van Niekerk and Turpie, 2012) and an overall health score of 80% considering factors such as water quality and biodiversity amongst others (see Department of Water and Sanitation [DWS], 2015). The present knowledge on the phytoplankton community composition of the Mlalazi Estuary is from a single summer sample in 2013 (see DWS, 2015) and previous studies on phytoplankton biomass proxied by chl *a* concentration in this estuary show contradictory seasonal trends. In 2010, biomass was lower in spring (November) than in autumn (May) (Ortega-Cisneros and Scharler, 2014), whereas in 2011, the trend was reversed (Vezi, 2013). The aim of this study is to characterise the phytoplankton community structure (composition, richness, abundance and biomass) in this estuary by answering the following questions, 1) are the patterns in phytoplankton community structure consistent with space and time? 2) What are the environmental parameters driving the observed phytoplankton community structure.

2.2. Materials and methods

2.2.1. Study area

The Mlalazi Estuary (31°49'22.897"E; 28°56'40.995"S; Fig. 2.1) is subtropical, and predominantly open estuary, closing only during major droughts, with a catchment area of 454 km^2, estuarine area of 95.86 ha and a depth of 1–2.5 m (Van Neikert et al., 2020; DWS, 2015; Van Niekerk and Turpie, 2012; Cyrus, 1988). The estuary receives agricultural and aquaculture runoffs from sugar cane plantations and a saltwater Tilapia respectively as a source of allochthonous nutrients (Mabaso, 2002). According to Resource Directed Measures for protection of water resources, DWA (2008), for estuarine <30 miles long, samples should be collected from at least four station; the upper, middle, lower reach and a site upstream of the furthest point of tidal exchange. Also, these sites should where possible, be sites that have been used for previous monitoring and/or research in order to ensure consistence and comparability with historical data (Cilliers and Adams, 2016). For this study, three sites which have been used for previous research (e.g., Ortega-Cisneros and Scharler, 2014; Ortega-Cisneros, 2013; Vezi, 2014) were chosen. The sites were in the upper, middle, and lower reaches of the estuary. The freshwater region was not sampled. However, estimated reference condition of salinity at the freshwater region is 2 and salinity at this region in winter of 2013 was 6 (DWS, 2015). At the furthest point of tidal reach at the head of the estuary, salinity ranging from 5 to 20 have been noted (Scharler unpub data).

The Sampling was conducted biweekly (once every two weeks) from the 6th of July 2014 to the 23rd of June 2015. Sampling dates were chosen to coincide with neap tides, in order to minimise tidal influence. However, due to weather conditions and unforeseen circumstances such as the boats' engine breaking down, there were more sampling dates (7) in autumn than the rest of the seasons which had 5 sampling dates each. To ensure that the uneven sampling did not introduce any bias to the result, the means between an even sampling period of five dates in autumn (without the two extra autumn dates, i.e., 16th of March and 14th of May 2015) were compared with the means from the uneven sampling (i.e., all seven dates) of the same season. Ideally, sampling started at the beginning of each season (beginning of the first month in that season), the last two dates in autumn (14th of May and 29th of May 2015) would ideally be the extra dates. However, this would exclude an entire month (May of 2015). Therefore, one date in May and a date in March which had three sampling sessions was chosen as the extra dates. These dates were chosen so that all months in the season be represented. The means were compared to ensure that the high abundance or richness reported for autumn was not due to the extra sampling effort.

Data from all sampling sessions were included in the plots and data analysis. Since sampling was conducted over one year, the data are interpreted over the temporal scale without drawing conclusions on seasonal uniqueness. The estuary mouth was open throughout this study period.

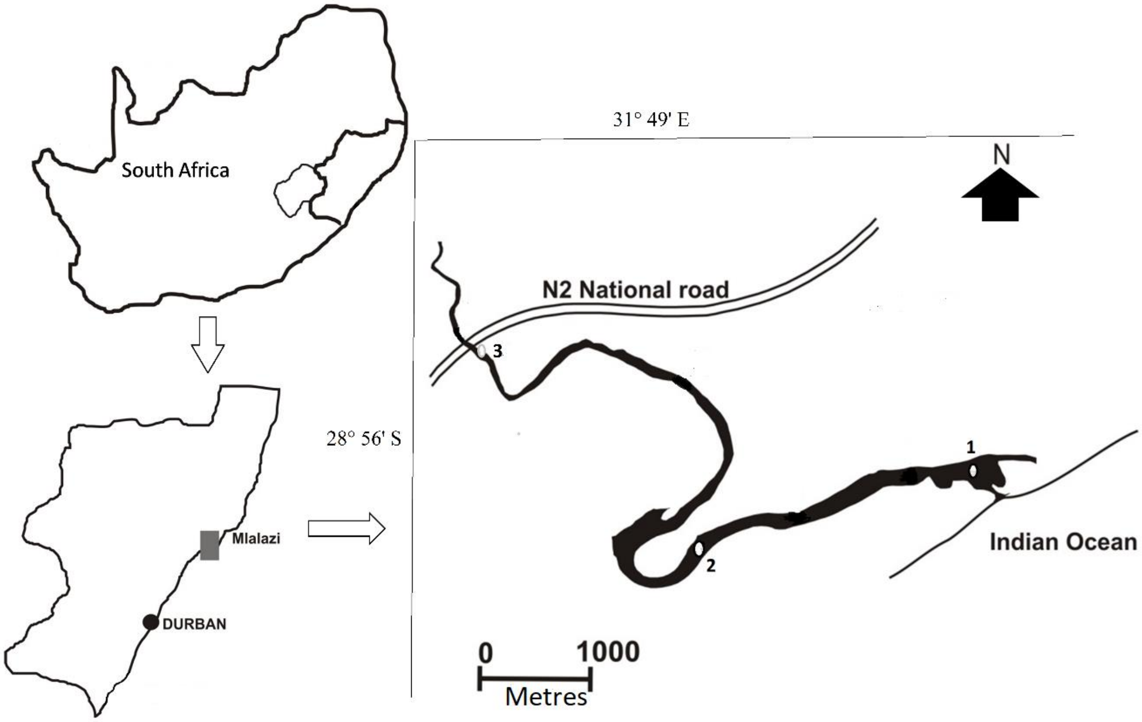


Figure 2. 1: Location of the Mlalazi Estuary and sampling sites, 1 (lower reaches), 2 (middle reaches) and 3 (upper reaches).

2.2.2. Physico-chemical measurements

The environmental variables salinity, temperature (ºC), dissolved oxygen (DO) concentration and turbidity (Nephelometric Turbidity Unit, NTU) were measured using a YSI 6920 multiprobe system. Duplicate subsurface (0.5 m) water samples were collected in one-litre acid-washed polyethylene bottles for the determination of phytoplankton biomass (using chlorophyll *a* [chl *a*] concentration as a proxy), total oxidised nitrogen (TON) and dissolved inorganic phosphorus (DIP) concentrations and the means reported. For total chl *a* determination, known volumes of the sample were filtered through a Whatman glass fibre filter (GF/F) of pore size 0.7 µm and 47 mm diameter, and extracted for 48 hrs in 90% acetone at -20 °C in the dark. Chl *a* concentration was measured with a Turner Trilogy laboratory fluorometer using the acidification method (Holm-Hansen and Riemann, 1978). For the determination of TON (nitrite + nitrate) and DIP (orthophosphate) concentrations, 100 ml of the water sample was filtered through a Whatman GF/F and filtrate stored at < -20 °C until analysed at the Analytic Laboratory of CSIR (Durban, KwaZulu-Natal) with a Scalar San ++ continuous flow analyser using the Mostert (1983) method. Due to equipment failure, ammonia was not analysed. Rainfall data were obtained from the Department of Water and Sanitation, South Africa, for station number W1E011 at the Eshowe station. This station was not within the catchment, however, it was the closest station to the catchment and study area with available reliable rainfall data.

2.2.3. Phytoplankton community structure

Resource directed measures for water protection in South Africa (DWA, 2008) specifies that phytoplankton samples be collected at 0.5 m depth intervals. Therefore, for the Mlalazi Estuary of depth 1-2.5 m should have at least two vertical profiles. However, due to the time-consuming nature of microscopic enumeration of phytoplankton communities, only samples from the surface (0.5 m) were considered for this study. Duplicate water samples were collected in 250 ml acid-washed polyethylene bottles at subsurface (0.5 m) water level, fixed immediately with 2% acid Lugol’s solution, then stored in the dark. For diatom identification and enumeration, 20-100 ml samples were settled depending on the cell density and the amount of detritus and sediment in the sample, using the Utermöhl method (Utermöhl, 1958) and viewed under a Nikon *ECLIPSE Ti Series* inverted microscope fitted with a DS-US camera powered by NIS-Elements BR software at magnifications of 400x to 1000x. To confirm the identification, diatom cells were digested using the acidification method by Taylor et al. (2007b) and prepared for scanning electron microscopy (SEM) according to Tillmann and Hoppenrath (2013). Taxa were identified to the lowest taxonomic level possible with the aid of relevant literature (Hallegraeff et al., 2010; Pienaar et al., 2007; Taylor et al., 2007a; Lange-Bertalot, 2000; Tomas, 1997; Round et al., 1990). All taxa were further checked on the World Register of Marine Science website for current terminology. See Appendix 2,3 and 4 for phytoplankton SEM and light micrographs. Transects or fields of view were counted for abundant and small (<5 µm of length) taxa. The whole chamber was counted for larger (>5 µm) cells. Filamentous cyanobacteria were counted as 100 µm units and individual cells were counted for colonial cyanobacteria (Helsinki Commission, 2006). Abundance was calculated as follows:

$$Abundance\ (Cells/L) = \frac{A\ (mm^2) * 1000}{a\ (mm^2) * V\ (ml)} * n$$

Here, *n* is the number of cells counted, *A* is the area of the whole chamber, *a* is the area counted, and *V* is the volume settled. Dominant taxa were defined as taxa representing ≥10% of the phytoplankton abundance in a sample. Taxon richness was used as a measure of diversity (Borics et al., 2020).

2.2.4. Data analysis.

The spatial and temporal variability in the phytoplankton community composition, based on the abundance of phytoplankton genera was determined using a Permutational Multivariate Analysis of Variance (PERMANOVA) with a two-way design. The abundance was first fourth root transformed, to reduce the effect of the dominant genera. A Bray-Curtis similarity index was used to quantify the difference in community structure between samples. The spatio-temporal variability in taxon richness was analysed using a Two-way Analysis of Variance (ANOVA). To meet the assumptions of the ANOVA, a Shapiro-Wilk normality test was used to test for normality of the residuals and a Levene's test for equality of variance.

To explore the temporal variability in the phytoplankton community structure (Objective 2), the coefficient of variation (CV), defined as the ratio of the standard deviation to the mean (CV = SD/$\bar{x}$), was used. A high CV represents high variation. The CV was also calculated for physico-chemical variables for each season.

The relationship between the measured physico-chemical variables (turbidity, salinity, temperature, DO concentration, rainfall, TON and DIP concentration) and abundance of the phytoplankton groups was analysed with a Canonical Correspondence Analysis (CCA). A Spearman's Rank correlation was conducted between chl *a* and the physico-chemical variables and between taxon richness and physico-chemical variables to assess any relationships.

The relationship between freshwater input (rainfall and river flow), water quality parameters (TON, DO and DIP concentrations, temperature, turbidity, and salinity), and phytoplankton attributes (chl *a* concentration, diatoms, dinoflagellate, chlorophytes, cyanobacteria, cryptophyte, and euglenophyte abundance) were investigated using Spearman's rank correlations. Spearman's rank correlation uses the rank values between variables to assess their relationship. Phytoplankton attributes include the abundance of important taxa such as *Prorocentrum triestinum*, the most abundant dinoflagellate species. Note that chlorophytes and cryptophytes are made up of single genera *Chlamydomonas* and *Cryptomonas* respectively. *Prorocentrum triestinum*, *Chlamydomonas*, and *Cryptomonas* were included because they were the most dominant taxa during near bloom events (abundance 1.0 x 10^6 Cells/L).

The ANOVA, Shapiro-Wilk normality test, Levene's test and Spearman's Rank correlation were conducted in the software R (R Core Team, 2013), the PERMANOVA in Plymouth Routines In Multivariate Ecological Research (PRIMER) 6 (Clarke and Gorley, 2005), and the CCA in PAST (Hammer et al., 2001).

2.3. Results

2.3.1. Physico-chemical environment

Expectedly, temperature and salinity peaked in summer, and they were lowest in winter and autumn, respectively. Temperature generally increased from the lower to the upper reaches and ranged from 15.85 to 31.74 °C (Fig. 2. 2). Salinity and dissolved oxygen (DO) concentration decreased from the lower to the upper reaches and ranged from 6.33 to 35.53 and 9.06 to 2.36 mg/L, respectively (Fig. 2. 3 and Fig. 2. 4). Both the highest and lowest DO concentrations were recorded in winter. Turbidity was generally highest in the middle reaches and lowest at the lower reaches (Fig. 2. 5). During the course of the year, turbidity was higher during summer compared to winter, with two peaks, coinciding with the sharp drops in salinity, apparent in autumn (March and April).

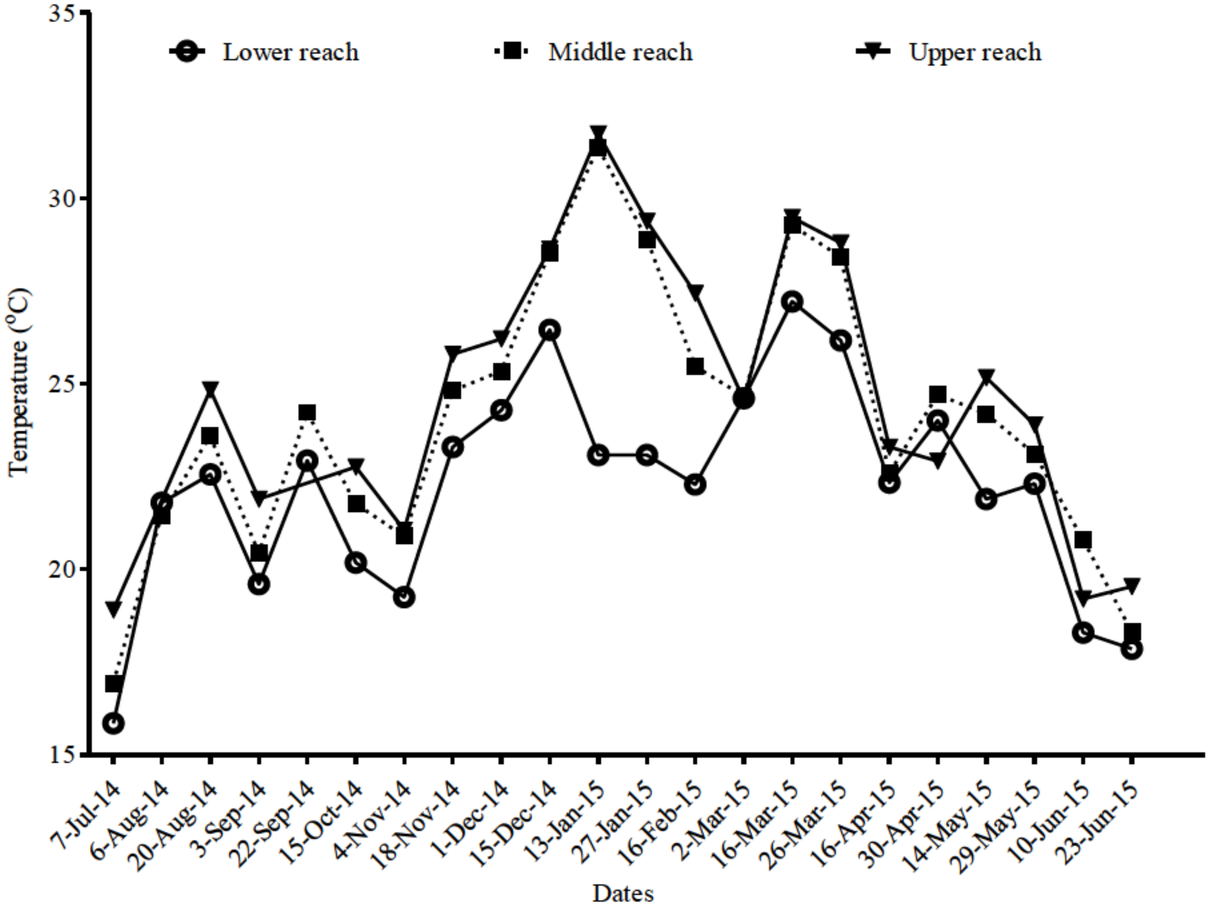


Figure 2. 2: Biweekly measurements of temperature (°C) from July 2014 to June 2015 at all reaches of the Mlalazi Estuary.

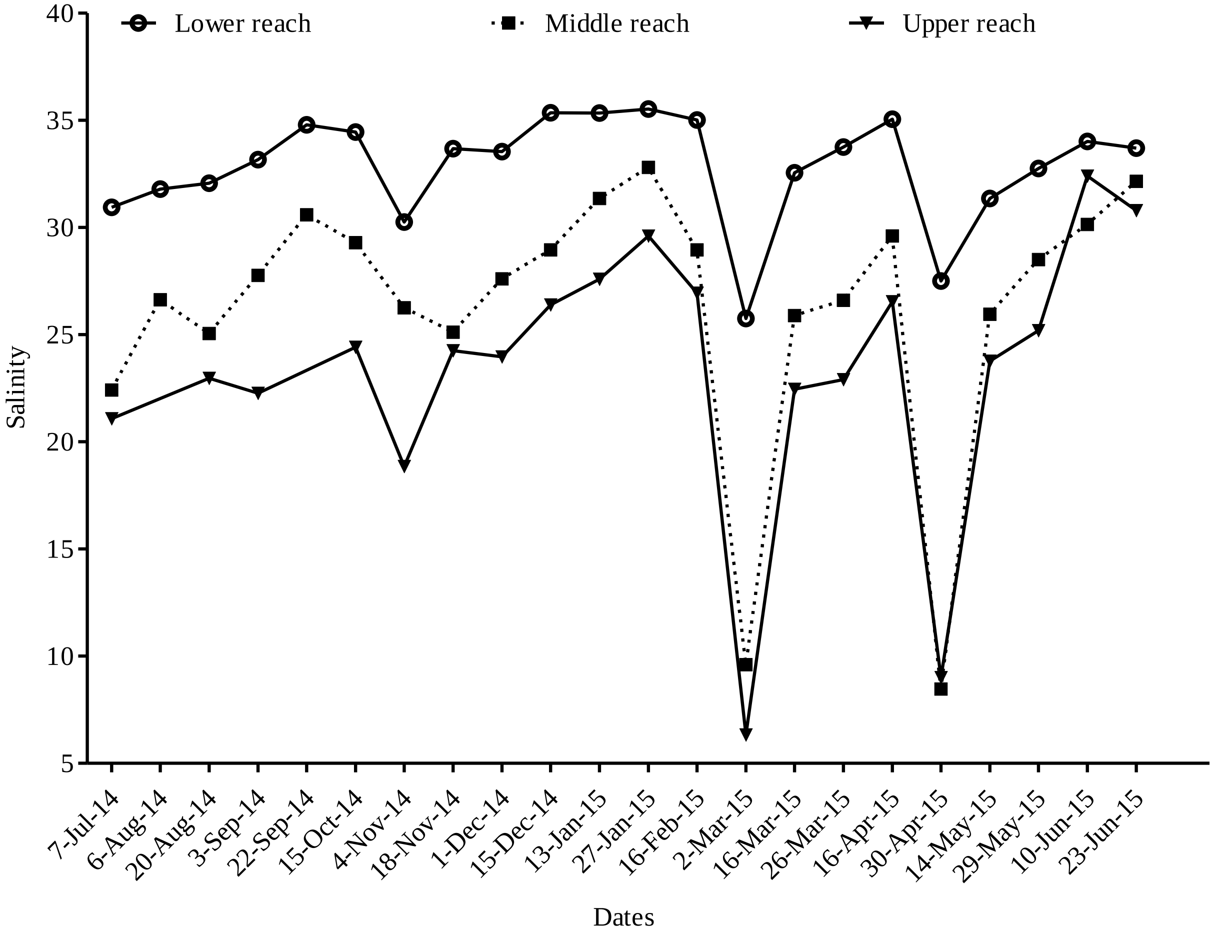


Figure 2. 3: Biweekly measurements of salinity from July 2014 to June 2015 at all reaches of the Mlalazi Estuary.

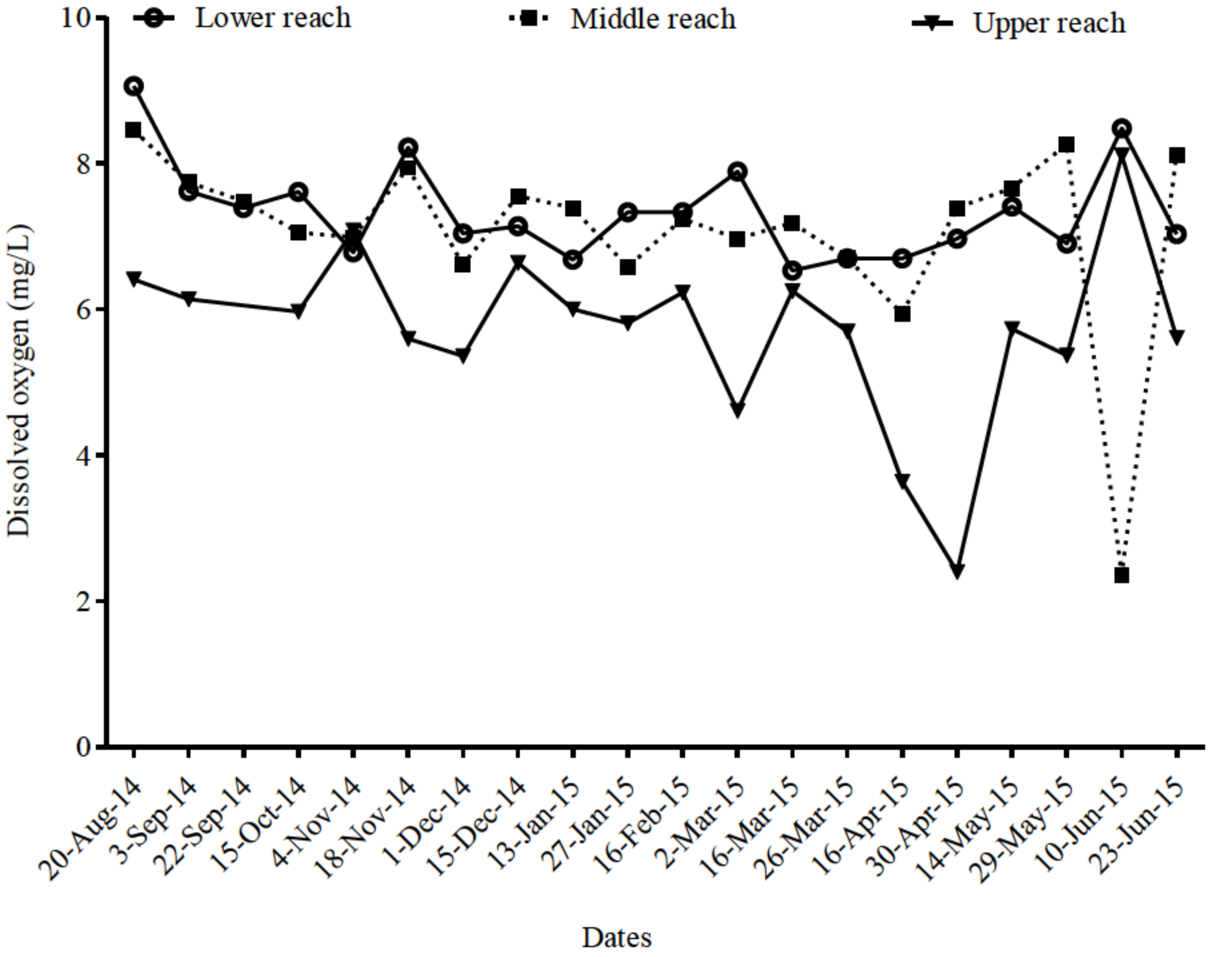


Figure 2. 4: Biweekly measurements of dissolved oxygen concentration (mg/L) from July 2014 to June 2015 at all reaches of the Mlalazi Estuary.

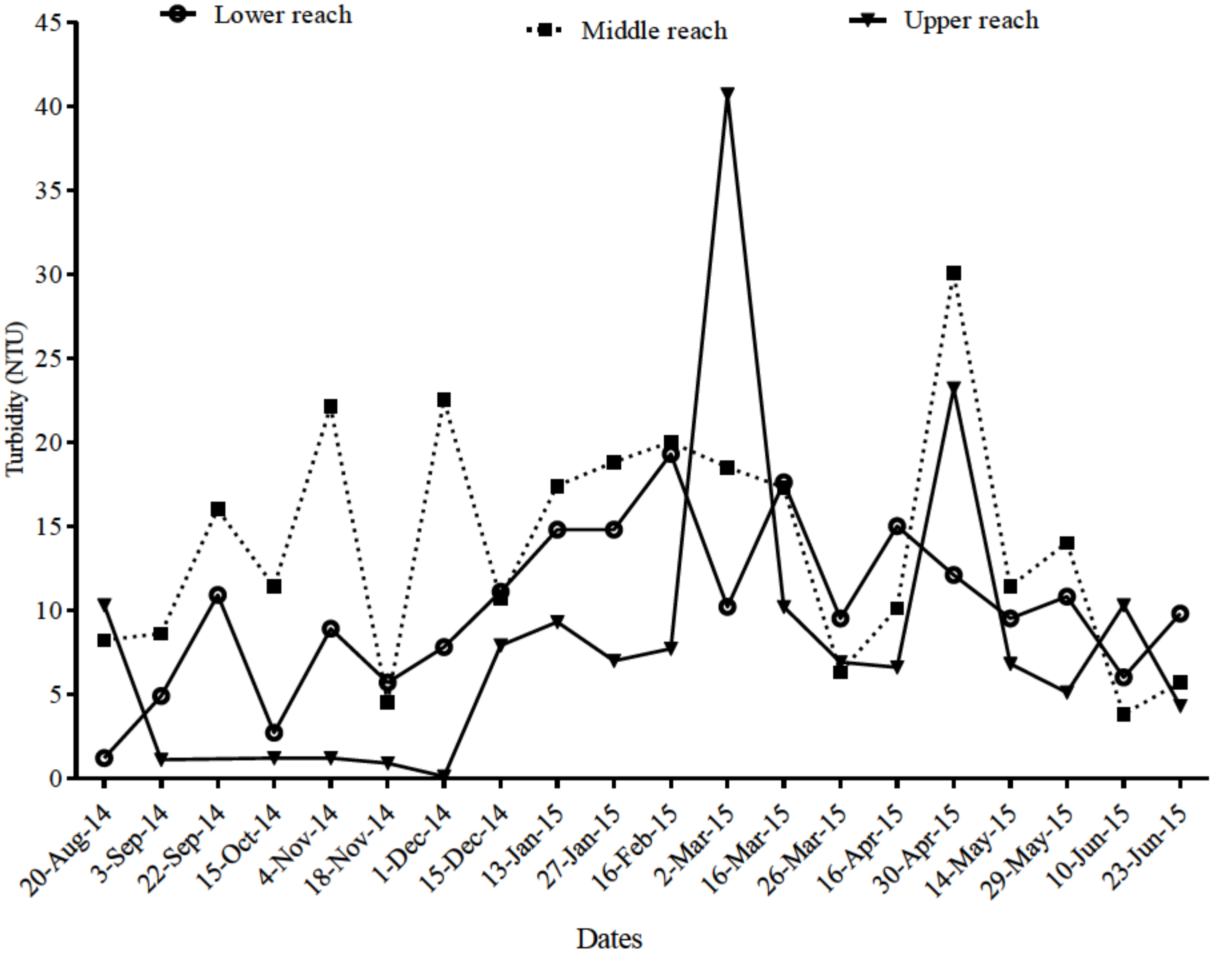


Figure 2. 5: Biweekly measurements of turbidity (NTU) from July 2014 to June 2015 at all reaches of the Mlalazi Estuary.

TON concentrations were otherwise generally low, increased from the lower to the upper reaches, and were highest in autumn, associated with two peak flows (March and April), especially at the upper reaches (Fig. 2. 6). Mean DIP concentration was highest at the lower reaches due to the single peak in summer, lowest at the middle reaches, and generally higher in summer with a peak in January (Fig. 2. 7). However, DIP concentration in the upper reaches was higher for most of the sampling sessions. Mean rainfall per season in the region was highest in winter due to a peak in July and lowest in spring (Fig. 2. 8). However, using salinity, a known rainfall/freshwater dependent in estuaries as a proxy for rainfall, the peaks occurred in early autumn (March) and autumn (April). Phytoplankton chl *a* concentration increased from the lower (0.82 to 8.18 µg/L) to the upper reaches (0.3 to 16.04 µg/L), and was generally highest in the spring months, and lowest in the autumn months (Fig. 2. 9). In autumn, the two extra sampling sessions led to an increase of 0 6 µg/L chl *a* concentration at the lower reaches, 0 4 µg/L at the middle reaches, and 0 5 µg/L at the upper reaches in autumn. However, this did not influence the overall pattern observed and was thus included in the plots and data analysis. Eutrophic conditions (> 20 µg/L chl *a* concentration) occurred at the middle reaches in spring (October and November) (Fig. 2. 9) but were not associated with any phytoplankton blooms.

The relationship between chl *a* and phytoplankton abundance especially when chl *a* was in the eutrophic range showed that an increase in chl *a* was not associated with an increase in total phytoplankton abundance, neither was it associated to a specific phytoplankton taxa. For example, in Autumn, when total abundance reached bloom concentrations (> 1.0 x 10^6 Cells/L), chl *a* concentration was 4 µg/L and cryptophytes represented 80% of the abundance. Also, in Summer, when total abundance reached bloom concentrations, chl *a* concentration was 9 µg/L and chlorophytes represented 80% of the abundance. In Spring, when chl *a* concentration reached bloom conditions (20 µg/L), total phytoplankton abundance was <1.0 x 10^6 Cells/L and cryptophytes dominated in abundance (see Fig 2.9 and Fig. 2.11)

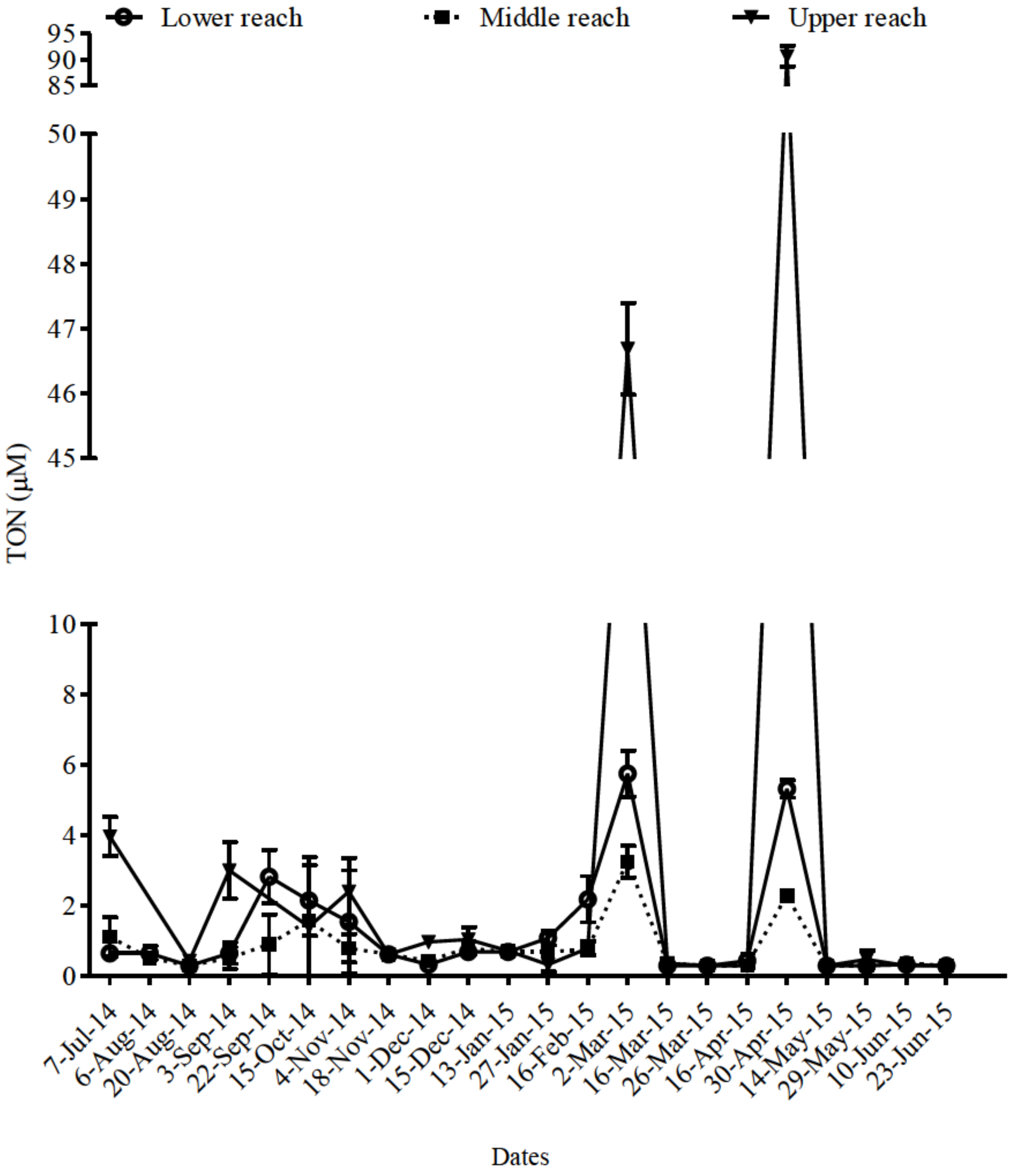


Figure 2. 6: Mean (± SD) biweekly measurements of TON (µM N in $NO_3^- + NO_2^-$) concentrations along the Mlalazi Estuary (3 reaches) from July 2014 to June 2015.

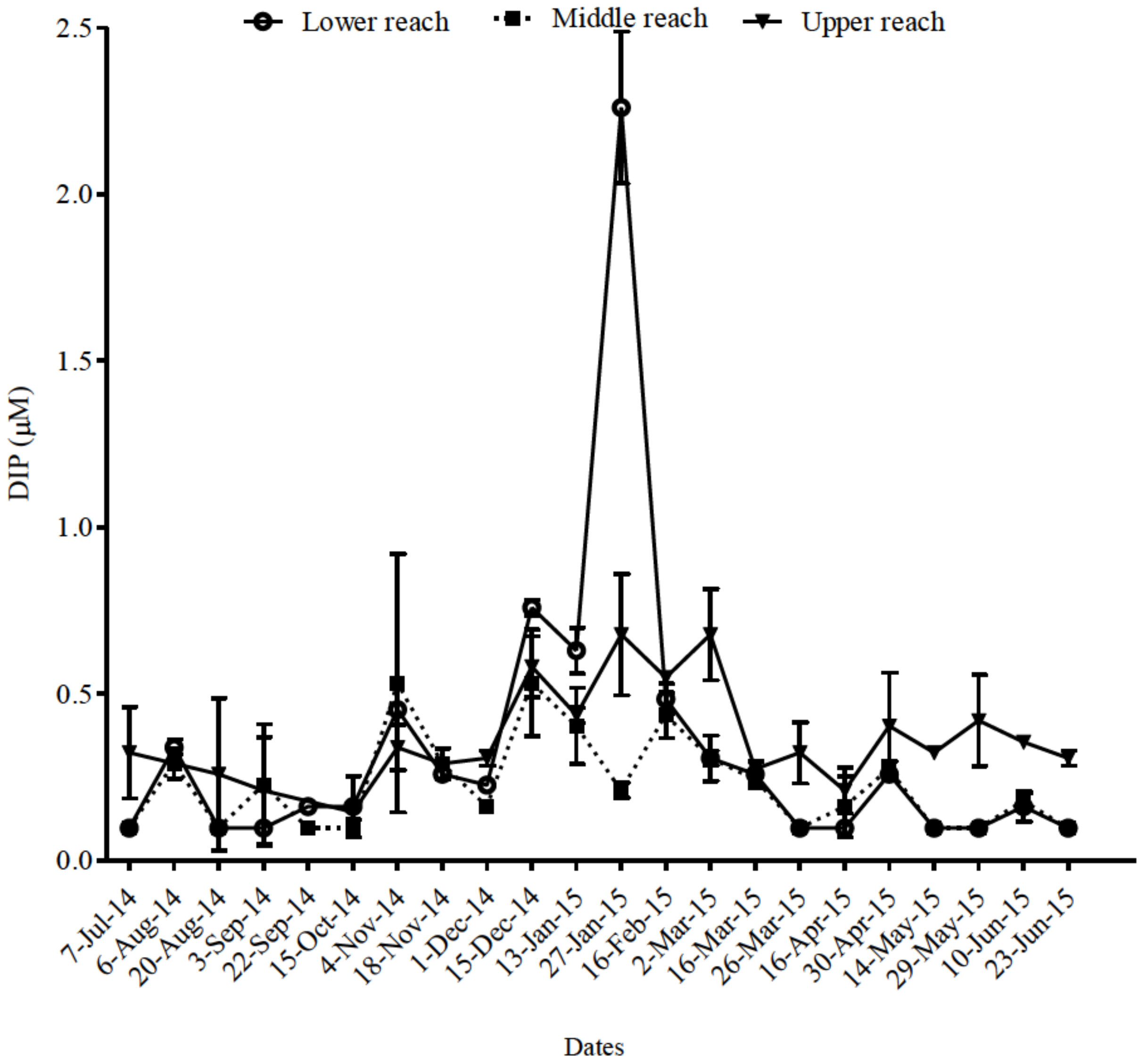


Figure 2. 7: Mean (± SD) biweekly measurements of DIP (µM P in PO_4^{3-}) concentrations along the Mlalazi Estuary (3 reaches) from July 2014 to June 2015.

Relationship between freshwater input and water quality parameters

Table 2.1: Relationship between freshwater input and water quality parameters represented by Spearman's rank correlation r values. Significant relationships are in bold where * is *p* = 0.05≤0.01, ** is p = 0.01≤0.001 and *** is p <0.0001. Sample size = 63.

Water quality parameter	Rainfall	River flow
Temperature (ºC)	-0.02	**0.45****
Salinity	**-0.25***	-0.09
DO (mg/L)	-0.06	**0.40****
Turbidity (NTU)	0.05	-0.14
TON (µM)	**0.34****	0.23
DIP (µM)	0.07	**0.53*****

There was no significant relationship (r = 0.16. *p* > 0.05, n = 21) between riverflow and rainfall. Both rainfall and river flow showed significant relationships with different variables. The results suggest that rainfall had a significant realationship with salinity and total oxidised nitrogen (TON) concentration. Increase in rainfall was related to a decrease in salinity and an increase in TON. River flow on the other hand had a significant positive relationship with temperature, dissolved oxygen, and dissolved inorganic phosphorus (DIP) concentration suggesting an increase in riverflow was related to an increase in temperature, dissolved oxygen and DIP concentration. Statistically, turbidity showed no significant relationship with rainfall and river flow (Table 2.1).

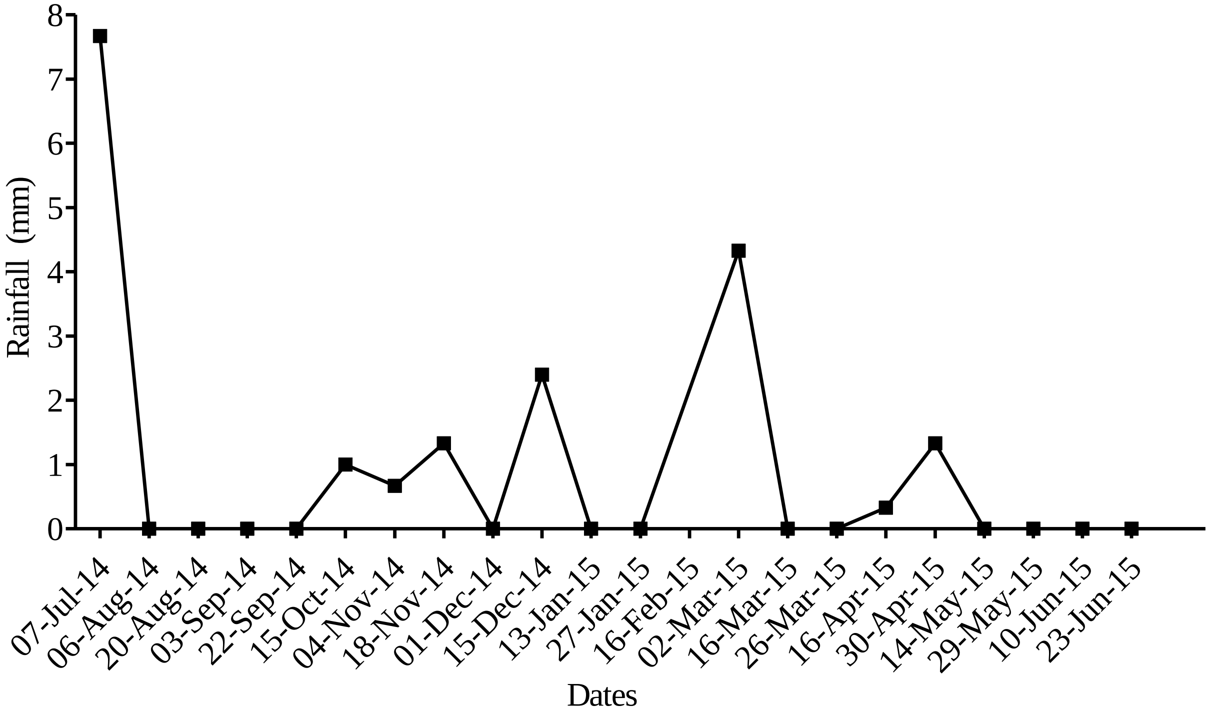


Figure 2. 8: Rainfall in the study area on the sampling dates from July 2014 to June 2015.

Table 2.2: Mean of physico-chemical variables measured during winter, spring, summer, and autumn from July 2014 to June 2015 at three reaches along the Mlalazi Estuary. Analysis of variance showing F value for chl *a* and Kruskal-Wallis analysis showing Chi-square values for all other variables with *p* values in brackets. Significant differences ($p < 0.05$) between seasons and sites are in bold.

Variables	Winter			Spring			Summer			Autumn			Kruskal-Wallis analysis, n = 64.	
	Lower	Middle	Upper	Lower	Middle	Upper	Lower	Middle	Upper	Lower	Middle	Upper	Site (df=2)	Season (df=3)
Salinity	32.51	27.28	26.81	33.27	27.81	22.45	34.96	29.94	26.90	31.25	22.09	19.46	**45.71 (<0.0001)**	**10.69 (0.014)**
Temperature (°C)	19.27	20.22	20.62	21.05	22.43	22.88	23.84	27.92	28.68	24.08	25.28	25.45	5.96 (0.113)	**44.94 (<0.0001)**
Dissolved oxygen concentration, mg/L	8.53	5.93	6.43	7.52	7.54	6.40	7.10	7.21	5.88	7.01	7.01	5.34	**29.62 (<0.0001)**	**13.28 (0.004)**
Turbidity (NTU)	3.64	7.22	6.25	6.62	12.52	0.37	13.56	17.88	6.40	12.10	15.39	14.21	**11.81 (<0.0001)**	**13.67 (<0.001)**
TON concentration (µM)	0.44	0.96	1.24	1.55	1.85	1.85	0.99	1.14	0.76	1.81	13.20	19.86	1.22 (0.748)	**16.90 (0.001)**
DIP concentration (µM)	0.16	0.28	0.31	0.23	0.15	0.25	0.89	0.40	0.51	0.17	0.30	0.38	**11.42 (0.01)**	**22.375 (<0.0001)**

Relationship between freshwater input, phytoplankton attributes

Table 2.3: Relationship between freshwater input and phytoplankton represented by Spearman's rank correlation r values. Significant relationships are in bold and * is $p = 0.05 \leq 0.01$. Sample size is 63.

Phytoplankton attributes	Rainfall	River flow
Chlorophyll *a* (µg/L)	0.14	0.02
Bacillariophyta	-0.12	0.17
Dinophyta	0.05	0.09
Cryptophyta	0.18	0.09
Chlorophyta	-0.15	0.07
Euglenophyta	**0.29***	**0.26***
Cyanophyta	**0.31***	0.11
Prorocentrum triestinum	0.02	0.29

The results from the Spearman's rank correlation between rainfall, river flow and phytoplankton attributes suggest that of all the six major phytoplankton groups, only euglenophytes and cyanobacteria showed a significant positive relationship with freshwater input. Increase in eugleonphyte abundance was related with an increase in river flow and rainfall while an increase in cyanobacteria was related to a rainfall only. Phytoplankton biomass (chl *a*) and *Prorocentrum triestinum* showed no sigificant relationship with rainfall or river flow (Table 2.1).

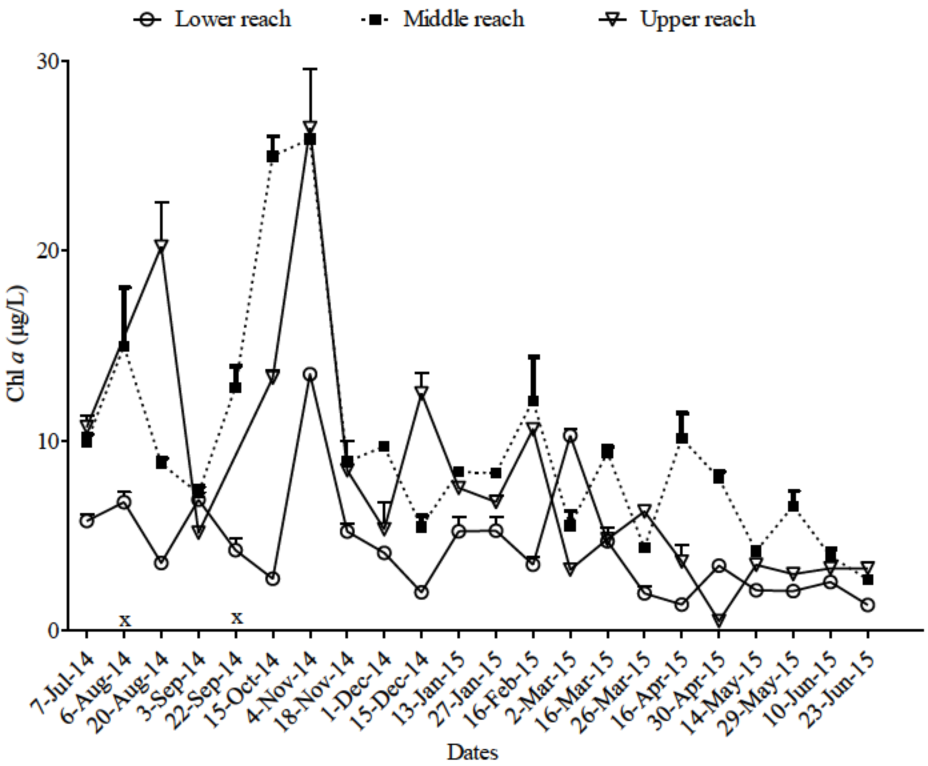


Figure 2. 9: Mean (± SD) biweekly measurements of chlorophyll *a* concentration (phytoplankton biomass) (µg/L) at all three reaches from July 2014 - June 2015.

Based on UNEP and CSIR (2009) and the Assessment of Estuarine Trophic Status (ASSETS) thresholds for DIP concentration (Bricker et al., 2003), the water quality at the lower and upper reaches was fair (0.01 to 0.1 mg L^{-1}, mesotrophic) while the water quality at the middle reaches was good (0 to 0.01 mg L^{-1}, oligotrophic). DO concentration was considered non-hypoxic (>2 mg L^{-1} [Bricker et al., 2003]). Based on the National Coastal Condition Assessment (NCCA) thresholds for chl *a* concentration (U.S. EPA, 2001) and Lemley et al. (2015), water quality at the lower reaches was good (0 to 5µg/L, oligotrophic) while water quality at the middle and upper reaches was fair (5 to 20 µg/L, mesotrophic). Overall, the water quality status along the estuary was good to fair based on the chl *a* concentration and DIP concentrations recorded during this study.

2.3.2. Phytoplankton community structure

A total of 97 phytoplankton genera were recorded. This included 68 diatoms (Bacillariophyta), 21 dinoflagellates (Dinophyta), three chlorophytes (Chlorophyta), three cyanobacteria (Cyanophyta), one cryptophyte (Cryptophyta) and one euglenophyte (Euglenophyta). Overall, cryptophytes had the highest mean (±SD) abundance ($1.97 \pm 2.82 \times 10^5$ Cells/L), followed by chlorophytes ($1.53 \pm 3.20 \times 10^5$ Cells/L), dinoflagellates ($8.05 \times 10^4 \pm 1.30 \times 10^5$ Cells/L), diatoms ($6.06 \pm 9.80 \times 10^4$ Cells/L), euglenophytes ($9.31 \times 10^3 \pm 3.20 \times 10^4$ Cells/L) and lastly cyanobacteria ($0.75 \pm 2.2 \times 10^1$ Cells/L). Therefore, although diatoms were the most diverse, cryptophytes and chlorophytes, represented by only one and three genera respectively, were far more abundant.

Phytoplankton community composition differed significantly (p (Perm) = 0.001, pseudo F = 8.45) among the three reaches of the estuary. The highest taxa richness was recorded at the lower reaches (80 genera), followed by the middle (65 genera) and upper (62 genera) reaches. Mean taxa richness, represented by the number of genera per season ranged from 16 to 37 genera and increased from the upper to the lower reaches (Fig. 2. 10). Taxon richness correlated with dissolved oxygen concentration ($p = 0.01$, R = 0.32, n = 63) and salinity ($p = 0.01$, R = 0.34, n = 63), variables (DO concentration and salinity) that also increased from the upper to the lower reaches. Rare species were more numerous at the lower reaches (28), than at the middle (22) and upper reaches (17). However, in a sample, at any time, the number of diatom taxa representing ≥10% of the total diatom abundance (dominant taxa) ranged from 1 to 7 and a mean of 3.7 to 4.4 per site. Richness was significantly different along the estuarine gradient ($p < 0.001$, F = 12.58, df = 2) but not season ($p > 0.05$). Phytoplankton abundance showed an opposite trend to richness, but the same trend to chl *a* concentration, as it increased from the lower ($1.97 \pm 2.38 \times 10^5$ Cells/L) to the upper reaches ($8.45 \pm 4.80 \times 10^5$ Cells/L). Variability in the abundance of phytoplankton groups was at the upper reaches (CV = 2.05) and lowest at the middle reaches (CV = 1.25).

At the lower reaches, a clear succession was apparent for the dominant groups. Dinoflagellates dominated in winter (49.1% of total abundance), cryptophytes in spring (36.1%) and diatoms in summer (58.0%) and autumn (44.5%) (Fig. 2. 12 and Fig. 2. 13). Dinoflagellates and diatoms were overall the most abundant phytoplankton group at this site, and the dominance of dinoflagellates was apparent from both (2014, 2015) winter seasons (Fig. 2. 11). In contrast, at the middle reaches, cryptophytes were the most abundant phytoplankton group in all four seasons by contributing >38% of the total phytoplankton abundance (Fig. 2. 13). Therefore, there was no seasonal change in the dominant phytoplankton group at this site. At the upper reaches, a shared dominance of cryptophytes and chlorophytes was noted throughout the year. Cryptophytes dominated in winter (46.9%), spring (83.9%) and autumn (52.3%) and chlorophytes dominated in summer (89.3%) (Fig. 2. 11 and Fig. 2. 13). Note that uneven sampling did not affect species richness but rather the mean abundance of species, precisely with cryptophytes. About 4% of cryptophyte abundance in autumn at the lower reaches was due to the uneven sampling effort. In the other sites, uneven sampling led to a minimal increase (0.7%) of the cryptophyte abundance. However, this does not influence the observed pattern.

Except for the upper reaches where cryptophytes dominated in all the spring months (three months) and chlorophytes dominated in all the summer months (three months), no phytoplankton group dominated in all months within a season. Rather, a phytoplankton group dominated from a single sampling session (two weeks) to over three months (Fig. 2. 11).

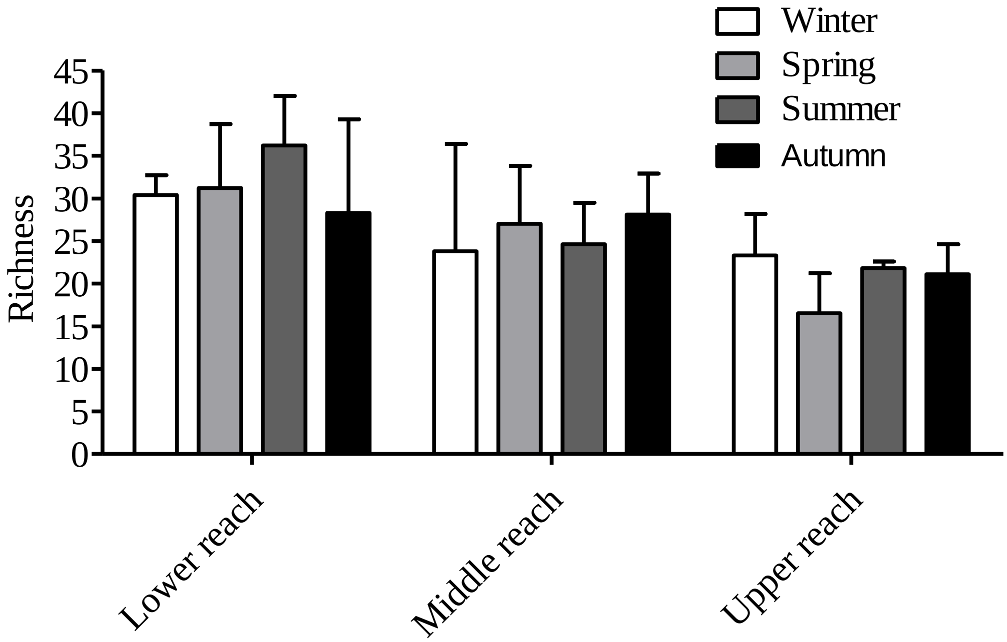


Figure 2. 10: Phytoplankton richness (mean ± SD) in different seasons along the salinity gradient from July 2014 to June 2015.

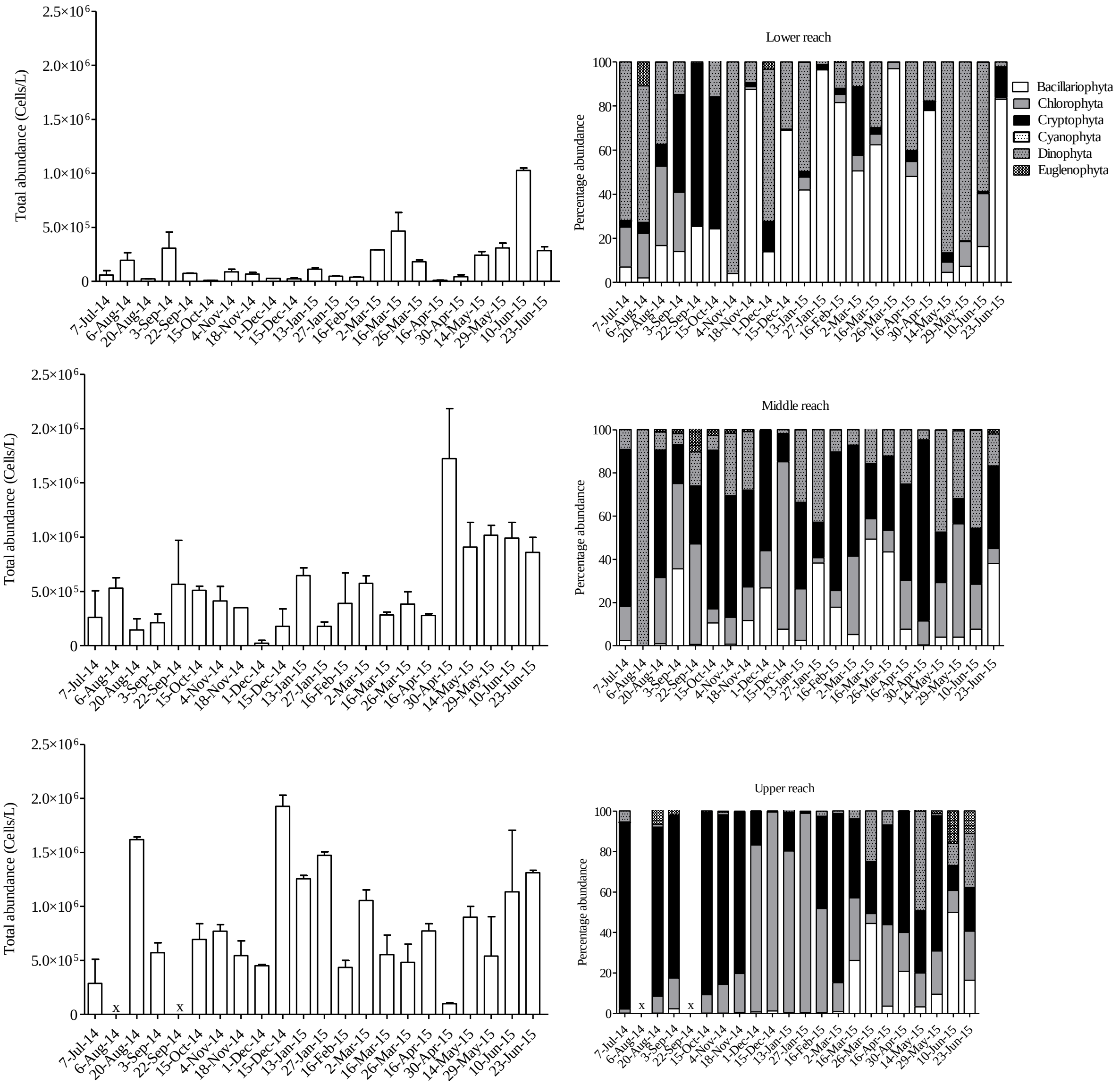


Figure 2. 11: Mean (± SD) of phytoplankton abundance from July 2014 to June 2015 (left) and the percentage contribution of each phytoplankton group to the total abundance from July 2014 to June 2015 (right) at all three sites of the estuary. X denotes missing data.

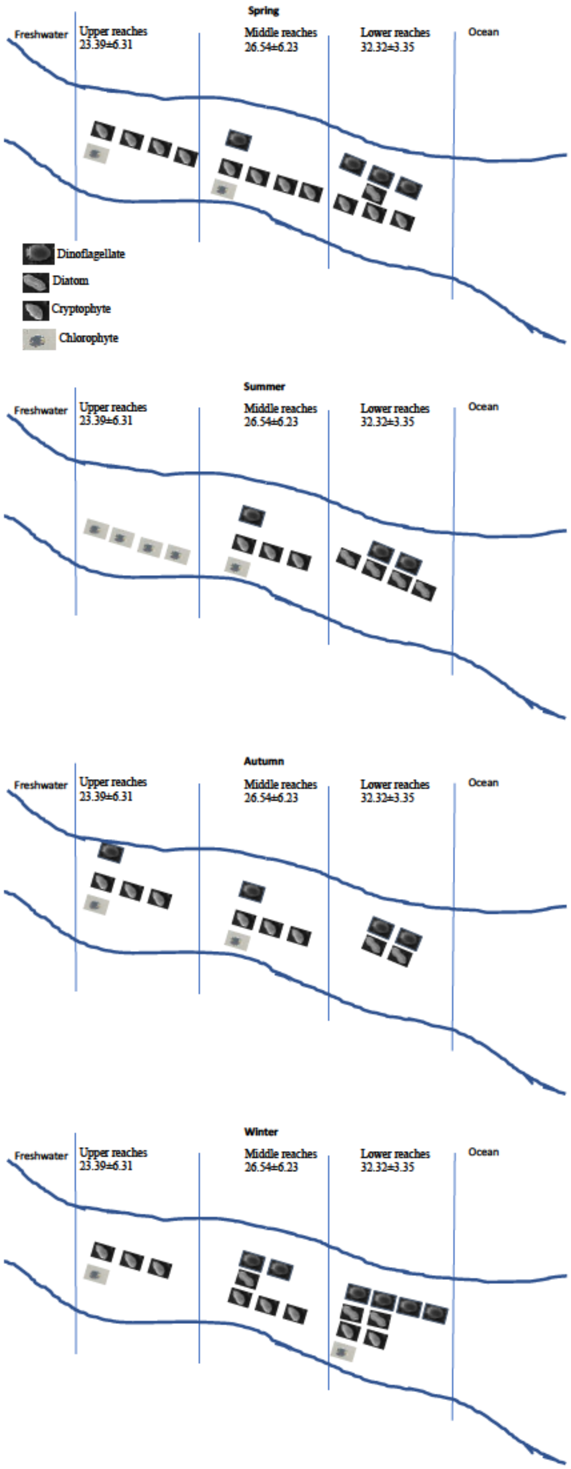


Figure 2. 12: Seasonal variation in the abundance of phytoplankton groups along the longitudinal salinity gradient.

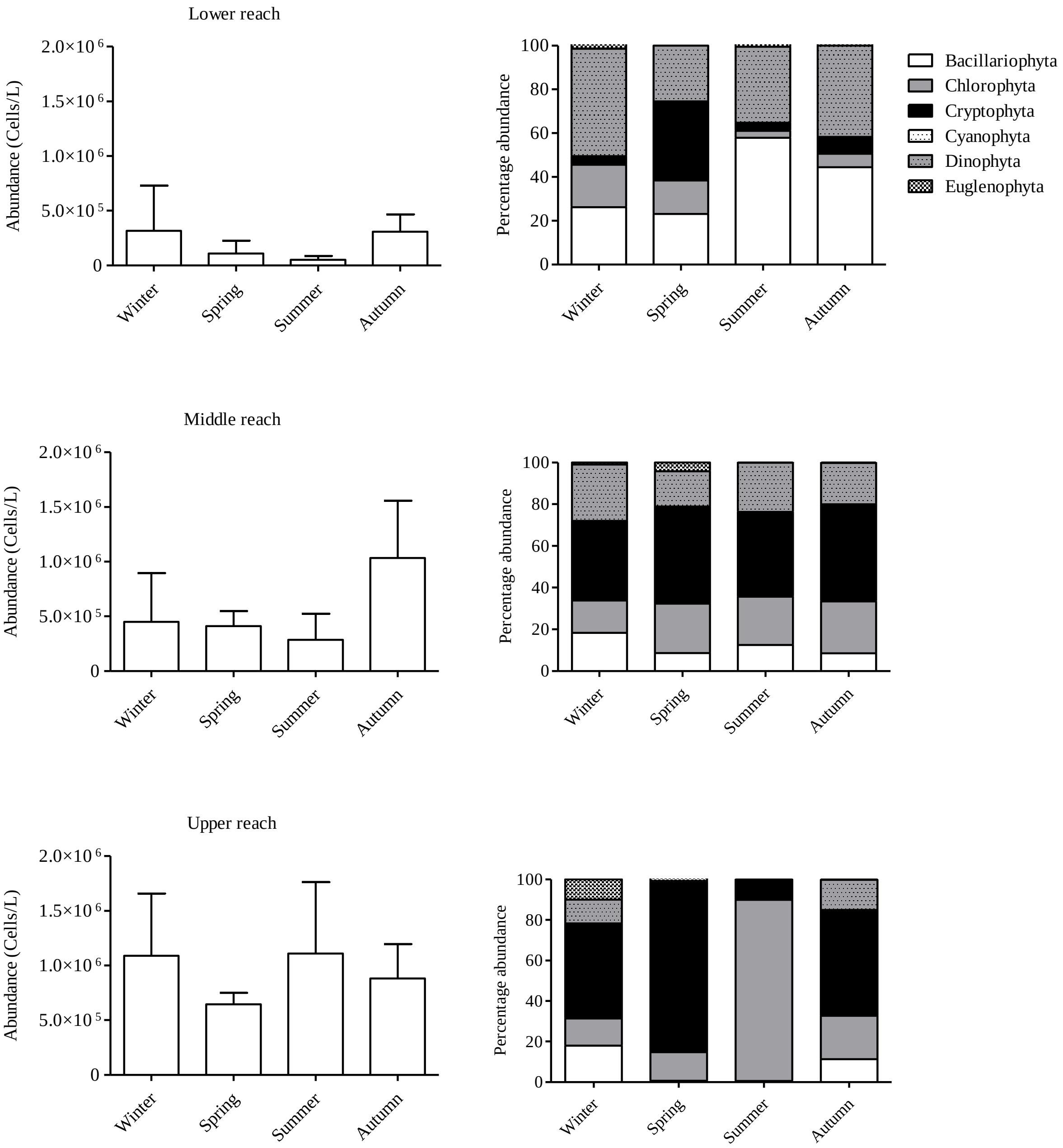


Figure 2. 13: Mean (± SD) of phytoplankton abundance in different seasons (left) and the percentage contribution of each phytoplankton group to the total abundance in each season (right) at all three sites of the estuary.

Phytoplankton abundance, chlorophyll *a* concentration and blooms

During this study, total abundance reached bloom concentrations (> 1.0 x 10^6 Cells/L) once at each site. In winter at the lower reaches when dinoflagellates represented 60% of the abundance and chl *a* concentration was 4 µg/L, in autumn at the middle reaches when cryptophytes represented 80% of the abundance and chl a concentration was 9 µg/L and in summer at the upper reaches when chlorophytes represented 80% of the abundance and chl *a* concentration was 12 µg/L (Fig. 2.11). On all three occasions, chl *a* was < 20 µg/L hence did not present bloom conditions (Fig. 2.9). The three taxa associated with this high abundance, *Cryptomonas* sp., *Chlamydomonas* sp. and *Prorocentrum triestinum* did not reached bloom conditions (<1.0 x 10^6 Cells/L) and showed no significant relationship with rainfall or river flow (Table 2.3). With respect to chl *a* concentration, a eutrophic or bloom condition (> 20 µg/L) was rather noted in spring at the upper reach when total phytoplankton abundance was <1.0 x 10^6 Cells/L and cryptophytes dominated (Fig 2.9 and Fig. 2.11).

The distribution of the major phytoplankton groups along the estuarine gradient was further corroborated by correlating physico-chemical parameters with phytoplankton community abundance using a canonical correspondence analysis (CCA). The CCA was performed using 6 phytoplankton groups and seven physico-chemical variables (Fig. 2.14). The eigen values for axis 1 (λ = 0.21) and axis 2 (λ = 0.13) accounted for 95.83% of the total data variation (Table 2. 4). A significant (*p* = 0.001, F = 0.36) canonical correspondence analysis (CCA) shows that salinity, dissolved oxygen concentration, temperature, rainfall, TON and DIP concentrations influenced the phytoplankton community of the Mlalazi Estuary (Table 2. 1). The important variables for axis 1 ordination were salinity, rainfall, DO and temperature and for axis 2, the variables were temperature, DIP concentration, rainfall, and salinity (Table 2.5). Higher temperature and low salinity were the drivers of the dominance of chlorophytes in the upper reaches in summer (Fig. 2. 14). The observed maximum abundance of cryptophytes at the upper reaches coincides with highest TON concentration, rainfall, lowest dissolved oxygen concentration and low salinity. Diatom abundance was positively related to DIP concentration and salinity (Fig. 2. 14). None of the measured physico-chemical variables were singled out to influence the other phytoplankton groups and all four main phytoplankton groups are far apart from each other.

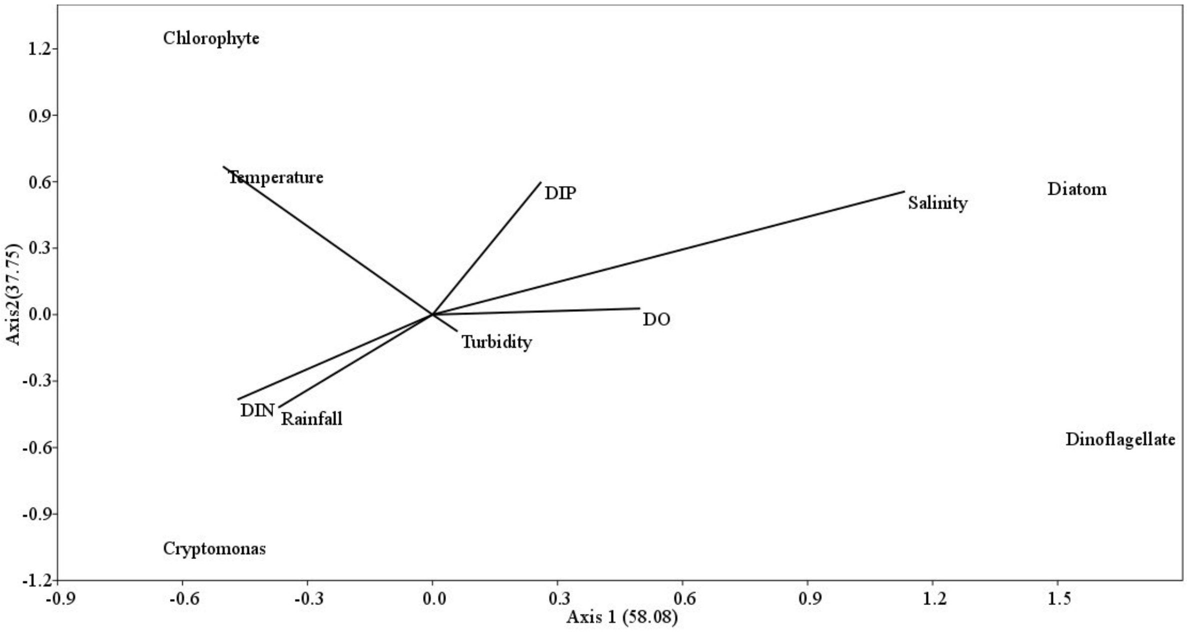


Figure 2. 14: Biplot of canonical correspondence analysis relating abundance of phytoplankton groups and physico-chemical variables.

Table 2.4: CCA Eigenvalues, significance, and percentage contribution of each axis. Significance level: 0.05, n = 64.

Axis	Eigen value	*P*	Percentage contribution
1	0.21	0.03	58.08
2	0.13	0.003	37.75
3	0.01	0.58	3.95
4	0.001	0.91	0.23
5	0.000	0.11	0.004

Table 2. 5: CCA Eigenvalues, contribution of each physico-chemical variable and phytoplankton group to the significant axes, axis 1 and axis 2. n = 64.

	Axis 1	Axis 2
Salinity	0.57	0.27
DO	0.24	0.01
Temperature	-0.24	0.34
Turbidity	0.04	-0.03
TON	-0.23	-0.19
DIP	0.15	0.30
Rainfall	-0.32	-0.28
Bacillariophyta	1.59	0.64
Dinoflagellate	1.49	-0.55
Cryptophyta	-0.66	-0.99
Chlorophyta	-0.65	1.30
Euglenophyte	1.42	0.22
Cyanophyta	-0.68	-2.71

2.4. Discussion

Phytoplankton undergo continual changes in their community structure in aquatic systems with dynamic environmental and biological variables (Lancelot and Muylaert, 2011; Cloern and Jassby, 2008; Reynolds, 2006). This study examined the spatio-temporal changes in the phytoplankton community structure along the horizontal salinity gradient of the subtropical, predominantly open, minimally anthropogenically impacted Mlalazi Estuary. The study yielded three main observations; 1) all major phytoplankton groups were present along the estuarine gradient from the lower to the upper reaches, 2) the middle reaches of the estuary were the least variable in terms of community composition over time, and 3) the dominant period of a single phytoplankton group differed substantially, lasting from less than two weeks to an entire season.

2.4.1: Environmental variables

As earlier mentioned, one of the most important factors influencing the functioning of estuaries is the river flow. Generally, it is expected that during high flow, the levels of turbidity are higher due to the disturbance of sediment, causing increased levels of total suspended solids (Chen et al., 2018; Froneman, 2002). However, in the Mlalazi Estuary, turbidity showed no significant relations with rainfall or river flow, neither was there any relationship between turbidity and chl *a* concentration. Concurring with other predominantly open estuaries in South Africa, such as Sundays Estuary, aMatikulu, Kariega, and Kromme (Vezi et al., 2020; Scharler & Baird, 2003; Grange et al., 2000), nutrient concentration was higher during high rainfall and river flow. Higher nutrient concentrations suggest that higher rainfall and river flow lead to nutrients from inland being brought into the estuary. However, rainfall correlated with TON concentration only, while river flow was associated with DIP concentration. The increase in TON and DIP concentration with increasing rainfall and river flow respectively suggest river flow as an essential source of nutrients to the Mlalazi Estuary. The relationship between nutrients and river flow shows the importance of policy regulating effluent discharge into the estuary and fertilizers for agricultural purposes along the estuary and its catchment. In periods of low freshwater input, the primary source of nutrients, especially nitrogen in estuaries, is seepage from the agricultural land at the upper reaches (Brooks et al., 2021; Van Niekerk et al., 2020; Snow et al., 2000). Higher phytoplankton biomass (chl *a*) was apparent at the upper reaches during a low flow period, indicating sufficient nutrients for phytoplankton in this region.

Due to the low river flow, longer residence time for adequate primary production, phytoplankton biomass was expected to be high. The upper reaches exhibited higher chl *a* concentration in spring (November) during low flow. Higher concentrations of nutrients promote primary productivity in predominantly open estuaries (Lemley and Adams, 2020; Perissinotto et al., 2003; Froneman, 2002). The highest concentration of chl *a* was observed during a low flow period in spring (November) rather than the high flow period in autumn (March and April), which had elevated nutrient concentrations, but lower residence time, not enough for phytoplankton to take up the nutrients. However, in the Mlalazi Estuary, chl *a* did not correlate with rainfall or river flow. Previous studies in the Mlalazi Estuary also attribute low chl *a* concentration during high river flow due to smaller residence time. The chl *a* concentration measured during this study was within the range of previously recorded values in the estuary during non-drought periods (DWS, 2015; Orterga-Cisneros and Scharler, 2014). The current study suggests that under similar environmental conditions as those experienced during this study, the Mlalazi Estuary has little potential for high primary production and blooms under the sampling conditions.

2.4.2: Phytoplankton

Phytoplankton community composition varied spatially between the different reaches of the estuary, with salinity as the main determinant. A typical pattern with chlorophytes dominant at the upper reaches characterised by low salinity, and dinoflagellates and diatoms at the lower reaches at higher salinities (Nunes et al., 2018; Bazin et al., 2014; Jendyk et al., 2014; Nursuhayati et al., 2013; Muylaert et al., 2009; Valdes-Weaver et al., 2006; Tomas, 1997) was apparent in this study.

High diatom and dinoflagellate diversities and high diatom abundance are generally recorded in estuarine environments irrespective of trophic status and contribute a very high proportion of all taxa. For instance, both groups combined represented 86% of taxa in the eutrophic Šibenik Bay (Bužančić et al., 2016) and 78, 95 and 95% of the taxa in the oligotrophic southern Bay of Bisca (Muñiz et al., 2018), Bay of Bengal (Vajravelu et al., 2018), and Kowie Estuary (Dalu et al., 2014), respectively. The high diatom diversity, their euryhaline nature and species-specific ecology (Armbrust, 2009) might explain the dominance of diatoms in estuaries (Muñiz et al., 2018). In concurrence with previous studies (Muñiz et al., 2018; Vajravelu et al., 2018; Bužančić et al., 2016; Dalu et al., 2014), diatoms and dinoflagellates were the most diverse phytoplankton groups in the oligo– to mesotrophic Mlalazi Estuary, representing 70 and 22% of the taxa, respectively. However, contrary to studies that suggest diatoms to be the most abundant taxa in estuaries overall (Muñiz et al., 2018; Vajravelu et al., 2018; Bužančić et al., 2016; Dalu et al., 2014), in this study, cryptophytes represented by a single genus, *Cryptomonas* was the most abundant. Though not detected in some South African estuaries (Dalu et al., 2018), cryptophytes have been noted in other South African estuaries (Nche-Fambo et al., 2015; Gama et al., 2005). In estuaries of North America and Northwestern Europe, cryptophytes are a minor component of the phytoplankton community, commonly present but rarely observed in high densities or blooms (Carstensen et al., 2015).

However, diatoms and dinoflagellates were the most dominant phytoplankton group during certain seasons in the lower reaches of the Mlalazi Estuary. Compared to other phytoplankton groups such as dinoflagellates, the fast-growing nature of diatoms allows diatoms to dominate during periods of higher nutrient concentrations (Reynolds, 2006; Chan et al., 2002; Gallegos, 1992), apparent at the lower reaches in summer. The slower-growing dinoflagellates, which thrive better than diatoms under lower nutrient concentrations (Reynolds, 2006; Chan et al., 2002; Gallegos, 1992), dominated under such conditions at the lower reaches in winter. Therefore, the salinity at the lower reaches may have favoured the dominance of diatoms and dinoflagellates. However, the seasonal difference in nutrient concentrations influenced the community compositions of diatoms and dinoflagellates.

Cryptophytes, the overall dominant group, typically experience the highest growth under low salinity and reach high abundances at estuaries' upper reaches (Johnson et al., 2018; Šupraha et al., 2014). Similarly, in another subtropical South African estuarine lake, St. Lucia, this group dominated under fresh to brackish conditions (4.6 to 22.4) at the freshwater region (Nche-Fambo et al., 2015). In this study, *Cryptomonas* accounted for up to 84% of the phytoplankton population in spring at all reaches, correlating with low salinity.

Nutrient concentrations in the estuary were generally low (<7.7 ± 22.04 µM TON and <0.37 ± 0.15 µM DIP). Small phytoplankton cells such as cryptophytes and chlorophytes with high surface-volume ratios are favoured under low nutrient conditions. However, the mixotrophic nature of the cryptophyte *Cryptomonas* (Yoo et al., 2017; Gama et al., 2005) gives it a competitive advantage over chlorophytes, possibly leading to the overall dominance of cryptophytes at the middle and upper reaches. Also, because chlorophytes have a competitive advantage under higher temperatures (Dalu et al., 2018; Haraguchi et al., 2018; Carstensen et al., 2015; Jendyk et al., 2014; Li et al., 2014), they outcompeted cryptophytes at the upper reaches in summer when the temperature was highest (28.68 °C). Chlorophytes' positive relationship with temperature and negative relationship with salinity (Fig. 2. 14) supports the finding that chlorophytes occur mainly at lower salinities. This is true for other South African estuaries (e.g., St. Lucia Estuary (Nunes et al., 2018; Nche-Fambo et al., 2015), Sundays Estuary (Kotsedi et al., 2012)), and other coastal water bodies worldwide (Tragin and Vaulot, 2018; Hamilton et al., 2000; Flameling and Kromkamp, 1994). The phytoplankton community responds to different environmental parameters in different ways, ensuring its diversity and persistence throughout the changing environment, thereby a continued presence of energy at the bottom of the food chain.

The response of phytoplankton to these environmental variables differ from estuary to estuary, reaches to reaches and time to time (Lancelot and Muylaert, 2011). Seasonal changes in phytoplankton communities, as well as spatial changes, have been noted (Dalu et al., 2018; Li et al., 2014; Rychtecky and Znachor, 2011; Thompson et al., 2008) although a lack of spatial variability has also been reported (dos Santos et al., 2017). In South Africa, of the four studies that reported on phytoplankton community composition in estuaries (Dalu et al., 2018, 2014; Nche-Fambo et al., 2015; Kotsedi et al., 2012), only one (Dalu et al., 2018) reported a change in dominant phytoplankton groups between seasons. Dalu et al. (2018) documented a shift from chlorophytes in winter to diatoms in spring and cyanobacteria in summer in oligotrophic micro-estuaries characterised by a typical salinity gradient (freshwater to marine). In this study, phytoplankton groups clearly responded to space and time. However, distinct seasonal variations were not apparent at the middle reaches where cryptophytes were the most dominant group in all four seasons. The dynamic nature of the longitudinal salinity gradient in estuaries with possible oligohaline regions in the mouth during elevated river discharge in summer and oligohaline region in upstream during drier months strongly influences phytoplankton dynamics. Due to this dynamic nature, the phytoplankton community's temporal changes at the lower and upper reaches of this study may have spilled into the middle reaches, thereby influencing the small seasonal variability observed at the middle reaches. This lack of seasonal variability at the middle reaches is evident in winter, spring, and autumn. Therefore, the spatial community composition variability along the estuarine gradient informed its temporal responses throughout the year. The change in composition with time is not reflected in taxon richness, which did not significantly differ between seasons. With seasonal changes in driving variables, taxa are replaced by others that could better tolerate the conditions, keeping a consistent richness but varying composition. Richness correlated with salinity and dissolved oxygen concentration. However, it is most likely that these variables did not influence taxon richness because, at the lower reaches where higher taxon richness was reported, the lowest number of dominant taxa and more of the rare taxa were also noted. The variables (salinity and DO concentration) did not make the lower reaches more conducive for more taxa to dwell. The Mlalazi Estuary mouth was open throughout this study. Tides may have been responsible for bringing in marine taxa from the sea, but the estuary was not conducive for growth, hence recorded as rare species.

In estuarine studies, despite the fast growth rate and short (hours to a few days (Marra, 2002) life spans of phytoplankton cells, shifts and dominance of phytoplankton groups are mostly reported either monthly or seasonally, e.g., in South Africa (Dalu et al., 2018, 2014; Lemley et al., 2017; Nche-Fambo et al., 2015; Gama et al., 2005) and internationally (Kouhanestani et al., 2019; Muñiz et al., 2018; Dos Santos et al., 2017; Shen et al., 2011). There is very limited information on the phytoplankton temporal variability beyond seasons, especially in South African estuaries. In the subtropical Skidaway Estuary, USA, in weekly samples, high variability obscures seasonal shifts in phytoplankton composition (Anderson and Harvey, 2019). Similarly, in this study, bi-weekly variations were more extreme than seasonal phytoplankton community shifts where the dominance period varied from two weeks to rarely an entire season. On a monthly scale where the dominant period varied from one month to three months, a clear shift in phytoplankton composition is noticed in some seasons (Nche-Fambo et al., 2015; Gama et al., 2005) as was in a single season in this study. There is no universal rule to describe the dominant time scale of phytoplankton variability (Cloern and Jassby, 2010). However, the use of smaller time scales (weekly and biweekly) reveals the temporal variability in phytoplankton composition and thus the existence or lack of seasonal dominance and seasonal shifts.

There is a typical seasonal trend of high phytoplankton biomass in spring and summer due to high temperatures, nutrient concentrations and light (Paczkowska et al., 2019; Adolf et al., 2006; D'Alcala et al., 2004; Cebrián and Valiela, 1999) and lower biomass in winter. This trend is true for temperate and subtropical South African estuaries due to the increased river flow in the rainy season (summer and spring) which increases the nutrient concentration (Dalu et al., 2018; Gama et al., 2005; Nozais et al., 2001; Perissinotto et al., 2000; Adams and Bate, 1999). Phytoplankton biomass differed seasonally and spatially, and contrary to previous findings in estuaries (Dalu et al., 2014; Barbosa et al., 2010; Rocha et al., 2002), the highest biomass was not in summer but instead winter. Previous studies in the Mlalazi Estuary reported lower phytoplankton biomass in the rainy season (summer and spring) due to reduced water residence time caused by high freshwater inflow (Vezi, 2013; Mabaso, 2002). Freshwater inflow is the main factor influencing residence time, and bloom development is often inversely correlated to freshwater inflow (Kenov et al., 2012). As such, estuaries with short residence times generally experience fewer water quality problems (Wang et al., 2019; Kenov et al., 2012). The fewer water quality problems are because residence time is essential for the assimilation of nutrients by the phytoplankton (Wang et al., 2019; Kenov et al., 2012; Lancelot and Muylaert, 2011; Perissinotto et al., 2002; Adams and Bate, 1999). Grazing pressure is another factor controlling phytoplankton biomass (Lürling, 2020; Alves-de-Souza et al., 2006; Chan et al., 2002; Walker et al., 2001). The zooplankton biomass in the Mlalazi Estuary is highest in summer (Ortega-Cisneros and Scharler, 2014) and zooplankton grazing could be partly responsible for the low phytoplankton biomass during this season.

In conclusion, the Mlalazi Estuary is an oligo– to mesotrophic system, characterised by low nutrient concentrations and the lack of phytoplankton blooms confirming the ecological status earlier reported (Van Nierkerk et al., 2020; Whitfield and Baliwe, 2013). The estuary has low potential of becoming eutrophic due to the absence of high phytoplankton biomass during periods of high rainfall and river flow. River flow is a main source of nutrients. However, during droughts and low river flow, primary productivity leading to bloom conditions is possible suggesting other sources of nutrients. The spatial variability of the main phytoplankton groups diatoms, dinoflagellates, chlorophytes, and cryptophytes recorded in this estuary were influenced by salinity. Meanwhile, temperature and nutrient concentration mostly influenced the temporal variability observed. These changes ensured a diverse phytoplankton community, and due to low nutrient concentrations, no taxa bloomed. In spring, the conditions in this estuary favoured the growth of cryptophytes, which, unlike most estuaries, were the most abundant group. Taxon richness did not change temporally, it differed with the site and was not influenced by any measured environmental variables. Instead, the ocean seemed to be the possible source of species leading to the spatial difference. With the dominant period of a phytoplankton group ranging from two weeks to three months (an entire season), the conventional seasonal sampling applied in most South African monitoring schemes would be inadequate in determining phytoplankton community changes in subtropical predominantly open estuaries. The temporal and spatial phytoplankton community structure characterised in this study provides a good basis for assessing monitoring frequency and future changes.

Chapter 3. SPATIO-TEMPORAL PATTERN OF THE DIATOM COMMUNITY ALONG A SALINITY GRADIENT IN A SUBTROPICAL ESTUARY

Abstract

With the current degradation of estuaries, there are very few near-pristine estuaries with a good ecological health status left. A study of the phytoplankton community in the Mlalazi Estuary of good ecological health status showed that diatoms were the most diverse group. Their overall abundance was related to increasing DIP concentration and salinity. A detailed diatom community structure (taxon richness, taxa composition and taxa abundance) along a salinity gradient, in conjunction with other physico-chemical parameters, was investigated in the subtropical Mlalazi Estuary of good to fair water quality status on the east coast of South Africa. Samples were collected biweekly at the upper, middle, and lower reaches of the estuary for one year to represent all four seasons. Nutrient concentrations were low, abundance was low (3.40×10^2 to 5.67×10^5 Cells/L), diatom richness was high (182 taxa), and 23 taxa represented >10% of the abundance at any of the sampling sessions but no blooms were recorded. Low nutrient concentrations, high taxa richness and low diatom densities are typical to estuaries with a good to fair water quality. In the absence of severe anthropogenic effects on the estuary's water quality, Canonical Correspondence Analysis revealed that salinity, turbidity, and dissolved inorganic phosphorus (DIP) concentration influenced the diatom community. Diatoms had a wide salinity tolerance. Pelagic chain-forming species *Cyclotella meneghiniana*, *Thalassiosira tenera*, *Rhizosolenia setigera*, and *Paralia sulcate* generally associated with incoming tides and high salinity dominated at the lower reaches while benthic pennate taxa with a wide salinity range from freshwater to marine waters and usually associated with turbidity dominated at the upper reaches. Changes in primary physico-chemical variables led to changes in diatom taxa or type (benthic, pelagic, centric, solitary or chain-forming) and such changes can be used as a gauge for varying freshwater inflow. Here, centric planktonic forms' dominance implies increasing tidal flow and pennate benthic forms implies an increase in freshwater inflow. Some of the dominant taxa (*Pseudo-nitzschia* sp., *Asterionellopsis glacialis* and *Chaetoceros* spp.) generally tolerate a wide salinity (freshwater to marine) and are common in eutrophic estuaries of poor water quality as well as good water quality. Under the current environmental regime, the oligo- to mesotrophic Mlalazi Estuary is not on the brink of becoming eutrophic, a finding supported by the diatom community (taxon richness and taxon abundance) study and the phytoplankton community (total abundance, total biomass, overall taxon richness) study.

Key words: Chain-forming diatoms, benthic diatoms, turbidity, good to fair water quality, Mlalazi Estuary.

3.1 Introduction

Estuaries usually have a higher nutrient concentration than their surrounding ecosystems, which increases their potential for primary production (Freeman et al., 2019; Elliot and McLusky, 2002). Phytoplankton are one of the main primary producers in estuaries and provide a link between inorganic dissolved nutrients and organisms at higher trophic levels and ultimately to top predators (Wilken et al., 2017; Miller et al., 1996). Phytoplankton community structure changes rapidly with changes in water quality parameters such as nutrient concentration, making them good indicators of water quality

Knowledge of the community structure of both benthic and pelagic phytoplankton is required to assess the ecological health of estuaries (DWS, 2015; CEC, 1991). According to the European Water Framework Directive (CEC, 2000, 1991), a “high ecological status is attained when the composition and abundance of phytoplankton taxa are consistent with undisturbed (pristine) conditions”. In estuaries, diatoms are the most diverse and important phytoplankton group (Muñiz et al., 2018; Malviya et al., 2016; Armbrust, 2009; Bates and Trainer, 2006). Diatoms, belonging to the class Bacillariophyta, are ubiquitous, very distinctive due to the presence of siliceous cell walls called frustules (Korsunsky et al., 2019; Round et al., 1990) and absorb nutrients more readily compared to other phytoplankton groups (Reynolds, 2006; Lomas and Glibert, 2000). Diatoms are considered the most preferred phytoplankton group for grazing (Sathish et al., 2020; Dortch et al., 2001) and are heavily grazed upon by a host of herbivores especially copepods (Alves-de-Souza et al., 2006; Chan et al., 2002; Walker et al., 2001).

Turbidity and nutrient concentration are important factors that influence diatom communities in estuaries. An increase in turbidity, mostly associated with tides, river flow in estuaries, leads to decreased diatom abundance (Thomson and Manoylov, 2019; Shi et al., 2017; Barbosa et al., 2010). Diatoms are photosynthetic, and an increase in turbidity leads to a decrease in light availability (Thomson and Manoylov, 2019). Turbidity is considered a limiting factor for diatom growth because even at high nutrient concentrations favourable for diatoms growth, high turbidity, hence reduced light availability, does not encourage growth.

The salinity regime is an important factor determining the changes in diatom species irrespective of nutrient concentration (Moser et al., 2017). Knowledge on the salinity preferences of diatom species and their changes due to natural seasonal variations is of utmost importance in diatom community structure (Moser et al., 2017). As good indicators of salinity changes, they can thus be used to measure changes in freshwater flow into estuaries for management purposes (Snow, 2016; Potapova, 2011; Potapova and Charles, 2007).

Due to the importance of diatoms, there has been extensive research on the identification and taxonomy of diatoms in South African aquatic systems (such as Taylor et al., 2007a; Taylor et al., 2007b; Hartley et al., 1996; Schoeman and Archibald, 1976; Giffen, 1970, 1966, 1963; Cholnoky, 1968, 1965, 1963, 1955). Although diatoms are always present and make a considerable contribution to the phytoplankton community in South African estuaries (Dalu et al., 2018; Kotsedi et al., 2012; Snow et al., 2000; Snow, 2000), knowledge on their environmental and ecological interactions are limited mainly to a few studies on epipelic or benthic diatoms (Dalu et al., 2015; Bate et al., 2013) and pelagic diatoms (Bate and Smailes, 2008; Minne, 2003; Walker et al., 2001). Generally, high diversity is associated with good water quality (Stirling and Wilsey, 2001). An earlier South African study (Archibald, 1972) showed that it is essential to identify species composition, especially dominant species and their drivers, such as nutrient concentration as a criterion for assessing water quality. However, due to the lack of thorough ecological assessments of the species within the microalgae communities and inadequate sampling periods (Lemley et al., 2016), very little is known about the diatom community structure in South African estuaries and there is no reference condition to near pristine estuaries.

The study aimed to characterise the spatial and temporal patterns of the diatom community structure in the Mlalazi Estuary, a subtropical predominantly open estuary with good ecological health status. The Mlalazi Estuary has an estuarine health score of 80% (DWS, 2015), implying the estuary is minimally affected by anthropogenic factors. The objectives were to examine the 1) spatial and temporal variability of the diatom community structure (composition, richness and abundance); and 2) the influence of other water quality variables on the diatom community structure.

3.2. Materials and methods

3.2.1. Study area

See section 2.2.1 and Figure 2. 1 for description of the study site.

3.2.2. Physico-chemical measurements

See Section 2.2.1 for methods used in the measurement of physico-chemical variables (salinity, temperature (ºC), dissolved oxygen (DO) concentration, turbidity (Nephelometric Turbidity Unit, N TU), total oxidised nitrogen (TON) and dissolved inorganic phosphorus (DIP) concentrations).

3.2.3. Diatom taxa and abundance

Duplicate water samples were collected in 250 ml acid washed polyethylene bottles at subsurface (0.5 m) water level, fixed immediately with 2% acid Lugol's solution, then stored in the dark. For diatom identification and enumeration, 20-100 ml samples were settled depending on the cell density and the amount of detritus and sediment in the sample, using the Utermöhl method (Utermöhl, 1958) and viewed under a Nikon *ECLIPSE Ti Series* inverted microscope fitted with a DS-US camera powered by NIS-Elements BR software at magnifications of 400x to 1000x. To confirm the identification, diatom cells were digested using the acidification method by Taylor et al. (2007b) and prepared for scanning electron microscopy (SEM) according to Tillmann and Hoppenrath (2013). All permanent slides were stored for future references and research. See Appendix 2 for diatom SEM and light micrographs. Taxa were identified to the lowest taxonomic level possible with the aid of relevant literature (Hallegraeff et al., 2010; Taylor et al., 2007b; Pienaar et al., 2007; Lange-Bertalot, 2000; Round et al., 1990; Tomas, 1997). All taxa were further checked on the World Register of Marine Science website for current terminology. Transects or fields of view were counted for abundant and small (<5 µm of lenght) taxa. The whole chamber was counted for larger (>5 µm) cells. Abundance (Cells per L) was calculated as follows:

$$Abundance\ (Cells/L) = \frac{A\ (mm^2) * 1000}{a\ (mm^2) * V\ (ml)} * n$$

Here, *n* is the number of cells counted, *A* is the area of the whole chamber, *a* is the area counted and *V* is the volume settled. Dominant taxa were taxa representing ≥10% of the diatom abundance in a sample on any of the sampling sessions. Abundance is presented as seasonal data comprised of the mean of the biweekly data for each season to address objective 1. Biweekly data were presented to explore in detail the variability of community structure within seasons, and to address objective 2.

3.2.4. Data analysis.

To examine the seasonal and spatial diatom community structure in an estuary (objective 1), a Kruskal-Wallis analysis was performed to assess differences in the physico-chemical variables salinity, temperature, turbidity, DO concentration, TON and DIP concentration, across the seasons (summer, autumn, winter, and spring) and the three reaches. The non-parametric test was chosen because the above physico-chemical variables did not meet the assumption of normality after the transformation required for a parametric test. A Permutational Multivariate Analysis of Variance (PERMANOVA) was conducted to determine whether the diatom community in terms of abundance differed with the season and reaches. Only the abundance of dominant diatom taxa was used in this analysis. Before the PERMANOVA analysis, the diatom abundance data were fourth root transformed to minimise the effect of rare taxa. A PERMANOVA pair-wise test was further conducted to determine where seasonal differences existed. To determine the diatom community's spatial and temporal patterns, a non-metric Multidimensional Scaling analysis (nMDS) was performed based on the abundance of all the diatom taxa at the different reaches and sampling occasions. To explore in detail, the variability in the diatom community structure within seasons (biweekly), a coefficient of variation (CV), defined as the ratio of the standard deviation to the mean was used ($CV = SD/\bar{x}$).

To investigate the influence of physico-chemical variables (salinity, temperature, turbidity, TON and DIP concentration) on the occurrence and distribution of diatom taxa in the estuary (objective 2), a Canonical Correspondence Analysis (CCA) was performed. Only dominant taxa were used for this analysis. A Two-way Analysis of Variance (ANOVA) was conducted to determine the presence of temporal and spatial differences in richness. A correlation was conducted between richness and the physico-chemical variables to determine any relationships. To meet the assumptions of the ANOVA, a Shapiro-Wilk normality test was used to test for normality of the residuals and a Levene's test for equality of variance. Existing salinity preference of diatoms was referred from Round et al. (1990). All taxa were further checked on the World Register of Marine Science website and Algae Base website for current salinity preferences.

The Kruskal-Wallis analysis, the Two-way ANOVA, Shapiro-Wilk normality test and Levene's test were performed in R (R Core Team, 2013) and CCA in PAST (Hammer et al., 2001). The PERMANOVA and nMDS were conducted using Plymouth Routines In Multivariate Ecological Research (PRIMER) 6 (Clarke and Gorley, 2005).

3.3. Results

3.3.1. Spatio-temporal changes in physico-chemical environment

A decrease in salinity coincided with a low diatom abundance in November at the upper reaches and a high abundance in March at all sites. Peaks in temperature in March also coincided with high diatom abundance, while trends in dissolved oxygen showed no relation to trends in diatom abundance. In March, increased turbidity and TON concentration coincided with high abundance. However, increased turbidity and TON concentration in April coincided with low diatom abundance.

3.3.2. ***Diatom community***

The diatom community of the Mlalazi Estuary was highly diverse with a total of 182 species belonging to 68 genera recorded across the four seasons for one year. The abundance was generally low, ranging from 3.40×10^2 to 5.67×10^5 Cells/L with 23 dominant genera (Table 3.1) of the 68 genera representing 99.1% of the total abundance. *Nitzschia* (18 species) and *Navicula* (10 species) were the most diverse genera of the dominant taxa. Others such as S*urirella* (9 species), *Amphora* (8 species) and *Achnanthes* (7 species) (Appendix 1 and 2) were also diverse but never accounted for more than 10% of the relative abundance in the sample when present. Mean taxa richness, represented by the number of genera, ranged from 16 to 26 genera per season, increased from the upper to the lower reaches (Fig. 3.1), and showed significant differences between reaches (p = <0.0001, F = 12.18, df = 2) but not between seasons. Rare species were higher in the lower reaches (20) than the middle (15) and upper reaches (12). However, in a sample, at any time, the number of diatom taxa representing ≥10% of the total diatom abundance (dominant taxa) ranged from 1 to 5 and a mean of 2.2 to 2.6 per site. Diatom richness was positively correlated with salinity (p = <0.007, R = 0.34, n = 64).

Table 3. 1: Dominant diatom taxa (accounted for ≥ 10% of the total phytoplankton abundance in a sample) recorded at three reaches of Mlalazi Estuary and their salinity ranges recorded between the 6th July 2014 to the 23rd of June 2015. L = lower reaches, M = middle reaches, U = upper reaches. D represents seasons

when the taxa was dominant. ^{b} = benthic, ^{p} = pelagic, ^{m} = marine and ^{f} = fresh, $^{m\text{-}f}$ = marine to fresh. Salinity (freshwater or marine) and habitat (benthic) preferences of taxa were determined from literature (see section: 3.2.4).

Diatom taxa	Winter			Spring			Summer			Autumn			Salinity range
	L	M	U	L	M	U	L	M	U	L	M	U	
Amphora spp.		D						D			D		27.8-30.0
Asterionellopsis glacialis ^{m}		D		D									23.7-34.0
Brachysira vitrea ^{f}				D					D				24.0-35.5
Cerataulina bicornis ^{m}							D			D			32.8-35.4
Chaetoceros spp. $^{m\text{-}f}$					D	D		D			D	D	22.3-29.0
Coscinodiscus oculoides ^{m}	D		D	D	D	D	D			D		D	18.9-35.0
Cyclotella meneghiniana $^{m\text{-}f}$		D			D		D			D		D	9.6-35.5
Cylindrotheca closterium ^{m}												D	6.33
Detonula pumila ^{m}			D				D						23.0-35.4
Diploneis spp. $^{m\text{-}f}$		D	D		D								18.9-34.8
Entomoneis spp. ^{m}	D												31.8-32.1
Eunotia bilunaris ^{f}			D		D				D				22.5-32.8
Fragilaria ulna ^{f}	D						D	D			D	D	30.9-35.5
Gomphonema pseudoaugur ^{f}								D					32.8
Gyrosigma spp.$^{m\text{-}f}$				D			D						18.9-33.5
Mastogloia spp. ^{m,f}							D						35.5
Melosira sp. ^{m,f}							D		D				23.0-26.9
Navicula spp. $^{m\text{-}f}$	D		D		D	D	D	D	D	D		D	6.3-35.5
Nitzschia spp. $^{m\text{-}f}$				D	D		D	D	D				22.5-35.4
Paralia sulcata ^{m}				D									21.1-35.1
Pseudo-nitzschia sp.^{m}		D	D	D			D						25.1-35.4
Rhizosolenia setigera $^{br,\,m}$	D	D	D										26.4-33.7
Thalassiosira tenera ^{m}					D		D	D		D	D	D	6.3-35.1

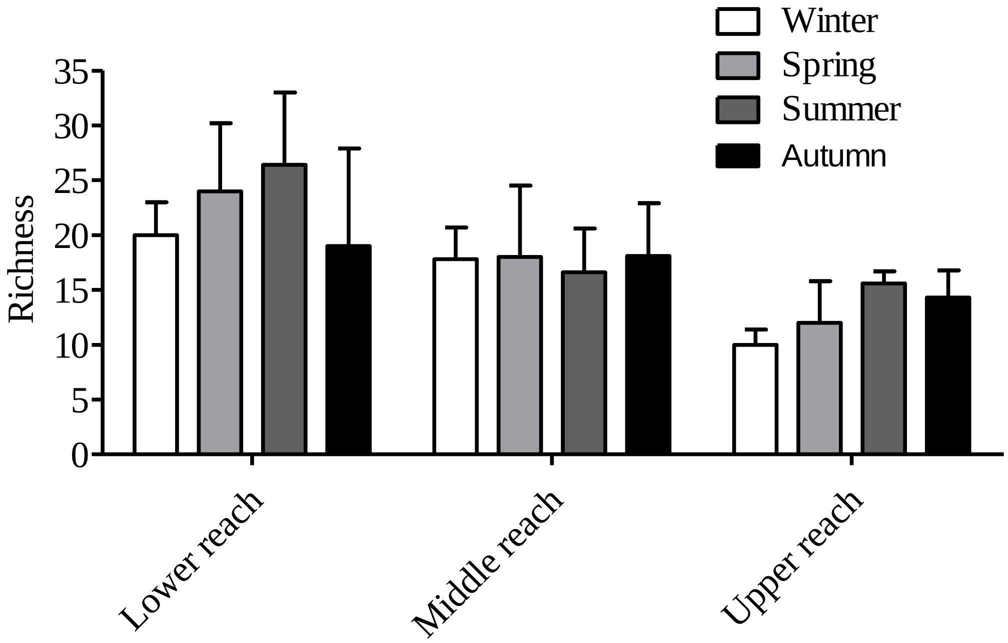


Figure 3. 1: Diatom richness (mean ±SD) in different seasons along the salinity gradient from July 2014 to June 2015.

3.3.3. *Temporal and spatial changes in the diatom community structure*

Taxon richness increased from the upper reaches (44 genera) and middle reaches (46 genera) to the lower reaches (58 genera) of the estuary while diatom density was higher in the upper reaches ($6.66 \times 10^4 \pm 1.36 \times 10^5$ Cells/L) than the lower ($6.22 \times 10^4 \pm 8.44 \times 10^4$ Cells/L) and the middle reaches ($5.20 \times 10^4 \pm 7.00 \times 10^4$ Cells/L) (Fig. 3. 2). Mean abundance was lowest in spring in all three reaches, and in the lower reaches, mean abundance was highest in autumn while in the middle and upper reaches, mean abundance was highest in winter (Figure 3.2). Overall, mean diatom abundance was higher in winter ($1.11 \times 10^5 \pm 1.68 \times 10^5$ Cells/L) than autumn ($7.61 \times 10^4 \pm 8.30 \times 10^4$ Cells/L), summer ($2.45 \times 10^4 \pm 2.46 \times 10^4$ Cells/L) and spring ($2.22 \times 10^4 \pm 2.61 \times 10^4$ Cells/L) (Fig. 3. 2). The nMDS plot shows that samples from the lower reaches were different from the upper and the middle reaches and that some similarity existed between the upper and middle reaches (Fig. 3. 2). Diatom community in terms of abundance differed statistically between reaches (*p* (Perm) = 0.001, pseudo F = 6.74, n = 64). A PERMANOVA pair-wise test shows that communities in all seasons differ significantly ($p \leq 0.05$) except between winter and spring, and winter and autumn (Table 3. 3). There was no observable trend in the biweekly abundance of the diatoms except that they should a high abundance in September and December of 2014, March, and June of 2015 (Fig. 3.2).

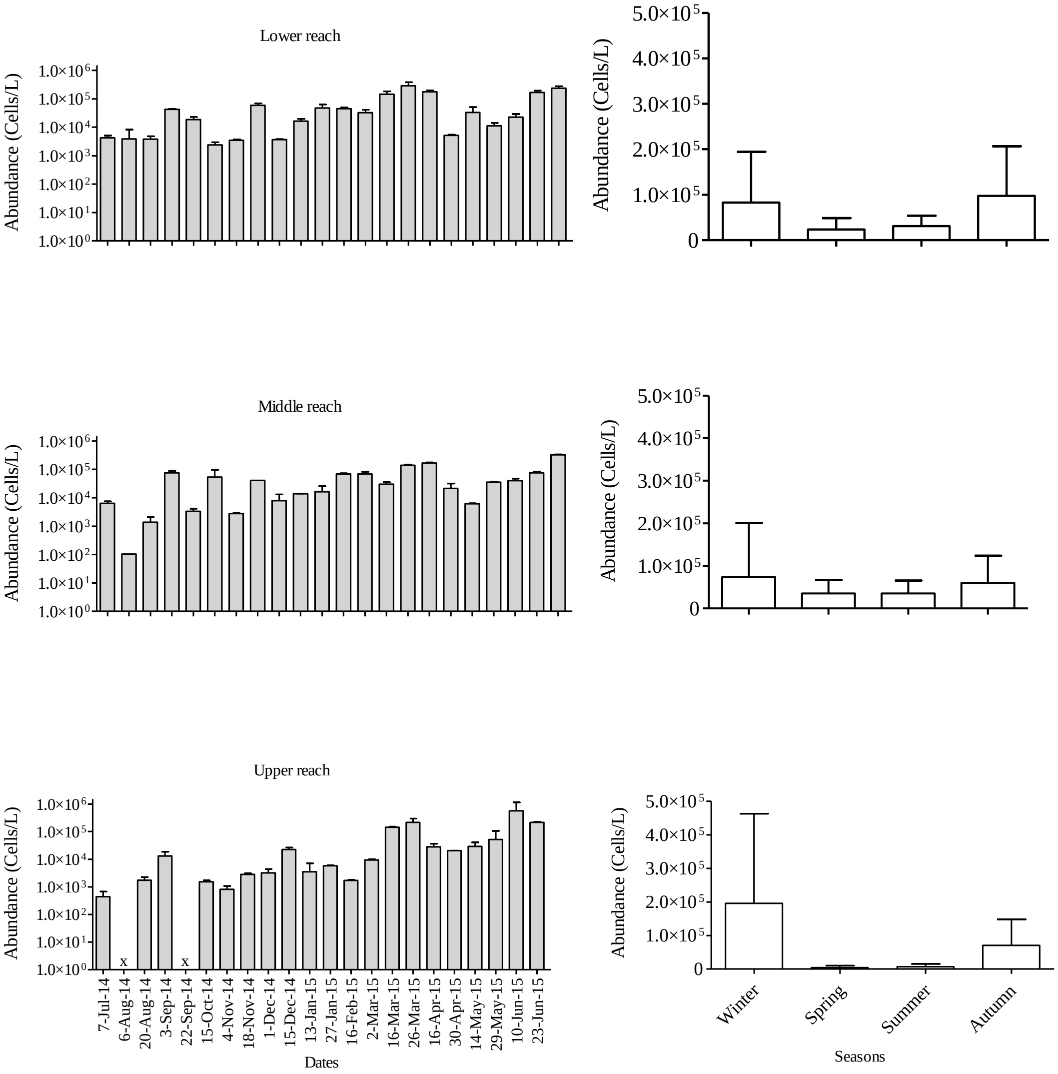


Figure 3. 2: Log$_{10}$ abundance (mean ± SD) of diatoms at all sites per sampling date. X represent dates on which samples were not collected (Left). Abundance (mean ± SD) of diatoms per season at all three reaches of the Mlalazi Estuary for a one-year period from July 2014 to June 2015.

Table 3. 2: Results of a PERMANOVA Pair wise test showing seasons with a significantly different ($p \leq 0.05$) and non-significantly different ($p > 0.05$) diatom abundance. Sample size n was 64.

Groups	t	*p* (Perm)
Winter, Spring	1.24	0.16
Winter, Summer	1.36	0.09
Winter, Autumn	2.10	0.002
Spring, Summer	1.64	0.007
Spring, Autumn	2.89	0.0001
Summer, Autumn	1.98	0.001

Spatially, the taxa accounting for up to half of the abundance in a sample when present varied. At the lower reaches, these taxa were centric, chain-forming (*Cyclotella meneghiniana*, *Thalassiosira tenera*, *Rhizosolenia setigera*, and *Paralia sulcata*), centric, solitary (*Coscinodiscus oculoides*) and colonial, araphid (*Asterionellopsis glacialis*) of which *Coscinodiscus oculoides* and *Paralia sulcata* were dominant taxa in all seasons. At the middle reaches, the taxa accounting for more than half of the diatom abundance were pennate, benthic, or planktonic (*Amphora* spp., *Diploneis* spp. and *Pseudo-nitzschia* sp.) as well as centric, chain-forming (*Chaetoceros* spp. and *Cyclotella meneghiniana*), of which *Cyclotella meneghiniana* was the dominant taxon at all reaches. At the upper reaches, centric, chain-forming (*Chaetoceros* spp. and *Thalassiosira tenera*), centric, solitary (*Coscinodiscus oculoides*), pennate, benthic, or planktonic (*Nitzschia* spp. and *Pseudo-nitzschia* sp.) accounted for more than half of the diatom abundance, as well as pennate, benthic (*Navicula* spp.), which was dominant taxon in all seasons. There was a change in the dominant (important) taxa from mostly centric chain-forming pelagic species at the lower reaches to the inclusion of pennate, benthic taxa at the middle and upper reaches.

A few taxa such as *Coscinodiscus* spp. and *Cyclotella* spp. showed a wide range of tolerance to salinity from the lower to the upper reaches. *Fragilaria ulna, Eunotia bilunaris* and *Gomphonema pseudoaugur* reported as freshwater taxa by Round et al. (1990) showed a wider salinity tolerance. For instance, *F. ulna* was a dominant taxon at all three sites at salinities ranging from 9.61 to 35.5, and high densities of *E.*

bilunaris and *G. pseudoaugur* were recorded at the middle reaches at salinities of 22.5 to 32.8 (Table 3. 1). Spatial patterns in diatom species distribution along the horizontal salinity gradient were obvious for non-dominant taxa (<10% of the total abundance in a sample) such as *Bellerochea malleus*, *Hyalodiscus laevis*, *Hantzschia* spp., *Plagiogramma* sp., *Planothidium delicatulum*, *Tabularia fasciculata* and *Terpsinoë* spp. that were restricted to the lower reaches.

There were five biweekly sampling sessions in the winter season, five in spring, five in summer and seven in autumn. There was high biweekly variability in abundance and species composition. The variability in total abundance was higher within winter than spring, autumn and summer with up to a six-fold increase of the abundance within summer, 16 fold within autumn, 19 fold within spring and 64 fold within winter. With the variability experienced within both a single month and season, sampling monthly or once per season does not provide an accurate record of the temporal changes in the diatom community given the fast growth rate and short lifespan (hours to a few days) of phytoplankton cells (Marra, 2002). Sampling biweekly provided a more realistic temporarily detailed record of the diatom community dynamics.

3.3.4. ***Influence of physico-chemical variables***

The CCA identified the main variables influencing the dominant diatom community composition (i.e., the 23 dominant genera) and abundance as salinity, temperature, turbidity, DO concentration, TON and DIP concentrations. Only axis one and two with eigenvalues 0.56 and 0.34 respectively were significant ($p <$ 0.05) (Table 3. 3) and together represented 74.26% of the variation explained by the CCA analysis. The CCA biplot (Fig. 3. 3) only shows 83% of the dominant diatom genera included in the analysis, however, Table 3. 4 shows all the genera included. The CCA analysis shows that the main physico-chemical variables that correlated with the diatom community and abundance were temperature (0.54), salinity (-0.44), turbidity (0.39), TON concentration (0.28) and DO concentration (0.26) for CCA 1, and DIP concentration (-0.7) and DO concentration (0.14) for CCA 2.

Individual taxa responded differently to the physico-chemical variables. The abundance of *Amphora* spp., *Thalassiosira* sp., *Cylindrotheca closterium*, *Fragilaria* spp., *Nitzschia* spp. and *Chaetoceros* spp. increased with a decrease in salinity and DO concentration and an increase in temperature and turbidity. The abundance of *Paralia sulcata*, *Pseudo-nitzschia* sp. and *Gyrosigma* spp. increased with an increase in salinity and a decrease in temperature. An increase in the abundance of *Detonula* sp. was associated with an increase in DIP concentration. Other taxa such as *Brachysira vitrea*, *Mastogloia* spp., *Melosira* spp, *Cerataulina bicornis*, *Rhizosolenia setigera*, *Entomoneis* spp. and *Navicula* spp. were not significantly associated with any of the variables included in this analysis (Fig. 3. 3).

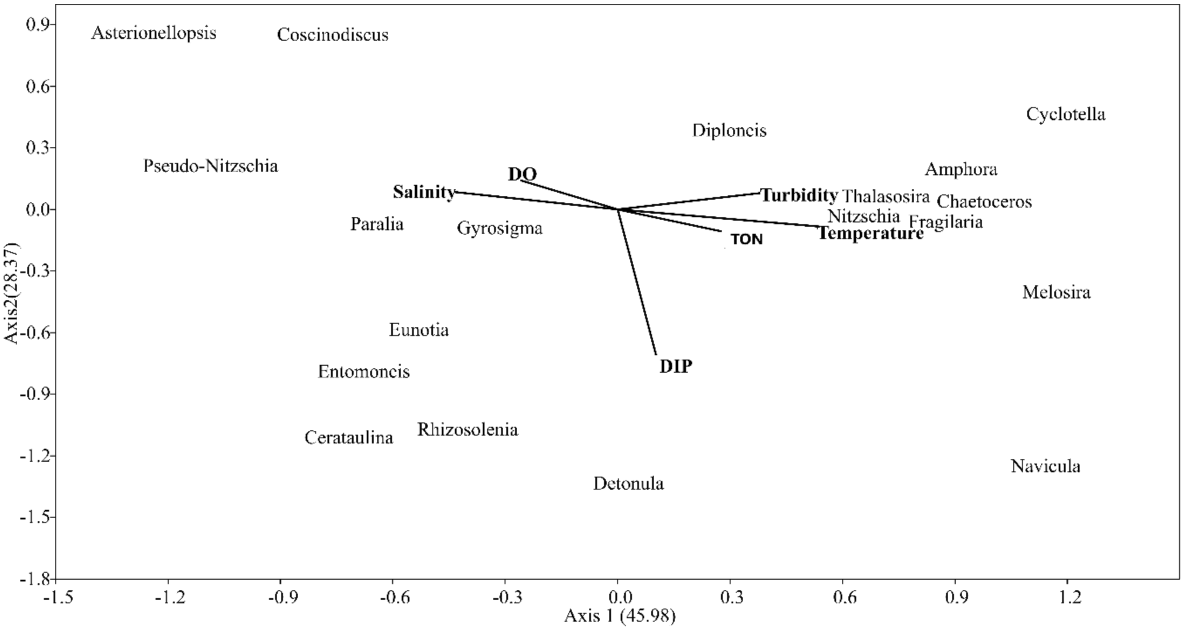


Figure 3. 3: Biplot of canonical correspondence analysis (CCA) relating abundance of dominant diatom taxa (>10% abundance) to physico-chemical variables.

Table 3. 3: CCA results showing the eigenvalues, significance, and percentage contribution of each axis. Here, an axis was significant when *p* was ≤ 0.05. Sample size, n = 64.

Axis	Eigen value	*p*	Percentage contribution
1	0.56	0.015	45.89
2	0.34	0.04	28.37

3	0.20	0.11	16.27
4	0.07	0.58	5.92
5	0.04	0.33	3.53
6	0.00	0.25	0.02

Table 3. 4: CCA results showing the eigenvalues, contribution of each physico-chemical variable and diatom taxa to the significant axes, axis 1 and axis 2. Sample size, n = 64.

	Axis 1	Axis 2
Salinity	-0.44	0.09
Temperature	0.54	-0.08
Turbidity	0.38	0.08
DO	-0.26	0.14
DIN	0.28	-0.11
DIP	0.10	-0.71
Amphora	0.81	0.25
Asterionellopsis	-1.41	0.91
Brachysira	-0.49	-10.12
Cerataulina	-0.84	-1.06
Chaetoceros	0.84	0.09
Coscinodiscus	-0.92	0.91
Cyclotella	1.08	0.52
Cylindrotheca	2.29	-0.64
Detonula	-0.07	-1.28
Diploneis	0.19	0.44
Entomoneis	-0.81	-0.74
Eunotia	-0.62	-0.53
Fragilaria	0.77	-0.01
Gomphonema	1.73	-0.42
Gyrosigma	-0.44	-0.04
Mastogloia	-0.51	-9.23
Melosira	1.07	-0.35
Navicula	1.04	-1.20

Nitzschia	0.58	-0.05
Paralia	-0.72	-0.02
Pseudonitzschia	-1.28	0.26
Rhizosolenia	-0.54	-1.02
Thalasosira	0.59	0.12

3.4. Discussion

This study was conducted to determine the pelagic diatom community structure, its spatial and temporal patterns and the factors influencing the diatom community composition in a subtropical predominantly open estuary of good to fair water quality status. The study showed that overall diatom richness was high, total abundance was low, and no blooms (a species abundance per sample $< 10^6$ Cells/L) were recorded. Abundance showed high variability within seasons and between seasons, with the lowest abundances recorded in winter and the overall abundance was highest in winter. Spatio-temporal variability of the dominant taxa was influenced by salinity, temperature, turbidity, DIP concentration and TON concentrations.

Previous studies on the pelagic diatoms in the Mlalazi Estuary were based on one sampling session in autumn which showed that the diatom community along the entire estuary from the lower to the upper reaches, consisted almost entirely of *Skeletonema*, a centric pelagic marine diatom genus (DWS, 2015) that was not detected in this study period (July 2014 to June 2015). The dominance of a single marine taxon, *Pseudo-nitzschia* sp., along the entire estuarine length was also experienced during this study. This taxon, however, was never dominant on any other sampling date. The biweekly changes in dominant taxa showed that the diatom community structure changes rapidly and cannot be entirely determined with monthly or less frequent sampling. This high variability justifies the importance of the high sampling frequency conducted in this study. Lemley et al. (2015) though analysing samples from once-off sampling trip, similarly, suggested a high sampling frequency in order to record phytoplankton species and understand how they dominate and respond to different environmental conditions.

After light, nutrients are the second most crucial factor needed for the biosynthesis of new phytoplankton cells (Lancelot and Muylaert, 2011). The magnitude with which these variables influence the diatom community and other phytoplankton groups are specific to each estuary (Klais et al., 2015; Lancelot and Muylaert, 2011). Given the shallow nature of the Mlalazi Estuary (1–2.5 m (DWS, 2015)), light is not considered a limiting factor for phytoplankton growth as with most South African estuaries (Nche-Fambo et al., 2015; Adams and Bate, 1999; Grange and Allanson, 1995). The response of diatoms to nutrient concentrations differs spatio-temporally from estuary to estuary (Lancelot and Muylaert, 2011). Total diatom abundance was positively related to DIP concentration (see Fig. 2. 11).

The role of freshwater inflow as a source of nutrients, which leads to high diatom abundance, has been documented for pelagic diatom communities in temperate estuaries (Muñiz et al., 2018; Barbosa et al., 2010; Cloern and Jassby, 2008), some of which from South African estuaries (Dalu et al., 2018; Adams and Bate, 1999). Contrary to the established relationship between summer rainfall, nutrient concentration and phytoplankton biomass, high diatom abundance due to elevated nutrient concentrations during high flow was not observed.

Chain-forming taxa dominated during this study and the previous study on the estuary (DWS, 2015). These taxa, such as *Pseudo-nitzschia* sp., *Chaetoceros* spp. and *Asterionellopsis glacialis* are not limited to good to fair water quality systems as they can dominate both under oligotrophic conditions (Al-Aidaroos et al., 2019; Muñiz et al., 2018; Liefer et al., 2009) as well as under high nutrient and eutrophic conditions (Schnetzer et al., 2013; Liefer et al., 2009; Parsons and Dortch, 2002). Considering other factors such as total abundance and richness, the Mlalazi Estuary is not at the brink of becoming eutrophic despite the presence of the taxa *Pseudo-nitzschia* sp., *Chaetoceros* spp. and *Asterionellopsis glacialis*. The presence of a diatom bioindicator of eutrophic conditions in an oligotrophic system does not always imply a change in ecological status. Chain-forming and colonial diatoms are difficult to ingest for some grazers (Kenitz et al., 2020; Bates and Trainer, 2006). This gives such diatoms a survival advantage over single-celled diatoms and may lead to their dominance in aquatic systems. This type of diatom is mostly marine and dominates if the estuary is tidal (Moser et al., 2017) as was the case with the Mlalazi Estuary, where the upper reaches had a mean salinity of 23.39.

Salinity is the main driving factor for pelagic diatom communities in estuaries (Vajravelu et al., 2018; Moser et al., 2017; Round et al., 1990). Compared to other phytoplankton groups, diatoms have a wider tolerance to different environmental variables (Nassar and Gharib, 2014; Margalef, 1978), hence their omnipresence in aquatic systems. Total diatom abundance was positively related to salinity (see. Fig. 2. 12). The only three freshwater taxa, *Fragilaria ulna*, *Eunotia bilunaris* and *Gomphonema pseudoaugur* (Round et al., 1990) recorded during this study were not restricted to lower salinities but showed a wider salinity tolerance and high densities of *E. bilunaris* and *G. pseudoaugur* were recorded at the middle reaches. Based on the salinity preferences, except for some non-dominant species, diatom taxa had a wide salinity range tolerating marine to brackish salinities, and no true freshwater species were encountered. However, under possible future conditions of decreased freshwater input or droughts leading to the closure of the estuarine mouth (DWS, 2015), there will be limited or no tidal intrusion, and a decrease in salinity as is the case with KwaZulu-Natal temporarily open/closed estuaries (Perissinotto et al., 2010, 2002; Froneman, 2002), possibly leading to a low salinity environment. Under such conditions, the diatom community would shift from predominantly marine and brackish species, as observed in this study to freshwater species not recorded during this study. During this study, richness was higher in the lower reaches, and most of the taxa were rare and marine.

In addition to salinity, the diatom community shows a clear pattern with respect to turbidity. Mixing of sediment from the estuarine floor results in high turbidity and resuspension of benthic diatom taxa. The dominance of such taxa in the water column of shallow estuaries is therefore mostly associated with high turbidity (Nche-Fambo et al., 2015; Brito et al., 2012; Allanson and Baird, 1999; MacIntyre and Cullen, 1996). Whereas, the chain-forming centric taxa that dominated at the lower reaches, was associated with low turbidity. More of the benthic taxa such as *Amphora* spp., *Nitzschia* spp. and *Navicula* spp., dominated at the middle and upper reaches where there was possible re-suspension of sediments. Taxon richness and abundance of benthic diatoms are typically highest in the middle reaches of South African estuaries characterised by high turbidity (e.g., Sundays Estuary (Kotsedi et al., 2012), Gamtoos Estuary (Snow and Adams, 2007) and Kowie Estuary (Dalu et al., 2015)). During periods of high rainfall, an increase in freshwater inflow into the estuary increases turbulence leading to higher turbidity, resuspension of benthic, pennate taxa and increase in freshwater taxa (Thomson and Manoylov, 2019; Walker et al., 2001; Adams and Bate, 1999). Wind-induced mixing is also causing resuspension of benthic sediment leading to high turbidities in shallow (<2m in depth) South African estuaries (Snow and Taljaard, 2007). In shallow estuaries such as the Mlalazi Estuary, resuspension of benthic taxa is possible. This explains why benthic taxa *Amphora* spp. and *Nitzschia* spp., which had a strong affinity to high (7.9 to 13.45 NTU) turbidity, dominated in the middle and upper reaches during summer and autumn when an increase in river flow may have suspended benthic taxa. Based on the results of chapter 2, turbidity is not the main driver of variability in the phytoplankton community as a whole and diatom abundance. Further exploration of the diatom community structure reveals turbidity as an important driver of the variations in the diatom community structure in this estuary.

Diatoms are an excellent food source to a host of herbivores, especially copepods (Alves-de-Souza et al., 2006; Chan et al., 2002; Walker et al., 2001). The role of zooplankton in the top-down control of phytoplankton abundance has been highlighted in many studies (Severiano et al., 2018; Chan et al., 2002; Griffin et al., 2001; Walker et al., 2001; Metaxas and Scheibling, 1996). The zooplankton abundance in the Mlalazi Estuary in summer is 15-fold higher than in winter (Ortega-Cisneros and Scharler, 2014). This higher grazing pressure on the diatoms in summer than winter might explain why the diatom density was highest in winter despite the lower nutrient concentrations in winter.

Several anthropogenic and natural factors influence the phytoplankton community in estuaries, difficult to select just one influencing variable in a system (Lancelot and Muylaert, 2011). In this study, the abundant taxa *Melosira* spp, *Cerataulina bicornis*, *Eunotia bilunaris*, *Entomoneis* spp., *Cerataulina bicornis* and *Rhizosolenia setigera* were not correlated with either axis one or two of the CCA. Implying these taxa were influenced by a combination of the variables the CCA could not detangle or that other factors not analysed in this study such as grazers, silica concentration and ammonia concentration played an important role in the occurrence and distribution of these taxa in the Mlalazi Estuary.

In conclusion, this study presents information on the variability of the spatio-temporal diatom community structure in the Mlalazi Estuary, a system of good to fair water quality status and quantifies the relationship between the diatom assemblage and the physico-chemical variables. Diatoms in this estuary have a wide salinity tolerance, and no dominant taxa could be used to separate salinity regimes within the estuary. Diatom taxon richness was consistent with seasons but varied by site due to the influx of marine taxa (rare) from the ocean. However, changes in freshwater inflow to the estuary, either anthropogenically through damming, abstraction, diversion and discharge or naturally through droughts and floods, will lead to a change in diatom composition. Planktonic centric taxa will dominate under open mouth conditions and increase tidal flow. Pennate benthic forms will dominate under conditions of increased freshwater inflow, leading to changes in phytoplankton productivity and estuarine processes. No blooms were recorded in the estuary during this study due to the low nutrient concentration. However, high species richness was reported, which is characteristic of estuaries with good water quality.

It should be noted that the diatom composition and species abundance may also be influenced by factors other than the six physico-chemical variables, salinity, temperature, turbidity, DO concentration, TON concentration and DIP concentrations analysed in this study. The information provided here is valuable for monitoring future changes in the estuary's water quality given the established knowledge of the distribution of diatom taxa spatially and temporarily in the estuary under good to fair water quality conditions. The high temporal variability in the diatom community structure experienced on a biweekly basis suggests that the diatom community structure cannot be entirely determined with a monthly or less frequent sampling schedule and justifies the importance of a higher sampling frequency, such as biweekly.

Chapter 4. THE SPATIO-TEMPORAL VARIABILITY OF THE DINOFLAGELLATE COMMUNITY IN A SUBTROPICAL SOUTH AFRICAN ESTUARY, WITH A FOCUS ON POTENTIALLY HARMFUL SPECIES

Abstract

Dinoflagellates are one of the most ecologically and economically essential phytoplankton groups due to their role as primary producers, consumers, and causative agents of harmful algae blooms. To understand the factors driving the dinoflagellate dynamics and the key indicator species for monitoring ecological change, the dinoflagellate community was investigated for one year in the Mlalazi Estuary, an oligo- to mesotrophic subtropical predominantly open estuary in South Africa. During this study, 40 dinoflagellate species were recorded. 25% were potentially harmful species of which six, *Gonyaulax spinifera*, *Karlodinium veneficum* (original name: *Gyrodinium estuariale*), *Prorocentrum cordatum*, *P. micans, P. triestinum* and *Scrippsiella trochoidea* individually accounted for more than 10% of the total dinoflagellate abundance in a sample. Other dominant species included *Diplopsalis lenticula, Peridinium quinquecorne, Protoperidinium* sp. 1 and *S. spinifera*. Total abundance was highest in winter and lowest in summer. Mean dinoflagellate abundance per reach ranged from 6.00 x 10^4 to 1.05 x 10^5 Cells/L and a canonical correspondence analysis (CCA) showed that the variability in the dinoflagellate community composition and species abundance observed was influenced by temperature, salinity, turbidity, and nutrient concentration. In the Mlalazi Estuary of good to fair water quality, the highest dinoflagellate abundance was represented by potentially harmful species from the *Prorocentrum* genus. However, due to the low nutrient concentration, which is characteristic of such water quality status, no blooms (>10^6 cells/L) were recorded. This study provides key species and a reference condition for dinoflagellates in estuaries minimally affected by anthropogenic factors. It highlights the importance of improving or maintaining the estuary's water quality, because anthropogenic nutrification, or mouth closure due to reduced freshwater inflow, could lead to bloom of these potentially harmful species, and decline in water quality.

Key words: Total Oxydised Nitrogen (TON), harmful algae, *Prorocentrum*, salinity, South Africa.

4.1. Introduction

Dinoflagellates are autotrophs, heterotrophs, mixotrophs and well-known causative agents of red tides and harmful algae blooms. They are one of the most ecologically and economically important phytoplankton groups in aquatic systems (Carty and Parrow, 2015). Compared to other phytoplankton groups, dinoflagellates are not a preferred choice for grazers (Gordon et al., 2011; Devlin et al., 2007; Domingues et al., 2005). However, some studies have shown that they provide a very good food source for organisms such as other dinoflagellates (Jeong et al., 2001), and zooplankton (Löder et al., 2011) including ciliates (Jeong et al., 2002; Stoecker et al., 1984). In turn, dinoflagellates prey on bacteria, nanoflagellates, microalgae, microzooplankton and injured metazoans (Hae et al., 2005; Jeong, 1999) and therefore provide an important trophic link in aquatic food webs.

The proliferation and presence of dinoflagellates in aquatic systems have been associated with toxic and harmful effects such as anoxia or hypoxia, and shell and finfish deaths (Dai et al., 2020; Wolny et al., 2020; Ndhlovu et al., 2017; Tango et al., 2005). However, potentially toxic dinoflagellate species have also been recorded in oligotrophic estuaries such as the Bay of Biscay (Muñiz et al., 2018). Dinoflagellate species such as *Prorocentrum cordatum* (Ostenfeld) J.D. Dodge [also called *P. minimum* (Pavillard) Shiller] (Heil et al., 2005) and *Dinophysis acuminata* (Moroño et al., 2003) are causative agents of Diarrhetic Shellfish Poisoning (DSP) while other species such as *Alexandrium catenella* and *Alexandrium minutum* are toxic to invertebrate larvae and cause Paralytic Shellfish Poisoning (PSP) (Bravo et al., 2008). It is imperative that in aquatic systems, the dinoflagellates, especially harmful species, are known, monitored, and their driving factors understood. For this reason, dinoflagellates are regularly monitored in some European countries, the United States of America and Japan (Alkawri and Ramaiah, 2010).

In South Africa, most dinoflagellate studies have been restricted to the nearshore marine environment, mostly on the west coast. These studies followed the occurrence of red tides, the impacts of which mostly included hypoxia, rock lobster walkouts and deaths as well as shell and finfish kills (Ndhlovu et al., 2017;

Cockcroft et al., 2008; Pitcher et al., 2007; Cockcroft, 2001). Phytoplankton studies from South African estuaries show that the dinoflagellate community composition varies under different water quality conditions such as in eutrophic conditions in the Sundays Estuary (Lemley et al., 2017; Hilmer and Bate, 1991) and in hypersaline conditions in the St. Lucia Estuary (Nche-Fambo et al., 2015). Dinoflagellates were not recorded under oligotrophic conditions in the Kwesani, Cunge, Mtwendwe and Mtendwe estuaries (Dalu et al., 2018).

Generally, dinoflagellate species have different habitat preferences defined by their adaptive strategies and ecological abilities to survive under specific local environmental conditions (Carstensen et al., 2015; Smayda and Reynolds, 2003; Edler and Olsson, 1985). While salinity and temperature determine the species composition (taxa) (Pitcher and Nelson, 2006), high nutrient concentrations are factors that positively influence the growth of dinoflagellates (Gettings et al., 2014; Alkawri and Ramaiah, 2010). In South Africa, the increase in the degradation of estuaries is associated with mining, agriculture, urbanisation and industrialisation due to human population growth (Van Niekerk and Turpie, 2012). Notably, the increase in algal abundance and biomass in South African estuaries has been associated with the inflow of nutrients from agricultural runoffs and wastewater treatment plants (Adams et al., 2020; Lemley et al., 2018, 2017; Van Niekerk and Turpie, 2012).

There is an increase in the global degradation of estuaries associated with the increase in anthropogenic sources of nutrients associated with algae growth [harmful algae blooms (HABs)] (Paerl and Scott, 2010). The subtropical, predominantly open Mlalazi Estuary of northern KwaZulu-Natal, South Africa (Fig. 2. 1), has been designated a good to fair water quality status (see Section 2.3.1), with low levels of anthropogenic nutrification (DWS, 2015). The water quality status of this estuary provides an opportunity to study the occurrence and drivers of dinoflagellates, including the potentially harmful species, under near-pristine estuarine conditions. The dinoflagellate community structure of the Mlalazi Estuary can provide a reference condition for comparison to future conditions should the water quality of the estuary change. This study aims to assess the seasonal changes to the dinoflagellate community and abundance of the potentially harmful (PH) species in the Mlalazi Estuary. The objectives are to 1, examine the temporal variability in dinoflagellate the community structure (composition, richness and abundance) along the estuary, and to 2) determine the physico-chemical variables influencing the variability in the dinoflagellate community including the PH species.

4.2. Materials and methods

4.2.1. Study area

See section 2.2.1 and Figure 2. 1 for description of the study site.

4.2.2. Physico-chemical measurements

See section 2.2.2.

4.2.3. Dinoflagellate community structure

Duplicate water samples were collected in 250 ml acid-washed polyethylene bottles at subsurface (0.5 m) water level, fixed immediately with 2% acid Lugol's solution, then stored in the dark. For dinoflagellate identification and enumeration, based on density, 20-100 ml samples were settled using the Utermöhl method (Utermöhl, 1958) and viewed under a Nikon *ECLIPSE Ti Series* inverted microscope fitted with a DS-US camera powered by NIS-Elements BR software at magnifications of 400x to 1000x. Taxa were identified to the lowest taxonomic level possible with the aid of relevant literature such as Hallegraeff et al. (2010), Hoppenrath et al. (2009), and Pienaar et al. (2007). All species names were further checked on the World Register of Marine Science website for current terminology. A sum of at least 500 cells was counted per sample following Hillebrand et al. (1999). Abundance was calculated using the equation: Abundance (Cells/L) = $[A(\text{mm}^2) \text{ x } 1000/(a(\text{mm}^2) \text{ x } V(\text{ml}))] \text{ x } n$, where n = number of cells counted, A = area of the whole chamber, a = area counted, and V = volume settled.

For further identification, dinoflagellates cells were prepared for scanning electron microscopy (SEM) according to Tillmann and Hoppenrath (2013). Dinoflagellate cells were isolated from the sample by micro-pipetting, transferred onto a 5 µm polycarbonate membrane (TTTP, Millipore) and washed with distilled water after which they were processed using the alcohol dehydration series, and further dehydrated using 1.1.1.3.3.3-Hexamethyldisilazane (HMDS) according to Tillmann and Hoppenrath (2013). Filters were then mounted on stubs, sputter-coated with gold and viewed using a scanning electron microscope. See Appendix 3 for SEM and light micrographs. Dominant species were regarded as those that contributed >10% of the total abundance of dinoflagellates in any sample, and potentially harmful species were those previously recorded as toxic and causative agents of harmful algae blooms.

4.2.4. Data analysis

A Permutational Multivariate Analysis of Variance (PERMANOVA) using a two-way design was conducted to determine the temporal variability in the dinoflagellate abundance along the estuary (Objective 1). A Bray-Curtis similarity measure was used to quantify the dissimilarity between samples. The abundance was fourth root transformed to reduce the effect of dominant species. The temporal variability of the dinoflagellate abundance and biomass within a site was then investigated from the biweekly abundance and biomass data from July 2014 to June 2015 using the coefficient of variation (CV). The equation used was $CV = SD/\bar{x}$, where SD is the standard deviation, and $\bar{x}$ is the mean of the abundance per sample. The spatio-temporal variability in taxon richness was analysed using a Two-way Analysis of Variance (ANOVA). To meet the assumptions of the ANOVA, a Shapiro-Wilk normality test was used to test for normality of the residuals and a Levene's test for equality of variance.

A Canonical Correspondence Analysis (CCA) was used to determine the physico-chemical variables influencing the dinoflagellate community's spatio-temporal dynamics (objective 2). The CCA was conducted to determine and visualise the relationship between the physico-chemical variables (turbidity, salinity, temperature, TON concentration and DIP concentration) and abundance of dominant dinoflagellate species. A Spearman's Rank correlation was conducted between taxon richness and physico-chemical variables to assess any relationships.

The relationship between freshwater input (rainfall and river flow), water quality parameters (TON, DO and DIP concentrations, temperature, turbidity, and salinity), and the abundance of the two most dominant dinoflagellate species, which are also potentially harmful species (*Prorocentrum triestinum* and

Prorocentrum cordatum) were investigated using Spearman's rank correlations. Spearman's rank correlation uses the rank values between variables to assess their relationship.

The PERMANOVA was calculated in Plymouth Routines In Multivariate Ecological Research (PRIMER) 6 (Clarke and Gorley, 2005). The Kruskal-Wallis and ANOVA were conducted in R (R Core Team, 2013). The CCA was conducted in PAST (Harmer et al., 2001). All data (physico-chemical and biological) was presented on a seasonal basis and on a biweekly basis to explore the influence of the temporal scale. The seasonal data comprises of all biweekly data collected within each season.

4.3. Results

4.3.1. Spatio-temporal variation in the physico-chemical environment

In January, when the temperature was higher (32) and the middle reaches and lower (23) at the lower reaches, *P. cordatum* dominated. In March, when salinity decreased, *P. cordatum* abundance was highest at all sites. While in April, when salinity also decreased, *G. estuariale* dominated in the upper reaches, *P. cordatum* and *G. estuariale* at the middle reaches, and *P. micans* at the lower reaches. *P. triestinum* dominated at the end of May and in June when temperature and turbidity were low, and salinity was high

4.3.2. Dinoflagellate community structure

Forty dinoflagellate species were recorded, 10 of which have been previously reported to produce toxins associated with harmful algae blooms (PH species). The highest total species richness (34) was recorded at the mouth and the lowest (22) at the estuary's upper reaches. Eighteen of the species were common to all three reaches, while 10 species were only recorded at the lower reaches (Table 4. 1).

Mean species richness, represented by the number of genera ranged from 6 to 11 genera per season and increased from the upper to the lower reaches (Fig. 4.1) and showed significant differences between reaches (p = <0.0002, F = 9.99, df = 2) and seasons (p = <0.04, F = 2.91, df = 3). There was no significant interaction between both factors (site and season). Seasonally, mean richness was highest in autumn (irrespective of the extra sampling effort) and lowest in spring (Fig. 4. 1). Rare species were highest at the lower reaches (10) than the middle (8) and upper reaches (4). However, in a sample, at any time, the number of dinoflagellate species representing ≥10% of the total dinoflagellate abundance (dominant species) ranged from 0 to 4 and a mean of 1.8 to 2.1 per site. There was no correlation ($r < 0.5$) between dinoflagellate richness and any of the measured environmental variables.

Dinoflagellate species *Diplopsalis lenticula, Peridinium quinquecorne, Protoperidinium* sp. 1 and *Scrippsiella spinifera* were the only non-potentially harmful species that were dominant at all three reaches. Six of the 10 PH species, *Gonyaulax spinifera, Karlodinium veneficum* (original name: *Gyrodinium estuariale), Prorocentrum cordatum, P. micans, P. triestinum* (formally called *P. redfieldii*) and *Scrippsiella trochoidea* dominated during this study and were recorded at all three sites of the estuary (Table 4. 1). Four of the PH species (*Akashiwo* 1, *Alexandrium catenella*, *Dinophysis acuminata* and *Dinophysis caudata*) were sporadic, occurred in trace amounts (<240 Cells/L) and were recorded at the lower reaches only, except for *Dinophysis acuminata* which was recorded at the middle reaches as well. The PH species *Prorocentrum triestinum* and *P. cordatum* were the most dominant of all recorded species in the estuary, making the genus *Prorocentrum* the most important in the Mlalazi Estuary.

Table 4. 1: The dinoflagellate species and their dominance per site in the Mlalazi Estuary recorded between July 2014 and June 2016. x = Present, D = Dominant species (accounted for ≥ 10% of the total dinoflagellate abundance in a sample) and (*) represents potentially harmful species.

Species	Lower reach	Middle reach	Upper reach
Akashiwo sp. 1*	x		
Alexandrium	x		
*Alexandrium catenella**	x		
Amphidinium crassum		x	
Amphidinium sp. 1	x	D	
Amphidinium sp. 2	D		x
Tripos furca^r	x		
Tripos lineatus		x	
Phalacroma	x		

_Dinophysis acuminata_*	x	x	
_Dinophysis caudata_*	x		
Diplopsalis lenticula	D	D	x
Durinskia capensis	D	x	D
_Gonyaulax spinifera_*	D	x	x
Gyrodinium sp. 1	x	x	D
Gyrodinium sp. 2	x	x	x
Karlodinium veneficum (original name: _Gyrodinium estuariale)_*	D	D	D
Kaneria sp. 1		D	x
Oxyrrhis marina	x	D	D
Oxytoxum sp. 1	x		
Peridinium quinquecorne	D	D	D
Preperidinium meunuri	x		
Prorocentrum sp. 1	x		
_Prorocentrum cordatum_*	D	D	D
_Prorocentrum micans_*[r]	D	D	x
_Prorocentrum triestinum_*	D	D	D
Protoperidinium sp. 1	D	D	D
Protoperidinium sp. 2	D	x	x
Protoperidinium sp. 3	D		
Protoperidinium sp. 4		x	x
Protoperidinium bispinum	x	D	x
Protoperidinium depressum	x		
Protoperidinium pentagonium	x	x	x
Protoperidinium steinii	x	x	D
Scrippsiella sp. 1	x	x	x
_Scrippsiella trochoidea_*	D	x	D
Scrippsiella spinifera	D	D	D
Unknown sp. 1		x	
Unknown sp. 2		D	
Unknown sp. 3	x		

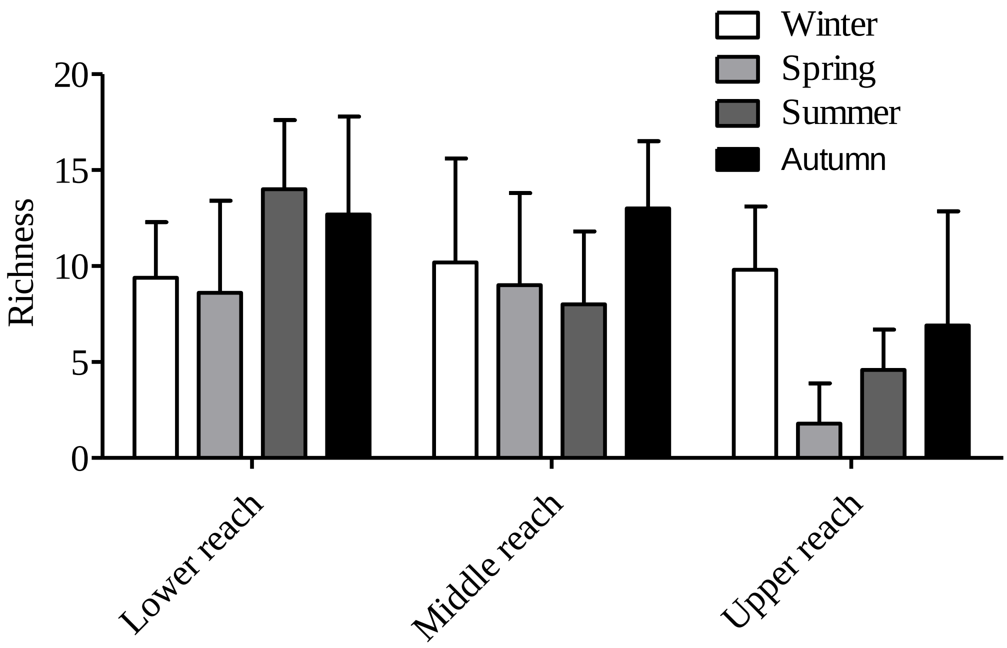


Figure 4. 1: Dinoflagellate richness (mean ±SD) in different seasons along the salinity gradient from July 2014 to June 2015.

4.3.2.1. Seasonal and spatial variability in dinoflagellate abundance

Total Dinoflagellate abundance ranged from 4.32 x 10^3 – 1.56 x 10^5 Cells/L (Fig. 4. 2) and showed significant seasonal and spatial differences (*p* [Perm] = 0.001, F = 2.8, n = 64; *p* [Perm] = 0.001, F = 2.83, n = 64 respectively).

In winter, at all reaches, the dinoflagellate community consisted predominantly of two PH species namely *Prorocentrum triestinum* and *P. cordatum* accounting for >90% of the total abundance with *P. triestinum* representing >56%. In spring, up to 60% of the community consisted of PH *P. triestinum*, *P. cordatum*, *Gonyaulax spinifera* and *Karlodinium veneficum* (original name: *Gyrodinium estuariale*) at the lower and middle reaches, where *P. cordatum* accounted for >38%. At the upper reaches, other dinoflagellates represented 89% predominantly consisting of *Gyrodinium* sp. 1, *Oxyrrhis marina* and *Peridinium quinquecorne* and PH accounted for only 11% of the population. In summer, PH species dominated at all reaches, accounting for >60% of the total abundance, represented predominantly by *P. cordatum* at the lower and middle reaches and by *Scrippsiella trochoidea* and *K. veneficum* (original name: *G. estuariale*) at the upper reaches. In autumn, PH species also dominated, accounting for >60% of the total dinoflagellate abundance, mostly represented by *P. cordatum* and *P. triestinum* at the lower and middle reaches and *P. cordatum* (77%) at the upper reaches (Fig. 4. 3).

Overall, mean abundance was highest at the upper and lower reaches in winter, and at the middle reaches in autumn. The lowest dinoflagellate abundance was reported at all sites in summer. PH species dominated the dinoflagellate abundance throughout the estuary in all seasons except at the upper reaches in spring. Despite the dominance of PH species, there were no blooms and abundance was generally highest at the middle reaches and lowest at the upper reaches (Fig. 4. 3). Variability in abundance was highest and lowest at the middle reaches (CV = 2.05) and upper reaches (CV = 1.25), respectively.

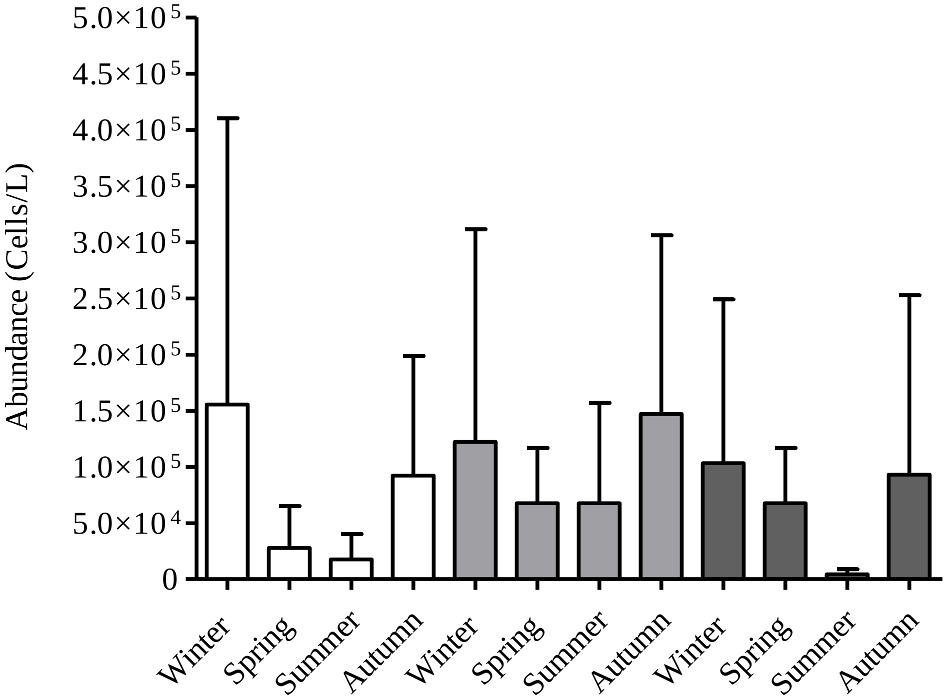


Figure 4. 2: Seasonal variability in dinoflagellate abundance (mean ± SD) per site. White bars = lower reaches; light grey bars = middle reaches; dark grey bars = upper reaches.

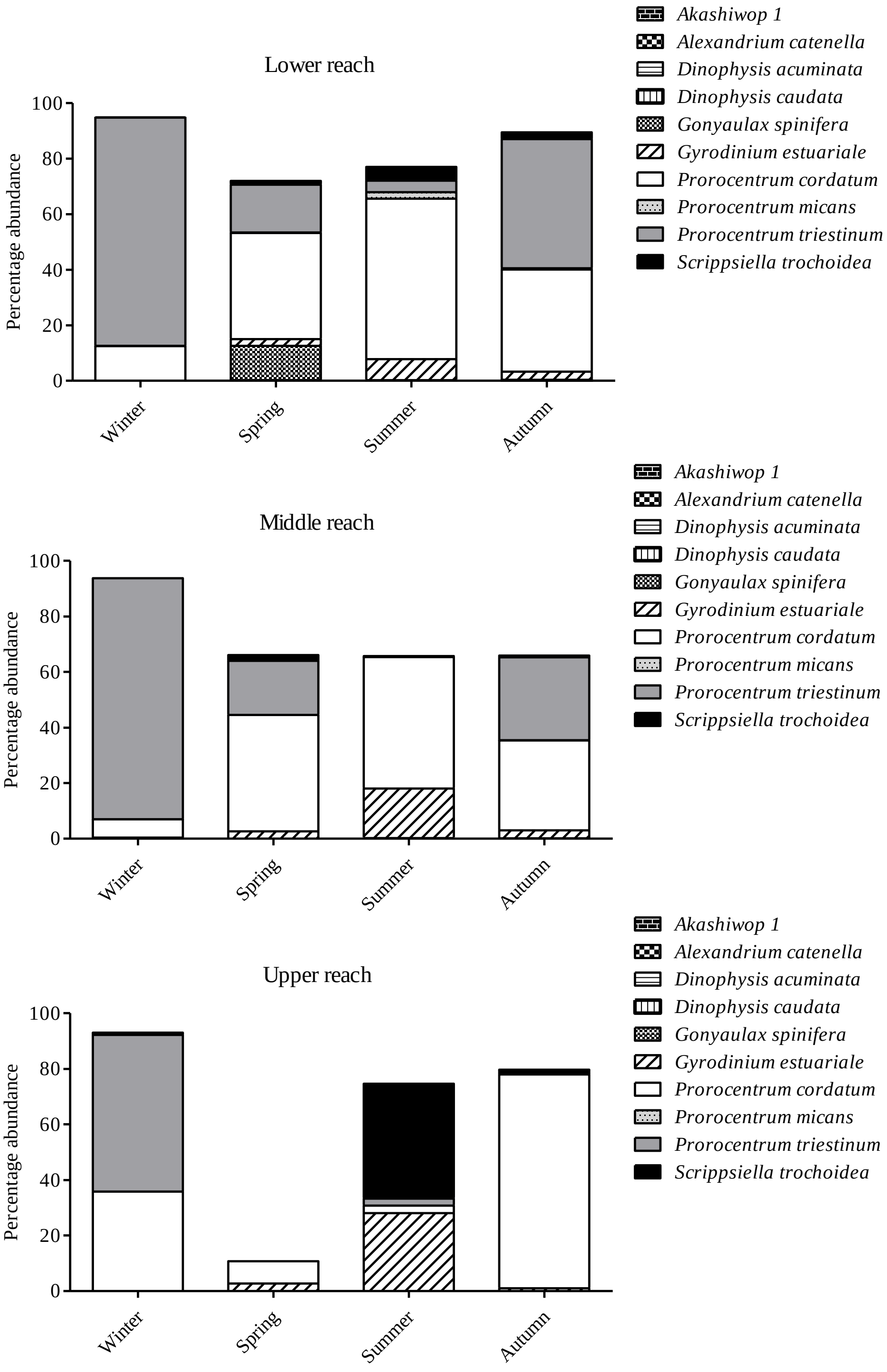


Figure 4. 3: Seasonal variations in percentage contribution of PH species to the total dinoflagellate abundance at all three sites from July 2014 - June 2015.

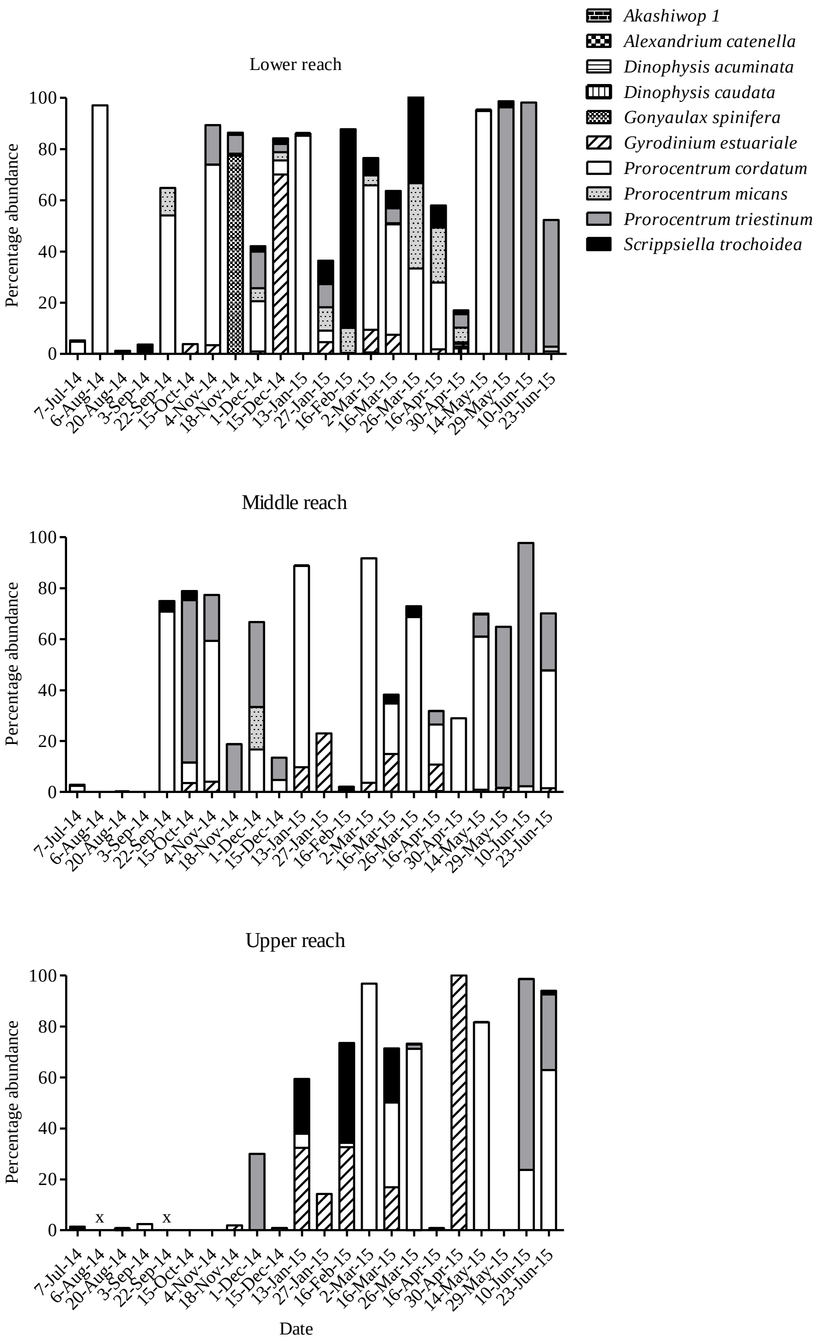


Figure 4. 4: Biweekly variations in percentage contribution of PH species to the total dinoflagellate abundance at all three sites from July 2014 - June 2015. X represent dates on which samples were not collected. Empty spaces represent dates PH species were not detected in samples and non-PH species dominated.

4.3.3. Relationship between dinoflagellate community and physico-chemical variables

The most abundant species, *Prorocentrum triestinum*, and *P. cordatum*, had no significant correlation with either rainfall or river flow ($p > 0.05$, n = 63). *P. triestinum* showed a significant positive relationship with temperature (r = 0.24, $p = 0.03$, n = 63). The CCA was used to relate the abundance of dinoflagellate species with physico-chemical variables and revealed a significant association ($p = 0.03$, F = 1.215), but only explained 15.41% of the variation in the abundance of the species with the two ordination axes (CCA1 and CCA2) accounting for 82.42% of the explained variability in the occurrence (Fig. 4. 5). The physico-chemical variables were temperature (-0.62), DIP concentration (-0.37), salinity (-0.29) and turbidity (-0.27), for CCA1 and TON concentration (0.51), turbidity (0.37) and salinity (0.34) for CCA2 (Fig. 4. 5). Because the CCA results explain only 15.41% of the observed variation in abundance and occurrence of the species, factors other than temperature, turbidity, salinity, TON concentration and DIP concentration used in the analysis might have influenced the dinoflagellate species recorded during this study. The CCA showed that individual species respond differently to these variables. The abundance of the PH species *Karlodinium veneficum* (original name: *Gyrodinium estuariale*), *Prorocentrum cordatum* and *Scrippsiella trochoidea* increased with increase in temperature and DIP concentration. The increase in abundance of the PH species *G. spinifera* and *P. triestinum* were associated with increased salinity and decreases in turbidity and TON concentration. *Prorocentrum micans* was not correlated with any of the physico-chemical variables included in the CCA. With regards to other dinoflagellate species, the abundance of species such as *D. capensis* and *D. lenticulate* increased with an increase in salinity and decreases in TON concentration and turbidity (Fig. 4. 5). The abundance of *Gyrodinium* sp. 1, *Oxyrrhis marina* and *Peridinium quinquecorne* were associated to high temperature and lower salinities. Increase in the abundance of *Protoperidinium* sp. 1 and *P. bispinum* was associated with an increase in temperature (Figure 4. 5) while *Protoperidinium* sp. 2 increased with increasing DIP concentration.

An observation of the shifts in some dominant dinoflagellate species showed no link between changes in their dominance with any specific environmental variable. For example, at the lower reach, change in the dominance of *P. cordatum* (75% of the total dinoflagellate abundance) from the 4th of November to *G.*

spinifera (79%) on the 18th of November was associated with a decrease in DIP (0.5 to 0.3 µM), TON (1.9 to 0.4 µM), turbidity (9 to 6 NTU), and an increase in DO (7 to 8.5 mg/L), salinity (29 to 33), and temperature (19 to 23 ºC). However, on this same site, no measured environmental variable was associated with the shifts from *P. cordatum* (90%) on the 14th of May to *P. triestinum* (90%) on the 29th of May. At the middle reach, the shift from *P. cordatum* (60%) on the 14th of May to *P. triestinum* (60%) on the 29th of May was also not associated with changes in any measured environmental variables.

At the upper reaches, shifts from *G. estuariale* (*Karlodinium venificum*) (98%) on the 30th of April to *P. cordatum* (80%) on the 14th of May was associated with a sharp decrease in TON (90 to 0.2 µM) and turbidity (24 to 8 NTU) and increase in salinity (9 to 22) and DO (2.5 to 6 mg/L). The shifts in dominant PH dinoflagellates on these dates showed that *P. cordatum* and *P. triestinum* dominate under similar environmental conditions while *G. spinifera* dominated under better water quality state (i.e., high DO, low TON concentrations) compared to *G. estuariale* (*K. venificum*), whose dominance was associated with poorer water quality (i.e., high TON, high turbidity, and low DO) (Fig. 2.5, 2.6, 2.7).

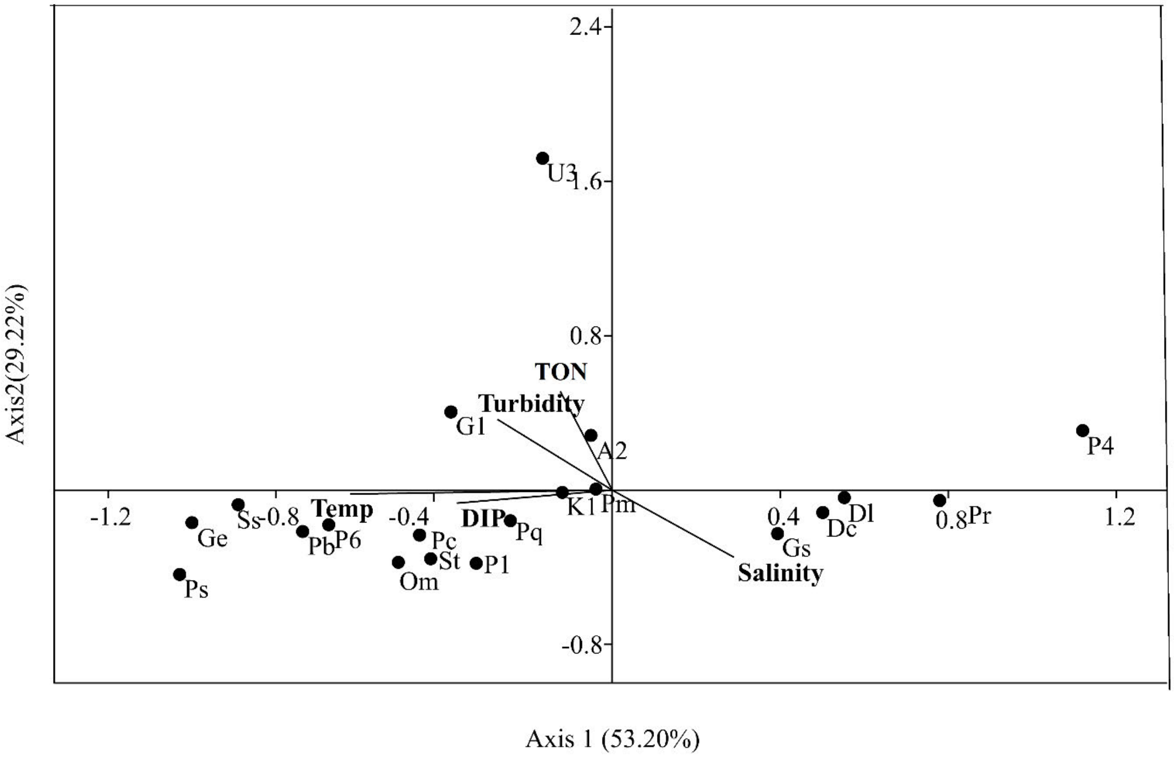


Figure 4. 5: Biplot of canonical correspondence analysis relating abundance of dominant dinoflagellate species (>10%) and physico-chemical variables.

TON- Total oxidised nitrogen concentration, DIP- Dissolved inorganic nitrogen concentration, Temp- Temperature, A1- *Amphidinium* 1, U3- Unknown 3, A2- *Amphidinium* 2, Ps- *Protoperidinium steinii*, Ge- *Karlodinium veneficum* (original name: *Gyrodinium estuariale*), Ss- *Scrippsiella spinifera*, Pb- *Protoperidinium bispinum*, P6- *Protoperidinium* 6, Om- *Oxyrrhis marina*, Pc- *Prorocentrum cordatum*, St- *Scrippsiella trochoidea*, P1- *Protoperidinium* 1, Pq- *Peridinium quinquecorne*, K1-*Kaneria* 1, Pm- *Prorocentrum micans*, Gs- *Gonyaulax spinifera*, Dc- *Durinskia capensis*, Dl- *Diplopsalis lenticulata*, Pr- *Prorocentrum triestinum* and P4- *Protoperidinium* 4.

4.4. Discussion

This study investigated the dinoflagellate community structure and the relationship between the abundance and various environmental variables along the horizontal salinity gradient of the subtropical predominantly open Mlalazi Estuary, which is minimally affected by anthropogenic influences and of good to fair water quality status. Using a data set of dinoflagellate community structure and physico-chemical parameters collected biweekly for one year period of drought, it was found that dinoflagellates varied in species richness, composition, and abundance over spatial and temporal scales.

Dinoflagellate taxon richness varied significantly between seasons and reaches. However, none of the measured environmental variables were significantly related to taxon richness. Spatially, species richness was highest at the lower reaches, with ten species restricted to that site. Most dinoflagellate species are marine, and about 10% of freshwater origin (Hoppenrath et al., 2009). Therefore, these findings agree with the overall trend of increasing dinoflagellate species richness from the upper to the lower reaches of estuaries found in other studies (Quinlan and Phlips, 2007; Nunes et al., 2018; Paerl, 1988). This trend suggests that dinoflagellate species richness in this estuary may be associated with proximity to the ocean. However, there was no relationship between salinity and richness ($r < 0.5$) as was the case with the diatoms

Abundance was highest in the middle reaches of the estuary. In the better studied permanently open Sundays Estuary in South Africa, phytoplankton abundance was highest in the middle reaches, coinciding with high nutrient concentrations (Van Vuuren and Taylor, 2015) as well as turbidity (Snow et al., 2000). Kotsedi (2012) recorded unidentified dinoflagellate blooms in the middle reaches of the Sunday's Estuary while Hilmer, (1990) reported dinoflagellate dominance in the stratified middle reaches of the estuary. Environmental conditions at all reaches, especially the middle reaches favored the growth of K*arlodinium veneficum* (original name: *Gyrodinium estuariale*). The middle reaches are more favourable for overall dinoflagellate growth. Nonetheless, some dinoflagellate species were most abundant at the mouth instead of the middle site such as the PH *Gonyaulax spinifera* and *P. micans.*

Seasonally, the highest cell density was recorded in winter and the lowest in summer, contrary to previous literature where the highest abundances were documented in summer (Roshith et al., 2018; Adolf et al., 2006; Reynolds, 2006; Chan et al., 2002; Gallegos, 1992). Studies have shown that nutrients especially Nitrogen species (ammonia, nitrite, and nitrate), are crucial in the growth and bloom of dinoflagellates (e.g., Lemley and Adams, 2020; Bravo et al., 2008). The slow-growing nature of dinoflagellates compared to other phytoplankton groups such as diatoms (Reynolds, 2006; Lomas and Glibert, 2000; Gallegos, 1992; Lomas et al., 1999) and chlorophytes have been put forward to support this theory. Slow-growing taxa tend to absorb nutrients better when in deficiency in the water. During this study, TON concentration was high in autumn, during which dinoflagellates compete with diatoms that absorb nutrients more than dinoflagellates under such conditions. This could explain why despite elevated TON concentration (maximum 13.20 µM) in autumn, dinoflagellate abundance was highest in winter when their slow-growing nature and ability to absorb nutrients under low concentrations (maximum 1.24 µM) gives them an advantage over other phytoplankton groups such as diatoms. Total dinoflagellate abundance was not directly related to any of the measured physico-chemical variables but instead increased with a decrease in temperature and TON concentration further substantiating the abundance of dinoflagellates in winter. This means that dinoflagellate species' presence or bloom does not indicate an onset of water quality degradation or increase in nutrient concentration in an estuary but rather an ongoing problem.

In this study, 25% of the dinoflagellate species encountered were potentially harmful. Potentially harmful dinoflagellate species are common in eutrophic estuaries (e.g., Lemley and Adams, 2020; Lemley et al., 2017; Bricker et al., 2007; Anderson et al., 2002). *P. triestinum* and *P. cordatum* were the most abundant dinoflagellate species in the estuary. The high total dinoflagellate abundance in winter was due to the PH *P. triestinum* and *P. cordatum*, which showed no relationship with nutrient concentration (see Table 4.2). *P. triestinum*, however, dominated the total dinoflagellate abundance and almost reached bloom densities ($2.02x10^5$ - $5.29x10^5$ Cell/L) at the mouth and middle reaches two weeks after peaks in TON concentration. Therefore, there possibly is a lag time required for this species to assimilate the nutrients as supported by the negative correlation between *P. triestinum* and TON concentration shown by the CCA (see Fig. 4. 4). Interestingly, this species was beyond detection at the upper site during this period even though TON concentration was also at its peak two weeks prior. The abundance and the number of times this species was recorded at the upper reaches was low, and an increase in the abundance of the species was associated with a decrease in salinity. This suggests that the growth of *P. triestinum* favours a spatial gradient of decreasing nutrient concentration and high salinity.

The invasive, cyst-forming *P. cordatum* might have reached high densities because of its opportunistic, high growing, mixotrophic nature, capable of utilizing both organic and inorganic sources of N, P and C nutrients and its ability to survive under a wide variety of temperatures and environmental conditions (Skarlato et al., 2017; Glibert et al., 2012; Heil et al., 2005; Tango et al., 2005). *P. cordatum* is problematic globally and indicates eutrophic conditions due to its association with an increase in anthropogenic sources of nutrients (Glibert, 2020; Lemley and Adams, 2020; Glibert et al., 2012).

Salinity is one of the main factors influencing the dinoflagellate community. However, this species is ecologically plastic in their response to changing salinity (Skarlato et al., 2017; Olenina et al., 2016), grows optimally in low to moderate salinities (brackish environments) (Carstensen et al., 2015; Tas and Yilmaz, 2015; Heil et al., 2005) and explains their dominance along the estuarine length. In South Africa, *P. cordatum* has been noted as a dominant species (>10% of the total abundance) in the St. Lucia Estuary (salinity, 4.62 to 22.44) (Nche-Fambo et al., 2015), in the Gamtoos Estuary (mean ± SD salinity; 28.2 ± 3.9) (Lemley and Adams, 2020) and in this study where it was most abundant in the upper reaches (salinity, 23.39). Therefore, salinity range in the Mlalazi Estuary during this study period was ideal for its growth. This species' ability to grow under a wide range of environmental conditions might explain why this species is not correlated with any of the physico-chemical variables measured in this study.

P. micans is rare and described as sporadic and opportunistic in well-studied estuaries (Sahraoui et al., 2013; Marshall et al., 2009; Reynolds, 2006). In the Mlalazi Estuary, the abundance of *P. micans* was not associated with any of the measured physico-chemical variables. In South Africa, populations of *P. micans* has only been reported in the hypersaline as False Bay of St. Lucia estuarine lake system, at salinities of 42 - 48 (Nche-Fambo et al., 2015; Perissinotto et al., 2013).

Despite the use of the presence of PH species as an indicator of eutrophic conditions (Lemley and Adams, 2020; Glibert et al., 2012), PH species have also been recorded in other oligotrophic systems such as the

Bay of Biscay (21 species, Muñiz et al., 2018). Under optimal salinity and temperature conditions, high nutrient (nitrate, ammonia and phosphates) concentrations characteristic of eutrophic systems are required for dinoflagellates to bloom (Glibert et al., 2012; Heisler et al., 2008; Heil et al., 2005; Tango et al., 2005). In the absence of such blooms, the estuary is considered oligotrophic (Glibert et al., 2012; Heil et al., 2005; Tango et al., 2005). The open estuary mouth, constant flushing and low nutrient concentration under the optimal salinity and temperature conditions of the Mlalazi Estuary could explain why none of the dominant dinoflagellate species reached bloom status. Also, why a low mean chl *a* concentration (<5.84 µg/L) was noted at all three reaches, representing the oligo- to mesotrophic state of the water in the estuary. This suggests that in the future, changes such as an increase in anthropogenic nutrification and a decrease in freshwater inflow which may lead to mouth closure, harmful blooms should be expected. Although *Prorocentrum* species have not been directly linked to toxic effects on humans and marine fauna, they cause adverse ecological effects such as dissolved oxygen depletion and food web structuring (Glibert, 2020; Lemley and Adams, 2020). Other PH species recorded in this study, such as *Alexandrium catenella*, *Karlodinium veneficum* (original name: *Gyrodinium estuariale*) and *Scrippsiella trochoidea*, are directly associated with toxicity in marine fauna (Dai et al., 2020; Wolny et al., 2020). Caution should be taken to improve or maintain the current good to fair water quality state of the estuary to avoid harmful blooms.

In conclusion, this study demonstrates the presence and dominance of PH dinoflagellate species in an oligo– to mesotrophic predominantly open estuary. Accounting for only 25% of the dinoflagellate species in the Mlalazi Estuary, PH species contributed >60% of the dinoflagellate population along the estuary during all seasons, except during spring in the upper reaches. The oligo– to mesotrophic subtropical Mlalazi Estuary provided ideal salinity and temperature conditions for the growth of the PH species. The high abundance of dinoflagellates such as the most abundant species; *P. cordatum* and *P. triestinum* provide a good food source for primary consumers without any harmful effects associated with blooms.

As earlier mentioned in chapter 2, an increase in river flow led to elevated nutrient concentrations. However, the absence of a relationship between river flow, rainfall and the most dominant dinoflagellate species showed that changes in the abundance of these species was not related to variations in river flow or rainfall. Though PH species were not related to the nutrient concentration in this estuary, the low nutrient concentration recorded was not ideal for bloom initiation. However, *G. estuariale* (*K. venificum*), whose dominance was associated with poorer water quality, elevated TON concentration of 90 µM, and low DO concentration of 2.5 mg/L) should be considered a key indicator of possible water quality degradation. Such high nutrient concentrations and near hypoxic oxygen concentrations should also be regarded as thresholds when *G. estuariale* (*K. venificum*) starts to dominate and a threshold for degrading water quality in the estuary. Globally, an increase in nutrient concentration has been associated with dinoflagellate blooms. In South Africa, the only reported harmful bloom of *P. cordatum*, at the predominantly open Gamtoos Estuary (Lemley and Adams, 2020) concurs with the global views that increased nutrient concentration leads to harmful algae blooms. This suggests that anthropogenic nutrient enrichment into the Mlalazi Estuary could potentially lead to harmful dinoflagellate blooms. Dinoflagellate species richness increased from the upper to the lower reaches, was highest in autumn and lowest in spring, but did not relate to any of the measured environmental variables. The dinoflagellate species, abundance and environmental conditions reported during this study provide reference conditions for dinoflagellates in an estuary with minimal anthropogenic effects and can be used by management to monitor future changes in water quality.

Chapter 5. PLANKTONIC CILIATES IN THE OLIGO– TO MESOTROPHIC SUBTROPICAL MLALAZI ESTUARY

Abstract.

Ciliates are ubiquitous protozoans that occur in aquatic systems and dominate the microzooplankton abundance in coastal systems. Ciliates rapidly respond to changing environmental factors. Hence some species are used as water quality bioindicators. To determine if the ciliate community in a subtropical predominantly open estuary could be used as an indicator of future ecological changes, attributes of the ciliate community structure (feeding habit, composition, abundance, and biomass) were investigated at three reaches along the salinity gradient biweekly for one year. A total of 24 ciliate taxa (17 families) were recorded in the predominantly open estuary, 65% of which were present at all three estuary reaches. Overall abundance and biomass differed significantly amongst reaches and seasons, however, the subclasses Choreotrichia and Oligotrichia, typical for good water quality conditions consistently dominated in terms of abundance and biomass. No seasonal trends were observed in taxon richness and diversity. Algivorous ciliates dominated especially under mesotrophic conditions when phytoplankton biomass was high. Temperature, turbidity, salinity, and total oxidised nitrogen concentration influenced the spatio-temporal variations in the ciliate community. However, predictable changes in dominant ciliate taxa could not be observed within the recorded chl *a* (oligotrophic lower reaches to the mesotrophic upper reaches) range. Accounting for the consistency in the ciliate community composition along the horizontal salinity gradient, allowing for taxa such as *Tintinnopsis* spp., *Rimostrombidium* sp., *Strobilidium* sp., *Spirotontonia* sp., *Stenosemella* sp., and *Strombidium* spp. to dominate at all times. The above taxa are therefore key indicators of both good to fair water quality conditions in a predominantly open estuary.

Key Words: Oligotrichs, choreotrichs, water quality, predominantly open estuary.

5.1. Introduction

Ciliates are ubiquitous protozoans that occur in aquatic systems (Agatha, 2011) and dominate the microzooplankton in coastal water bodies (McManus and Santoferrara, 2013; Wasserman et al., 2013; Ojaveer et al., 2010; Kibirige et al., 2002). The very high metabolic rates, rapid growth and high turnover rates of ciliates (Du et al., 2012; Pfister et al., 2002) allow them to rapidly respond to changing environmental factors, hence, some species are used as water quality bioindicators (Basuri et al., 2020; Pfister et al., 2002; Park and Marshall, 2000). The ecological preference of most ciliates is broad (Weisse, 2017), however, certain ciliate species' distribution are known to be influenced by one or more environmental variables (Basuri et al., 2020; Pfister et al., 2002; Kalavati et al., 1997). For example, the species in the order Oligotrichida are more adapted to oligotrophic environments (Beaver and Crisman, 1989). Some species such as *Oxytricha setigera* and *Aspidisca cicada* prefer nutrient-rich environments (Xu et al., 2011; Kchaou et al., 2009; Pfister et al., 2002). Also, Pfister et al. (2002) and Munawar et al. (2020) showed that abundance and biomass tend to be higher under eutrophic conditions (poor water quality) due to high algae biomass when compared to oligotrophic conditions (good water quality).

Food availability measured as chl *a* concentration and bacteria load in aquatic systems influences the ciliate functional groups (based on feeding behaviour) that dominate the system (Munawar et al., 2020; Dolan, 2010; Gonzalez, Juan et al., 1990). Low chl *a* concentration seems to limit the occurrence of algivorous and omnivorous species such as *Loxodes striatus* and *Urotricha furcate.* Algivorous ciliates tend to dominate under high chl *a* concentration condition (Kchaou et al., 2009; Pfister et al., 2002). Bacteria populations under conditions of low dissolved oxygen concentration related to the decomposition of organic material during eutrophication can sustain bacterivorous ciliates (Gonzalez, Juan et al., 1990). However, a species may have more than one feeding mode (Lynn, 2010; Pfister and Arndt, 1998).

Along an estuary with a typical salinity gradient from marine to oligohaline reaches, the highest abundance of ciliates mostly occurs in the brackish regions (Li et al., 2019; Pfister et al., 2002), whereas highest species richness occurs at the estuary's mouth due to the intrusion of marine species, accompanied by a lower abundance (Li et al., 2019; Muylaert et al., 2009; Day, 1981). Xu et al. (2014) and Pfister (2002) suggest that low salinity (<5) restricts marine species, making salinity a vital factor influencing the spatial community structure of ciliates. Seasonally, changes in salinity and temperature influence the ciliate community composition (Onda et al., 2017) while nutrient concentration and chl *a* concentration affect the abundance rather than species type (Pfister et al., 2002). Understanding the ciliate taxon richness, abundance, biomass of ciliates, and the dynamics is important to determine the ecology and state of estuarine systems' water quality.

In addition to water quality properties, ciliates play an essential role in grazing, nutrient regeneration and secondary production in estuarine ecosystems (Anderson and Harvey, 2019; Grinienė et al., 2011; Urrutxurtu et al., 2003) which are one of the most productive on Earth. Phytoplankton (primary producer) as a food source has been shown to control the abundance and biomass of ciliates (Nche-Fambo et al., 2016; Grinienė et al., 2011; Carrasco and Perissinotto, 2012; Urrutxurtu et al., 2003) and changes in the ciliate biomass (carbon) in an estuary influences the biomass of other organisms such as rotifers, cladocerans, and copepods (Azémar et al., 2007; Urrutxurtu et al., 2003), and other higher trophic level organisms. Assessing the spatial and temporal changes in the ciliate community structure (composition, abundance, and biomass) and functional groups in an estuary is therefore crucial in understanding the estuary's ecology.

According to Foissner et al. (2008), ciliates represent the most wholly studied free-living heterotrophic protists due to their large cell size and importance in aquatic food webs. This is, however, not true for South African estuarine systems. So far, of 290 South African estuaries, the community structure and dynamics of ciliates have been reported in one estuary only, the St. Lucia Estuary, under hypersaline conditions (Nche-Fambo et al., 2016). However, certain aspects of ciliates as a group or one of its species have been reported in a few studies. Ciliate abundance was investigated in the Kariega Estuary (Eastern Cape Province) (Froneman and McQuaid, 1997) and the Mpenjati Estuary (KwaZulu-Natal Province) (Kibirige et al., 2002). The role of ciliates in planktonic food webs was investigated in the Kasouga Estuary (Eastern Cape Province) (Wasserman et al., 2013) and the importance of *Fabrea salina* in the planktonic food web of the St. Lucia estuarine lake (Carrasco and Perissinotto, 2012) has been established under hypersaline conditions.

In addition to mesozooplankton, that have generally been well studied in South African estuaries (Cyrus and Blaber, 2015; Montoya-Maya and Strydom, 2009), this study aims to add another piece to the plankton puzzle by contributing knowledge to the spatio-temporal variability of the ciliate community structure in South African estuaries. The study area is a subtropical predominantly open estuary characterised by low nutrient concentrations and good ecological health status (DWS, 2015), representing near-natural conditions and a reference point to monitor future water quality changes.

The following questions were investigated; 1) how does the ciliate community structure change with time along a subtropical predominantly open estuary, and 2) which physico-chemical factors influence the ciliate community structure under oligotrophic to mesotrophic conditions?

5.2. Materials and methods

5.2.1. Study site

See section 2.2.1 and Figure 2.1 for description of the study site.

5.2.2. Physico-chemical measurements

See section 2.2.2 on how the physico-chemical variables (salinity, temperature (ºC), turbidity (Nephelometric Turbidity Unit, NTU), total chlorophyll *a* (chl *a*) concentration, total oxidised nitrogen (TON) and dissolved inorganic phosphorus (DIP) concentrations were measured. In addition to total chl *a*, microplankton and nanoplankton chl *a* size fractions were measured by filtering known volumes of water samples through 20 µm Nitex and 2.0 µm Millipore filters, respectively.

The water quality status of the estuary was assessed using the indicator parameters chl *a*, dissolved oxygen concentration and DIP concentrations and threshold values as per the environmental protection agency for national coastal assessment (EPA NCA) (US EPA, 2001) and the assessment of estuarine trophic status (ASSETS) (Bricker et al., 2003) due to its applicability to South African estuaries (Lemley et al., 2015). Water quality was defined according to US EPA (2001) and Bricker et al. (2003) as good if it was oligotrophic (0 – ≤ 5 µg/L chl *a*), had a DO concentration of >5 mg/L and had a DIP concentration of 0 - ≤ 0.01 mg/L (0 - ≤ 0.11 µM). Fair water quality was defined as mesotrophic (> 2 to ≤ 20 µg/L chl *a*), had a

DO concentration of >2 but ≤5 mg/L and DIP concentration of 0.01 - < 0.1 mg/L (0.11 - < 1.05 µM) (also see Lemley et al., 2015).

5.2.3. Ciliate community structure

Subsurface (0.5 m) duplicate water samples were collected biweekly at the upper, middle, and lower reaches in 250 ml acid-washed polyethylene bottles at subsurface (0.5 m) water level at each site, fixed immediately with 2% acid Lugol's solution, then stored in the dark. For ciliate identification and enumeration, 100 ml of the water sample were settled using the Utermöhl method (Utermöhl, 1958) and viewed under a Nikon *ECLIPSE Ti Series* inverted microscope fitted with a DS-US camera powered by NIS-Elements BR software at magnifications of 400x to 1000x. Ciliates were prepared for scanning electron microscopy (SEM) according to Tillmann and Hoppenrath (2013). Scanning electron micrographs were used to identify cells to the lowest taxonomic level possible using Agatha and Sai (2008), Jiang et al. (2012), Kim et al. (2002) and Lynn (2010) as taxonomic guides. See Appendix 5 for ciliate SEM and light micrographs. For enumeration, the whole Utermöhl chamber was scanned to ensure all cells were recorded. Abundance (Cells per L) was calculated as follows:

$$Abundance\ (Cells/L) = \frac{A\ (mm^2) * 1000}{a\ (mm^2) * V\ (ml)} * n$$

Here, n is the number of cells counted, A is the area of the whole chamber, a is the area counted, and V is the volume settled.

To determine cell volume, linear dimensions (length, width or diameter) of at least 25 cells per taxon per sample were measured using the NIS-Elements BR software and fitted into geometric models according to Vadrucci et al. (2007). Total biovolume per taxon per litre (μm^3/L) was calculated by multiplying the mean cell biovolume for each taxon by the abundance of that taxon per litre. Carbon biomass (pg C/L) was determined by converting total biovolume using a C: biovolume ratio of 0.19 which applies to ciliates of all cell sizes and takes cell shrinkage due to Lugol as a preservative into account (Putt and Stoecker, 1989).

Ciliates were classified into five size classes of <20, 20–35, 35–50, 50–100 and >100 μm based on their longest linear dimension (Müller et al., 1991; Gaedke and Wickham, 2004).

5.2.4. Data analysis

To address question 1, a Permutational Multivariate Analysis of Variance (PERMANOVA) using a two-way design was conducted to determine the differences in the ciliate abundance and biomass between sites and seasons. A Bray-Curtis similarity measure was used to quantify the difference between samples. The abundance was fourth root transformed to reduce the effect of dominant genera encountered. The Shannon diversity index (1-D) was used to examine diversity changes along the horizontal salinity gradient and across time. The temporal variability of the ciliate abundance and biomass within a site was then investigated from the biweekly abundance and biomass data from July 2014 to June 2015 using the coefficient of variation (CV). The equation used was $CV= SD/\bar{x}$, where SD is the standard deviation and $\bar{x}$ is the samples' mean value.

To address question 2, a two-way analysis of variance (ANOVA) was conducted using square root normalised chl *a* data and a non-parametric Kruskal-Wallis analysis was performed on those physico-chemical variables that could not be normalised after transformation (salinity, temperature, turbidity, TON and DIP concentration) to assess their differences between seasons (summer, autumn, winter, and spring) and the three reaches. A distance-based linear model (DISTLM) using the environmental variables (salinity, temperature, turbidity, TON concentration, DIP concentration and chl *a*) and ciliate biomass analysed to determine the physico-chemical variables influencing the variability in the ciliate community structure (Question 2). The physico-chemical variables were normalised using the square root method, and a Euclidian matrix was formed before the DISTLM analysis. The ciliate biomass was fourth root transformed to reduce the effect of dominant genera encountered, and a Bray Curtis similarity matrix was formed before the DISTLM analysis. The stepwise method was used for this model so that only the significant influencing variables were included in the final model. Chl *a* size fractions were correlated against ciliate size fractions to check for relationships.

The PERMANOVA and DISTLM were calculated in Plymouth Routines In Multivariate Ecological Research (PRIMER) 6 (Clarke and Gorley, 2005) and the Kruskal-Wallis analysis was conducted in R (R Core Team, 2013). To answer question 1, all data (physico-chemical and biological) was presented on a seasonal basis and biweekly. This was to explore and better understand the ciliate community's temporal variability given the rapid growth, high turnover rates, and quick response of ciliates to changing environmental conditions (Du et al., 2012; Pfister et al, 2002). This also allows for variations in the ciliate community structure missed from a routine seasonal sampling to be recorded. Seasonal data are the mean of the biweekly data collected within a particular season.

5.3. Results

5.3.1. Temporal variations in physico-chemical properties along the estuary

At the lower reaches, total ciliate abundance was high when the temperature was lower in September 2014 and June 2015. However, choreotrichs dominated in September while oligotrichs and choreotrichs dominated in June. No clear trends in temperature and ciliate abundance were noticed at the middle and upper reaches. For salinity, there was an increase in ciliate abundance with a decrease in salinity in March and April 2015. However, this was only evident at the middle reaches. No apparent trend was noticeable with dissolved oxygen and DIP concentration. There was an increase in ciliate abundance with spikes in TON concentration and turbidity in autumn at the middle reach only.

5.3.2. Abundance and biomass

Although taxa composition was similar throughout the estuary (at all three study sites), abundance and biomass showed clear spatial patterns, differing between sites (Fig. 5. 1) (*p* Perm = 0.001, F = 10.17 and *p* Perm = 0.001, F = 10.16, for abundance and biomass respectively, n = 62). On a biweekly basis, the temporal variability in ciliate abundance and biomass was lowest at the upper reaches with a CV of 0.98 and 1.05 for abundance and biomass, respectively. Abundance and biomass had the highest variability at the middle reaches with a CV of 2.11 and 2.64, respectively. During the study period, seasonally, ciliate abundance was highest in winter and lowest in summer and differed significantly between seasons (*p* Perm = 0.001, F = 2.93, n = 62). During this study, biomass had the same seasonal trend (*p* Perm = 0.001. F = 3.20, n = 62) except in autumn when biomass was highest at the lower reaches and lowest at the upper reaches (Figure 5. 1). Ranging from 0 to 4.05 x 10^4 Cells/L and 0 to 8.22 x 10^6 pg C/L, respectively, abundance and biomass were generally highest in the middle reaches and lowest at the lower reaches (Fig. 5. 1).

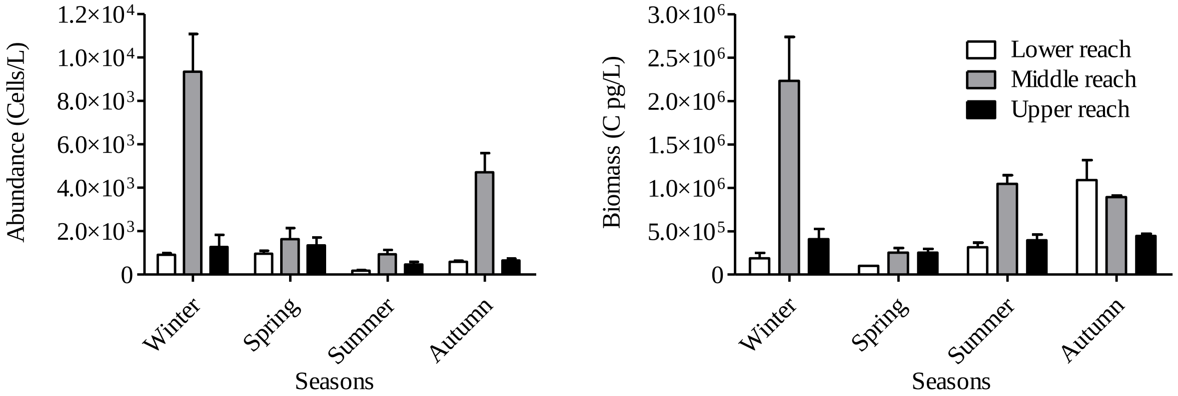


Figure 5. 1: Seasonal variations in ciliate abundance (mean ± SD) and biomass (mean, ± SD) at all three reaches from July 2014 - June 2015.

At the lower reaches, abundance and biomass ranged respectively from 0 to 3.63 x 10^3 Cells/L (Fig. 5. 2) and 0 to 5.82 x 10^6 pg C/L (Fig. 5. 3). Although abundance and biomass varied biweekly, the ciliate taxa at the lower reaches mostly consisted of the subclass Choreotrichia throughout the year except on the 20th of August 2014 and the 23rd of June 2015 in winter when Oligotrichia dominated. Therefore, although taxa composition changed with time, taxa were mostly replaced by others from the same subclass making the single subclass Choreotrichia dominate the ciliate abundance and biomass more than 86% of the time at this site (Fig. 5. 2 and Fig. 5. 3). At the lower reaches, except for hypotrichs in winter of 2014, similar species dominated in winter of 2014 and winter of 2015. The hypotrich, *Euplotes* sp., and haptorian, *Monodinium* sp., represented 6% of the abundance and 1% of the biomass at this site. On the 27th of January 2015 (summer), only the tintinnids *Stenosemella* sp., *Tintinnopsis* spp. And *Tintinnidium* sp. were recorded at the mouth. All three tintinnids are typical marine taxa (Warren, 2018).

At the middle reaches, abundance and biomass ranged from 2.66 x 10^2 to 4.05 x 10^4 Cells/L and 6.45 x 10^4 to 8.22 x 10^6 pg C/L respectively (Fig. 5. 2 and Fig. 5. 3). Here, choreotrichs and oligotrichs accounted on average for >80% of the abundance and biomass. However, on the 7th of July 2014 (winter of 2014) benthic haptorians *Litonotus* sp., *Mesodinium* sp. and *Monodinium* sp. represented 90% of the abundance and biomass. It is not clear why benthic species dominated in this sample. Choreotrich and oligotrichs still dominated in the winter of 2015. Some species were thus unique in the winter of 2014 and absent in the winter of 2015. With no clear biweekly trend, choreotrichs and oligotrichs were dominant in terms of abundance except for samples on the 1st of December 2014 to the 16th of February 2015 when only choreotrichs dominated throughout at this site (Fig. 5. 2). For biomass, choreotrichs dominated throughout the year except in spring samples and most of autumn when the growth of oligotrichs was favoured (Fig. 5. 3). High abundance and biomass were recorded on the 2nd of March 2015 accompanied by a decrease in salinity and an increase in TON concentration. On this date, the taxa *Strombidium* sp. 4 and *Eutintinnus* sp. were most dominant in terms of abundance while *Spirotontonia* sp. dominated in biomass.

The abundance and biomass were generally low at the upper reaches, ranging from 5.0 x 10^1 to 3.04 x 10^3 Cells/L and 1.61 x 10^3 to 1.29 x 10^6 pg C/L, respectively. Also, choreotrichs and oligotrichs dominated, accounting for >90% of the abundance and biomass, respectively (Fig. 5. 3). The coefficients of variation in the biweekly abundance and biomass at this site were lower than at the other study sites. Contrary to the middle reaches, the spikes in TON concentration in autumn in the upper reaches on the 2nd of March 2015 coincided with lower abundance and biomass (Fig. 5. 2). With regards to taxa composition, the benthic *Uronema* sp., known to swim fast when disturbed (Pan et al., 2010) and possibly resuspended into the water column, was recorded only at this site in autumn, on the 2nd of March 2015, accompanied by high turbidity when it represented 21.4% of the total ciliate abundance. At the upper reaches, similar species dominated in the winter of 2014 and winter of 2015.

The subclasses Choreotrichia and Oligotrichia dominated the ciliate community more than 80% of the time along the subtropical estuary, and overall, abundance and biomass differed spatially and showing seasonal differences. With regards to taxon richness, 65% of the taxa were found at all three sites, 25% consisted of benthic ciliates (*Didinium*, *Euplotes* and *Litonotus*) restricted to the middle reaches and 15% consisted of pelagic hyaline taxa (e.g., *Favella* and *Proplectella*) restricted to the upper reaches.

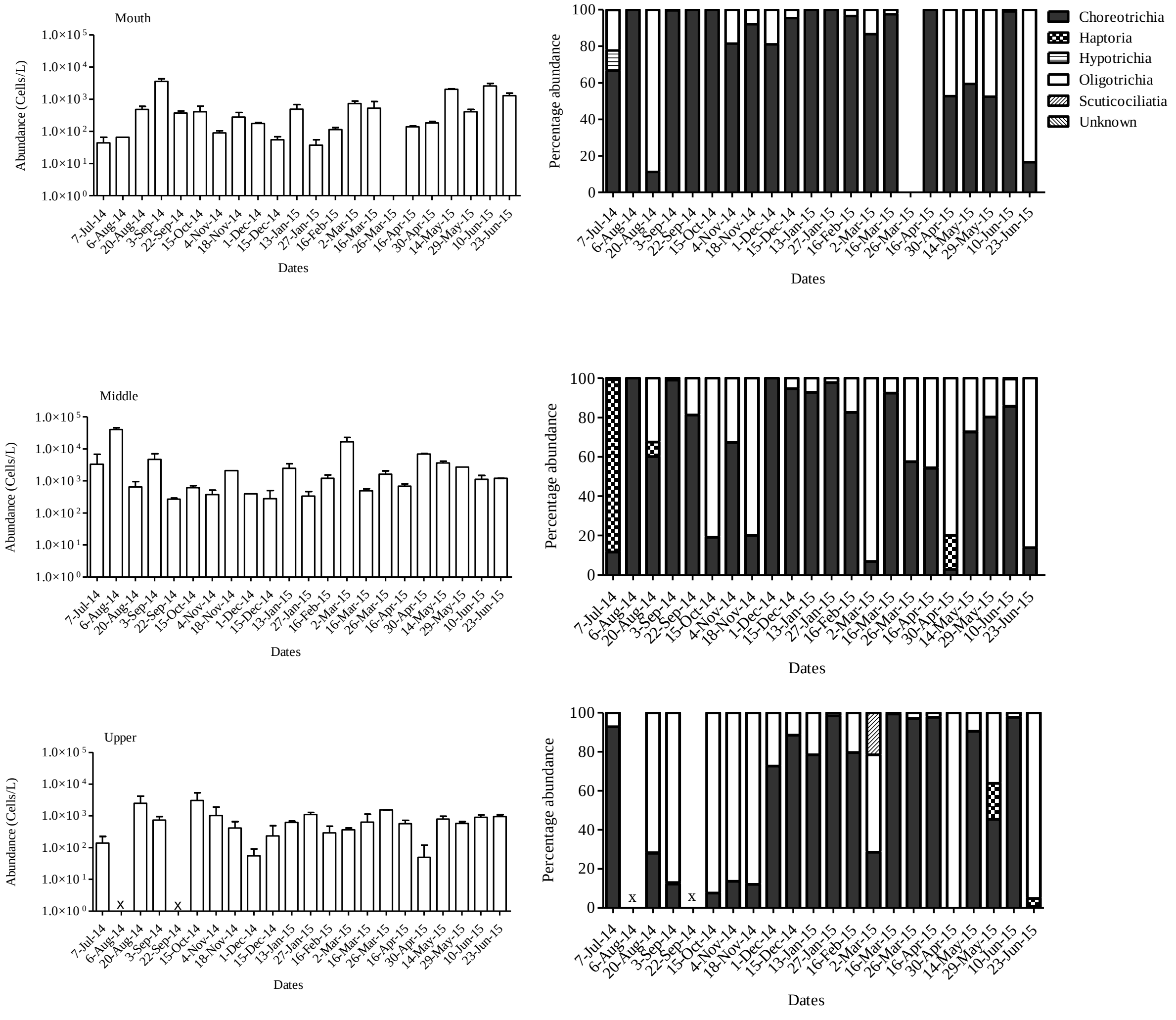


Figure 5. 2: Biweekly variations in total ciliate abundance (log$_2$, mean ± SD, left) and biweekly variations in percentage contribution of ciliate subclasses to the total abundance (right) at all three reaches from July

2014 to June 2015. X represents dates on which samples were not collected and the empty spaces represents when ciliates were not detected in samples, hence the abundance was zero.

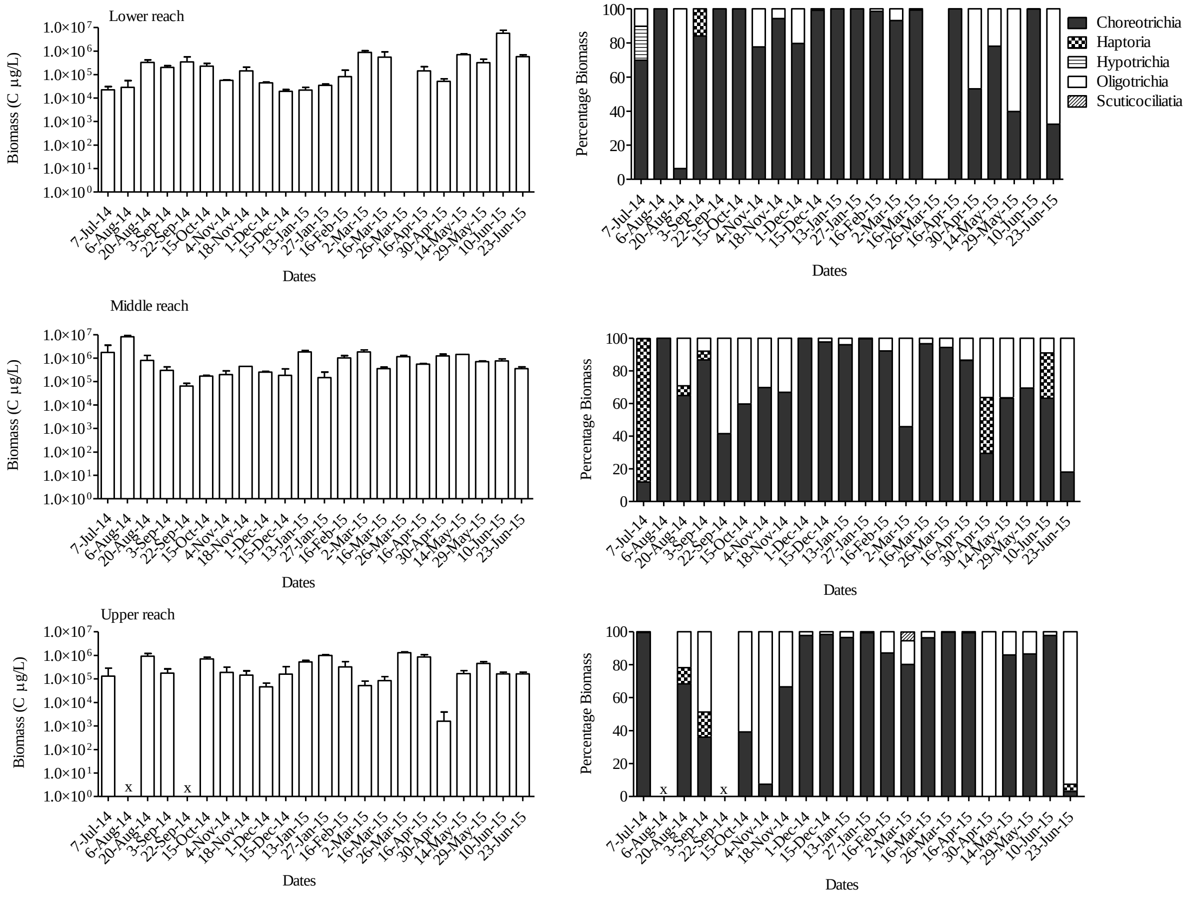


Figure 5. 3: Biweekly variations in total ciliate biomass (log$_2$, mean ± SD, left) and biweekly variations in percentage contribution of ciliate subclasses to the total biomass (right) at lower, middle and upper reaches from July 2014 to June 2015. X represent dates samples on which were not collected, and the empty spaces represents when ciliates were not detected in samples, hence an abundance of zero was used.

5.3.3. Ciliate taxa composition and diversity

A total of 24 taxa belonging to 17 genera and five subclasses were identified during this study (Table 5. 1). 65% of the taxa were found at all three reaches. Resuspended benthic taxa (*Didinium*, *Euplotes* and

Litonotus) were found at the middle reaches only and the pelagic taxa *Favella* sp. And *Proplectella* sp. were restricted to the upper reaches.

Taxon richness and diversity showed different trends and varied both spatially and within each site. Taxon richness was highest in autumn at all reaches (irrespective of the extra sampling effort), and lowest in spring at the lower reaches, winter of 2014 and 2015 for the middle reaches and summer at the upper reaches. Diversity, on the other hand, was highest in summer at all reaches, lowest in spring at the lower reaches and lowest in winter of 2014 and 2015 at the middle and upper reaches (Fig. 5. 4, Fig. 5.5). The biweekly data showed that richness and diversity varied within a season. For example, in autumn, the number of taxa at the lower reaches varied between 0 and ≥10 on two different dates. (Fig. 5. 5). At the middle reaches the highest number (13) of ciliate taxa and lowest number of taxa (1) in that site were recorded within winter of 2014 (Fig. 5. 5). Also, at the upper reaches the lowest taxon richness (1) and highest number of taxa (6) for this site was recorded in autumn (Fig. 5. 5). No taxon was highlighted as characteristic of a specific season. There was no significant relationship ($p > 0.05$) between taxon richness and total abundance on a temporal scale at any of the study sites while diversity-related negatively with the total abundance (R = -0.56, $p = 0.01$, n = 64) at the middle reaches. The highest number of taxa (21) and Shannon-Wiener diversity index (1-D) (0.53 ± 0.25) was recorded at the middle reaches and the lowest (15 taxa and 1-D of 0.30 ± 0.23) at the upper reaches. (Fig. 5. 4 and Fig. 5. 5).

Table 5. 1: Ciliate taxa of the Mlalazi Estuary recorded between July 2014 to June 2015. L = lower reaches, M = middle reaches, U = upper reaches and * represents the first records of ciliate taxa in South African estuaries. Functional groups were determined from Lynn (2008).

Taxa	Subclass	Site	Size class (µm)	Trophic functional group
Cyrtostrombidium sp.*	Oligotrichia	L,M,U	50-100	Algivorous
Didinium sp.	Haptoria	M	35-50	Carnivorous
Euplotes sp.	Hypotrichia	M	35-50	Omnivorous

Eutintinnus sp.*	Choreotrichia	L,M	35-50	Bactivorous, algivorous
Favella sp.*	Choreotrichia	U	50-100	Bactivorous, algivorous
Litonotus sp.	Haptoria	M	50-100	Omnivorous
Mesodinium sp.	Haptoria	M,U	20-25	Algivorous
Monodinium sp.*	Haptoria	L,M	20-25	Carnivorous
Proplectella sp.*	Choreotrichia	U	<20	Bactivorous, algivorous
Rimostrombidium sp.*	Choreotrichia	L,M,M	35-50	Algivorous
Strobilidium sp.	Choreotrichia	L,M,U	35-50	Algivorous
Spirotontonia sp.*	Oligotrichia	L,M,U	50-100	Algivorous
Stenosemella sp.*	Choreotrichia	L,M,U	20-25	Bactivorous, algivorous
Strombidium 1	Oligotrichia	L,M,U	20-25	Algivorous
Strombidium 3	Oligotrichia	L,M,U	20-25	Algivorous
Strombidium 4	Oligotrichia	L,M,U	<20	Algivorous
Tintinnidium 1*	Choreotrichia	L,M,U	<20	Bactivorous, algivorous
*Tintinnopsis tocantinensis**	Choreotrichia	M	50-100	Bactivorous, algivorous
*Tintinnopsis uruguyaensis**	Choreotrichia	L,M,U	20-25	Bactivorous, algivorous
Tintinnopsis 1	Choreotrichia	L,M,U	<20	Bactivorous, algivorous
Tintinnopsis 2	Choreotrichia	L,M,U	50-100	Bactivorous, algivorous
Tintinnopsis 3	Choreotrichia	L,M,U	20-25	Bactivorous, algivorous
Uronema sp.	Scuticociliatia	U	<20	Bactivorous
Unknown 1	Haptoria	M	50-100	Carnivorous

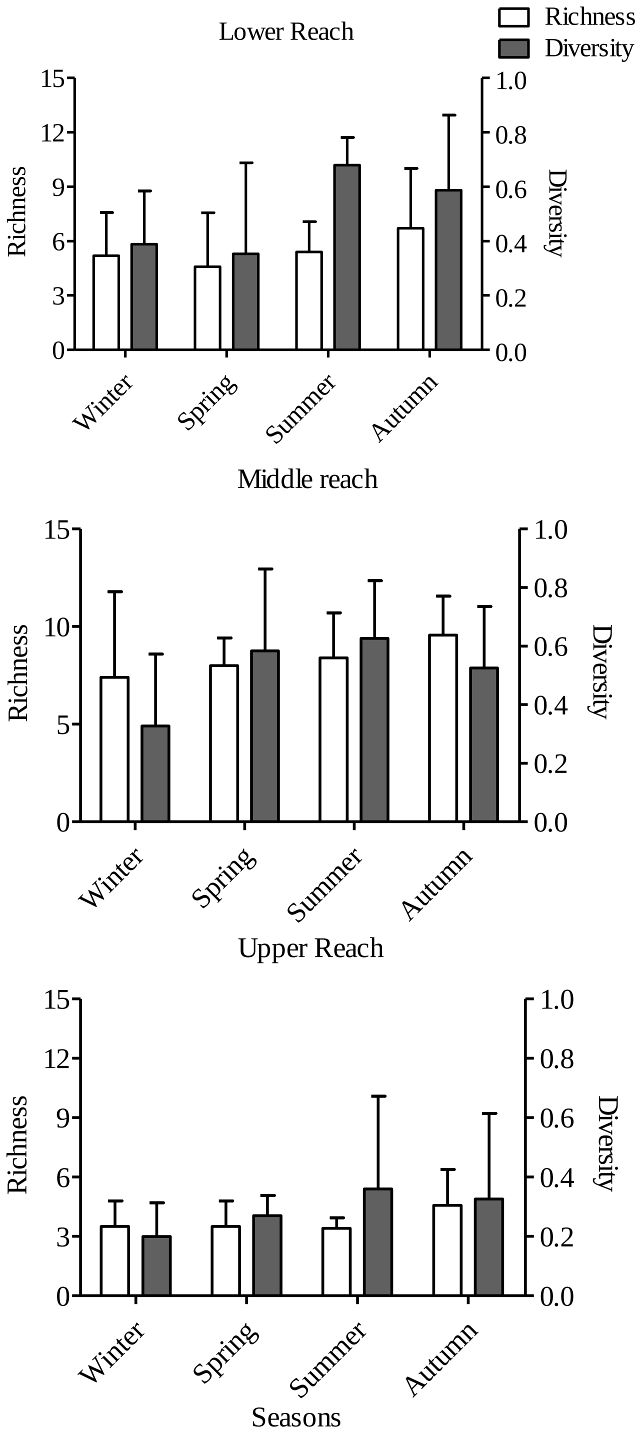


Figure 5. 4: Seasonal variations in ciliate taxon richness (left *y*-axis) and diversity (right *y*-axis) at all three reaches from July 2014 to June 2015.

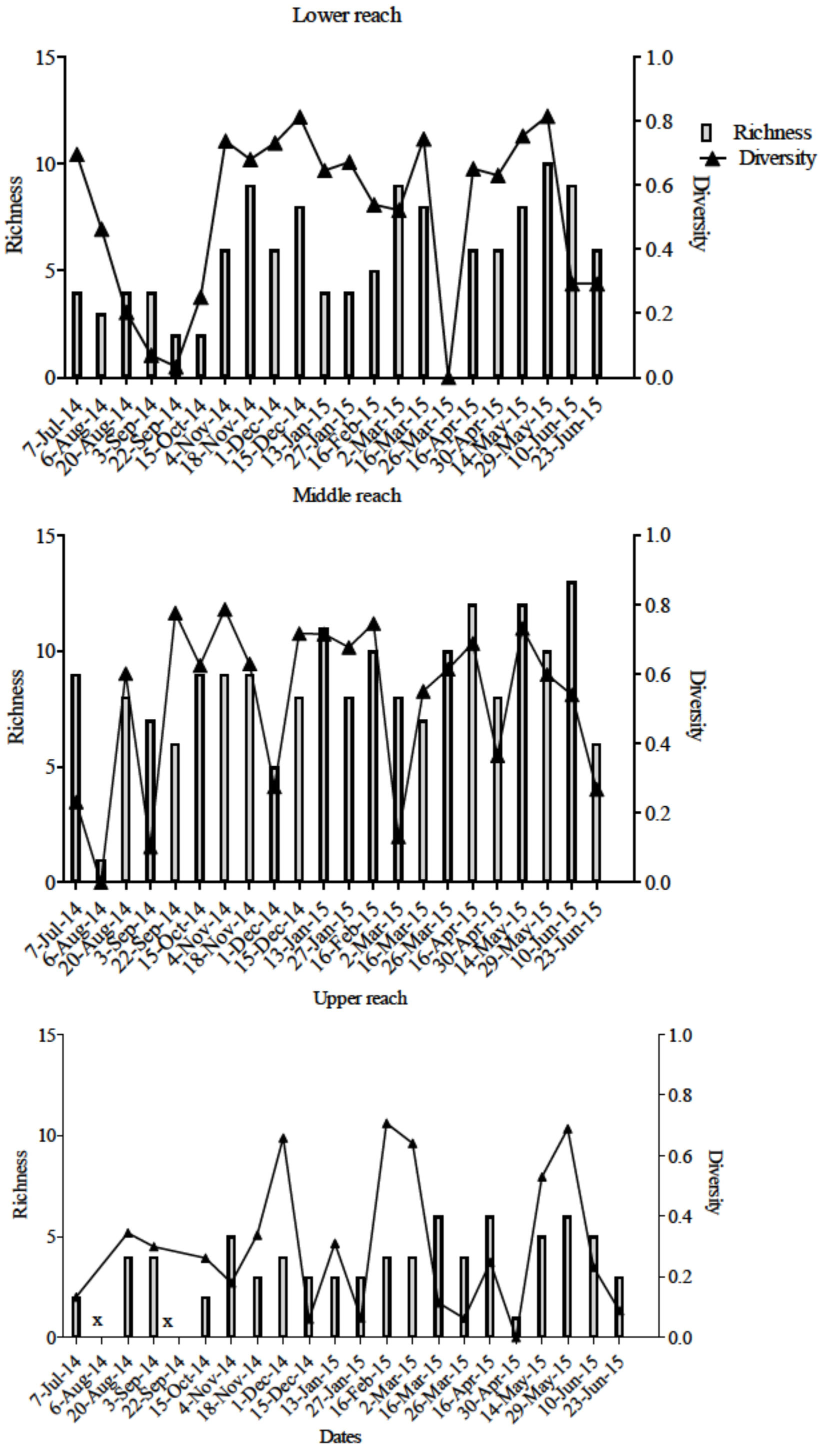


Figure 5. 5: Biweekly variations in taxon richness (left y-axis) and diversity (right y-axis) at all three reaches from July 2014 to June 2015. X represent dates on which samples were not collected.

5.3.4. Relationship between ciliates community structure and physico-chemical variables

Of the physico-chemical variables (chl *a*, salinity, temperature, turbidity, TON concentration and DIP concentration) used in the distance-based linear model (DISTLM), correlations with salinity, temperature, TON concentration and turbidity were significant (Table 5. 2), accounting for 25.6% of the variation observed in the ciliate community in the composition in terms of biomass. This suggests that factors other than the variables above also influenced the ciliate community. Axis one (dbRDA1) (55.5%) and two (dbRDA2) (21.7%) accounted for 77.2% of the explained variation. The variation in the ciliate community was best explained by salinity (0.76), temperature (0.51) and to a lesser extent by TON concentration (0.37) and turbidity (0.15) (Table 5. 3). As previously found, the influencing variables differed with the season, and only turbidity and salinity differed spatially (see Table 2. 1). At the lower reaches, high temperatures and high salinity led to an increase in ciliate biomass. In contrast, high temperatures, high turbidity, and low TON concentration are correlated with the increase in ciliate biomass at the middle reaches. At the upper reaches, high temperatures, low salinity, and low TON concentration led to an increase in the ciliate biomass (Fig. 5. 6). This analysis showed that the total ciliate biomass in the Mlalazi Estuary was not influenced by total phytoplankton biomass (chl *a*). Correlation analysis between the five ciliate and the three chl *a* size class shows only one significant relationship (p = 0.02, R = 0.30, n = 63), which was positive, between small-sized ciliates (20 – 35 µm) and the microplankton size class (2 ≤ 20 µm).

As mentioned above, ciliate taxa were similar in the winter of 2014 and 2015 at all reaches except of the occurrence of hypotrichs in the lower reaches and the dominance of haptorians at the middle reaches in the winter (July) of 2014. Examining shifts in dominant ciliate taxa from haptorians in July 2014 to choreotrichs in August 2014 showed that haptorians flourished in slightly lower temperatures (16.93 ºC) and salinity (22.42) and elevated nutrient levels (3.18 µM) while choreotrichs dominated under conditions of higher temperature (21.46 ºC), salinity (26.63) and lower nutrient concentrations (0.5 µM). Environmental conditions in August of 2014 where like those in June 2015 when choreotrichs and oligotrichs dominated. Shifts in the dominance from choreotrichs to oligotrichs in winter 2014 (6th and 20th of August) and winter

2015 (10th and 23rd of June), were not associated with changes in the measured physico-chemical variables at the lower reaches.

Table 5. 2: Sequential test showing significant physico-chemical variables included in the distance-based linear model.

Variable	Sum of squares	Pseudo F.	*p*	Residual df
Temperature	10882	4.83	0.001	61
Salinity	8322	3.87	0.001	60
TON concentration	10549	5.25	0.001	59
Turbidity	7615	3.98	0.001	58

Table 5. 3: Contributions of the significant physico-chemical variables to the dbRDA axes.

Variable	dbRDA1	dbRDA2
Temperature	0.521	-0.039
Salinity	-0.758	-0.368
TON	-0.365	0.37
Turbidity	0.145	-0.852

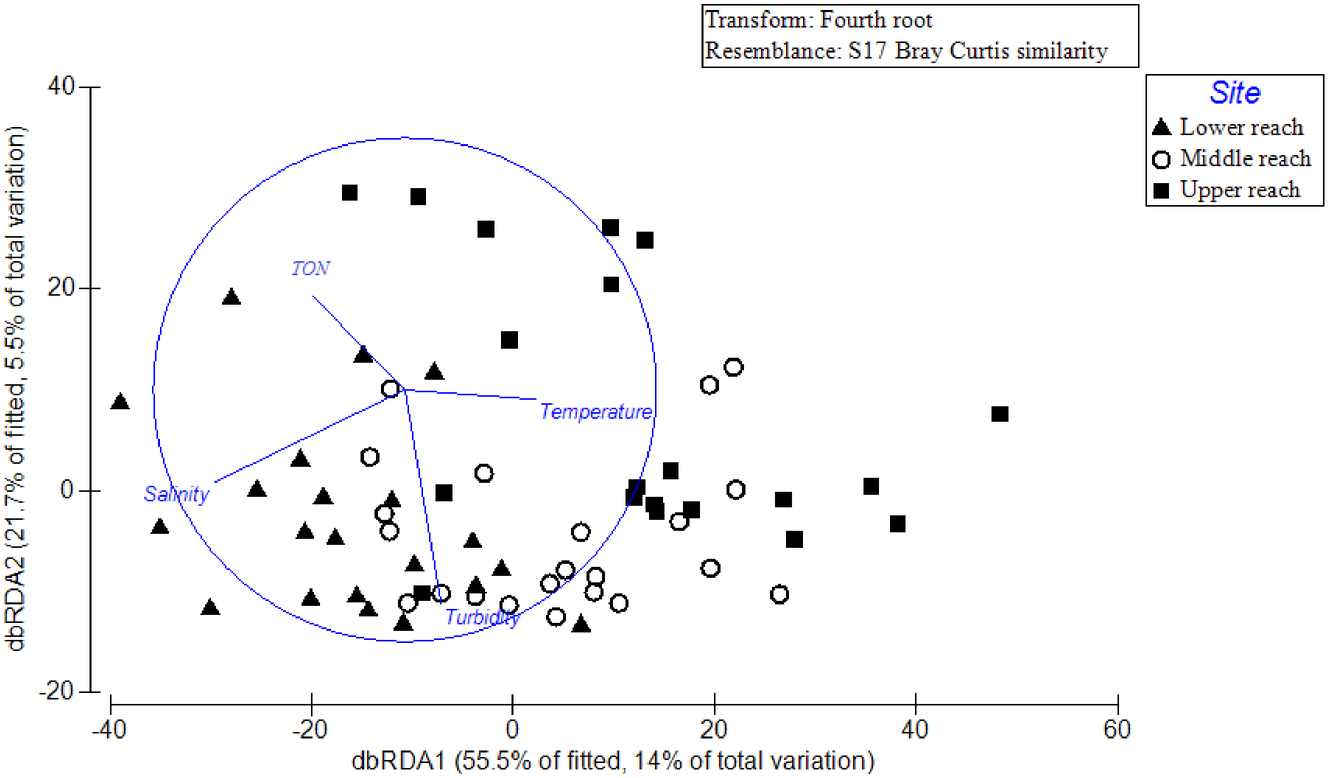


Figure 5. 6: Two-dimensional biplot of the relationship between the significant physico-chemical variables and ciliate biomass with the two primary axes from a distance-based redundancy analysis (dbRDA) of a multivariate multiple regression analysis (DISTLM).

5.4. Discussion

The temporal variation in the ciliate community structure (composition, abundance, and biomass) along the salinity gradient of an oligotrophic to mesotrophic predominantly open estuary was investigated. The ciliate community showed significant spatial and temporal variations, influenced by changes in temperature, salinity, turbidity, and TON concentration. However, 65% of the dominant ciliate taxa were present along the entire estuary and in all seasons.

Ciliates respond rapidly to environmental factors that influence their distribution in aquatic systems (Wu et al., 2018; Pfister et al., 2002; Kalavati et al., 1997). Taxon richness in this study was higher at the lower reaches than the middle and upper reaches, concurring with literature (Li et al., 2019; Lynn, 2010; Lei et al., 2009; Elloumi et al., 2006). This richness trend has been attributed to the high salinity preference of ciliates and the proximity of the lower reaches to the ocean enabling marine taxa to enter the estuary (Lei et al., 2009; Elloumi et al., 2006). Though spatially, richness and diversity were higher at a particular reach, temporally, a high or low richness and diversity could not be associated to any season as within seasons, these attributes fluctuated, recorded both highest and lowest values within the same season.

An increase in taxon richness in this study was associated with an increase in turbidity. High turbidity can result from mixing and resuspension of sediment, which also resuspends benthic ciliate taxa (Telesh et al., 2008; Garstecki et al., 2002). Benthic taxa or non-typical planktonic ciliates such as *Litonotus* sp., *Euplotes* sp. And *Didinium* sp. (Lynn, 2010) found at the middle reaches have previously been associated with resuspension of benthic sediments (e.g., Kchaou et al., 2009). The high relative contribution to biomass (21.4%) of the benthic *Uronema* sp. at the upper reaches, coinciding with a peak in turbidity also confirms its possible resuspension. Due to the estuary's shallow nature [1-2.5 m (DWS, 2015)], resuspension of benthic taxa is possible. 65% of the taxa were present at all reaches. Findings of a South African study showed the spatial distribution of ciliate communities along a large salinity gradient (5 to 157) is mainly influenced by salinity (Nche-Fambo et al., 2016). The narrower salinity range (19.46 – 34.39) from the upper to the lower reaches during this study could explain the lack of unique ciliate communities at each site along the salinity gradient.

The trophic status of an estuary and the phytoplankton size class are other factors influencing the ciliate community structure in an estuary by determining the dominant ciliate size class (Gaedke and Wickham, 2004; Johansson et al., 2004). Large ciliates feed on larger phytoplankton (Johansson et al., 2004) while smaller ciliates dominate when smaller phytoplankton communities dominate (Nche-Fambo et al., 2016; Johansson et al., 2004). Other studies suggest that phytoplankton (prey) type does not influence ciliate diversity and community but rather only prey cell size matters in selecting food (Grattepanche et al., 2019). In the Mlalazi Estuary during this study, a relationship was observed only between the small-sized ciliates (20 – 35 µm) and the microplankton medium size class ($2 \leq 20$ µm). This suggests ciliates in the Mlalazi preyed on phytoplankton similar or larger than their body size and that other ciliate size classes such as the smallest (<20 µm) and large (>50 µm) ciliates had sources of prey in addition to phytoplankton.

Although most ciliates have a broad ecological niche, environmental factors such as nutrient concentration and trophic status (in terms of chl *a* concentration) of the estuary can influence the taxa composition within an estuary. A lower taxon richness is noted in eutrophic areas with high nutrient concentration (poor water quality) as opposed to more types of taxa flourishing in oligotrophic areas with low nutrient concentration (good water quality) (Wu et al., 2018; Xu et al., 2011; Dopheide et al., 2009). It has previously been shown that the dominating ciliate subclass in terms of abundance changes with water quality. For example, under hypersaline conditions, heterotrichs dominate in biomass (Nche-Fambo et al., 2016; Lei et al., 2009), under eutrophic conditions gymnostomatids and scuticociliatids (Xu et al., 2011), and under oligotrophic conditions choreotrichs and oligotrichs (this study, Elloumi et al., 2015; Xu et al., 2011; Beaver and Crisman, 1989). Spirotrichs have been shown as indicators for oligotrophic systems (Kchaou et al., 2009; other references within). However, this group dominates in eutrophic systems as well (e.g., Dhib et al., 2013). In this study, only ~300 Cells/L of a single scuticociliatids taxon, *Uronema* sp., an indicator of poor water quality was recorded once during autumn on the 2nd of March 2015 at the upper reaches only. At this site on that date, TON concentration was high (90 µM) (Fig. 2. 6). The choreotrichs and oligotrichs dominated overall, which are indicators of good water quality in estuaries (Elloumi et al., 2015; Xu et al., 2011). Therefore, the variations in water quality (good to fair) were insufficient for dominant ciliate taxa changes to be observed.

Ciliate abundance and biomass were lowest in summer while ciliate abundance and biomass were highest in winter. This is contrary to previous studies (Zhang et al., 2015; Mironova et al., 2012; Johansson et al., 2004), where abundance was highest in summer rather than winter. Factors other than salinity, turbidity, temperature, and nutrients might also influence the ciliate community, leading to the low ciliate biomass reported in summer. Top-down pressure in the form of predation is a factor known to influence ciliate abundance and biomass in estuaries (Nche-Fambo et al., 2016; Wasserman et al., 2013; Dolan and Gallegos, 2001). Copepods were shown to be the main predators of ciliates in the Kariega Estuary with the biomass and abundance of ciliates increasing in their absence (Wasserman et al., 2013). The zooplankton abundance in the Mlalazi Estuary in summer (wet season) was 15-fold higher than the zooplankton abundance in winter (dry season) (Ortega-Cisneros and Scharler, 2014), implying that there is a higher grazing pressure on the ciliates in summer than in winter. This could explain why ciliate abundance and biomass was highest in winter in the Mlalazi Estuary and not in summer.

Ciliate abundance and biomass increase from oligotrophic to eutrophic conditions (Wu et al., 2018; Auer et al., 2004; Pfister et al., 2002; Pace, 1986). In this study, although ciliate biomass was not correlated to chl *a* concentration (phytoplankton biomass), ciliate abundance and biomass were higher at the mesotrophic middle reaches than the oligotrophic lower reaches. Lower abundance at the lower reaches is representative of oligotrophic marine conditions (Moritz et al., 2006; Lynn and Montagnes, 1991). Ciliate abundance in this study was higher than those in other oligotrophic coastal bodies such as the Gulf of Gabes, Tunisia (Kchaou et al., 2009) (Table 5. 4). Although this system was oligotrophic to mesotrophic, the abundance recorded was within the range of the ciliate abundance in some eutrophic (poor water quality) estuaries such as the Bahia Estuary, Argentina (Pettigrosso and Popovich, 2009), Neva Estuary, Finland (Mironova et al., 2012) and Ghar El Melh Lagoon, Tunisia (Dhib et al., 2013) (Table 5. 4). Ciliate biomass in this study was lower compared to some estuarine eutrophic systems [e.g., Bahia Estuary, Argentina (Pettigrosso and Popovich, 2009)] and Ghar El Melh Lagoon, Tunisia (Dhib et al., 2013) but higher than biomass in the other eutrophic estuaries such as the Neva Estuary, Finland (Mironova et al., 2012) (Table 5. 4). The ciliate abundance and biomass in the Mlalazi Estuary during this study were lower than those of the hypersaline St Lucia Estuary [1.20 x 10^5 Cells/L and 3.20 x 10^9 pg C/L respectively (Nche-Fambo et al., 2016)]. However, the abundance in this study was higher than that of the oligotrophic Kariega Estuary in South Africa where aloricate ciliate abundance was 4.50 x10^2 to 1.95 x 10^3 Cells/L of and loricate ciliate abundance was less than 1.00 x 10^2 Cells/L (Froneman and McQuaid, 1997). Based on the ciliate composition and physico-chemical variables, the Mlalazi Estuary was oligotrophic to mesotrophic during this study. However, the ciliate abundance and biomass recorded during this study were within the range of international eutrophic coastal bodies. In contrast, within South Africa, the ciliate abundance was higher than that of the only recorded oligotrophic estuary.

Table 5. 4: Ciliate abundance and biomass ranges in estuaries of varying trophic status

Coastal body	**Trophic Status**	**Abundance (Cells/L)**	**Biomass (pg C/L)**	**Reference**
Mlalazi Estuary, South Africa	Oligo-mesotrophic	0 to 4.05×10^4	0 to 8.22×10^6	This study
Gulf of Gabes, Tunisia	Oligotrophic	9.4×10^2 to 6.29×10^3	Not reported	Kchaou et al., 2009
Bahia Estuary, Argentina,	Eutrophic	2.00×10^2 to 5.0×10^3	5.5×10^5 to 8.4×10^7	Pettigrosso & Popovich, 2009
Neva Estuary, Finland,	Eutrophic	1.02×10^2 to 1.03×10^4	3.0×10^2 to 5.33×10^3	Mironova et al., 2012
Ghar El Melh Lagoon, Tunisia,	Eutrophic	0 to 5.35×10^3	0 to 4.26×10^7	Dhib et al., 2013

In conclusion, in the subtropical predominantly open Mlalazi Estuary, the dominance of Choreotrichia and Oligotrichia in over 80% of the samples throughout the study period and the low total chl *a* and nutrient concentration supports the oligo- to mesotrophic status of the estuary. The distribution of the dominant ciliate taxa, e.g., *Tintinnopsis* spp., *Rimostrombidium* sp., *Strobilidium* sp., *Spirotontonia* sp., *Stenosemella* sp., and *Strombidium* spp. were not restricted to space and were associated to all seasons. The lack of a shift in ciliate community composition at a subclass level with sites and the presence of 65% of the taxa at all the reaches suggest that the ciliates grow under oligotrophic and mesotrophic conditions. The temperature and chl *a* concentration ranges were too narrow for the ciliates to experience changes in the dominating taxonomic groups temporally and along the salinity gradient, which allowed for the same group of ciliates to dominate temporarily and spatially. Therefore, variations in temperature and phytoplankton biomass (chl *a* concentration) wider than 22.3 to 24.8 °C and 4.47 to 9.63 µg/L, respectively, required to record changes in ciliate community composition. Findings of this study indicate a positive relationship between temperature and ciliate biomass at all sites, a positive relationship between salinity and ciliate biomass at the lower reaches but a negative relationship with ciliate biomass at the upper reaches. As shown in this study and stated by Wu et al. (2018), the relationships between ciliate communities and environmental variables are subject to local conditions of the systems studied. In addition to temperature and salinity, changes in other ciliate community attributes (taxon richness, abundance, and biomass) were influenced by the differences in turbidity and TON concentration along the estuary's salinity gradient. Except for the Scuticcociatids, *Uromena* sp. which can be used as an indicator of declining water quality (TON concentration of 90 µM), the dominant ciliate taxa present, their abundance and biomass in this study can be used a guide of what to expect in an oligo– to mesotrophic predominantly open subtropical estuaries with limited anthropogenic influences.

Chapter 6. GENERAL CONCLUSIONS AND SUGGESTIONS FOR FUTURE RESEARCH

Microplankton (phytoplankton and ciliates) community structure (composition, abundance, and biomass) respond rapidly to changes in the aquatic environment, making them good bioindicators of aquatic ecosystem health (Grinienė et al., 2011; Paerl et al., 2009; Blanco et al., 2008; Pfister et al., 2002). Despite the global increase in the water quality degradation of estuaries (Paerl and Scott, 2010), accompanied by harmful algae blooms, only a few studies in South African estuaries (most of which are already degraded systems) have evaluated the phytoplankton community and assessed the effects of physical and chemical water properties on the community (e.g., Lemley and Adams, 2020; Lemley et al., 2018, 2017; Kotsedi et al., 2012; Van Niekerk and Turpie, 2012). The studies examining non-degraded South African coastal systems have greatly been limited to the country's south-east coast (warm temperate coastal region) (Lemley and Adams, 2020; Dalu et al., 2018, 2014; Gama et al., 2005).

In South Africa, estuarine systems are one of the most important, the most threatened, and the most difficult ecosystems to protect (Skowno et al., 2019). The Mlalazi Estuary, in the subtropical coastal region, located within the Umlalazi nature reserve and under the protection of the Ezemvelo KwaZulu-Natal Wildlife, a provincial conservation agency was classified as having a good ecological status (DWS, 2015). 53% of the subtropical predominantly open estuaries in South Africa are highly vulnerable (Whitfield and Baliwe, 2013), implying possible future degradation due to a combination of anthropogenic influences. The Mlalazi Estuary provided a good study area for investigating the spatio-temporal variability of the microplankton community structure and its drivers in an aquatic system of good to fair water quality status, with no severe anthropogenic influences. Given that phytoplankton forms the base of the estuarine food web, ciliates play a vital role in the microbial loop and both ciliates and phytoplankton can be used as bioindicators of ecological changes in estuaries (Konoplya and Soares, 2011; Ramdani et al., 2009; Fielding et al., 1991), understanding the spatio-temporal variability in the microplankton community attributes (phytoplankton biomass, composition, species richness, and abundance, ciliate biomass, composition, abundance, size structure and feeding mode) is essential in using microplankton as a measure of possible future changes in the water quality of an estuary and provides insights on estuarine functioning.

Phytoplankton biomass [chlorophyll *a* (chl *a*)] and phytoplankton abundance measurements are methods of quantitatively examining phytoplankton community structure (Karlson et al., 2010). Phytoplankton species that dominate in abundance do not necessarily dominate in biomass (Nche-Fambo et al., 2015) and here, phytoplankton biomass and abundance showed different seasonal patterns. Therefore, despite the common use of phytoplankton biomass and or phytoplankton abundance to evaluate water quality changes, biomass does not always reflect abundance or the species blooming, which may be toxic. This study combined phytoplankton attributes; species composition, the abundance of individual taxa and total biomass and showed a reflection of the changes in water quality parameters. Therefore, in addition to phytoplankton biomass, which is easier to assess, the enumeration of phytoplankton species, should be done to best evaluate water quality changes and determine baseline information (species and abundances) in the use of phytoplankton as water quality indicators for future changes in estuaries.

Phytoplankton richness in the estuary showed spatial, but no temporal differences and the mean number of dominant species were always consistent across time and space, despite the change in the dominant taxa's composition with time and space. A few taxa, always ≤7, dominated at any time. The same trend in phytoplankton richness was reported for diatoms, but dinoflagellates showed both spatial and temporal differences in taxon richness. No environmental variable was highlighted as a filter for dinoflagellate richness as well. Therefore, at a local scale, within the Mlalazi Estuary, none of the measured environmental variables influenced phytoplankton richness significantly and the spatial trend in richness was due to the estuary's proximity to the sea. In the Mlalazi Estuary, total phytoplankton richness cannot be used as a proxy for water quality, instead, the richness of dinoflagellates can be used.

Shifts in phytoplankton community structure, leading to changes in the planktonic food web and other biological processes, are usually influenced by salinity variations due to freshwater discharge (Cloern et al., 2017; Hemraj et al., 2017). Diatom species have been used as indicators of the different salinity zones in estuaries, i.e., marine, brackish, and freshwater zones (Liu, 2008). However, some species are resilient and have a wide salinity tolerance. Here, the changes in diatom taxa type from centric, chain-forming taxa at the lower reaches to an inclusion of more benthic, pennate taxa at the upper reaches indicated a variation in the primary physico-chemical variable influencing the community (especially salinity and turbidity). Regarding microplankton composition, in subtropical estuaries with a narrow water quality range (good to fair water quality), the ciliate composition cannot determine changes in water quality within the estuary. Still, the species *Tintinnopsis* spp., *Rimostrombidium* sp., *Strobilidium* sp., *Spirotontonia* sp., *Stenosemella* sp., and *Strombidium* spp. are indicators of a good to fair water quality. The dominance of choreotrichs and oligotrichs, the lack of blooms, and the high phytoplankton diversity are expected in estuaries of good water quality. The presence of *Prorocentrum triestinum* and *P. cordatum*, *Pseudo-nitzschia* sp., *Chaetoceros* spp., and *Asterionellopsis glacialis* in the Mlalazi Estuary does not imply Mlalazi is eutrophic or at the brink of becoming eutrophic as supported by other phytoplankton attributes such as biomass, abundance, and richness.

Potentially harmful species such as *P. cordatum* also occur in oligotrophic coastal water bodies such as the Southeastern Bay of Biscay (Muñiz et al., 2018). In subtropical South African estuaries, PH dinoflagellates are present in non-eutrophic estuaries (e.g., St Lucia Estuary (Nche-Fambo et al., 2015) and this study) while in the warm temperate region, these species have not been found in four oligotrophic estuaries (Dalu et al., 2018) but reported in its eutrophic estuaries (Lemley and Adams, 2020; Lemley et al., 2017; Kotsedi et al., 2012). Therefore, the presence of PH species did not imply that the water quality is degraded or poor. As bioindicators, emphasis should be laid on the abundance and toxicity of this PH species rather than their presence or absence in a system. Note, however, that dinoflagellates have been analysed and recorded only in two estuaries in the South African subtropical region. More estuaries in this region should be studied to confirm this suggestion.

Freshwater input was the primary source of nutrients in the Mlalazi Estuary. However, elevated nutrient concentrations (primary triggers of blooms) can be achieved anthropogenically. Globally, the growth in the human population has caused unprecedented land and freshwater use, with agriculture accounted for ca.

70% of the global freshwater use (Intergovernmental Panel on Climate Change, IPCC, 2019). A common problem evident in South African estuaries is limited freshwater (Lemley and Adams, 2020; Turpie, 2004). This problem, made worse by human alterations for agricultural purposes, commonly causing mouth closure and accumulation of nutrients, is one of the main factors leading to the degradation of South African estuaries (Lemley and Adams, 2020; Lemley et al., 2017; Van Niekerk and Turpie, 2012). The effects of this problem on the Mlalazi are the higher nutrient concentration and near low dissolved oxygen concentration at the upper reaches. In other South African estuaries, it included the survival of salt-tolerant species such as *Dunaliella salina* and *Cyanothece* sp. (Nche-Fambo et al., 2015; Muir and Perissinotto, 2011) under hypersaline conditions and the growth of algae, including harmful and nuisance species such as *P. cordatum* during closed mouth conditions in temporarily open-closed estuaries (Lemley and Adams, 2020; Thomas et al., 2005; Nozais et al., 2001).

Climate change also alters precipitation patterns, increasing the frequency of extreme weather conditions such as drought frequency and intensity, rainfall intensity and floods, sea-level rise, and wave action amongst others (IPCC, 2019). These climate changes influence temperature, turbidity, salinity and nutrient concentration, factors that influence the variations in phytoplankton communities. The drought experience during this study did not significantly influence the microplankton community or the water quality parameters. The presence and dominance of PH dinoflagellates in the Mlalazi Estuary with limited anthropogenic influences imply that South African subtropical estuaries of good to fair water quality are also in danger of developing harmful algae blooms should there be an increase in nutrient concentration either anthropogenically or naturally.

Conclusion.

The characterisation of phytoplankton community composition is necessary for the classification of aquatic systems' ecological status (Cabecinha et al., 2009; Ferreira et al., 2007) and there is no 'one size fits all' method to characterise phytoplankton community for its use within monitoring networks. In subtropical predominantly open estuaries, phytoplankton community structure (composition, richness, abundance, and biomass) is suitable for evaluating water quality, rather than a single phytoplankton parameter as biomass, and monitoring programs should adopt a biweekly sampling schedule. However, in a country like South Africa and other developing countries with limited resources, monitoring programs encompassing all reaches on a biweekly scale is unlikely. For the Mlalazi Estuary and other subtropical predominantly open estuaries a biweekly sampling schedule should be adopted. In the case of the Mlalazi Estuary, during this study, the of key species to be monitored at all three sites of the estuary are, *Gonyaulax spinifera*, *Karlodinium veneficum* (original name: *Gyrodinium estuariale*), *Prorocentrum cordatum*, *P. micans*, *P. triestinum*, *Scrippsiella trochoidea* and the Scuticcociatids, *Uromena* sp. However, the presence and dominance of potentially harmful (PH) dinoflagellate species highlight the importance of maintaining the estuary's water quality.

Examining the total phytoplankton abundance, phytoplankton groups and phytoplankton biomass (chapter 2) showed that during the study period from July 2014 to June 2015, the Mlalazi Estuary was an estuary with good to fair water quality with minimal potential for blooms or poor water quality under such conditions. An examination into the diatom community also showed a high species richness, no blooms, and no species singled out to indicate poor water quality. However, the changes in diatom types (centric or benthic, chain-forming or solitary, and pelagic or benthic) could be used to gauge varying freshwater input. Despite the absence of blooms, an examination into the dinoflagellates of the system showed the dominance of potentially harmful species, highlighting the importance of maintaining good water quality as these species could take advantage of increased nutrients and bloom. In this study, the ciliates community was typical of good water quality and *Tintinnopsis* spp., *Rimostrombidium* sp., *Strobilidium* sp., *Spirotontonia* sp., *Stenosemella* sp., and *Strombidium* spp. could be used as indicators of good water quality. Despite the concurrence between all chapters on the good water quality state of the estuary and the use of its community stcuture as a reference point for possible future changes in the estuary, results from the dinoflagellate chapter (chapter 4) provide grounds for the importance of improving or maintaining the estuary's water quality. This is because anthropogenic nutrification, or mouth closure due to reduced river flow, could lead to a decline in water quality and blooms of the potentially harmful species, *Gonyaulax spinifera*, *Karlodinium veneficum* (original name: *Gyrodinium estuariale)*, *Prorocentrum cordatum*, *P. micans*, *P. triestinum*, and *Scrippsiella trochoidea*.

Due to the influence of seasons on the system variables (nutrient concentration, temperature, salinity, and turbidity) that affect phytoplankton community structure, even in the absence of severe anthropogenic interference (a significant cause of poor water quality in estuaries), the Mlalazi Estuary of high conservation status should still be monitored for variations in phytoplankton community structure or at least the key species. These variations could still lead to poor water quality and impairment of ecosystems.

This current study showed that the Mlalazi Estuary is not impacted and is good to fair water quality. This ecological state is reflected in the water quality, species richness, and abundance of microplankton communities. Most planning processes and estuary assessments are low confidence due to the lack of essential information for assessing the water quality health and microalgae health components (Skowno et al., 2019; Niekerk et al., 2019; DWS, 2015). The water quality health component depends on TON, DIP and DO concentration, turbidity, and salinity. The microalgae health component relies on the richness of phytoplankton species, phytoplankton abundance, and the composition of phytoplankton species (DWS, 2015). Even during a drought, the good water quality of the Mlalazi Estuary, its low potential for blooms or eutrophic conditions and its protected status makes it an ideal source for baseline information needed to define ecological categories. The information generated by this study is also required to define ecological categories and quantify ecological water requirement scenarios such as in the presence (DWA, 2004). For example, this data can be used to simulate scenarios of different river flow levels and be applied to assess the estuarine health index (EHI). This study provides reference conditions and the ecological state of the Mlalazi Estuary during a drought.

Future Research

To fully understand the influence of salinity in determining phytoplankton community structure and biological processes thereof, the uppermost reaches of the estuary where tidal influence is minimal, and salinity is within freshwater limits (<5) should be sampled in a future study. Such a portion of the estuary was not sampled during this study, and variations in phytoplankton community structure under low salinities in good to fair water quality conditions are lacking. Given the spatial variability of the microplankton community shown in this study, and in a country (South Africa) with freshwater limitations, it is crucial that future studies be conducted at the uppermost freshwater segment of the estuary to fully understand the influence of salinity and freshwater input to the ecosystem of an estuary.

Dinoflagellate blooms were not recorded. However, various factors such as temperature (Ashton et al., 2003) and presence of grazers (Bergkvist et al., 2008) influence the toxicity of potentially harmful dinoflagellate species despite their low abundance (absence of a bloom). Therefore, it is imperative that further studies measuring the level of toxicity of the potentially harmful species under the different environmental conditions be conducted to understand the threat that PH species provide to the ecosystem.

Humans manipulate a critical factor such as nutrient concentration related to natural factors such as rainfall and freshwater inflow to estuaries for agriculture and aquaculture practices. However, it is challenging to tease natural from anthropogenic influences because phytoplankton community composition is influenced by multiple factors simultaneously, as shown in this study. Also, variables analysed in the study did not fully explain the observed variability in the microplankton community, suggesting that other factors could be influencing the microplankton variability. Literature suggests that other factors not included in this study such as water flow, water retention period and predation influence microplankton communities in South African estuaries (Lemley and Adams, 2020; Kibirige and Perissinotto, 2003; Walker et al., 2001; Adams and Bate, 1999) as well as internationally (Bargu et al., 2019; Alves-de-Souza et al., 2006; Chan et al., 2002). It is therefore essential that further studies evaluate the effects of these other parameters (water flow, water retention period and predation) on the microplankton community.

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APPENDICES

Appendix 1: The List of all diatom taxa recorded in the Mlalazi Estuary from the 6th of July 2014 to the 23rd of June 2015. Authority of the genus is used for unidentified species.

Species name
Achnanthes brevipes var intermedia (Kützing) Cleve
Achnanthes fimbriata (Grunow) R. Ross
Achnanthes longipes Agardh
Achnanthes minutissima var gracillima (Meister) Lange-Bertalot
Achnanthes oblongella Strup
Achnanthes sp. 1 Bory
Achnanthes sp. 2 Bory
Achnanthidium exiguum (Grunow) Czarnecki
Achnanthidium sp. 1 Kützing
Actinopcyclus sp. 1 Ehrenberg
Actinoptychus sp. 1 Ehrenberg
Actinoptychus sp. 2 Ehrenberg
Amphiprora pseudoduplex (Osada & Kobayasi) Hällfors
Amphiprora sp. 1 Ehrenberg
Amphora coffeaeformis (Agardh) Kützing
Amphora gacialis Smith
Amphora laevis Gregory
Amphora ocellata Ehrenberg
Amphora soninkhishigae Edlund, Shinneman & Levkov
Amphora sp. 1 Ehrenberg ex Kützing
Amphora sp. 2 Ehrenberg ex Kützing
Amphora sp. 3 Ehrenberg ex Kützing
Asterionellopsis glacialis Round
Aulacoserra granulata (Ehrenberg) Simonsen
Bacillaria paxillifera (Müller) Marsson
Bellerochea malleus (Brightwell) Van Heurck
Bidulphia alterans (Bailey) Van Heurck
Bidulphia reticulum (Ehrenberg) Boyer
Bidulphia rigida Schmidt

Bidulphia sp. 1 Gray
Bidulphia tuomeyi Gray
Brachysira vitrea (Grunow) Ross
Campylodiscus clypeus (Ehrenberg) Ehrenberg ex Kützing
Campyloneis pseudolineata (Geifter) Lange-Bertalot
Campyloneis sp. 1 Ehrenberg
Cerataulina bicornis (Ehrenberg) Hasle
Cerataulina pelagica (Cleve) Hendey
Chaetoceros lorenzianus Grunow
Chaetoceros sp. 1 Ehrenberg
Chaetoceros sp. 2 Ehrenberg
Chaetoceros subtilis Cleve
Cocconeis krammeri Lange-Bertalot & Metzeltin
Cocconeis placentula var euglypta (Ehrenberg) Grunow
Cocconeis pseudolineata (Geifter) Lange-Bertalot
Cocconeis sp. 1 Ehrenberg
Cocconeis sp. 2 Ehrenberg
Coscinodiscus oculoides Karsten
Coscinodiscus sp. 1 Ehrenberg
Craticula sp. 1 Grunow
Cyclotella meneghiniana Kützing
Cyclotella sp. 1 (Kützing) Brébisson
Cylindrotheca closterium (Ehrenberg) Reiman & Lewin
Cymbella sp. 1 Agardh
Detonula pumila (Castracane) Gran
Diadesmis sp. 1 Kützing
Petrodictyon gemma (Ehrenberg) Mann
Diploneis bombus (Ehrenberg) Ehrenberg
Diploneis elliptica (Kützing) Cleve
Diploneis incurvata (Gregory) Cleve
Diploneis interrupta (Kützing) Cleve
Diploneis sp. 1 (Ehrenberg) Cleve
Diploneis sp. 2 (Ehrenberg) Cleve

Encyonema minutum (Hilse) Mann
Encyonema sp. 1 Kützing
Encyonema sp. 2 Kützing
Entomoneis plaudosa var plaudosa (Smith) Reimer
Entomoneis paludosa (Smith) Reimer
Entomoneis sp. 1 Ehrenberg
Eunotia bilunaris (Ehrenberg) Schaarschmidt
Eunotia sp. 2 Ehrenberg
Fallacia pygmaea (Kützing) Stickle & Mann
Fallacia sp. 1 (Kützing) Stickle & Mann
Fallacia sp. 2 (Kützing) Stickle & Mann
Fragilaria crotonensis Kitton
Fragilaria sp. 1 Lyngbye
Fragilaria sp. 2 Lyngbye
Fragilaria ulna var. acus (Kützing) Lange-Bertalot
Gomphonema parvulum (Kützing) Kützing
Gomphonema pseudoaugur Lange-Bertalot
Grammatophora oceanica Ehrenberg
Grammatophora sp. 1 Ehrenberg
Grammatophora sp. 2 Ehrenberg
Gyrosigma balticum (Ehrenberg) Rabenhorst
Gyrosigma Fasciola (Ehrenberg) Griffith & Henfrey
Gyrosigma obscurum (Smith) Griffith & Henfrey
Gyrosigma sp. 1 Hassall
Gyrosigma sp. 2 Hassall
Gyrosigma sp. 3 Hassall
Gyrosigma sp. 4 Hassall
Gyrosigma sp. 5 Hassall
Hantzschia amphioxys (Ehrenberg) Grunow
Hantzschia distinctepuntata (Hustedt) Hustedt
Hantzschia virgata var virgata Hustedt
Haslea sp. 1 Simonsen
Hyalodiscus laevis Ehrenberg

Licmophora sp. 1 Agardh
Lyrella hennedyi (Smith) Stickle & Mann
Lyrella lyra (Ehrenberg) Karajeva
Mastogloia binotata (Grunow) Cleve
Mastogloia exigua Lewis
Mastogloia smithii Thwaites ex Smith
Melosira arctica Dickie
Melosira nummuloides Agardh
Melosira sp. 1 Agardh
Navicula sp. 1 Bory
Navicula sp. 2 Bory
Navicula sp. 3 Bory
Navicula sp. 4 Bory
Navicula arenaria var rostellata Lange-Bertalot
Navicula cf. pellcidula (Brébisson ex Kützing) Hisle
Navicula cryptocephela Kützing
Navicula libonensis Schoeman
Navicula mollis (Smith) Cleve
Navicula Subrhynchocephala Hustedt
Nitschia compressa var compressa (Bailey) Boyer
Nitschia gracilis Hantzsch
Nitschia lancettula Müller
Nitzschia clausii Hantzsch
Nitzschia dissipata (Kützing) Rabenhorts
Nitzschia etoshensis Cholnoky
Nitzschia filiformis (Smith) Van Heurck
Nitzschia hungarica Grunow
Nitzschia longissimi (Brébisson) Ralfs
Nitzschia lorenziana (Grunow) Cleve & Möller
Nitzschia palea (Kützing) Smith
Nitzschia panduriformis var panduriformis Gregory
Nitzschia sigma (Kützing) Smith
Nitzschia sp. 1 Hassall

Nitzschia sp. 2 Hassall
Nitzschia sp. 3 Hassall
Nitzschia sp. 4 Hassall
Nitzschia spathulata Brébisson ex Smith
Odontella aurita (Lyngbye) Agardh
Odontella cf sinensis (Grenville) Grunow
Odontella obtuse Kützing
Odontella regia (Schultze) Simonsen
Odontella sp. 1 Agardh
Paralia sulcate (Ehrenberg) Cleve
Petroneis marina (Ralfs) Mann
Petroneis monilifera (Cleve) Stickle & Mann
Pinnularia cf. yarrensis Grunow
Pinnularia sp. 1 Ehrenberg
Pinnularia sp. 2 Ehrenberg
Pinnularia sp. 3 Ehrenberg
Pinnularia sp. 4 Ehrenberg
Placoneis sp. 1 Mereschkowsky
Plagiogramma sp. 1 Greville
Planthothidium delicatulum (Kützing) Round & Bukhtiyarava
Pleurosigma sp. 1 Smith
Pleurosigma sp. 2 Smith
Pleurosigma sp. 3 Smith
Podosira stelligera (Bailey) Mann
Pseudonitzschia sp. 1 Peragallo
Rhizosolenia setigera Brightwell
Rhizosolenia imbricata Brightwell
Rhizosolenia sp. 1 Brightwell
Rhopalodia gibberula (Ehrenberg) Müller
Seminavis sp. 1 Mann
Seminavis sp. 2 Mann
Seminavis sp. 3 Mann
Staurosirella pinnata (Ehrenberg) William & Round

Surirella amoricana Peragallo & Peragallo
Surirella scalaris Giffen
Surirella sp. 1 Turpin
Surirella sp. 2 Turpin
Surirella sp. 3 Turpin
Surirella sp. 4 Turpin
Surirella sp. 5 Turpin
Surirella sp. 6 Turpin
Surirella sp. 7 Turpin
Tabularia fasciculata (Agardh) William & Round
Terpsinoe americana (Bailey) Ralfs
Terpsinoe sp. 1 Ehrenberg
Thalassiosira tenera Proshking-Lavrenko
Trachyneis aspera (Ehrenberg) Cleve
Trachyneis sp. 1 Cleve
Trachyneis sp. 2 Cleve
Triceratium sp. 1 Ehrenberg
Tropidoneis sp. 1 Cleve
Tryblionella coarctata (Grunow) Mann
Tryblionella levidensis Smith

Achnanthes brevipes var. intermdeia (Kutzing) Cleve
L-B Iconographia Diatomolgica vol. 7
p 86 pl. 43 fig.4

slide 2.3

33µ long with 14 striae in 10µ

Achanathes fimbriata (Grunow) Ross
L-B Iconographia Diatomologica vol.7
p. 88 pl. 51 fig. 26

Slide 1.1

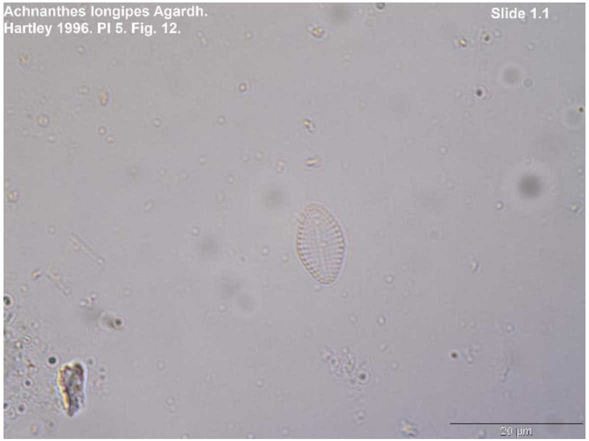
33.5µ x 12µ

2548

Achnanthes minutissima var. gracillima (Meister) Lange-l Bertalot

Slide 1.1

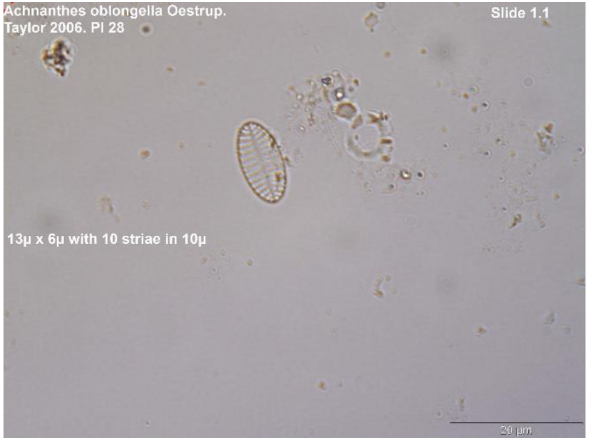
32µ x 3.5µ



Achnanthes longipes Agardh.
Hartley 1996. Pl 5. Fig. 12.

Slide 1.1

2549



Achnanthes oblongella Oestrup.
Taylor 2006. Pl 28

Slide 1.1

13µ x 6µ with 10 striae in 10µ

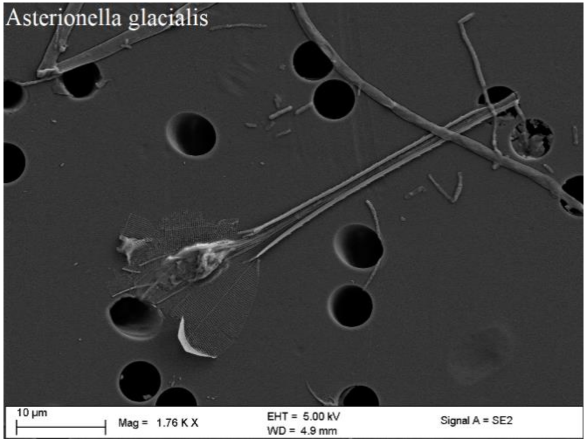
Achanathes sp. Bory

slide 2.2

39µ x 6µ with 10 striae and 10 punota in 10µ

2550

2551



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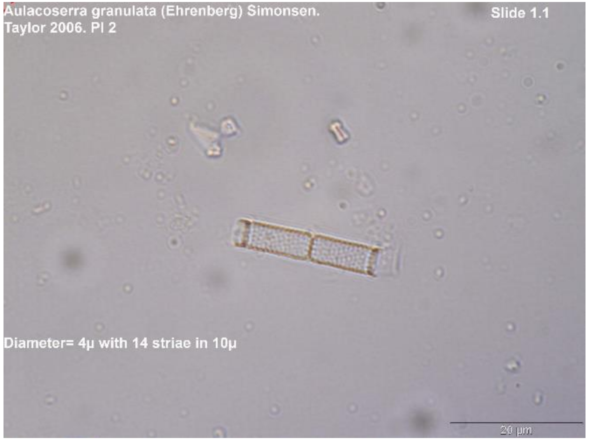

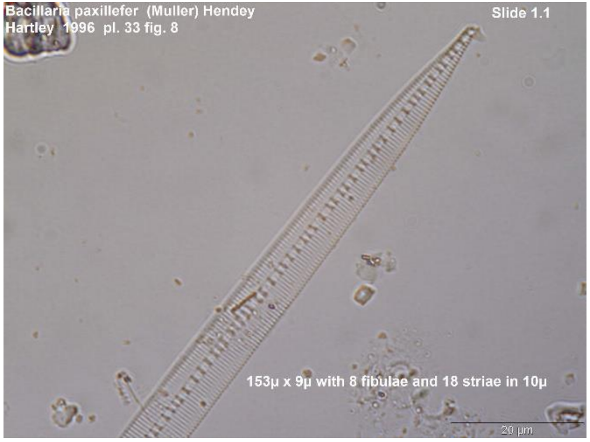

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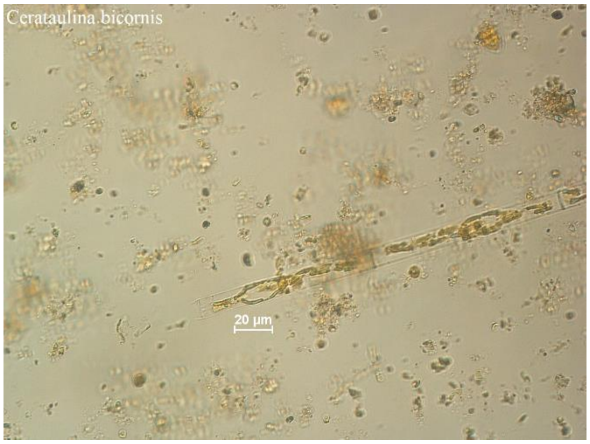

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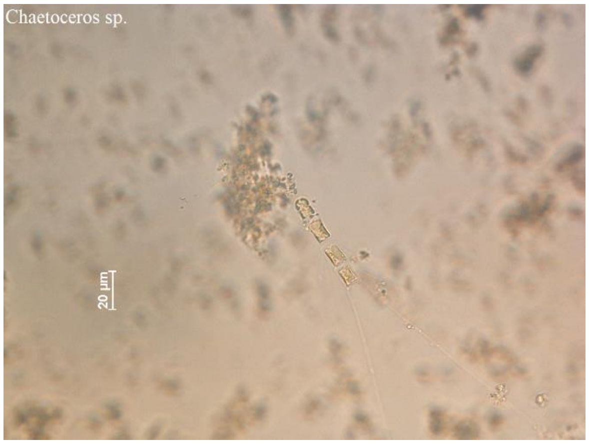

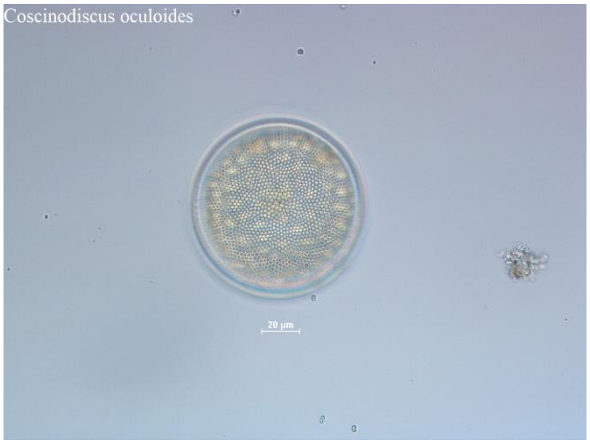

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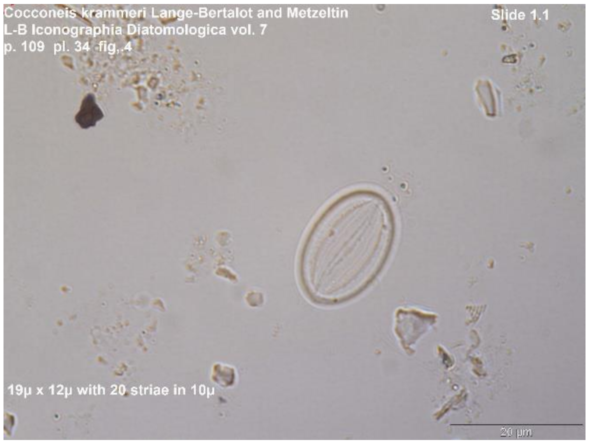

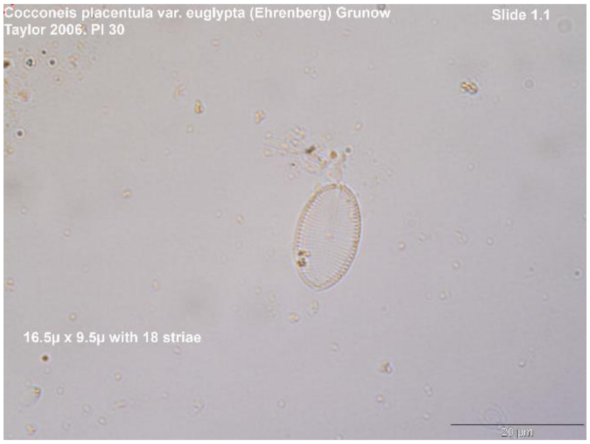

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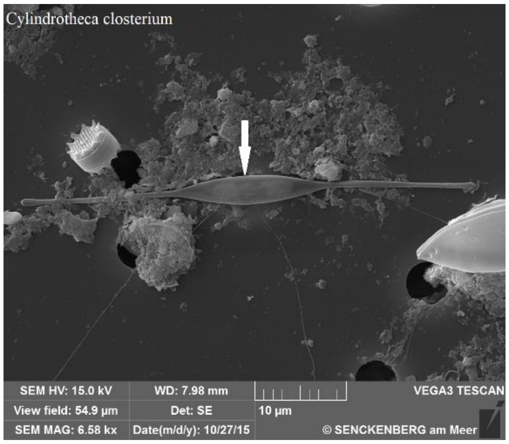

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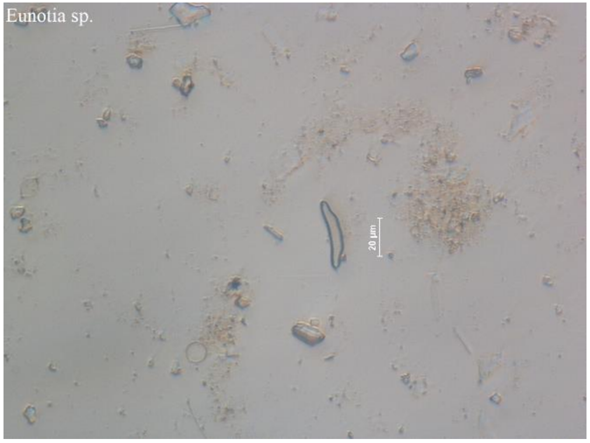

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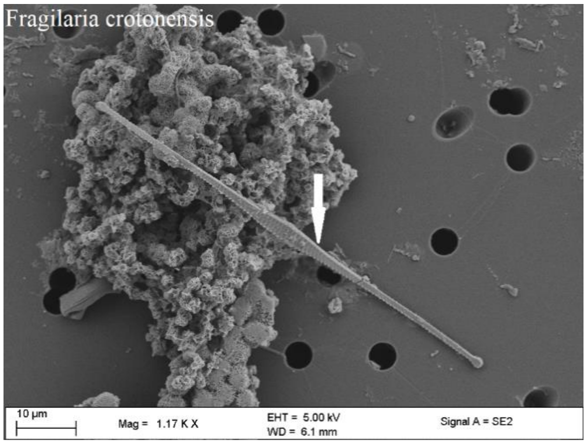

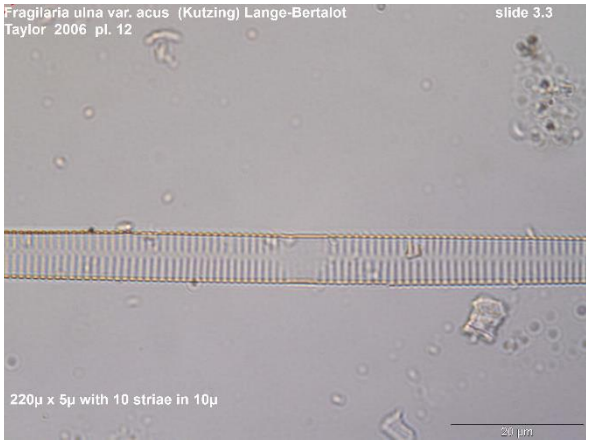

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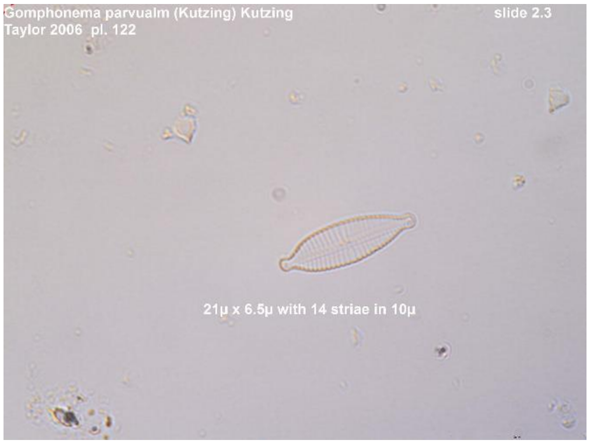

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Gyrosigma obscurum (W.Smith) Griffith and Henfrey
Archibald 1983 Diatoms of the Sundays and Fish Rivers
p. 117 pl. 13 fig. 223
Slide 1.1

152µ x 11µ


Gyrosigma obscurum (W.Smith) Griffith and Henfrey
Archibald 1983 Diatoms of the Sundays and Fish Rivers
p. 117 pl. 13 fig. 223
Slide 1.1

152µ x 11µ with 20 striae in 10µ

2567


Hantzschia distinctepunctata Hustedt
Taylor 2006 pl. 137
slide 2.3

43µ x 5µ with 12 striae in 10µ


Hantzschia amphioxys (Ehrenberg) Grunow
Taylor 2006 pl. 137
Slide 1.1

122µ x 12µ with 11 striae and 5 fibulae in 10µ

2568


Hantzschia virgata var. virgata (Roper) Grunow
L-B Iconographia Diatomologica vol. 7
p. 364 pl 175 fig. 10
Slide 1.1

55µ x 5µ with 6 fibulae and 12 striae nad 16 puncta in10µ


Hyalodiscus laevis Ehrenberg
Hartely 1996 pl. 119 fig. 3
slide 3.3

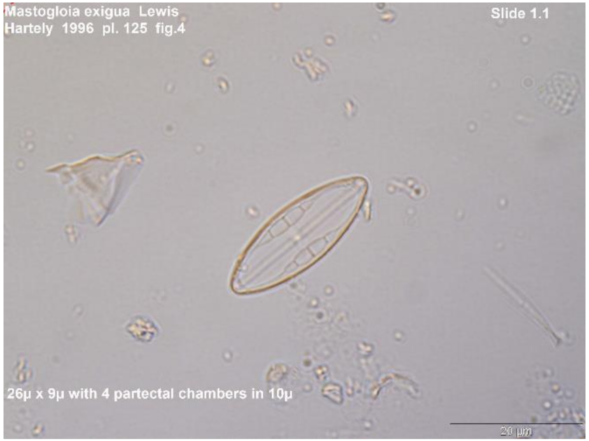
diameter = 98µ

2569


Lyrella hennedyi (W.Smith) Stickle and Mann
Hartely 1996 pl. 144 fig. 3
slide 2.2
41µ x 20.5µ with 10 striae in 10µ


Mastogloia binotata (Grunow) Cleve
L-B Icongraphia Diatomologica vol. 7
p. 240 pl. 75 fig. 15
Slide 1.1
29µ x 18µ with 12 striae in 10µ

2570


Mastogloia exigua Lewis
Hartely 1996 pl. 125 fig.4
Slide 1.1
26µ x 9µ with 4 partectal chambers in 10µ


Mastogloia smithii Thwaites
Hartley 1996 pl. 126 fig. 5
Slide 1.1
27µ x 9µ with 5 partectal chambers in 10µ

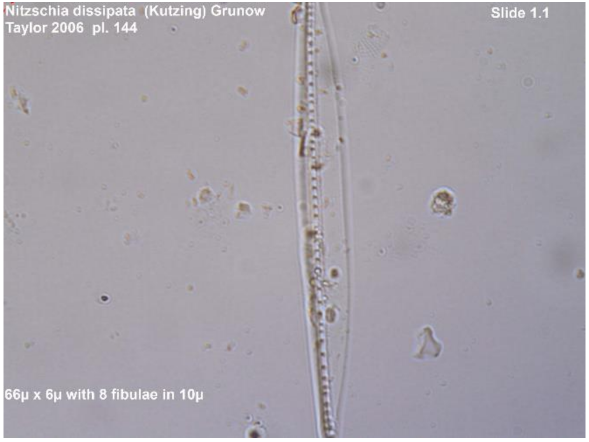
2571


Melosira arctica


Navicula arenaria var. rostellata Lange-Bertalot
L-B Iconographia Diatomologica vol. 7
p. 267 pl. 116 fig. 20.
Slide 1.1
56µ x 11µ with 12 striae in 10µ

2572

2573



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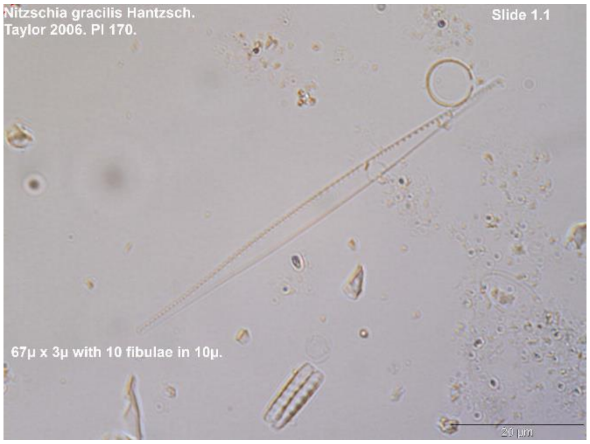

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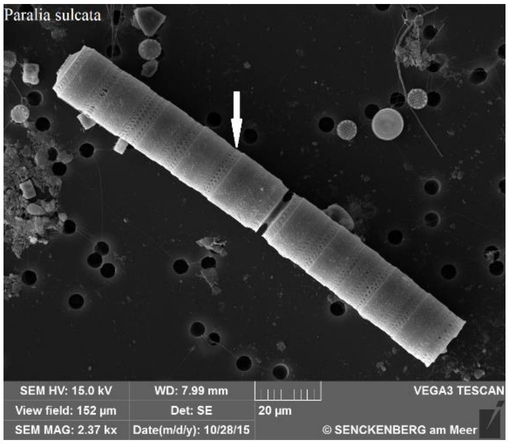

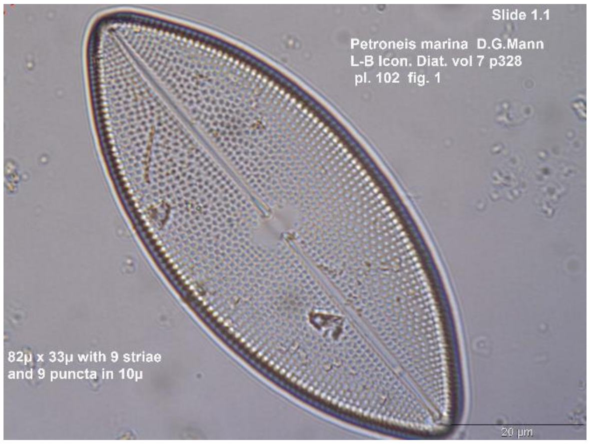

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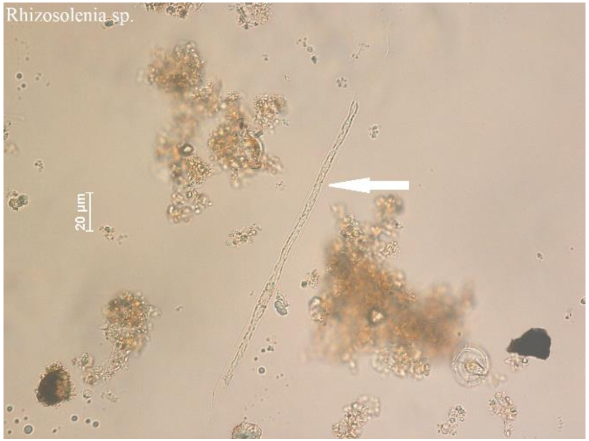

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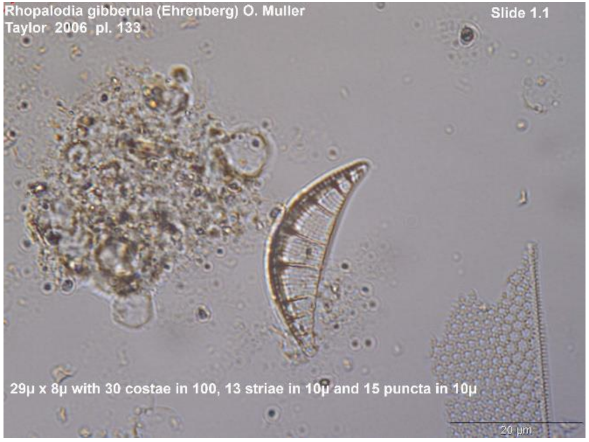

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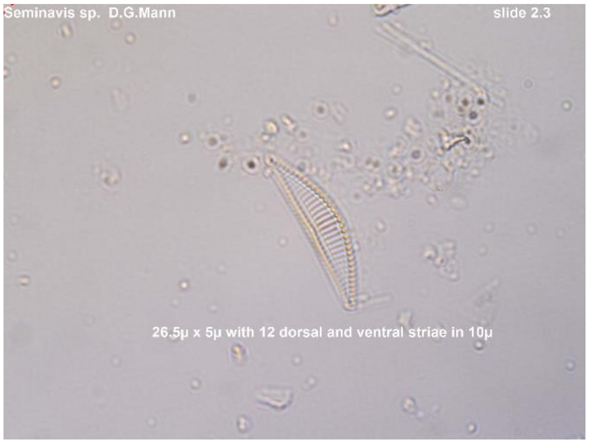

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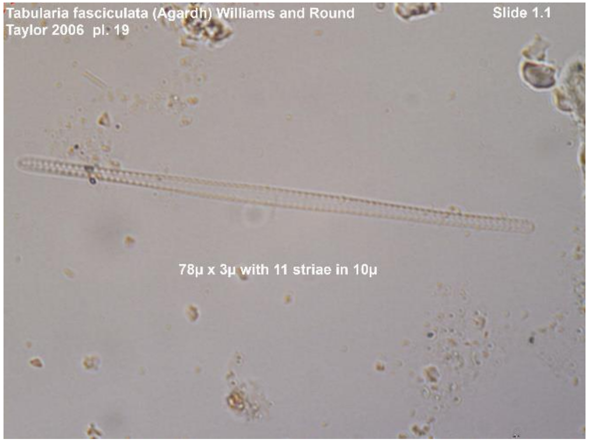

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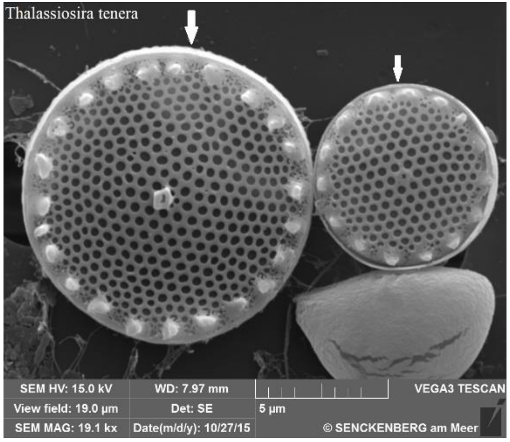

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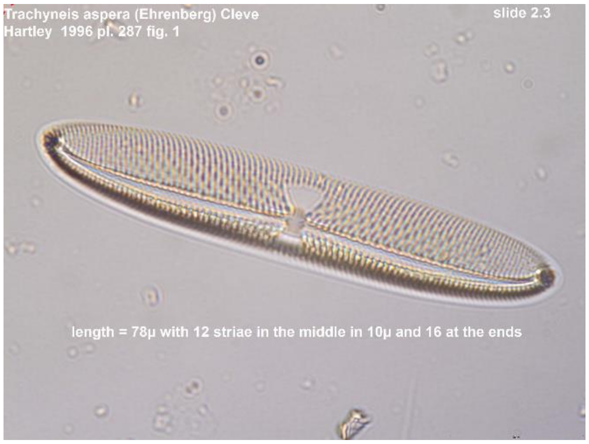
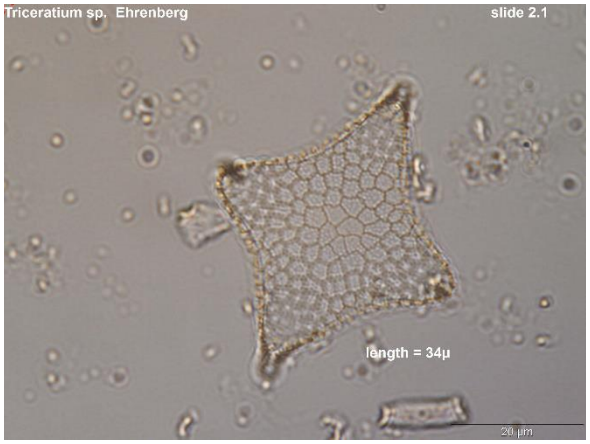
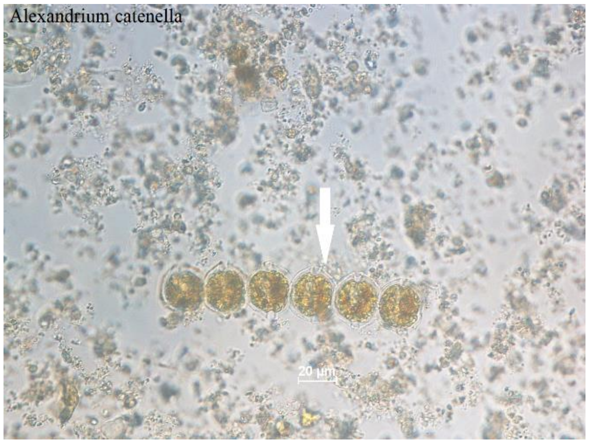
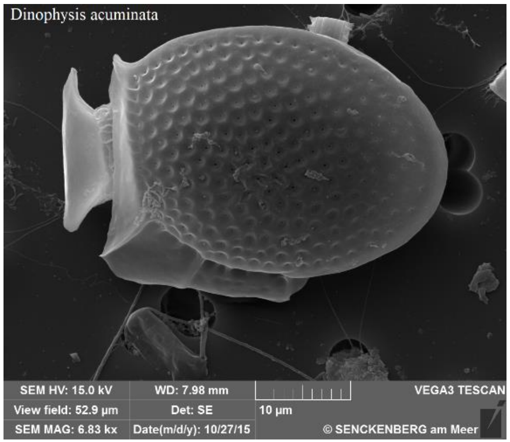
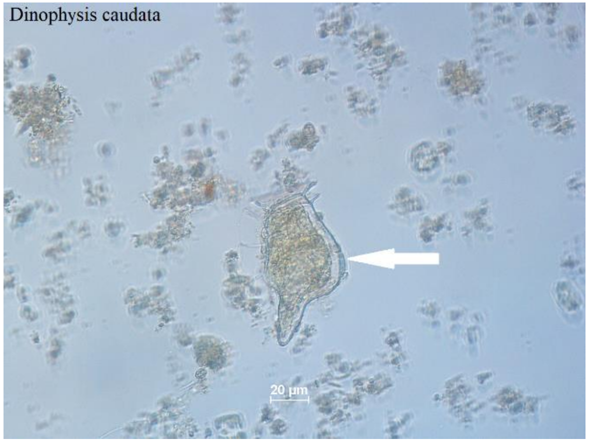


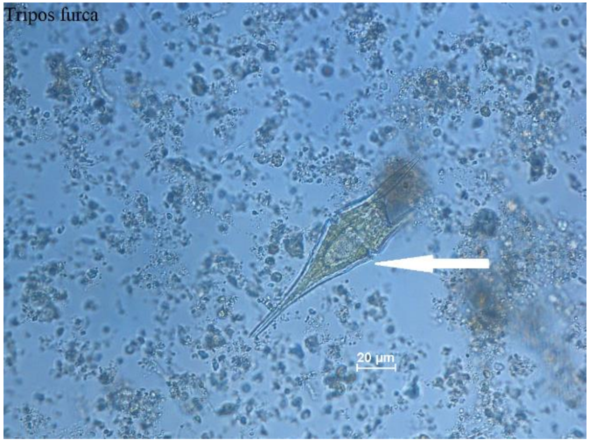


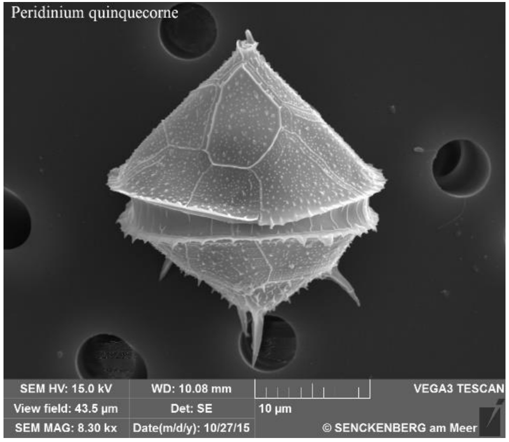
Appendix 2: Plates showing some of the diatoms (including all dominant taxa) recorded in the Mlalazi Estuary from July 2014 to June 2015. Plates includes permanently mounted slide numbers and identification source material (listed in section 3.2.3) for future reference.

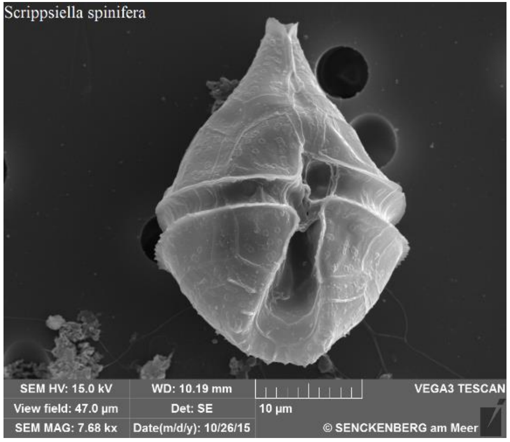

Alexandrium catenella

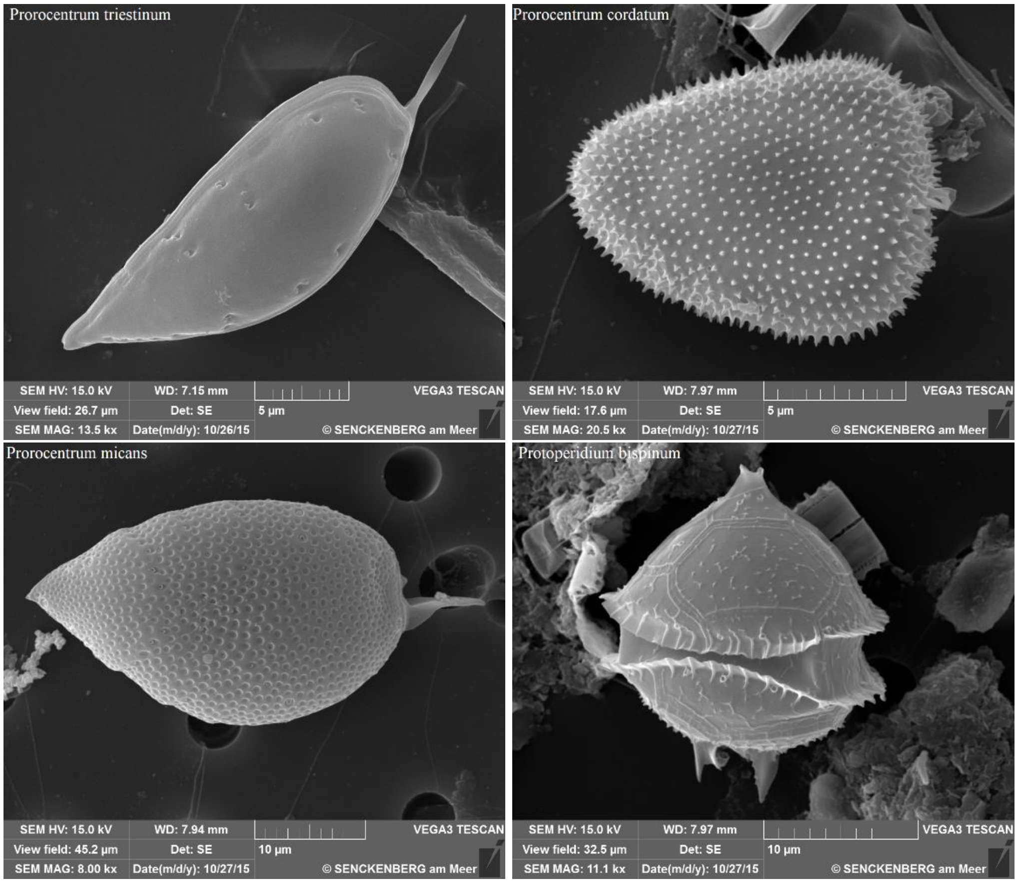

Dinophysis acuminata


Dinophysis caudata

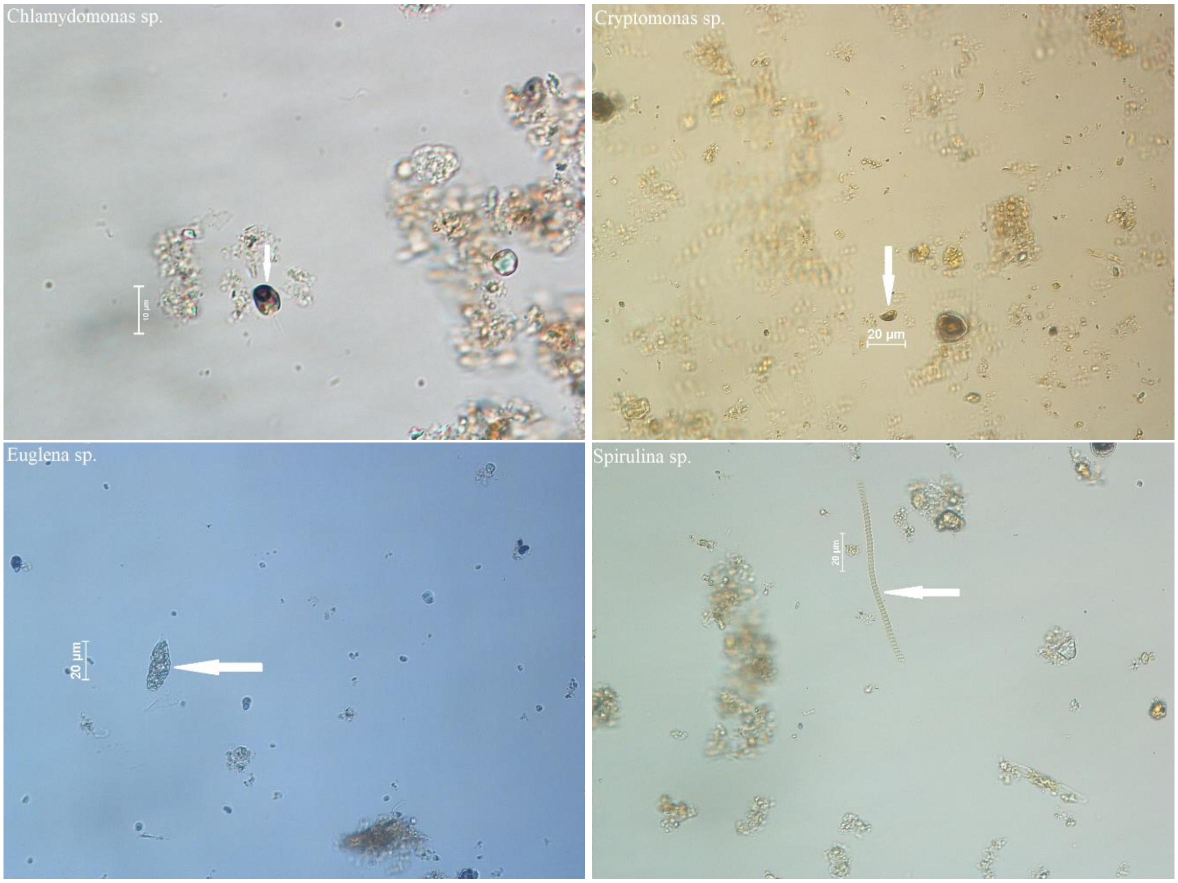

Tripos furca


Peridinium quinquecorne

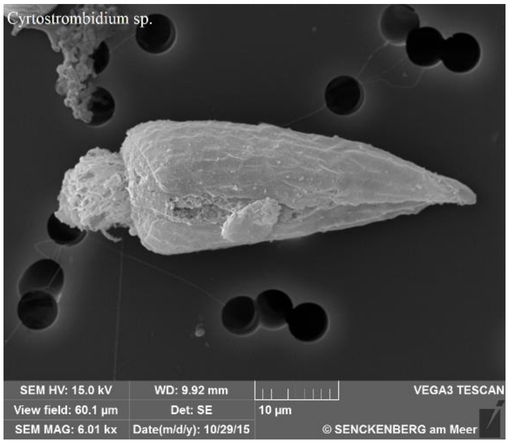

Scrippsiella spinifera

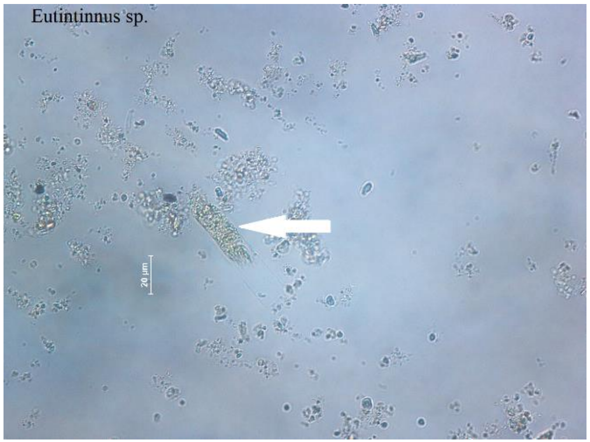


Appendix 3: Plates showing some of the dinoflagellate taxa recorded in the Mlalazi Estuary from July 2014 to June 2015.

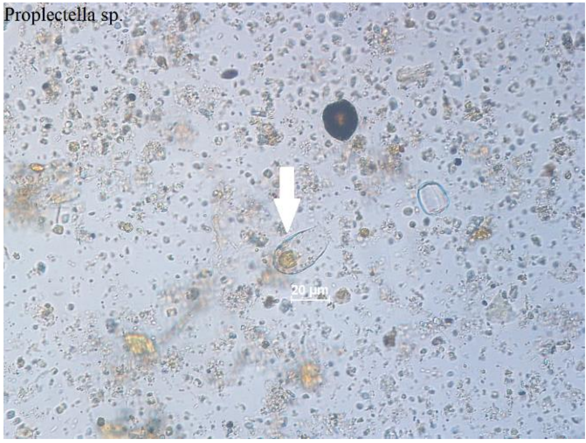


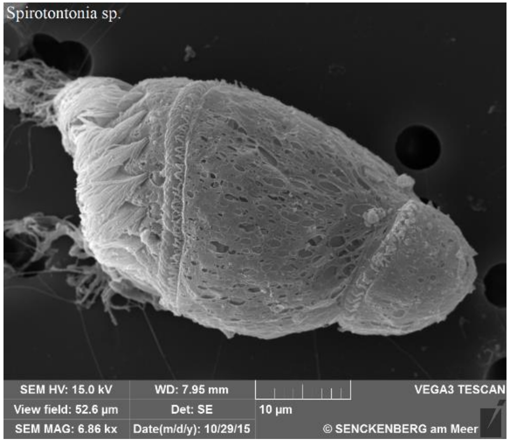
Appendix 4: Plates showing some taxa from other phytoplankton groups recorded in the Mlalazi Estuary from July 2014 to June 2015.


Cyrtostrombidium sp.

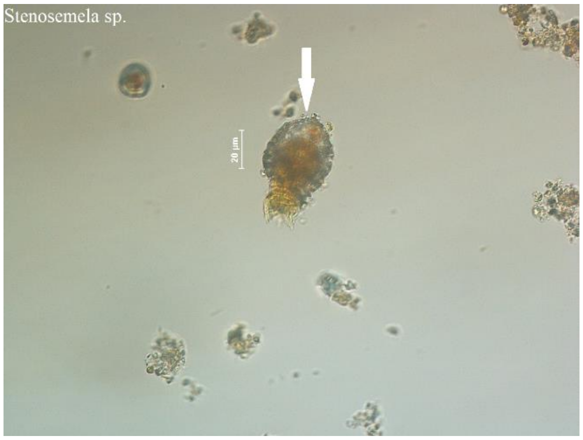

Eutintinnus sp.

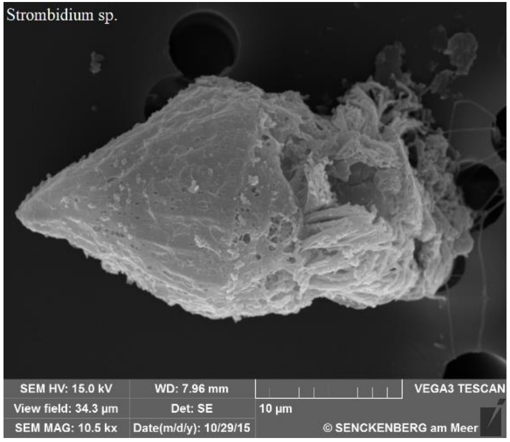
2602


Proplectella sp.

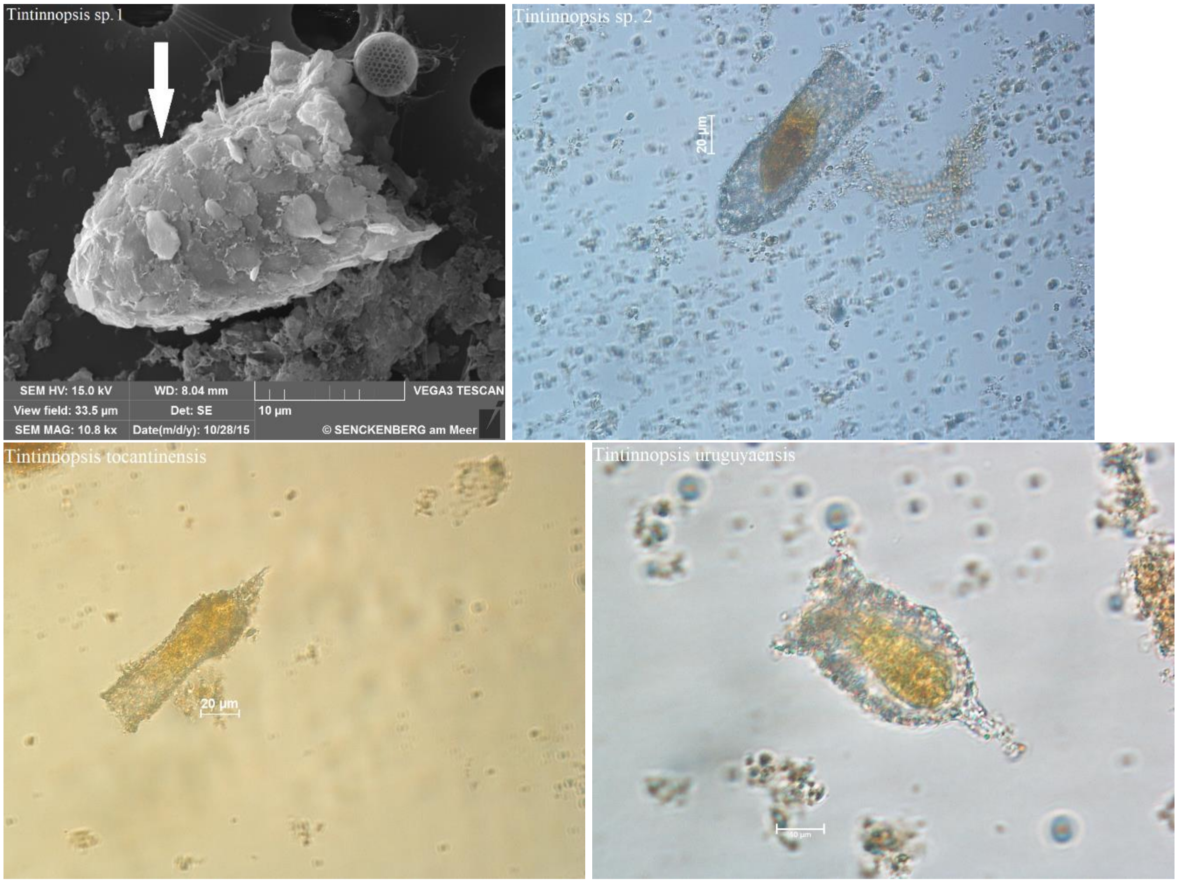

Spirotontonia sp.

2603


Stenosemela sp.


Strombidium sp.

2604



Appendix 5: Plates showing some of the ciliates recorded in the Mlalazi Estuary from July 2014 to June 2015.