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The response of microalgal biomass and community composition to the chemical and physical dynamics of two Eastern Cape estuaries

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requirements for the degree of

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DEPARTMENT OF BOTANY

by

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Declaration

I, **Phumlile L. Cotiyane**, certify that this thesis (MSc Botany) has not been submitted for a degree in any other university besides Rhodes University, Grahamstown, South Africa. This is the author's original work.

Signature: .....

Date: .....

Summary

Water quality characteristics of estuaries are influenced by both natural and anthropogenic activities. Estuaries situated in coastal urban areas are exposed to more perturbations than those in rural settings. This study determined the drivers of phytoplankton biomass and community composition in two Eastern Cape estuaries and evaluated the anthropogenic activities that influence the overall health of each estuary. The estuaries were sampled in summer and winter (2014, 2015). The water quality of the estuaries was determined by measuring the variability in physico-chemical parameters (salinity, temperature, dissolved oxygen and pH), nutrients, phytoplankton biomass and composition and faecal bacteria. Results show that both Mngazana and Nahoon are well oxygenated (~ 6.0 mg l^{-1}) and are saline systems (~ 35 ppt) due to low freshwater inputs into both estuaries. Mngazana Estuary exhibited low nutrient inputs along the length of the estuary including Creeks 1 and 2 with low chlorophyll *a* (4.0 ± 0.2 µg Chl-*a* l^{-1}) being recorded during this study while Nahoon Estuary had an overall chlorophyll *a* of 3.5 ± 0.3 µg Chl-*a* l^{-1}. The two estuaries were dominated by flagellates with phytoplankton blooms recorded seasonally. Possible eutrophic conditions were evident along the upper reaches of Nahoon indicated by nutrient accumulation and by the presence of cyanobacteria. This also this reflected the possible anthropogenic nutrient inputs originating from the Nahoon catchment despite the low freshwater inflow. The presence of faecal bacteria counts along both estuaries indicates the need for further investigation into the source of faecal contamination. The use of nutrient analyses and phytoplankton composition during this study enabled a clear description of the water characteristics of the investigated estuaries. Furthermore, the need for the adherence to freshwater flow requirements of estuaries to limit the dominance of marine waters was clearly illustrated. Urban runoff due to impervious surfaces increases nutrient inputs into estuaries while rural use of estuarine waters introduces contaminants into the system; coupled with low freshwater inputs and eutrophic conditions, the deterioration of estuarine water quality over time demonstrates the need for effective monitoring of these systems.

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MaNguni, booThukela, ooMnchumane, ndiyanibulela ngeentsikelelo.

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Chapter 1. General Introduction

1.1 Background

Estuaries are biologically productive ecosystems with high ecological and economical importance. They are landward extensions of the marine ecosystems and form the link between the terrestrial drainage system and the sea (Harrison *et al.* 2000, Turpie & Clark 2007). These systems are important geochemical (i.e. flocculation), biochemical (i.e. remineralisation) and biological (i.e. primary production) 'reactors', filtering and transforming nutrients passing from the river catchment to coastal shelf waters (Church 1986). Due to increased human-induced alterations, estuarine habitats have become one of the most threatened habitats in the country and the world at large (Allanson & Baird 1999, Elsdon *et al.* 2009).

Anthropogenic activities and pressures on South African estuaries include freshwater abstraction, coastal development, land-use change, agricultural and industrial pollution and the over-exploitation of living resources (Lubke *et al.* 1997, Whitfield *et al.* 2012, Van Niekerk *et al.* 2013). In terms of physicochemical characteristics and biological variability, South African estuaries differ considerably along the coast driven by the country's highly variable climate (Colloty *et al.* 2002, Vorwerk *et al.* 2008, Taljaard *et al.* 2009). Therefore, the extent of anthropogenic activities on estuaries differ depending on the location of a particularly estuary and resource availability. Contaminated commercial and industrial stormwater runoff is a major concern for urban estuaries while the clearing of floodplains for agricultural activities endangers a number of estuaries in the rural Eastern Cape (i.e. Mtata Estuary) (Wooldridge & Deyzel 2012, Van Niekerk *et al.* 2013).

Developments within catchments are known to alter the hydrologic regimes of natural systems and water abstraction is mostly used for consumption, irrigation and industrial use (Heinz Center 2008). According to the United Nations (UN) (2014a) report, many major cities are built in the coastal zone and 44% of the world's population lives within 150 km of the coast. Rapid urbanisation leads to increased stormwater outlets and the increase in impermeable surfaces has been linked to declining habitat and low water quality in aquatic ecosystems (Brabec 2009, Marinoni *et al.* 2013). Human microbial pathogens enter the estuarine environment via several points and sources that include surface runoff, wildlife excrement, septic tank outputs and storm and sanitary sewer overflows (Malham *et al.* 2014). Anthropogenic activities exerted on the environment eventually feedback and impact

our well-being (Cardinale *et al.* 2012), this emphasises the need to look into the aspect of human health when evaluating the health of aquatic ecosystems.

The coastal land in South Africa is under increasing threat from large-scale urban development, mostly residential and recreational developments. Most of the undeveloped north-eastern, Eastern Cape Province coastline (Wild Coast) regions are located within marine and/or nature reserves, so development is restricted but pressure is mounting to 'open up' these areas for economic exploration (DEAT 2006). South Africa has approximately 300 functional estuaries according to the recent National Biodiversity Assessment (NBA) 2011 (Van Niekerk & Turpie 2012). In the Eastern Cape, a total of 139 estuaries are found along the 970 km coastline (35 % of South Africa's coastline) (Berliner & Desmet 2007). While some are situated in the urban/developed areas, approximately 76 estuaries can be found along the Wild Coast in undeveloped areas (Whitfield 2000, Colloty *et al.* 2002).

Health is a term used to describe an estuary's condition. Real time or current conditions of an estuary are referred to as Present Ecological Status (PES). The Present Ecological Status of an estuary is *a measure of its present condition or 'ecological state'*, and should be defined on the basis of Estuarine Health (Van Niekerk & Turpie 2012). According to Rapport *et al.* (1998), ecosystem health is defined as *"the state, condition or performance of an ecosystem as defined in term of some defined endpoint"*. Ecosystem health is therefore, defined at the ecosystem level to capture the whole of the environment, both living organisms (including transients) and the non-living components of a landscape (Fairweather 1999).

The South African National Water Act (No. 36 of 1998) makes provision for the protection of water resources through the application of the Resource Directed Measures (RDM) method. South Africa is known as a semi-arid country, and the demand for water resources necessitates the construction of impounds on large rivers to supply the needs of a growing population (Reddering 1988a), therefore, the inception of RDMs was to ensure the protection of water resources and in a sense protection of ecosystem functioning and maintaining a desired state of health (integrity or condition) of aquatic and groundwater-dependent ecosystems (DWA 2010). Cooper *et al.* (1994) devised the Estuarine Health Index (EHI), a sum of three separate indices; biological health (Cooper *et al.* 1993), water quality (House 1989) and aesthetic health index. According to DWA (2010) the Estuarine Health Score represents the degree to which an estuary resembles its pristine ecological state. Such a

score thus influences the category of Present Ecological Status assigned to a particular estuary.

1.2 Significance of the study

The dynamic nature of estuaries is primarily driven by climate in regions where they occur and the state of the connections between the river, the estuary and the sea. To determine the impacts of urbanisation and resource use around estuaries, two permanently open estuaries in the Eastern Cape were identified to better understand the relationship between water quality (biological state) and developments around them. The Nahoon Estuary is located in the warm temperate region within the city of East London, Buffalo City Municipality and is surrounded by vast human developments while the Mngazana Estuary is located in the subtropical region 18 km south of Port St Johns (closest municipality) and is in a near-pristine environment and contains the third largest mangrove forest in South Africa. While these two estuaries lie in different biogeographical regions, their plant communities are similar.

Campbell *et al*. (1991) investigated the phytoplankton biomass, but not community composition, along the Nahoon Estuary and between 2002 and 2005 this kind of information was recorded for Mngazana Estuary. The objective of the current research was to determine the drivers of phytoplankton biomass and community composition in each estuary and to evaluate any human-induced impacts that influence the overall health of each system. Nationally, this study adds to the current information on estuaries and will influence the future management of these systems. Internationally, this study will allow for future comparisons of estuarine environments in the southern hemisphere in evaluating the influence of climate change (i.e. sea level rise), increase in freshwater abstraction, and catchment transformation due to increased human pressures.

The specific objectives of this study were;

- To determine the physico-chemical characteristics of the surface water and to identify nutrient point sources and pollution inputs into each estuary.

- To relate these characteristics to phytoplankton biomass and community composition.

- To determine the distribution and concentrations of the bacteria *Escherichia coli* (*E. coli*) (Migula 1985) Castellani and Chalmers (1919) along each estuary.

- To inform and support the current management initiatives and to further develop management recommendations for each estuary in relation to human activities.

1.3 Thesis Layout

Chapter 1 provides the background, significance and the specific objectives of this study and the general layout of the thesis.

Chapter 2 evaluated the available literature on estuaries in South Africa, appropriate definitions and the aspects of water quality are discussed. The potential of phytoplankton groups as tools/indicators of change was assessed leading to the importance and use of environmental assessments in the South African context.

Chapter 3 details the materials and methodology used during this study.

Chapter 4 provides an update on the microalgal dynamics of an estuary in a rural setting - Mngazana Estuary compared to work by Ngesi (2010). This study noted the level of anthropogenic influences on the estuary and its subsequent response.

Chapter 5 investigates the water quality characteristics of an estuary in an urban setting - Nahoon Estuary and illustrated the timeline of human-related disturbances along the estuary.

Chapter 6 summarises the overall outcome of the present study including the similarities and differences between the rural and urban estuaries under investigation. Possible management options (using the DPSIR framework) in conjunction with the existing individual management plans were outlined and the pathway for future assessment-like studies along South African estuarine systems was discussed.

Chapter 2. Literature Review

2.1 Estuaries of South Africa

The South African coast spans three biogeographical regions, namely the cool-temperate west coast, warm-temperate south coast and the sub-tropical east coast (Fig. 2.1). The coastline stretches for approximately 3 400 km from the Orange River mouth (28° 38'S; 16° 27'E) on the west coast to Kosi Bay (26° 54'S; 32° 48'E) on the east, and is intersected by an estimated 300 functional estuaries (Brown & Jarman 1978, Whitfield 2000, Harrison 2004, Van Niekerk *et al.* 2013) . According to Kennish (1986), estuaries are classified by their geomorphology, physiography, hydrology, tidal patterns, salinity characteristics, sedimentation and ecosystem energetics. The different estuarine types recognised in South Africa are; permanently open estuaries (POE), temporarily open/closed estuaries (TOCE), river mouths, estuarine lakes and estuarine bays (Whitfield 1992, Whitfield & Lubke 1998, Whitfield 2000). Approximately 75 % of the country's estuaries are temporarily open/closed estuaries while only 25 % are permanently open and are characterised by a moderate tidal prism with a horizontal salinity gradient between the head and the estuary mouth (Whitfield 1992, Breen & McKenzie 2001, Turpie 2004).

The estuaries in South Africa are predominantly microtidal systems (tidal range < 2 m), highly dynamic and shallow with an average depth of two meters (Van Niekerk & Turpie 2012). Whitfield (1992) predicted that changes in the structure and functioning of these systems due to natural drivers (i.e. sea level rise - SLR) and human influences could have future impacts on the characterisation of these systems. Scharler & Baird (2003) argued that most of South Africa's estuaries are located on the east and south coasts of the country where major rainfall occurs but also mentions that most of the water abstraction also takes place in this region. Recently, it was noted that 13 % of South Africa's estuaries are currently under human-induced pressures (i.e. habitat modification and/or development) with 4 % experiencing major pressure due to lack of freshwater inflow. Ultimately, the diversity and species composition within these estuaries are negatively affected by these pressures (Van Niekerk & Turpie 2012).

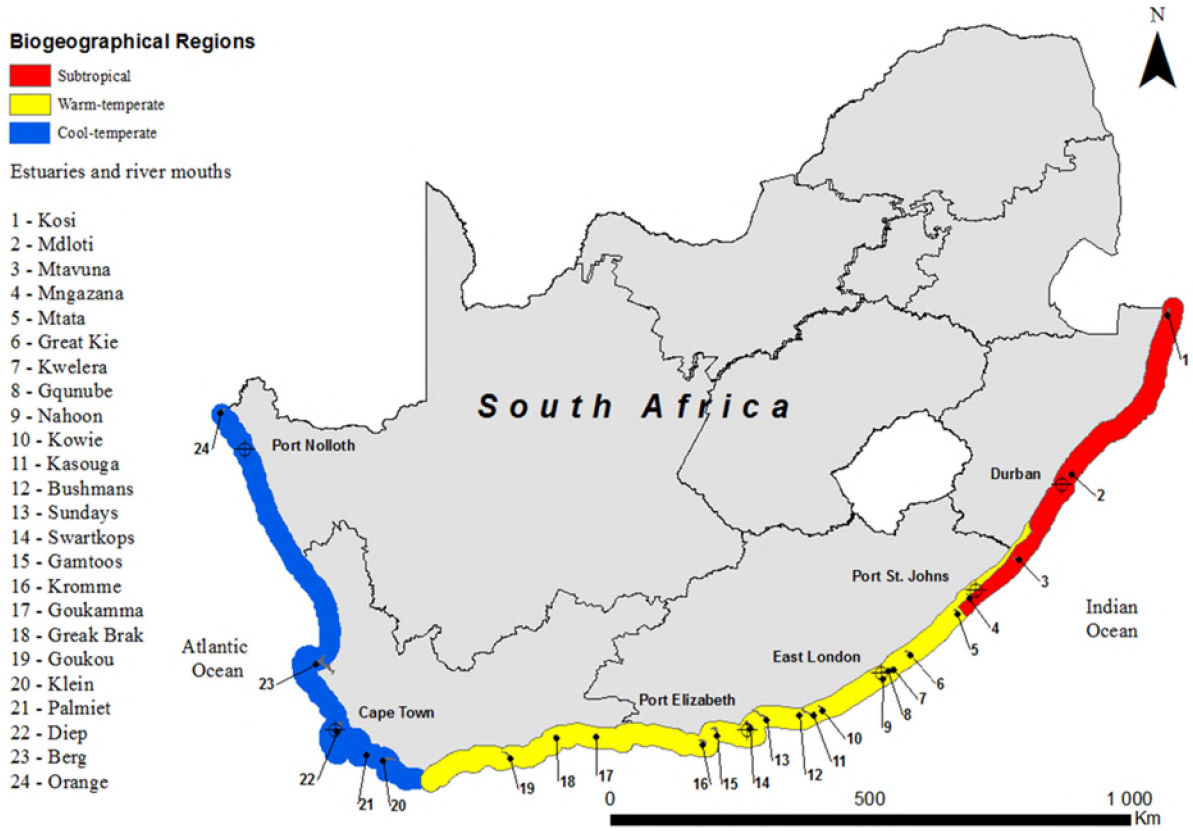

Figure 2.1: Map of South Africa illustrating the biogeographical regions along the coast and location of estuaries mentioned in the text.

2.1.1 Estuarine Definitions

Defining what an estuary is has been an ongoing process, according to Day (1980) the combination of small size and low runoff, coupled with extreme environmental conditions, such as droughts, has ultimately lead to various definitions being proposed for South African estuaries. Whitfield & Elliott (2012) provided the most recent definition of an estuary based on prior definitions. They defined an estuary as "*a semi-enclosed coastal body of water which is connected to the sea either permanently or periodically, has a salinity that is different from that of the adjacent open ocean due to freshwater impacts and includes a characteristic biota".* This definition is an amendment from that proposed by Pritchard (1967) where he defined an estuary as "*a semi-enclosed coastal body of water, which has free connection with the open sea, and within which sea water is measurably diluted with freshwater derived from land drainage*". Day (1980) had previously adjusted this definition since it did not recognise the existence of closed estuaries. Day (1980) then defined an estuary as "*a partially enclosed coastal body of water which is either permanently or periodically open to the sea and within which there is a measurable variation of salinity due to the mixture of sea water with freshwater derived from land drainage".*

However, the most comprehensive definition in South Africa reads that an estuary is "*that portion of a river system which has, or can from time to time have, contact with the sea. Hence, during floods an estuary can become a river mouth with no seawater entering the formerly estuarine area. Conversely, where there is little or no fluvial input an estuary can be isolated from the sea by a sandbar and become a lagoon which may become fresh, or hypersaline, or completely dry*" (Van Niekerk 2007). Despite all these competing definitions, the South African government, according to the National Water Act (No. 36 of 1998) defines an estuary as "*a partially or fully enclosed water body that is open to the sea permanently or periodically, and within which the seawater can be diluted, to an extent that is measurable, with freshwater drained from the land*" (Van Niekerk 2007). Brito (2012) maintains that further discussions are fundamental to achieve a consensual definition that is both detailed and widely applicable. Therefore, globally, there is no single definition that captures all types of estuaries.

2.1.2 Water quality

Estuarine water quality and quantity is driven by the interplay between different processes both abiotic and biotic coupled with human activities along the water course. Water quality describes the "*physical, chemical and aesthetic properties of water that determine its fitness*

for a variety of uses and for the protection of the health and integrity of aquatic ecosystems" (DWAF 1996). Natural processes such as hydrological (i.e. mixing, flushing and retention) and climatic conditions along with anthropogenic influence (i.e. municipal wastewater discharge) alter the water chemistry in estuaries (Taljaard *et al.* 2009, Pretorius 2014). Allanson (2001) maintains that the chemistry of estuaries is dependent upon tidal pulses, river flow, and on the hydrodynamic and autochthonous biological processes within them. Taljaard *et al.* (2003) argued that the quantity of river water inflow and the state of the tides affect water quality. The variability in freshwater inflow influences spatial and temporal variability within the estuarine system (Kimmer 2002), subsequently, the fluctuations in environmental parameters and nutrient inputs contribute to the water quality of aquatic environments. The temperature difference in climatic regions affects other hydro-climatic factors i.e. dissolved oxygen (DO). Iriarte *et al.* (2015) argued that temperature is an important potential climatic driver of DO and DO-saturation decreases considerably due to related temperature increase. VishnuRadhan *et al.* (2015) suggested using DO as an indicator of health of a water body since its adequate levels determine the type and composition of organisms living in an aquatic environment therefore, emphasising the issue of water quality monitoring in estuaries. South African estuaries are characterised by limited freshwater inflow hence salinity usually varies from 0 to 35 ppt (parts per thousand) with hypersaline conditions (> 35 ppt) occurring from time to time (Allanson & Baird 1999). Water clarity is argued to be a key indicator of water quality and is closely linked to biological activities such as chlorophyll *a* and nutrient levels (Liu *et al.* 2013). Water clarity and water chemistry are therefore closely linked, the water quality and ecological integrity of estuaries closely reflect activities within the entire upstream catchment (Lemley *et al.* 2015). Numerous other factors affect the dynamic nature of estuaries, which in turn influences the overall water quality. These include turbidity (Ohrel & Register 2006, Snow & Taljaard 2007), salinity and pH (Ohrel & Register 2006), dissolved oxygen (Best *et al.* 2007) and a relationship between nutrient dynamics and biological response has been studied in estuaries (Snow & Taljaard 2007).

2.2 Faecal Bacteria

Faecal indicator bacteria (FIB) signals the presence of pathogens in water, this ultimately affects water users. Faecal microorganisms are transported to urban water bodies via discharge of domestic and industrial wastewater while in rural areas, non-point sources (surface runoff), wild animals and livestock facilitate the addition of faecal microorganisms in rivers and estuaries. Kashefipour *et al.* (2006) emphasised that various sources of faecal indicator bacteria exist in estuarine and coastal waters, including effluent outfalls, combined

sewer outflows (CSO) and diffuse source inputs. Therefore, the microbial quality of surface waters has been studied due to illnesses attributed to waterborne pathogens (Mallin *et* al. 2000, Stewart *et al* 2008, Shelton *et al*. 2014). Human sewage pollution is among the greatest concerns in aquatic systems particularly for human health due to the known risk of exposure to human waste (Harwood *et* al. 2014). A microbial indicator of faecal contamination thus has to follow a set criteria; (1) it should be present in faeces of humans and warm-blooded animals, (2) its potential for growth in the aquatic environment should be minimal, (3) it should be readily detectable by simple means and produce unique and characteristic reactions to provide unambiguous identification of the group, (4) it should always be present when pathogens are present, and (5) the threat to human health is exposed when bacterial levels are above the limit due to the probability of other disease causing organisms being present (Elmund *et al*. 1999, Neill 2004). The presence of *Escherichia coli*, (Migula 1985), Castellani and Chalmers (1919) is considered a specific indicator of faecal contamination and reflects the possible presence of enteric pathogens (APHA *et al*. 1995 cited in Elmund *et al*. 1999). According to Harrison *et al*. (2000) faecal coliforms are included as indicators in water quality indices as categories to test suitability of water bodies for human contact. This study will focus on *E. coli* than other pathogens due to the inexpensive methodology involved and based on preceding studies conducted (Neill 2004).

According to DWAF (2002) *E. coli* and other faecal coliforms are non-conservative inferring that rapid change is expected independently of how much was initially added to the surface waters. Changing physico-chemical conditions and nutrients influence the growth or survival of bacterial organisms in estuaries. Bacterial counts for total coliforms and *E. coli* in estuaries are normally greater at the freshwater end than at the seaward end (Neill 2004). Rozen & Belkin (2001) noted that the survival of *E. coli* increased with decreasing salinity, and while high temperatures induce growth, extreme pH (~8) conditions intensify the bacterial decay (DWAF 2002). Study by Mallin *et al*. (2000) recorded low *E. coli* concentrations (90 CFU/100 mL) under high salinity conditions (~35 ppt). Furthermore, *E. coli* was found to be significantly correlated to nitrates and orthophosphate concentrations unlike other faecal coliforms tested. The experimental results from Shelton *et al*. (2014) illustrated temporal variation in growth response of *E. coli* to nutrient spikes.

The presence of microbial organisms in water bodies alters their health and functioning thus impacting on their users. Pathogens affect water quality and further impart significant impacts on commercial and recreational activities in estuaries with great implications for human health. The anthropogenic activities along the land-seawater interface have a strong

potential for contributing toward both ecological and human health problems (Mallin *et al.* 2000, Malham *et al.* 2014).

Due to the impracticality and expensiveness of conducting regular sampling of disease-causing bacteria, the use of "microbiological indicators" is usually adopted. The recommended target values for microbiological indicators in South African marine waters are presented in Table 2.2 and these are said to apply to the water column only. The microbiological water quality guidelines for recreational areas in South Africa are documented despite the lack of clear guidance on implementation (RSA DEA 2012).

Table 2.1: South African Risk-based ranges for *Escherichia coli* for recreational waters in the coastal marine environment.

Category	Estimated risk per exposure	E.coli (counts 100 ml^{-1})
Excellent	2.9 % gastrointestinal (GI) illness risk	≤ 250 (95 percentile)
Good	5 % GI illness risk	≤ 500 (95 percentile)
Sufficient or Fair (minimum requirement)	8.5 % GI illness risk	≤ 500 (90 percentile)
Poor (unacceptable)	> 8.5 % GI illness risk	> 500 (90 percentile)

Source: RSA DEA (2012)

2.3 Phytoplankton

Phytoplankton are microscopic, free-floating, unicellular microalgae which are suspended in the water column. Water motion controls their spatial distribution in horizontal and vertical planes despite some having independent locomotion (Adams & Bate 1999). These microalgae are vital contributors to primary production, this validates their importance in estuaries since they directly support and shape food webs and further facilitate the cycling of carbon, nutrients and oxygen (Paerl *et al.* 2010). The presence of some biological entities in water bodies is often seen as a sign of change. Phytoplankton biomass in South African estuaries is said to be dominant in large channel-like estuaries which have large catchments and a high mean annual runoff (Adams & Bate 1999). Phytoplankton biomass is measured as chlorophyll *a* concentrations in estuaries (Chuks & Wim 2010) and used as an estimate of microalgal biomass, which, when correlated with phytoplankton growth rate, yields the total estimate of primary productivity (Boyer 2009, Cloern & Jassby 2010, Cloern *et al.* 2014).

The dynamics of phytoplankton are affected by factors such as nutrient availability and water motion. The gradients in physico-chemical variables in estuaries are influenced by

freshwater inflow along with the intrusion of marine waters at the mouth (Pritchard 1967) and the variability of the inflow affects sediment, nutrient and organic loading into estuaries (Russel *et* al. 2006). Biological response to physico-chemical variables and nutrient flux often indicate changes in the estuarine environment, such response is governed by hydrodynamics of within estuaries. Therefore, water retention or residence time, which is defined as "*the time necessary to replace any given conservative quantity in a given volume of an estuary at the rate at which the quantity is being injected into the volume*" (Officer 1983) determines the phytoplankton development in estuaries. Snow (2000) illustrated that a residence time of 42 days under 0.8 $m \cdot s^{-1}$ and 1.2 $m \cdot s^{-1}$ flow rates in the Gamtoos Estuary yielded a maximum phytoplankton chlorophyll *a* biomass. Palmiet Estuary was reported with low (~10 µg Chl-*a* l^{-1}) water column chlorophyll *a* concentration due to short water residence time since the system is well-flushed (Adams & Bate 1999).

An increase in anthropogenic influences on the environment is expected to affect the physical and chemical processes in aquatic ecosystems with a consequential effect on water biogeochemistry (van de Waal *et al*. 2010). Smith (2006) argued that the stoichiometry of (Nitrogen to Phosphorus) N:P supplies to the water column has a central and essential control on phytoplankton primary production in freshwater, estuarine and coastal marine ecosystems. The particulate N:P ratio as suggested by Redfield (1958) is 16:1. Downing *et al*. (1997) suggested that the magnitude of phytoplankton growth responses to N is expected to be in nutrient rich waters with a low N:P ratio. Furthermore, the magnitude of growth responses to P should be high in near pristine waters with a, high N:P ratio. Dissolved inorganic nitrogen (DIN) and dissolved inorganic phosphate (DIP) are vital forms of inorganic nutrients in estuaries and low concentrations in the water column can affect primary production (Snow & Taljaard 2007). Nitrogen and phosphorus are basic elements found in all living matter thus an increase in these particular nutrient types induces an increase in microalgal biomass and a possible change in community structure since (Adams & Bate 1999). The rapid microalgal response to nutrient budgets validates the use of microalgal groups as good indicators of changing conditions (Ohrel & Register 2006). However, when evaluating eutrophic conditions in estuaries, one needs to adopt a holistic and adaptive approach since the effectiveness and relevance of indicator parameters are influenced by inter-system variability along with spatial and temporal scale differences (Cloern 2001, Lemley *et al*. 2015). According to Ohrel & Register (2006) the accumulation of microalgal biomass depends on suitable environmental conditions and often, algal blooms lead to a single species dominating the community structure (Coutinho *et al*. 2012).

Microalgal groups (i.e. diatoms, cyanobacteria etc.) make up the community structure. Community structure assessments can be advantageous in conveying information about the biological state of an estuary since they allow the consideration of heterotrophic species that are not represented in chlorophyll *a* measurements (Domingues *et al.* 2008, Garmendia *et al.* 2013). Ohrel & Register (2006) found that diatoms dominate estuarine waters with relatively low nutrients. However, Kotsedi *et al.* (2012) illustrated that diatom species indicated brackish, nutrient-rich water (*Cylindrotheca closterium* (Ehrenberg) Reimann and J.C. Lewin 1964*, Cyclotella atomus* Hustedt 1937 and *Cyclostephanus dubius* (Hustedt) Round 1988). Phytoplankton community structure in most South African estuaries is dominated by flagellates (Adams & Bate 1994, Snow *et al.* 2000, Snow & Adams 2006, Snow 2007, Kaselowki & Adams 2013). Indicator species (i.e. flagellate *Phaeocystis* sp. and cyanobacteria, *Microcystis* sp.) and larger cells (i.e. diatoms and dinoflagellates) can be used to evaluate the ecosystem state and function of estuaries (Snow & Adams 2006, Lemley *et al.* 2015). Hilmer (1990) reported phytoplankton blooms in Sundays Estuary consisted of dinoflagellate - *Katodinium rotundatum* (Lohmann) Loeblich III 1965 and chlorophyte - *Micromonas pusilla* (Butcher) I. Manton & M.Parke 1960. Phytoplankton community composition is vitally important when assessing the response of estuaries to change i.e. modified hydrology, nutrient status etc. These microalgal groups are crucial in illustrating the spatial and temporal changes in estuarine environments and can be used as valuable indicators to illustrate changing environmental conditions (Table 2.1). To accurately and broadly assess changes at planktonic level, indicators that can characterise structural and functional responses of phytoplankton groups are important and the use of morphological characteristics (for phytoplankton groups) enables the rapid identification and quantification of the relative composition in response to diverse anthropogenic and natural perturbations (Paerl *et al.* 2003)

Table 2.2. A summary of studies that linked environmental conditions with phytoplankton groups.

Phytoplankton group	Controlling factors
Flagellates	High flow conditions[6,8,9]; Low nutrient loading[1,2,4,6]; Reduced temperatures[9]; Cosmopolitan distribution along estuaries[9]
Dinoflagellates	High residence time (low flow)[4,9,10]; Stable, stratified conditions[7,9,10]; Warm temperatures (spring and summer)[2,6,7]; High nutrients, but low Si[6,7,8,10]
Diatoms	Present in marine and freshwater[8]; Low residence time (high flow)[4,5,8]; Low nutrient loading[2,5,6]; Good water clarity[2,5]; High N:P ratio, and high Si[2,3,5,7,8]; Spring and winter blooms[3,4,9,]
Chlorophytes	Freshwater conditions[2,4,8]; Low residence time (high flow)[5,8,9]; Cool temperatures (winter)[5,8]; High N:P, but low Si[3,5,7,11]
Cyanobacteria	High optimum temperature[2,4,6,8]; High nutrient inputs[5,7,8,10,11]; Low N:P, and low Si[3,7,8,11]; High residence time (low flow)[2,4,5,7,8]

[1]Snow *et al*. 2000; [2]Paerl *et al*. 2003; [3]Domingues *et al*. 2005; [4]Paerl *et al*. 2006; [5]Barbosa *et al*. 2010; [6]Paerl *et al*. 2010; [7]Domingues *et al*. 2011; [8]Gordon *et al*. 2011; [9]Kotsedi *et al*. 2012; [10]Kaselowski & Adams 2013; [11]Pinto & O'Farrell 2014

Source: Lemley 2015 and modified

2.4 Microphytobenthos

Benthic microalgae are regarded as important contributors to primary production in shallow aquatic ecosystems and tend to dominate estuaries with large intertidal regions (Adams & Bate 1999). Benthic microalgae are referred to as microphytobenthos (MPB) and its biomass and distribution are affected by light availability, salinity, hydrodynamics, sedimentary disturbance and nutrients inputs (Adams & Bate 1999, Nozais *et al.* 2001, Aktan *et al.* 2014). Furthermore, water velocity tends to influence the sediment texture via high flow rates that can lead to nutrient poor sediments. Sandy silt and exposed sandy habitats were noted to support lower MPB biomass than sheltered sites dominated by fine cohesive sediment (MacIntyre *et al.* 1996, Adams & Bate 1999, Underwood & Kromkamp 1999).

South African studies have reported a decrease in MPB biomass in permanently open estuaries (i.e. 60 and 98 % in Mdloti Estuary (Nozais *et al.* 2001)). This is attributed to hydrological events such as high flow and/or rapid flushing events which increase turbidity and thus hinder MPB production and biomass accumulation (Snow *et al.* 2000, Nozais *et al.* 2001). Thus, constant tidal exchange coupled with low residence time in POEs affects MPB biomass but differences can be expected under normal flow conditions. In this regard, TOCEs of South Africa have been found to have higher MPB biomass than POEs with values ranging from 1.5 to 616 $mg \cdot m^{-2}$ (Perissinotto *et al.* 2002). These high values were recorded during closed mouth states of TOCEs since calm conditions allow accumulation of MPB. However, caution is to be exercised when evaluating these high values since the methodology for measuring MPB is not standardised and the dynamic nature of estuaries creates different conditions across both POEs and TOCEs, hence differences in MPB values are evident. Due to the above mentioned differences, the current study did not evaluate the MPB biomass and community structure of both Mngazana and Nahoon estuaries. However, MPB biomass was included in Ngesi (2010) for Mngazana Estuary.

2.5 Estuary health assessments

It terms of the assessment of estuarine function and health, the understanding of the effects of biotic and abiotic parameters on the current state of an estuary is vital in ensuring the maintenance of the ecosystem health (Turpie *et al.* 2012). Borja *et al.* (2011) argues that ecological status assessments should incorporate ecosystem structure, function and processes through linking natural physical, chemical, geographic and climatic factors. The anthropogenic impacts within the system concerned should then be integrated (Van Niekerk *et al.* 2013). Biological indicators respond to altered physical and chemical conditions and

thus provide a combined assessment of environmental conditions in aquatic systems that are spatially and temporally variable. This makes monitoring aquatic biota more sensible since it tends to be costly to chemically analyse every pollutant in a sample of water and also interpret the results in terms of severity (Stevenson & Pan 1999, de la Rey *et al.* 2004, Ohrel & Register 2006). Baseline studies and health assessments of estuaries are crucial in describing estuaries in their present states and further quantifying their health in terms of the Estuary Health Index (EHI) (Turpie *et al*. 2012).

Present Ecological Status (PES) is a measure of the health of a resource based on a comparison between the current state and the reference condition. The health of estuary is determined via the EHI, its scores correspond to the ecological categories; Category A – unmodified/natural; B – largely modified with few modifications; C – moderately modified; D – largely modified; E – seriously modified; and F – critically/extremely modified. Data from assessment studies are meaningful via association with the different management classes, namely; Excellent, Good, Fair and Poor). The Recommended Ecological Category (REC) is thus allocated on the basis of the importance score using the PES, to indicate the level of protection required for the system of concern (DWAF 1999, 2008).

According to DWAF (1999) the loss of dynamic function in an estuary can be interpreted as an important indication of declining estuarine health. The National Biodiversity Assessment (NBA) 2011 report (Van Niekerk & Turpie 2012) outlines the health status of South African estuaries. The health statuses of the investigated estuaries are stated in each of the individual chapters as reported by Van Niekerk & Turpie (2012). The monitoring of both abiotic and biotic parameters is fundamental to validating the findings of the country-wide assessments (Van Niekerk *et al*. 2013). Therefore, microalgal dynamics (i.e. biomass, species composition) of the estuaries investigated in this study will illustrate the role of chemical and physical dynamics of these estuaries and further highlight the human-related pressures associated with them. The information gathered will be a good addition for future reference and enhancing estuarine health assessments of these two estuaries.

Chapter 3. Materials and Methods

Data were collected over four sampling trips; two summers (January 2014, 2015) and two winters (June 2014, 2015) at the two estuaries. Sampling stations were located along the length of the estuary from the mouth to the head to cover the longitudinal gradient of salinity. In total, 24 sites were assessed, 14 from Mngazana Estuary and 10 from Nahoon Estuary. At Nahoon Estuary, only five sites were sampled during January 2014 due to heavy rain and risky sampling conditions. The water column of each estuary was analysed for physico-chemical parameters, nutrients, *E. coli* counts, and phytoplankton biomass and community composition. The sea and river samples were collected near the surface and were analysed for nutrients only as these would be important sources of nutrients.

3.1 Physico-chemical variables

Physico-chemical variables were recorded at the sub-surface and thereafter at 0.5 m depth intervals until the bottom was reached. A YSI 5560 Professional Plus multiprobe was used to record physico-chemical variables at each site within each estuary; salinity (parts per thousand - ppt), dissolved oxygen (DO: $mg \cdot l^{-1}$), pH, temperature (°C) and electrical conductivity (EC: mS/cm^{-1}). Secchi depth was measured at each site using a Secchi disc. Water samples were collected in triplicate at the sub-surface and near-bottom waters for phytoplankton biomass using a weighted pop-bottle and the filtrate was further analysed for nutrients. The depth at which the near-bottom samples were collected was determined by the bathymetry of the estuary and is indicated in Tables 4.1 for Mngazana Estuary (Chapter 4) and 5.1 for Nahoon Estuary (Chapter 5) respectively.

3.2 Inorganic nutrient concentrations

Water samples collected at the sub-surface and near-bottom waters at each site in each estuary were gravity-filtered through glass-fibre filters (Whatman © GF/C) and further filtered through a cellulose acetate sterile membrane with a 0.45 µm pore-size syringe filters. The filtrates were stored in 150 ml acid washed pharmaceutical bottles and frozen until analyses could commence. The samples were analysed for soluble reactive phosphorus (SRP) and ammonium (NH_4^+) using standard spectrophotometric methods as described by Parsons *et al.* (1984). Total oxidised nitrogen (TOxN: NO_3^- and NO_2^-) was analysed using the reduced copper cadmium method as described by Bate & Heelas (1975). Reverse osmosis (RO) water was used during all analyses. Silicate analyses were not performed for this study.

Soluble reactive phosphorus (SRP) was determined by rapidly mixing 2.5 ml of sample (including standards) with 0.25 ml of mixed reagent. After 5 minutes, absorbance was read on a UV/VIS spectrophotometer at 885 nm. The mixed reagent was made up using the following solutions: 5 ml ammonium molybdate (15 g in 500 ml H_2O), 12.5 ml sulphuric acid (140 ml concentrated sulphuric acid in 900 ml H_2O), 5 ml ascorbic acid (1.35 g in 25 ml H_2O) and 2.5 ml potassium antimony tartrate (0.34 g in 250 ml H_2O). In order to make a standard series, first a 6 mM stock solution was made up by placing 0.816 g of anhydrous potassium dihydrogen phosphate in 1 litre of H_2O. Next, a 100 ml diluted stock solution (72 µM) was prepared by placing 1.2 ml of the stock solution in H_2O. From the diluted stock, the following volumes were taken out and diluted to 100 ml with H_2O: 1.5, 3, 6, 12, 24, 48 and 96 ml. The resultant standard series concentrations were as follows: 0 (blank), 1.08, 2.16, 4.32, 8.64, 17.28, 34.56 and 69.12 µM.

Ammonium (NH_4^+) was determined by first adding 0.1 ml of phenol solution (20 g crystalline analytical grade phenol in 200 ml 95% ethanol) to 2.5 ml of sample (including standards). Next, 0.1 ml of sodium nitroprusside solution (1 g in 200 ml H_2O) was added. Lastly, 0.25 ml of oxidising solution (mixture of an alkaline reagent solution [20 g sodium citrate + 1 g sodium hydroxide in 100 ml H_2O] with a sodium hypochlorite solution [10-14%]) was added to the sample, before allowing colour to develop in the dark for between 1 and 24 hours. Absorbances were read at 640 nm. In order to make a standard series, a 10 mM stock solution (0.535 g of ammonium chloride in 1 litre of H_2O) was made up. From this, a 100 ml diluted stock solution (73 µM) was prepared by placing 0.73 ml of the stock solution in H_2O. From the diluted stock, the following volumes were taken out and diluted to 100 ml with H_2O: 1.5, 3, 6, 12, 24, 48 and 96 ml. The resultant standard series concentrations were as follows: 0 (blank), 1.095, 2.19, 4.38, 8.76, 17.52, 35.04 and 70.08 µM.

Total oxidised nitrogen (TOxN) was analysed by initially adding 2 ml of a buffer solution (21.4 g of ammonium chloride in 1 litre of H_2O, adjusted to pH 9.6 using ammonium hydroxide) to 3 ml of sample (including standards). Next, *ca.* 2 g of copper cadmium (stored under weak acid-EDTA solution in a sealed, air-tight flask) was added to the samples and agitated for 10 minutes. After this, 1 ml of sulfanilimide solution (1 g in 100 ml 1.5 N HCl) was added to 1 ml of sample. Lastly, 1 ml of diamine hydrochloride solution (0.02 g in 100 ml H_2O) was added, before allowing colour to develop in the dark for 5 minutes. Absorbances were subsequently read at 540 nm. In order to make a standard series, a 5 mM stock solution (0.51 g of potassium nitrate in 1 litre of H_2O) was made up. From this, a 100 ml diluted stock solution (144 µM) was prepared by placing 2.88 ml of the stock solution in H_2O. From the diluted stock, the following volumes were taken out and diluted to 100 ml with H_2O:

1.5, 3, 6, 12, 24, 48 and 96 ml. The resultant standard series concentrations were as follows: 0 (blank), 2.16, 4.32, 8.64, 17.28, 34.56, 69.12 and 138.24 µM. All absorbance values of the standards were used to calculate nutrients concentrations (adapted from Lemley 2015).

3.3 Phytoplankton

3.3.1 Phytoplankton biomass (Water column chlorophyll *a*)

The phytoplankton samples were immediately gravity-filtered through glass-fibre filters (Whatman © GF/C) and frozen until analyses could commence. Chlorophyll *a* was extracted by placing the frozen filters into glass vials containing 10 ml of 95 % ethanol (Merck 4111). The samples were stored in a cold and dry room at 1 to 2 °C for 24 hours. After extraction, the contents of the vials were once again filtered and spectrophotometric determinations of chlorophyll *a* were performed according to Nusch (1980). Absorbance was measured at 665 nm before and after acidification with 0.1 N HCl. Chlorophyll *a* biomass was calculated according to the following equation:

$$Chl\ a\ (\mu g\ l^{-1}) = (E_{b665} - E_{a665}) \times 29.6 \times (v/(V \times l))$$

Where:

E_{b665} = absorbance at 665 nm before acidification

E_{a665} = absorbance at 665 nm after acidification

v = volume of solvent used for the extraction (ml)

V = volume of the sample filtered (l)

L = path of spectrophotometer cuvette (cm)

29.6 = constant calculated from the maximum acid ratio (1.7) and the specific absorption coefficient of Chlorophyll *a* in ethanol (82 $g \cdot l^{-1}\ cm^{-1}$)

3.3.2 Phytoplankton community composition

Water samples of 200 ml were collected from the sub-surface and near-bottom waters at each sampling site within each estuary. The samples were preserved with 1 ml of 25% Glutaraldehyde solution. Phytoplankton identification was completed using the Coulon & Alexander (1972) method where 60 ml of the preserved sample was settled overnight in 26.5 mm diameter settling chamber and two drops of Rose Bengal were added to stain the cells and left to settle for 24 hours. After settling, a Zeiss IM 35 inverted microscope was used to count and identify the microalgal groups at maximum magnification of 630X during which

either a minimum of 200 frames (3.142 mm^2 in diameter) or 200 cells were counted. The cells were classified according to different algal groups i.e. diatoms, flagellates, dinoflagellates, blue-green algae (cyanobacteria), and green algae (chlorophytes). The actual counts for the different groups were calculated using the following equation (Snow 2007):

$$Cells\ ml^{-1} = ((\pi r^2)/A) \times C/V$$

Where:

r = radius of the settling chamber (mm)

A = area of each frame (mm^2)

C = number of cells in each frame

V = volume of sample in settling chamber (m*l*)

3.4 Faecal bacteria

Three replicate water samples of 40 ml were collected from the sub-surface at each sampling site from each estuary. The samples were laboratory filtered using Metrice Grid sterilised membrane and each membrane filter was then placed on a plate with pre-prepared *Endo-type agar medium* (m-Tec agar). The filters were inverted and incubated for 22 to 24 h at 45 ± 0.5 ºC. Faecal bacteria counts were calculated using the following equation and expressed as counts per 100 ml^{-1} (Source: SM 2012).

$$\frac{Coliform\ colonies\ counted\ x\ 100}{mL\ sample\ filtered} = \text{No. CFU}/100\text{m}l$$

3.5 Statistical Analyses

The Shapiro-Wilks test including the skewness and kurtosis of the data were tested to determine the normality of the data. Non-parametric analyses were performed if data were not normally distributed ($p < 0.05$) using the Mann-Whitney Rank Sum test for the comparison between years and seasons in each year. A Kruskal-Wallis Anova was used to determine significant differences between sites. The Spearman Rank Order Correlation was used to determine the relationship and strength between physicochemical factors and biological variables. If data were normally distributed ($p > 0.05$), One-Way ANOVA along with Tukey HSD post hoc test were used. Statistical analyses were run using Statistica (Version 12, 2014) and all significances were determined at $p < 0.05$.

Contour plots for salinity, temperature and dissolved oxygen were produced using Grapher (Golden Software) Version 6. Contour XY Data Map was used to construct the plots.

CANOCO for Windows Version 4.5 was used to determine where biological species were constrained by environmental (physico-chemical) variables over time and between sites using Canonical Correspondence Analysis (CCA) plots. Monte Carlo permutation tests (499 permutations) were used to assess the significance of the canonical axis showing the relationship between phytoplankton groups and environmental variables. The constrained ordination axes correspond to the greatest set of variability that can be explained by the environmental variables (Lepš & Šmilauer 2003). CCA results were plotted as two-dimensional graph using CANODRAW for Windows Version 4.5. Physico-chemical variables were plotted as arrows originating from the centre of the CCA ordination. The direction and length of the arrows indicated an increase in the value and importance of each variable. The closer species are to a specific variable, the stronger the correlation with that variable. Statistical results are indicated in tables below the CCA plots (ter Braak & Šmilauer 2002).

Chapter 4. Microalgal and nutrient dynamics of the Mngazana Estuary: a comparison between 2002/2003 and 2014/2015.

4.1 Introduction

Estuaries of the former Transkei are situated in a rugged and rural region extending from the Great Kei Estuary (32º 41' S, 28º 23' E) in the south to the Mtamvuna Estuary (31º 04' S, 30º 11' E) in the north which also serves as the boundary between the Eastern Cape and KwaZulu-Natal provinces (Colloty *et al.* 1999; 2002) and the area is commonly known as the Wild Coast or the former Transkei coast. It is approximately 270 kilometres in length and includes a transition zone between the warm temperate and subtropical biogeographical regions (Colloty *et al.* 2002). Branch and Grindley (1979) have previously stated that the Transkeian coastline is a mosaic of forest and grassland with numerous estuaries. The Wild Coast has 17 permanently open estuaries out of 120 river outlets that occur along the coast (Whitfield 1992).

Estuaries situated in rural areas are usually impacted by freshwater abstraction and return flow from agricultural practices; primarily stock grazing or subsistence agriculture since the land is seen as fertile (Elsdon *et al.* 2009). The catchments are usually still covered by natural forests with sections transformed to communal land and farming (Mtetwa & Schutte 2002). Human dependency on estuaries in general has increased over the years (Huppert *et al.* 2003). Turpie *et al.* (2006) maintains that the significant value of estuaries is reflected in the amount of goods and services they provide and are categorised into a number of groups including; supporting (i.e. nutrient recycling), regulating (i.e. carbon sequestration), provisioning (i.e. fisheries) and cultural services (i.e. aesthetic beauty) (Potts *et al.* 2014). Increased impacts on or around estuaries such as human settlements, inadequate sanitation, reduced access to freshwater, elevated turbidity (via catchment erosion) and abstraction for agriculture have resulted in a decline in water quality and quantity (i.e. water – provisional service) and increase in eutrophication (Bouvy *et al.* 2010) amongst other impacts.

Mngazana Estuary has a mean annual runoff (MAR) of 46.5 x 10^6 m^3 with a low monthly average flow of 0.34 m^3 s^{-1} (Yang *et al.* 2014). According to the recent NBA assessment, Mngazana has a Present Ecological State (PES) of Category B [1] and a Recommended

[1] Largely natural with few modifications: A small change in natural habitats and biota may have taken place but the ecosystem functions and processes are essentially unchanged.

Ecological Category (REC) of B (Van Niekerk & Turpie 2012). This estuary has been subjected to minor human developments (i.e. holiday cottages, small community housing). Restricted access to some parts of the Eastern Cape coast including the former Transkei has allowed for undisturbed aesthetic scenery along the South African coastline, however this may now be at risk due to the proposed road network that will be built along this area (CCA 2009).

Harrison (2004) reported the following physico-chemical variables at Mngazana; mean temperature (23.5 °C), salinity (29 ppt) and dissolved oxygen (5.9 $mg \cdot l^{-1}$). Emmerson (2005) evaluated the physico-chemical characteristics at Mngazana (main channel) and found the mean temperature to be 23.4 °C (17.1 – 31.2 °C) with summer values significantly higher than winter. Average salinity was 25.3 ppt with no variation between seasons (summer: 25 ppt, winter: 25.6 ppt). The average depth was measured at 91.2 cm with no seasonal difference. The estuary was found to be well oxygenated (7.9 $mg \cdot l^{-1}$). A strong marine influence was evident with salinity ranging from 30 – 35 ppt in both Creeks 1 and 2. A longitudinal salinity gradient was recorded in the main channel with increasing distance from the mouth. Ngesi (2010) measured the nutrient content along the main channel and creeks in the Mngazana Estuary during 2002 and 2003. Low nutrient concentrations enter the estuary from the catchment via freshwater inputs. Soluble reactive phosphorus (SRP) concentrations ranged from 0.1 to 18.9 µM with little variation between the creeks and the main channel while main channel total oxidised nitrogen (TOxN) concentrations were generally between 0.1 and 8.7 µM. The highest ammonium (NH_4^+) concentrations were recorded 5.6 km upstream along the main channel (92.8 µM) and in Creek 2 (50.6 µM) (Ngesi 2010). The elevated concentrations of nutrients in Creek 2 are likely due to seepage from the anoxic mangrove sediments.

Ngesi (2010) revealed that the overall water column chlorophyll *a* at Mngazana showed no variation between the surface and bottom of the estuary and no seasonal differences were reported. During this time the phytoplankton community was dominated by flagellates (Relative Abundance: 60 %) with diatoms making up the remainder. No evidence of a unique microalgal community was reported while phytoplankton biomass was significantly correlated to DIN (Dissolved Inorganic Nitrogen) in Creek 2 of the estuary (Ngesi 2010).

Estuarine salinity remains high as Mngazana has a relatively large tidal prism, with good tidal mixing (Branch & Grindley 1979) which is characteristic of a permanently open system (Whitfield 1992). Emmerson (2005) stated that, the water clarity of the estuary decreases under high river flow conditions when turbid water flows in. Seasonal differences can be

expected at Mngazana Estuary due to the high summer rainfall which is expected to induce high freshwater inflow. However, according to Ngesi (2010), Mngazana Estuary is an oligotrophic system limited by phosphorus and nitrogen availability therefore nutrient fluctuations that influence phytoplankton biomass are generated within the system (i.e. mangrove porewater nutrients) since freshwater inflow into the estuary was previously recorded as low.

This study aimed to evaluate the effect of variations in physico-chemical and nutrient dynamics on microalgal biomass and composition between 2014 and 2015 and to compare this to prior study by Ngesi (2010).

4.1.1 Study Hypotheses

Physical variables

- Mngazana Estuary will be well oxygenated (>6.0 $mg \cdot l^{-1}$) along the main channel and Creeks 1 and 2.

Chemical variables

- Nutrient concentrations - soluble reactive phosphorus (SRP), ammonium (NH_4^+) and total oxidised nitrogen (TOxN) - will be higher along the main channel than Creeks 1 and 2.

Biological variables

- Water column chlorophyll *a* for the entire system will be low (< 10 µg Chl-*a* l^{-1}) due to low freshwater inflow.
- Mngazana Estuary will be dominated by flagellates along the main channel and Creeks 1 and 2 as recorded by Ngesi (2010).
- Faecal bacterial contamination will be high (> 2000 CFU 100 ml^{-1}) along the upper reaches of the estuary due to cattle presence.

4.1.2 Study area

The Mngazana Estuary (31° 42`S; 29° 25`E) is a permanently open estuary and is situated approximately 18 km south of Port St. Johns (the closest town) (Rajkaran & Adams 2007). It lies in the transition area between the warm and subtropical biogeographical region of the Eastern Cape, South Africa. The estuary is relatively short, approximately 7 km in length and has two major creeks that support the main mangrove populations. The tidal influence is hindered by a road bridge upstream of the estuary (Fig 4.1). The Mngazana River is approximately 150 km in length, it delivers freshwater into the estuary and the river arises from a small but steeply incised catchment estimated at about 275 km^2 in area (Branch & Grindley 1979). Branch (1976) noted that Mngazana Estuary is located in the coastal escarpment with low and restricted agricultural activity thus causing the estuary to be relatively silt free. The connection with the sea is permanently maintained via prominent dolomite based rocky promontory along the mouth through a strong tidal exchange (Wooldridge 1977, Theron 2007) this translates to the estuary being marine-dominated (salinity fluctuations ranging from 30 to 35 ppt) (Branch & Grindley 1979). The Mngazana Estuary has a high national conservation importance score of 84.5 (Turpie *et al.* 2002) and based on its geomorphology; unique geology and tidal flat formation, Colloty *et al.* (2002) classified the estuary as a Class 3B.

According to Harrison *et al.* (2001), 50% of the Mngazana catchment had been classified as natural and 24% as degraded. In the last 10 – 13 years, catchment use has been expected to increase as a consequence of community expansion and proposed infrastructure upgrading (Fig. B1, Appendix). The agricultural practices around Mngazana Estuary mostly consist of livestock farming and cultivation of crops (Deyzel 2012). Such activities have direct impact on estuarine water quality (i.e. pollution via cattle manure – faecal coliforms, fertiliser runoff). Grant (2007) predicted that due to the scenic beauty of this area, tourism and recreational activities will increase dramatically in the future. There are three settlements in the vicinity of the Mngazana estuary: Madakeni, Cwebeni and Mtalala villages. Closer to the mouth, there are a number of holiday cottages, some of which after a moratorium on land grants were constructed illegally in the 1990s (de Wet 2004). According to Mlangeni (2007) there are at least ten villages within in a 10 km radius of the estuary mouth. The different users of the Mngazana Estuary include; recreational use (i.e. fishing), subsistence use (i.e. bait collection and farming) and commercial use (i.e. boarding and lodging). However, the people around the Mngazana Estuary along with tourists seem to mostly utilise the estuary for recreational fishing and to support their livelihoods (Mlangeni 2007).

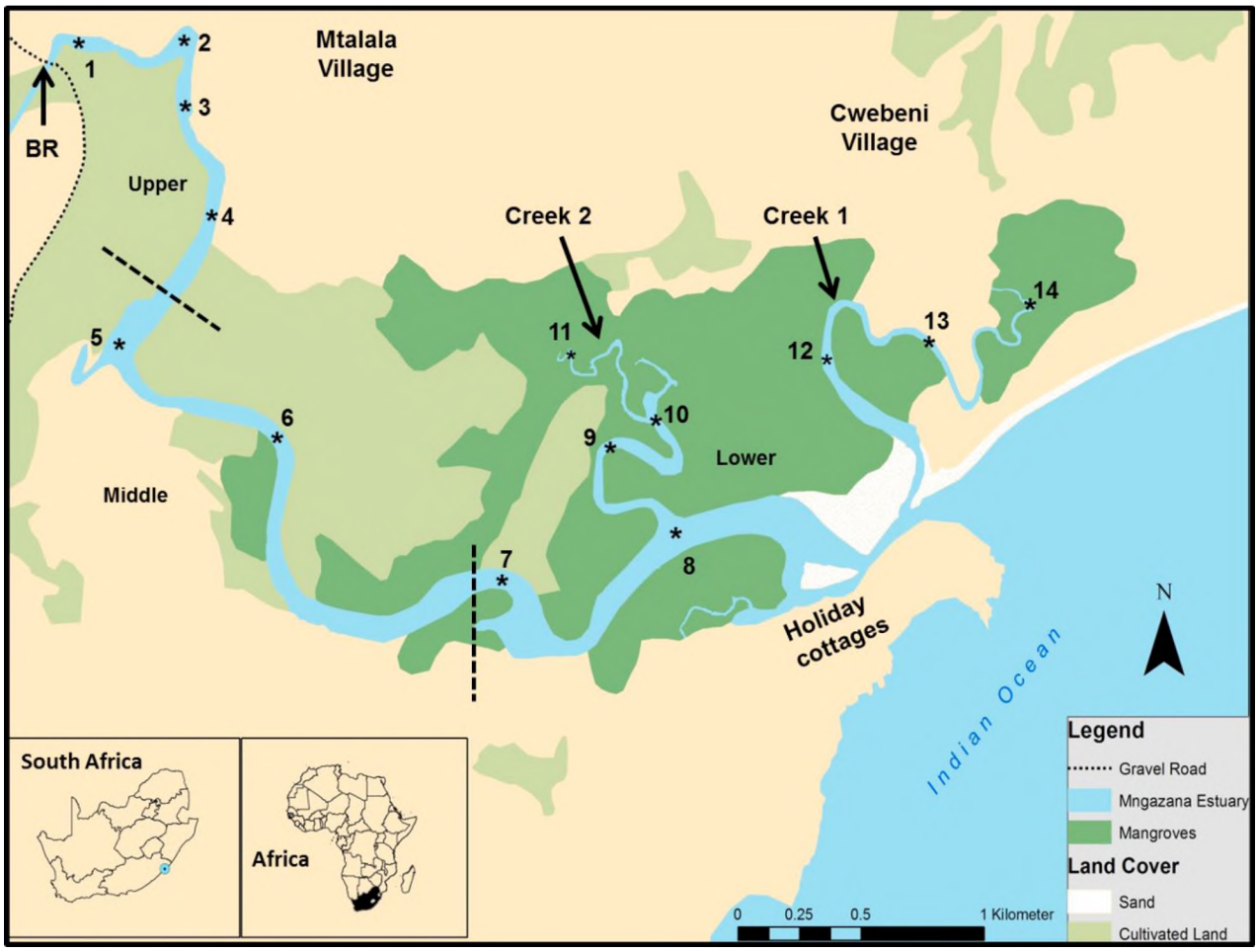

Figure 4.1: Location of the Mngazana Estuary along the east coast of South Africa. Asterisks (*) indicate sampling sites along the main channel (1 – 8), Creek 2 (9 – 11) and Creek 1 (12 – 14). **BR** – indicates the location of the bridge upgrade and dashed line (- - -) delineates between estuarine reaches.

The weather data were obtained from Weather SA (South African Weather Services) and were collected at Port St Johns (18 km north of Mngazana – Fig. 4.2). Rain occurs all year round in this region accompanied by floods, mostly in the months of March and December. This estuary is in the transitional region with minimum temperatures ranging from 8.3 to 22.4 ºC while the maximum temperatures range from 18.7 to 29.6 ºC (over the 57 years period). Mngazana Estuary has an average (± SE) monthly rainfall of 81.7 ± 2 mm month^{-1} and experiences less winter (average ± SE: 44.2 ± 3.2 mm) than summer rainfall (average ± SE: 107.4 ± 4.2 mm) (Fig 4.3). The mean annual rainfall, over a 60-year period (1955 – 2015) is 956 mm.

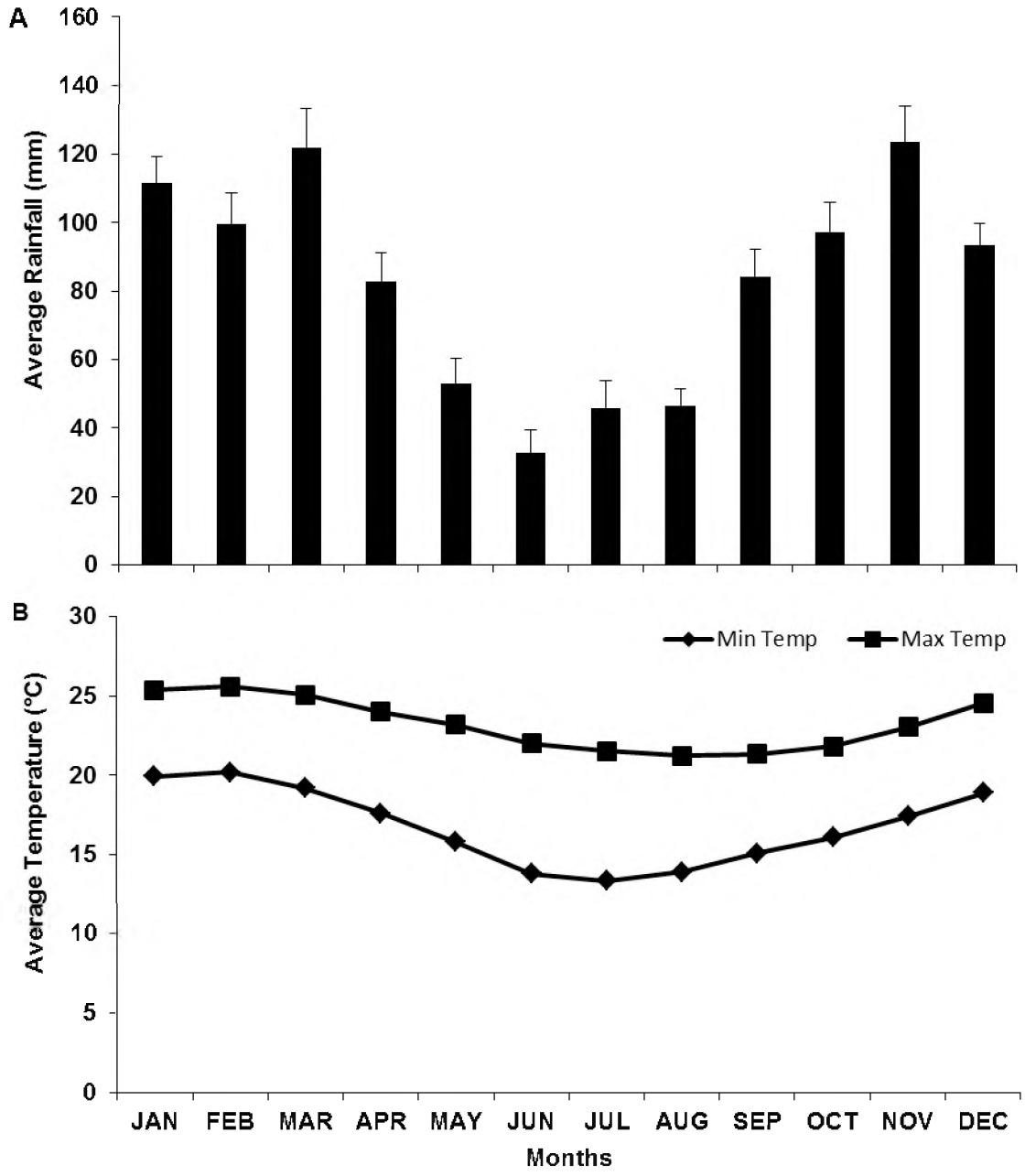

Figure 4.2: Historical (a) rainfall (1955 to 2015) and (b) temperature (1959 to 2015) measurements collected monthly at Port St Johns (18 km north of Mngazana Estuary). (Average ± SE present but in some cases not visible).

The estuary harbours the third largest mangrove forest in South Africa covering an estimated area of 118 ha (Hoppe-Speer 2012). The forest is characterised by the largest stand of red mangrove (*Rhizophora mucronata* Lam) in South Africa but it is still dominated by white mangroves (*Avicennia marina* Forssk. Vierh). Black mangrove (*Bruguiera gymnorrhiza* L. Lam) is also present. Other biota found in the Mngazana Estuary include salt marsh species such as; *Triglochin striata, Sarcocornia perennis, Sarcoconia natalensis, Bassia diffusa* (Thunb.) Kuntze*, Coluta filifolia, Salicornia meyeriana* and *Limonium linifolium* var. *maritimum* (Colloty *et al*. 2002). Due to the complexity of this system, it supports a diversity of invertebrates and fish species. Mbande (2003) identified 66 fish species compared to 62 identified by Branch & Grindley in (1979); Sesarmid crabs have been previously identified in Mngazana (Ngesi 2010) and it also harbours three crab species of the Red Data list (Sgwabe *et al*. 2004). Four of the five *Uca* crab species of South Africa; *U.* (*Austruca*) *annuipes* (H. Milne Edwards, 1837), *U.* (*Tubuca*) *urvillei* (H. Milne Edwards, 1852), *U.* (*Gelasimus*) *hesperiae* (Linnaeus, 1758) and *U. (Paraleptuca) chlorophthalmus* (H. Milne Edwards, 1837) are resident of the Mngazana Estuary (Emmerson 1994, Peer *et al*. 2015). The diverse flora and fauna contribute to the botanical and conservation importance of this estuary (Rajkaran & Adams 2012).

The tidal creeks at Mngazana both meander approximately 1.5 km north-east through dense mangrove forests: Creek 1 connects with the lower estuary approximately 150 m from the mouth. The inlet is a narrow (<8 m wide at HT) and shallow (<1.5 m at HT) channel that continually shifts in position as it crosses the sandy delta near the mouth. The rest of the creek is shallow (mean depth <1 m) except for the upstream section (Deyzel 2012). Creek 2 links with the main channel approximately 1.15 km from the mouth. Creek 2 inlet is characterised by finer sediments and organic material deposited by outflowing currents. The main channel with water depth that ranges from 2 to 3 m and even deeper in some areas meanders through the catchment and long-term erosion by the characteristic strong tidal currents has resulted in the formation of vertical estuary banks, extending up to 2 m above high tide water level (Deyzel 2012).

The upper reaches of the estuary (near Site 4) become very shallow (<1.5 m), where subtidal sediments comprise of coarse pebbles and large rocks between pockets of fine silts and degrading organics material. The elevated causeway at the top of the estuary results in unidirectional river flow preventing tidal exchange from extending further upstream (Branch and Grindley 1979, Deyzel 2012) but during extreme springs high tides, flow can be reversed above the causeway. The varying depths and distance of sampling sites are summarised in Table 4.1.

Table 4.1. Characteristics of the sampled sites at Mngazana Estuary, the same sites were used by Ngesi (2010).

Sampling station	Depth (m)	Distance from mouth for main channel sites & from the main channel for sites in the Creeks (km)	Observed impacts
Main channel			
Site 1	0.5	6.2	Clothes washing/detergent introduction into the system.
Site 2	1.0	5.7	Clothes washing.
Site 3	1.0	5.3	No visible activities. Cattle close by.
Site 4	1.3	4.9	Cattle sitting along the channel (during low tide).
Site 5	2.0	4.3	Entrance of small creek – water abstraction (Fig. B2 Appendix). There is a jetty located near the site.
Site 6	3.0	3.5	Erosion along the northern bank. Signs of bait collection on the opposite bank
Site 7	3.5	2.0	Signs of erosion.
Site 8	3.0	1.0	Recreational finishing near entrance of creek 2 and signs of bait collection.
Creek 2			
Site 9	1.0	0.5	Fishing taking place.
Site 10	2.0	1.1	Cattle presence.
Site 11	2.0	1.8	Possible cattle presence and harvesting.
Creek 1			
Site 12	1.0	0.8	Fishing taking place.
Site 13	1.0	1.6	Cattle grazing adjacent to the channel.
Site 14	1.0	2.5	No visible impacts but there are signs of harvesting and a half constructed fish trap in the area.

4.2 Results

4.2.1 Physico-chemical variables

4.2.1.1 Salinity

Salinity did not vary between sampling periods along the main channel during 2014 ($U = 299$; $p > 0.05$; $n = 52$) and 2015 ($U = 265$; $p > 0.05$; $n = 50$). Main channel salinity decreased with distance from the mouth, ~15 ppt was recorded at Site 2 (5.7 km) in the upper reaches during summer 2014 (Fig. 4.3A). However, the estuary was mostly saline with an average salinity of ~30 ppt during summer and winter 2015 (Fig. 4.3C &D). Creek 2 (summer 2014) was significantly more saline than summer 2015 ($U = 18$; $p < 0.05$; $n = 19$) with the lowest salinity recorded during summer 2015 (20 ± 4 ppt) along this creek (Fig. 4.4A-D). Creek 1 (winter 2015) was significantly more saline ($U = 15$; $p < 0.05$; $n = 16$) than 2014 with an average of 36 ± 0.9 ppt (Fig. 4.5 A-D).

4.2.1.2 Temperature

Temperature differed seasonally, with summer temperatures significantly warmer than winter along the main channel during 2014 ($U = 0$; $p < 0.05$; $n = 52$) and 2015 ($U = 3$; $p < 0.05$; $n = 50$). Summer 2014 was significantly hotter than summer 2015 ($U = 181$; $p < 0.05$; $n = 54$) along the main channel and Creeks 1 and 2 ($U = 0$; $p < 0.05$) and ranged from 26 ± 0.6 to 29 ± 0.4 °C (Fig. 4.3, 4.4 and 4.5E – H). The lowest temperature (~18.7 ± 0.1 °C) was recorded at 1 km (Site 8) from the mouth during summer 2015. Winter temperatures were significantly higher in 2015 along the main channel ($U = 90$; $p < 0.05$; $n = 48$) and Creek 2 ($F = 6.15$; $p < 0.05$; $n = 19$) with the highest winter temperature being ~18 °C across the entire estuary. Temperature varied along the estuary length and was correlated with distance from the mouth in summer 2015 (Table A3, Appendix).

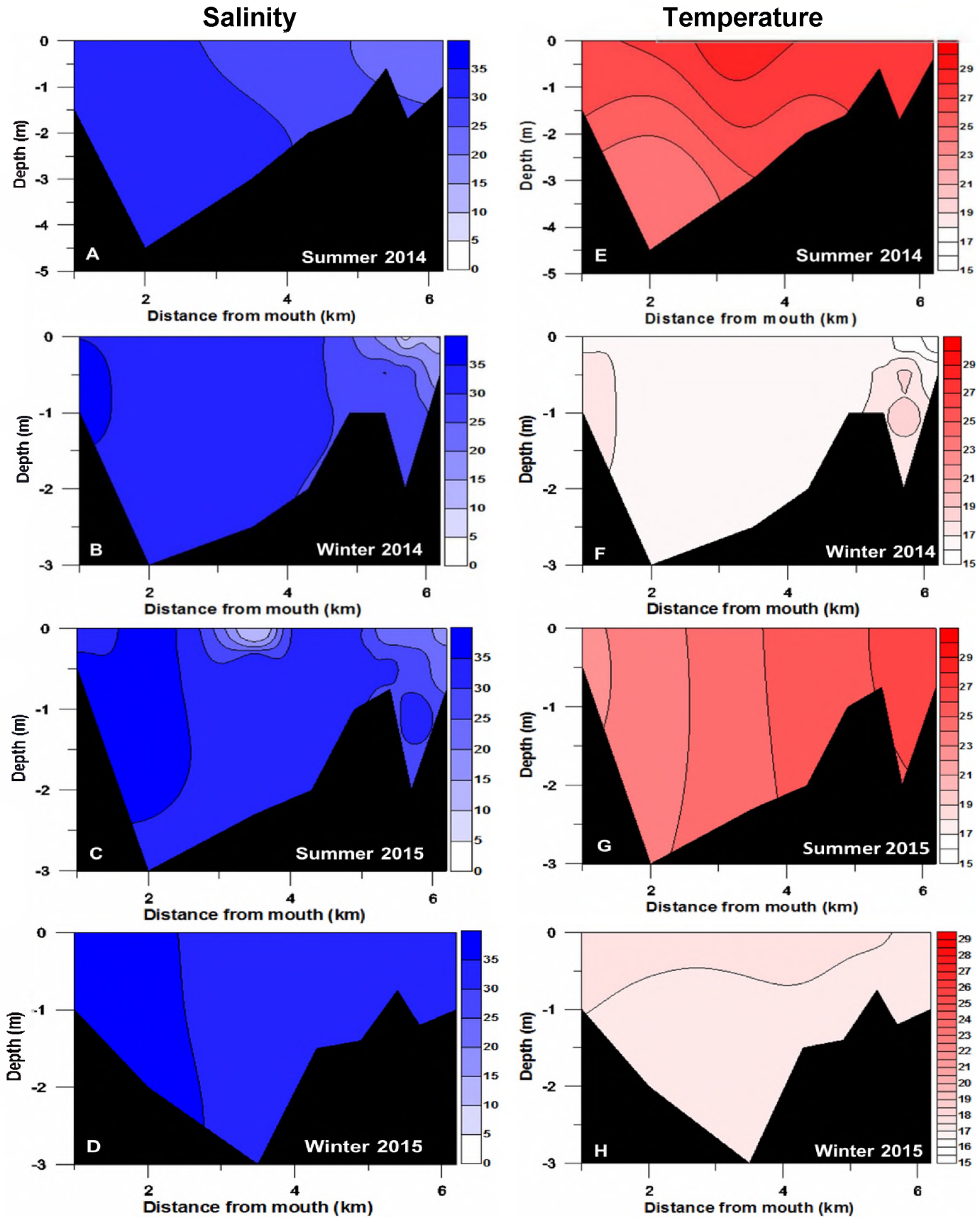

Figure 4.3: Salinity (ppt) (A – D) and temperature (°C) (E – H) profiles along the Mngazana main channel during the study period.

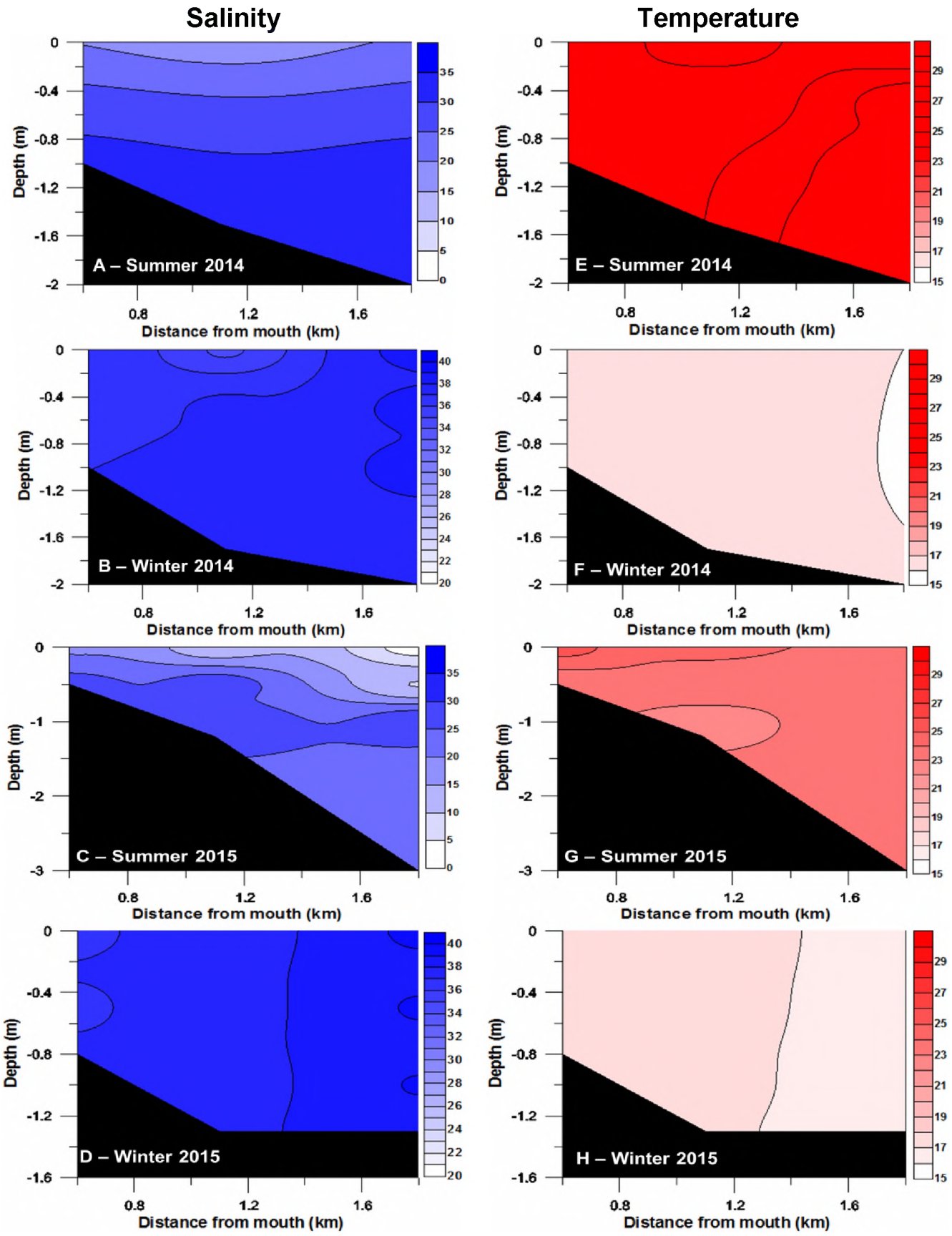

Figure 4.4: Salinity (ppt) (A – D) and temperature (°C) (E – H) profiles along Creek 2 at Mngazana during the study period. .

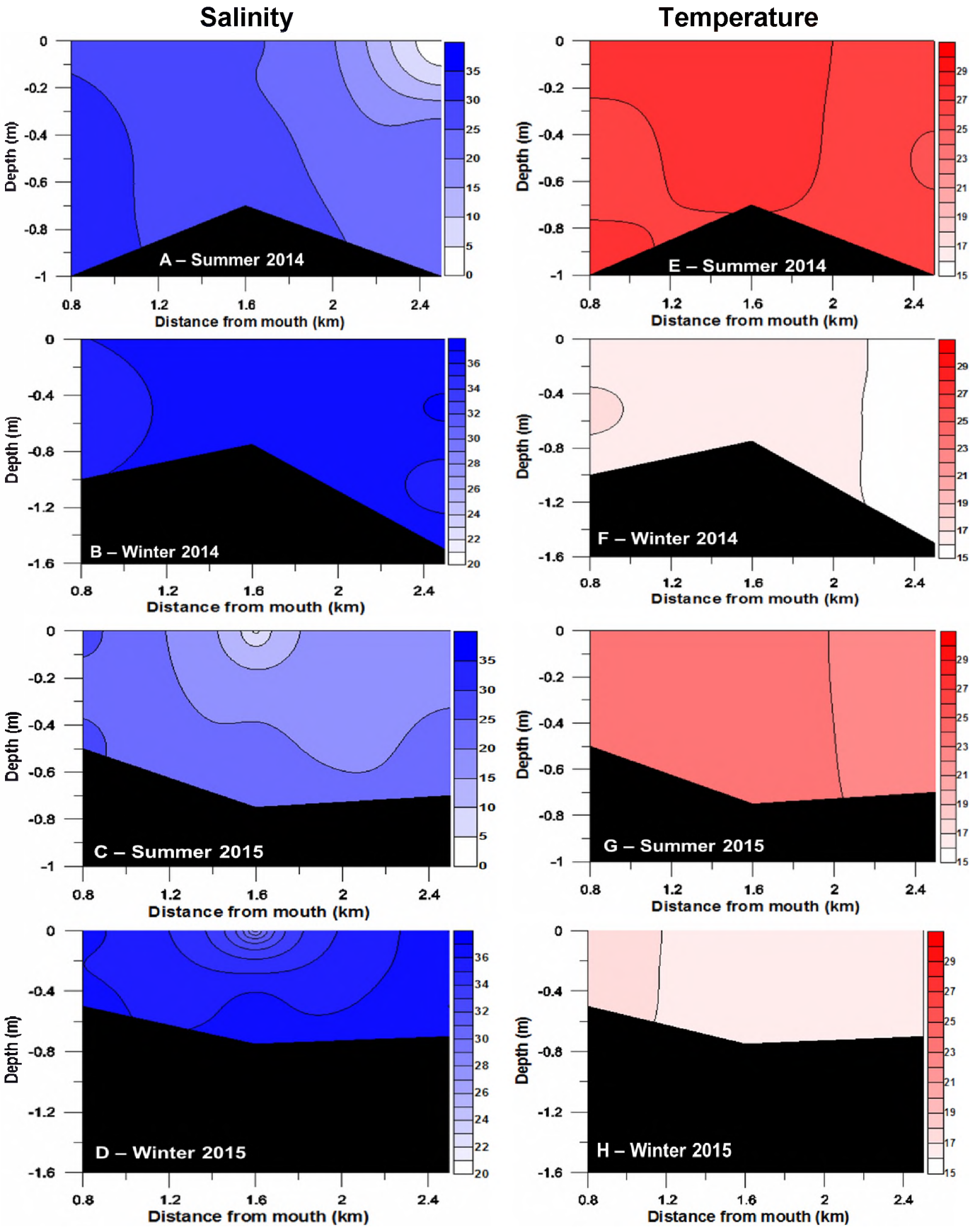

Figure 4.5: Salinity (ppt) (A – D) and temperature (°C) (E – H) profiles along Creek 1 at Mngazana during the study period.

4.2.1.3 Dissolved Oxygen and pH

Dissolved oxygen (DO) varied along the length of the estuary with summer 2014 (~7.7 ± 0.2 $mg \cdot l^{-1}$) concentrations being significantly higher than 2015 (~6.1 ± 0.2 $mg \cdot l^{-1}$) ($F = 26.9$; $p < 0.05$; $n = 54$) while no significant differences were found in Creek 1 and 2 ($p > 0.05$). DO levels were higher ($p < 0.05$) in the upper reaches of the main channel particularly 6.2 km from the mouth during the summer (10 ± 0.8 $mg \cdot l^{-1}$) and winter 2014 (9.4 ± 0.2 $mg \cdot l^{-1}$) (Fig. 4.6A & B). The lowest recorded DO level (2.7 ± 0.4 $mg \cdot l^{-1}$) was 1.8 km (Site 11 in Creek 2) with water stratification evident along the Creek (Fig. 4.7A-C). The highest DO level (10.6 ± 0.9 $mg \cdot l^{-1}$) was 0.8 km (Site 12 in Creek 1) with possible stratification in upper reaches during summer 2014 and mid-channel DO entrapment in Creek 1 (Fig. 4.8A & C). DO was positively correlated with salinity in summer 2014 (Table A1, Appendix), correlated with distance from the mouth and negatively correlated to salinity during winter 2014 (Table A2, Appendix).

pH of the water column was significantly higher during summer 2014 than summer 2015 ($U = 174$; $p < 0.05$; $n = 54$) along the main channel and Creek 2 ($F = 6.45$; $p < 0.05$; $n = 19$) (Fig. 4.9A & B). Measurements were more alkaline in summer 2014 compared to winter 2015 along the main channel ($F = 37.5$; $p < 0.05$), Creek 1 and 2 ($F = 8.68, 16.3$, $p < 0.05$). pH was highest along the main channel water column (~8) than the creeks for the duration of the study and was significantly correlated with dissolved oxygen during summer 2014 (Table A1, Appendix), 2015 (Table A3, Appendix) and winter 2014 (Table A2, Appendix) respectively. Furthermore, it was correlated to temperature in winter 2014 and 2015 (Table A2 & 4, Appendix) and with distance from the mouth during winter 2014 and summer 2015 respectively.

In terms of light penetration into the water, Secchi depth fluctuated throughout the sampling period with clear waters (low turbidity) persisting along the main channel during summer and winter 2014 (Depth: 60 – 100 cm) (Fig. 4.10C). Highly turbid conditions were recorded along Creek 2 during summer 2014 with increasing distance from the main channel while the highest turbidity (lowest Secchi depth - 25 cm) was evident along Creek 1 from 1.6 km from the channel.

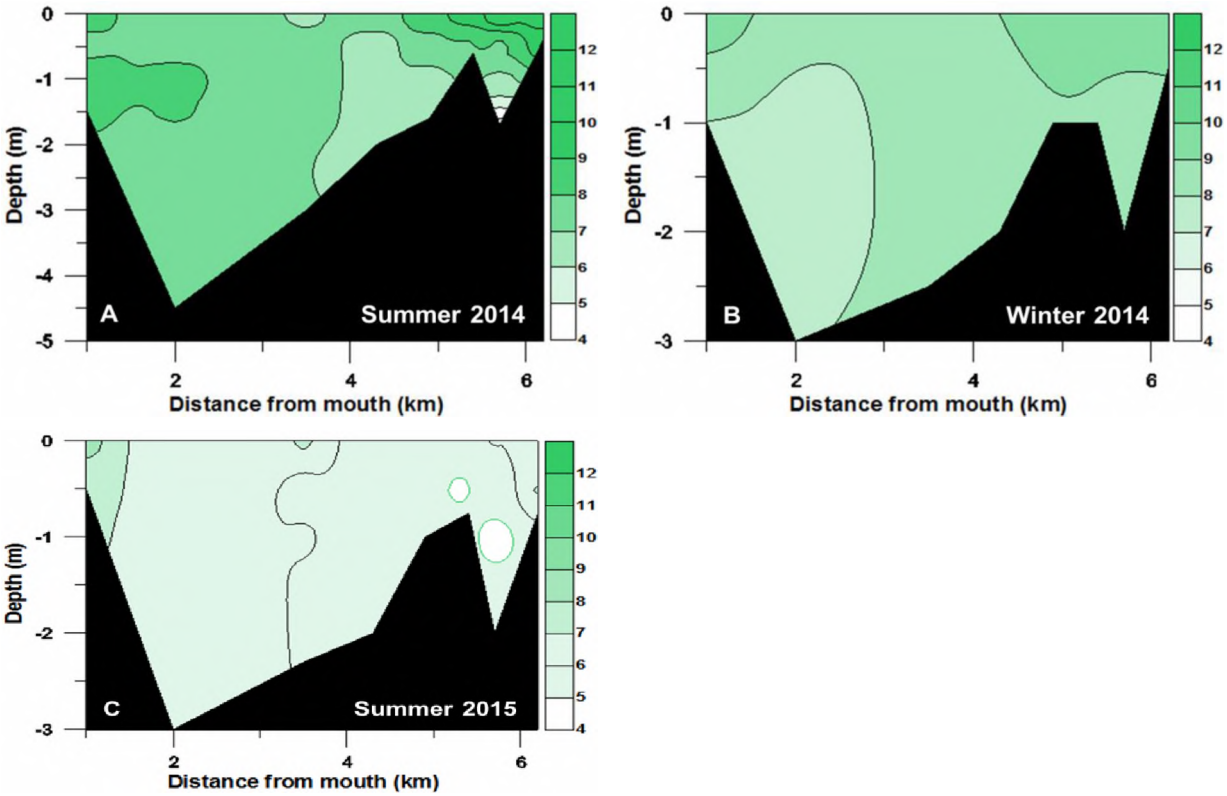

Figure 4.6: Dissolved oxygen (mg·l^{-1}) along the Mngazana main channel during the study period. **No dissolved oxygen data in winter 2015 as the meter was faulty**.

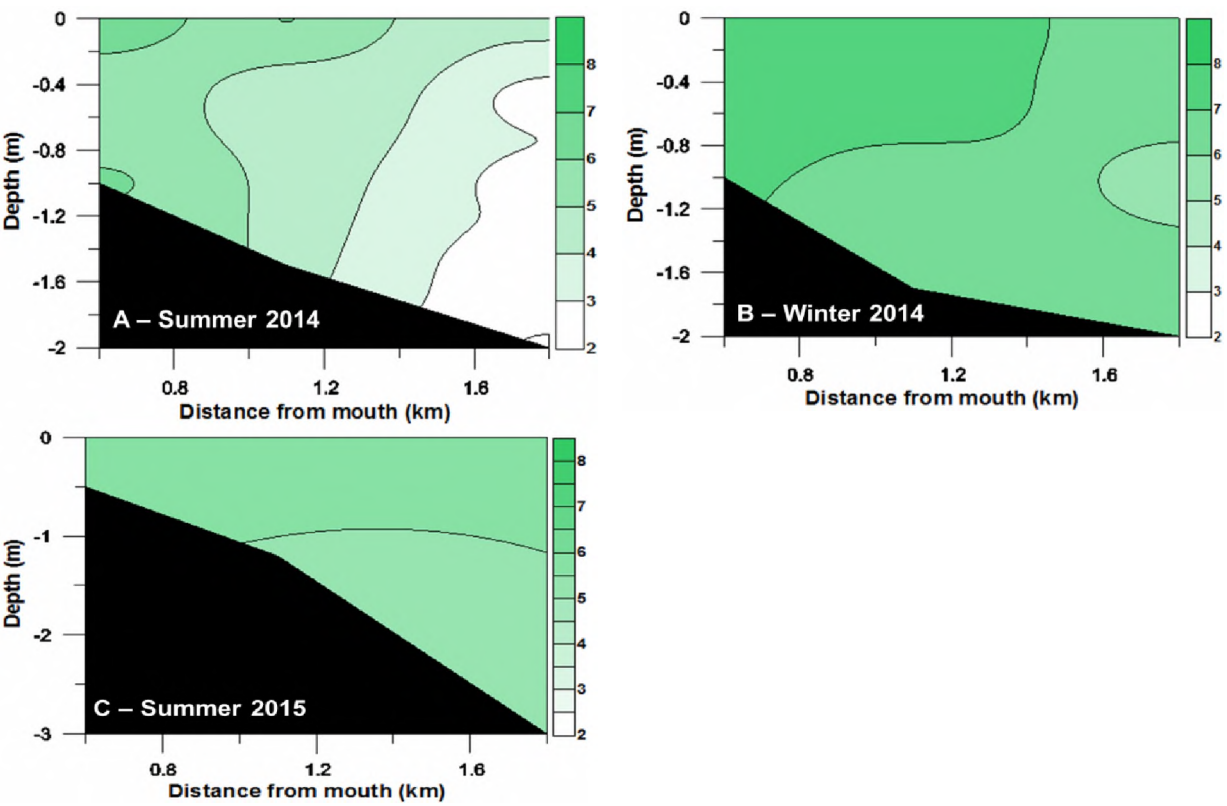

Figure 4.7: Dissolved oxygen (mg·l^{-1}) along Creek 2 at Mngazana during the study period. **No dissolved oxygen data in winter 2015 as the meter was faulty**.

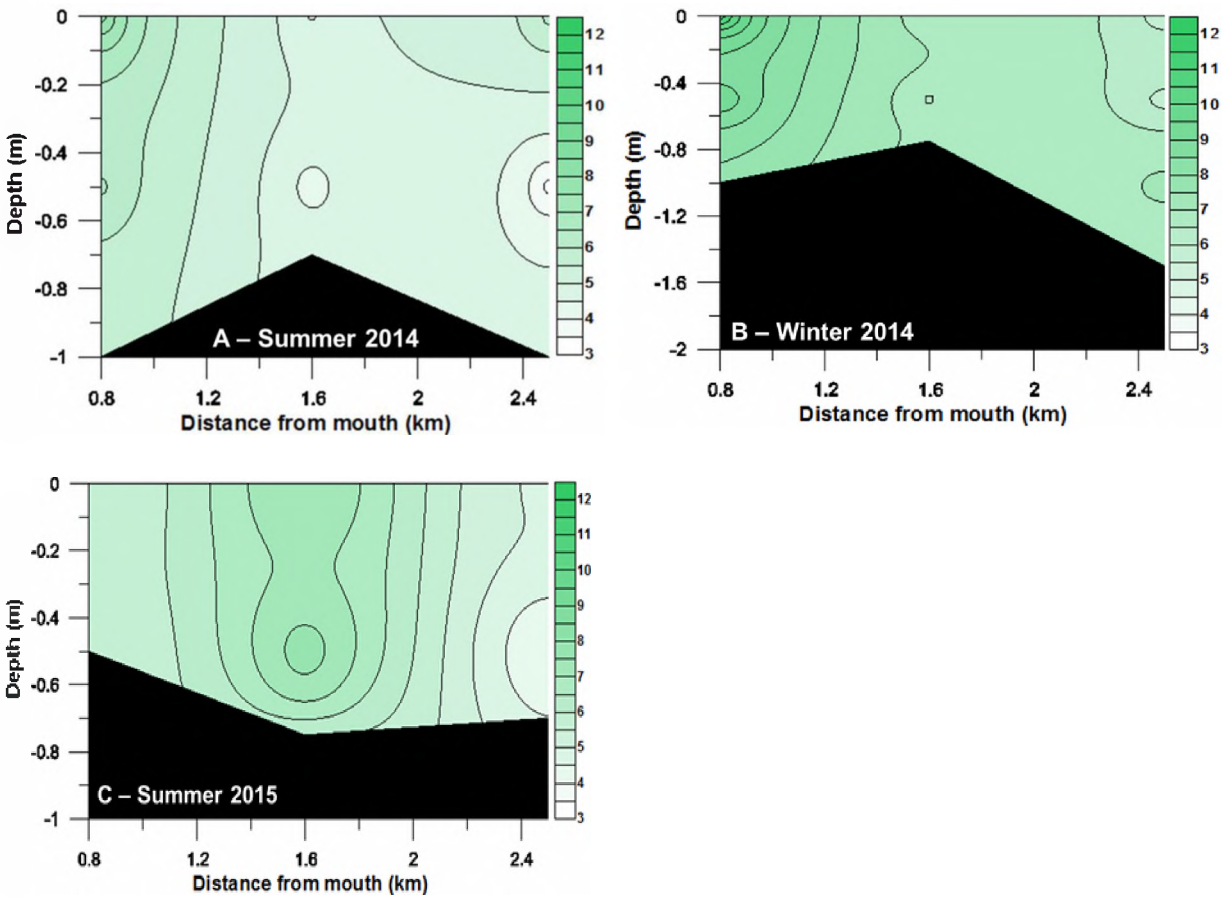

Figure 4.8: Dissolved oxygen ($mg \cdot l^{-1}$) along Creek 1 at Mngazana during the study period. **No dissolved oxygen data in winter 2015 as the meter was faulty**.

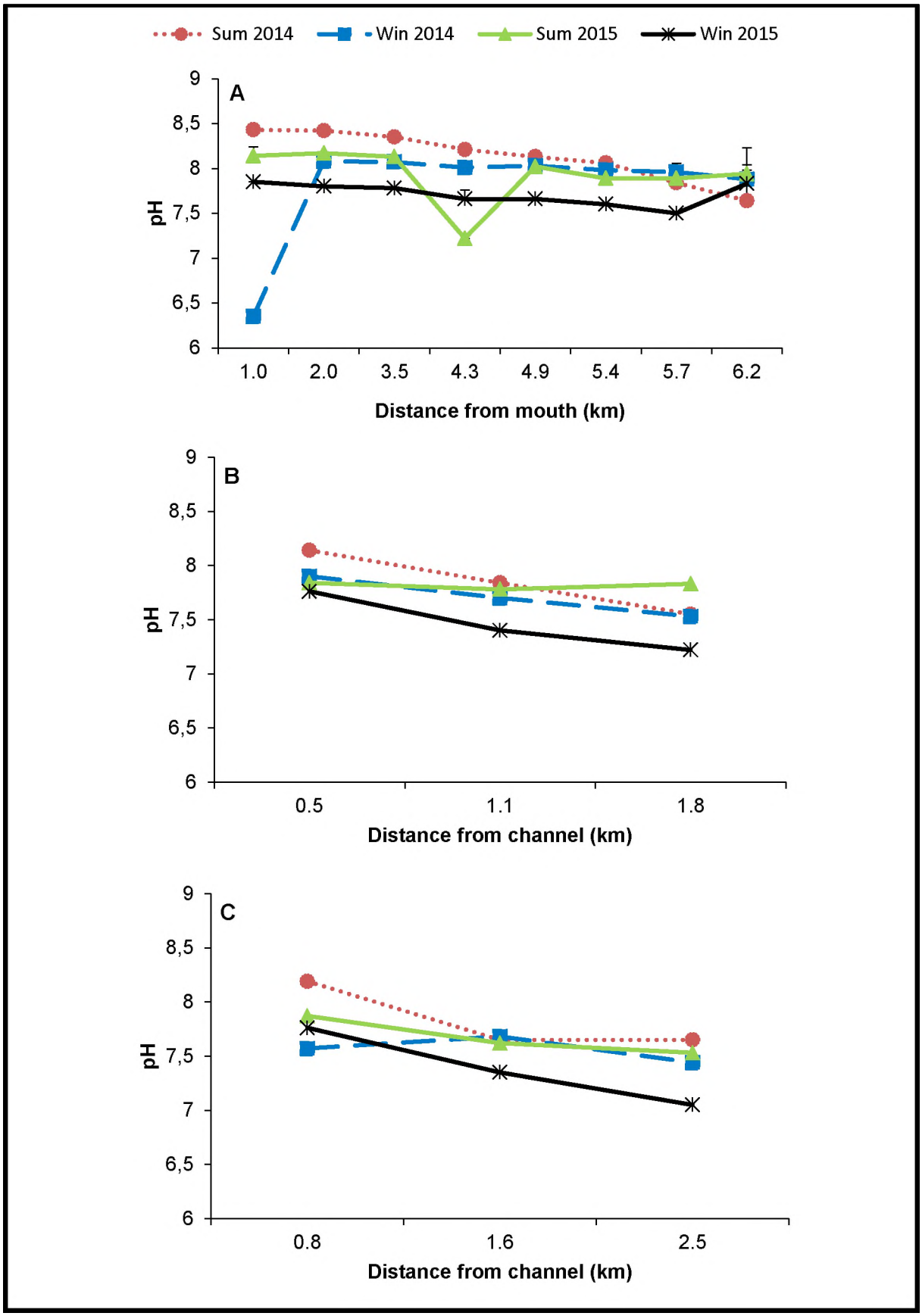

Figure 4.9: pH (± SE present but not visible) recorded at Mngazana Estuary along A - Main channel, B - Creek 2 and (C) Creek 1 during the sampling period.

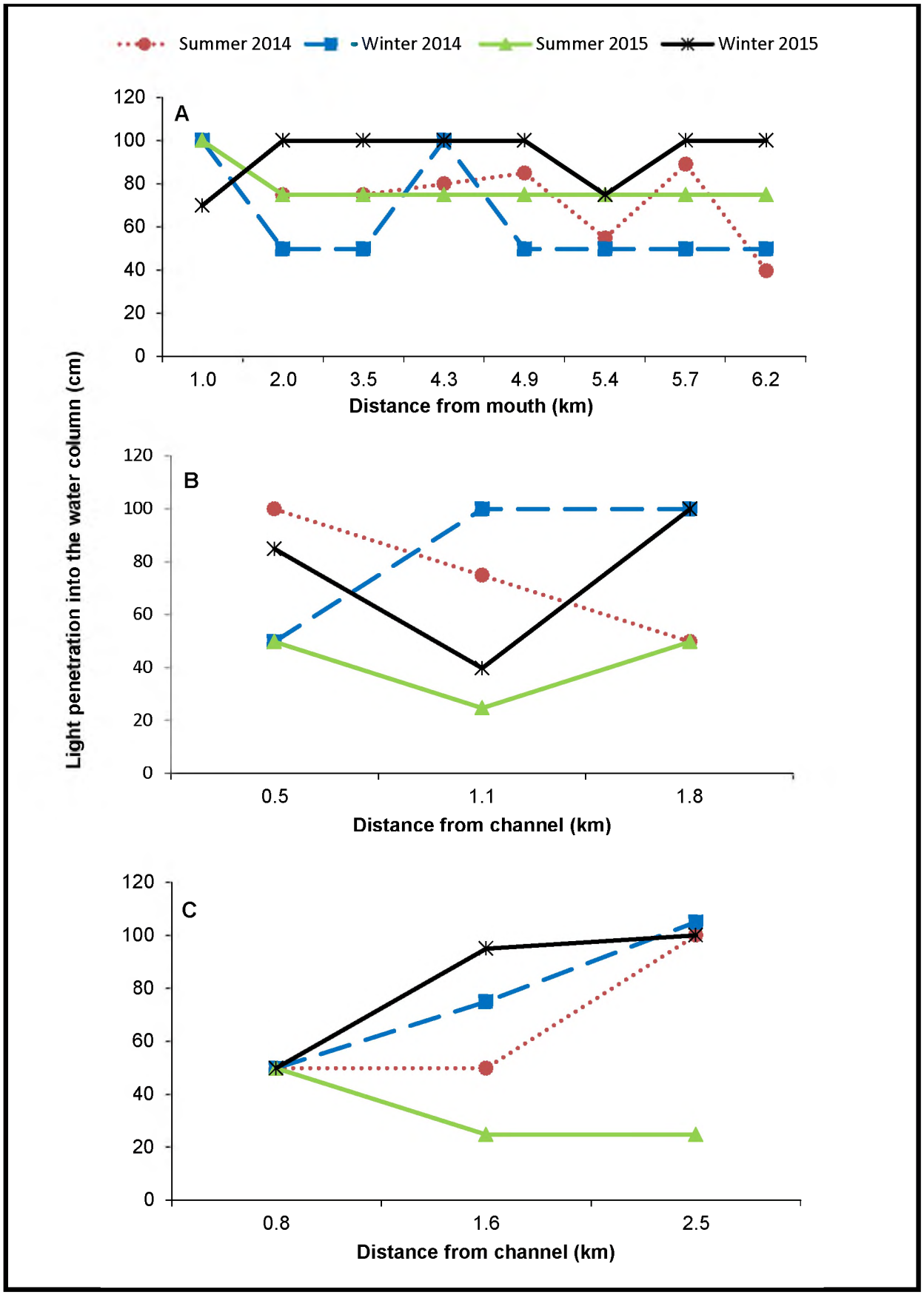

Figure 4.10: Secchi depth at the Mngazana Estuary along A – Main channel, B – Creek 2 and C – Creek 1 during the sampling period.

4.2.2 Inorganic nutrient concentrations

4.2.2.1 Ammonium (NH_4^+)

Ammonium (NH_4^+) concentrations varied significantly across the different sampling seasons in each year; 2014 (U = 694; $p < 0.05$; n = 93) and 2015 (U = 42; $p < 0.05$; n = 84). Ammonium in the main channel was higher in summer 2014 than summer 2015 (U = 618, $p < 0.05$; n = 96) but was similar along Creek 1 and 2 (U = 63 and 107; $p > 0.05$) respectively. It was significantly higher in winter 2015 along all water bodies in this estuary than winter 2014 (MC – U = 55, Creek 1 – U =0, Creek 2 – U =32; $p < 0.05$) (Fig 4.11 – 13A & B). Surface concentrations were only significantly higher during winter 2014 along the main channel and Creeks 1 and 2 (MC – U = 140, Creek 1 – U = 16, Creek 2 – F = 32; $p < 0.05$) respectively. The highest NH_4^+ concentration ~ 32.3 µM during the entire study period was recorded along Creek 2 at 1.8 km (Site 11) from the main channel (Fig 4.12A). NH_4^+ concentrations were correlated with dissolved oxygen in winter 2014 (Table A2, Appendix) and negatively correlated to salinity during summer 2015 (Table A3, Appendix).

4.2.2.2 Total oxidised nitrogen (TOxN)

Total oxidised nitrogen (TOxN) varied significantly across the sampling seasons, 2014 (U = 330; $p < 0.05$; n = 72) and 2015 (U = 361; $p < 0.05$; n = 105). Concentrations were similar between summer 2014 and 2015 along Creek 2 (U = 78; $p > 0.05$; n = 33) while increased with distance from the mouth in Creek 1 (Fig 4.13C). In winter 2015, TOxN concentrations were significantly higher than winter 2014 along Creek 1 (F = 31; $p < 0.05$; n = 36), Creek 2 (F = 20.1; $p < 0.05$; n = 36) and the main channel (U = 361; $p < 0.05$; n = 105). Furthermore, no significant differences were found between surface and bottom concentrations except in Creek 2 in summer 2015 (U = 13; $p < 0.05$; n = 18). TOxN concentrations were negatively correlated with distance from the mouth during summer 2014 (Table A1, Appendix), winter 2014 and 2015 (Table A2 & A4, Appendix).

4.2.2.3 Soluble reactive phosphorus (SRP)

Soluble reactive phosphorus (SRP) concentrations varied significantly during 2015 (U = 1041; $p < 0.05$; n = 108). Concentrations were significantly higher in summer 2014 than summer 2015 (U = 732; $p < 0.05$; n = 96) along the main channel while no differences were found in Creek 1 (U = 105; $p > 0.05$; n = 33) (Fig. 4.11 – 13E & D). Surface concentrations were significantly higher (U = 14; $p < 0.05$; n = 18) along Creek 1 during summer 2015. SRP

concentrations were negatively correlated with distance from the mouth and dissolved oxygen during summer 2014 (Table A1, Appendix). In winter 2014, SRP was s correlated with temperature (Table A2, Appendix) while correlated with pH in 2015 (Table A6, Appendix).

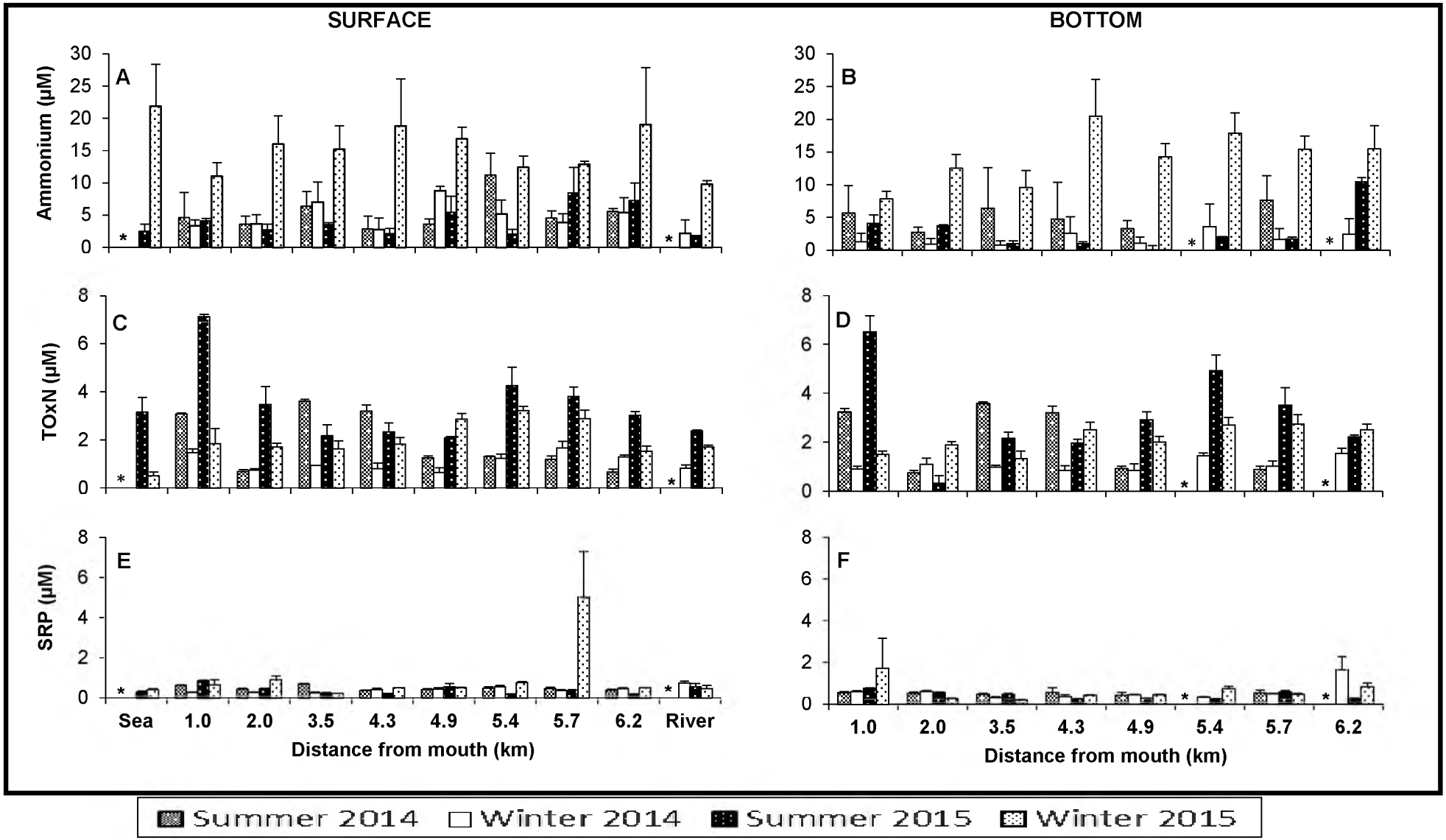

Figure 4.11: Nutrient concentrations of ammonium (A-surface & B-bottom), TOxN (C-surface & D-bottom) and SRP (E-surface & F-bottom) along the main channel of the Mngazana Estuary during the study period. Asterisk (*) indicates no data.

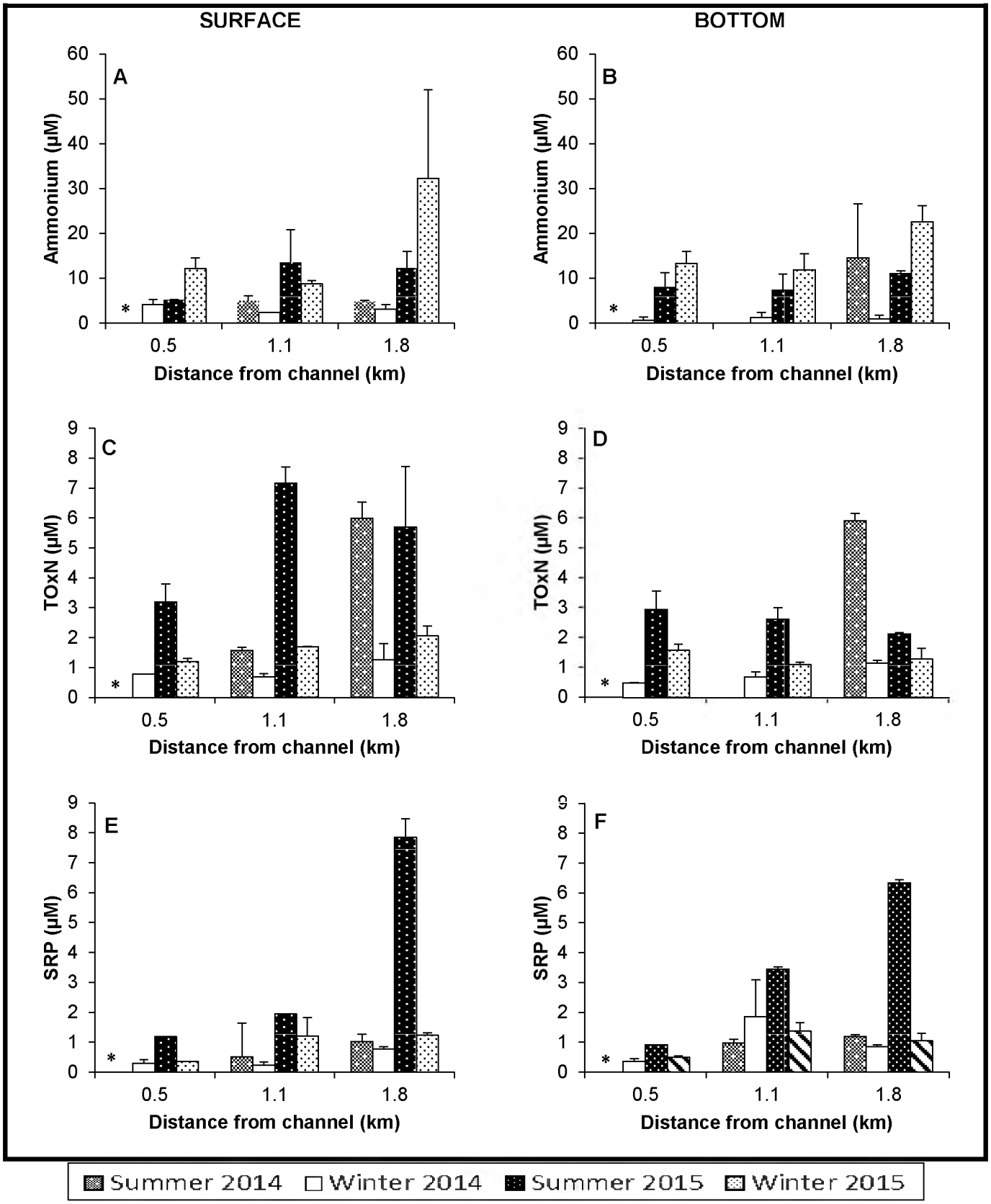

Figure 4.12: Nutrient concentrations of ammonium (A-surface & B-bottom), TOxN (C-surface & D-bottom and SRP (E-surface & F-bottom) along Creek 2 at Mngazana Estuary during study period. Asterisk (*) indicates no data.

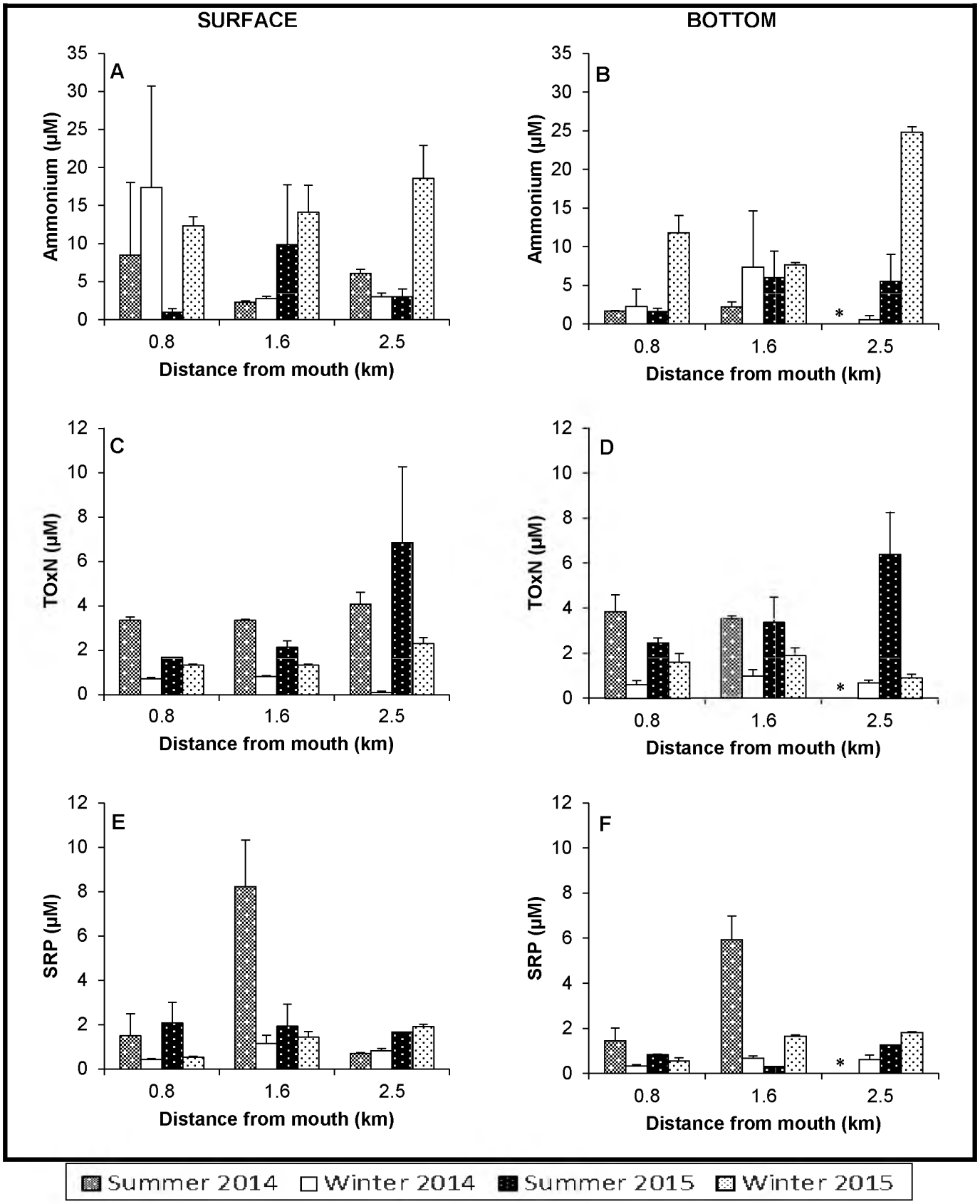

Figure 4.13: Nutrient concentrations of ammonium (A-surface & B-bottom), TOxN (C-surface & D-bottom) and SRP (E-surface & F-bottom) along Creek 1 at Mngazana Estuary during the study period. Asterisk (*) indicates no data.

4.2.3 Phytoplankton

Phytoplankton biomass

Phytoplankton biomass in the water column was measured (chlorophyll *a* was used as a proxy) and it varied significantly along the main channel between summer and winter in 2014 ($U = 309$; $p < 0.05$; $n = 96$) and 2015 ($U = 394$; $p < 0.05$; $n = 96$) (Fig. 4.14A). The maximum water column chlorophyll *a* recorded was during summer 2015 (20.1 µg Chl-*a* l^{-1}) along the water column and significantly differed between sites along the main channel ($p < 0.05$) (Fig 4.16A). Phytoplankton biomass bloom conditions (≥ 20 µg Chl-*a* l^{-1}) were only observed in summer 2015 in the upper reaches but no significant differences were observed between surface and bottom chlorophyll *a* ($p > 0.05$) across the sampling period.

Main channel Chl *a* biomass was highest during 2015 and winter biomass was 50 % less than recorded during summer. Chl *a* concentrations were significantly higher ($U = 457$; $p < 0.05$; $n = 96$) during winter 2015 (3.8 ± 0.5 µg Chl-*a* l^{-1}) than winter 2014 (1.5 ± 0.1 µg Chl-*a* l^{-1}) along the main channel (Fig 4.16D). Water column biomass significantly increased with increased distance from the mouth ($H = 25$; $p < 0.05$; $n = 48$) with the highest values recorded at 6.2 km from the mouth in summer 2015.

Creek 2 Chl *a* concentrations were significantly higher ($U = 37$; $p < 0.05$; $n = 36$) in summer 2014 than 2015 with the highest value recorded at 0.58 km but concentrations were similar between winter sessions ($U = 110$; $p > 0.05$; $n = 36$) (Fig. 4.15B). Creek 1 concentrations were also significantly high during summer sessions ($U = 93$; $p < 0.05$; $n = 36$) (Fig. 4.15 & 16C). Chl *a* concentration was positively correlated with distance from the mouth and temperature (Table A4, Appendix). Correlation between chlorophyll *a* and distance from the mouth was also observed during winter 2014 (Table A2, Appendix).

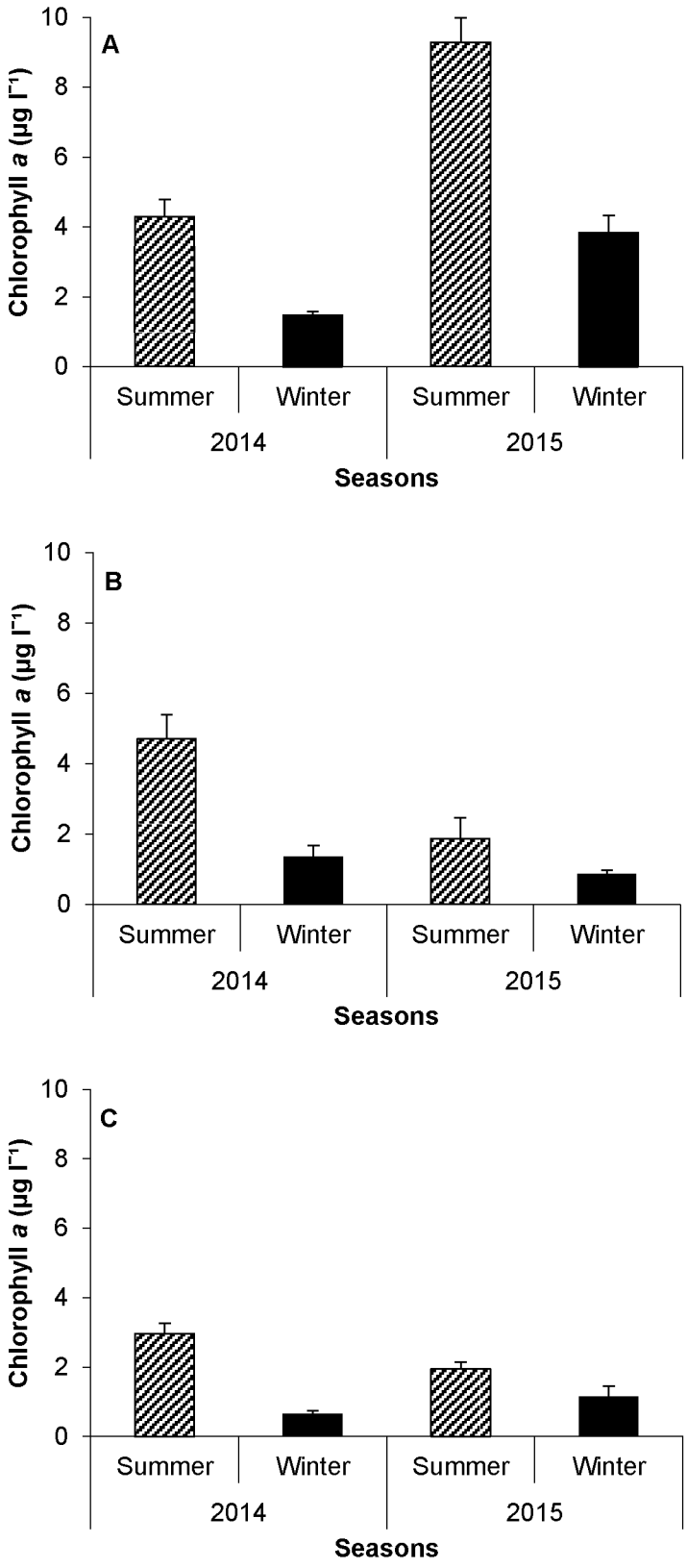

Figure 4.14: Seasonal mean phytoplankton biomass (chlorophyll *a*) recorded during the study period (mean ± SE) for (A) Main channel, (B) Creek 2 and (C) Creek 1.

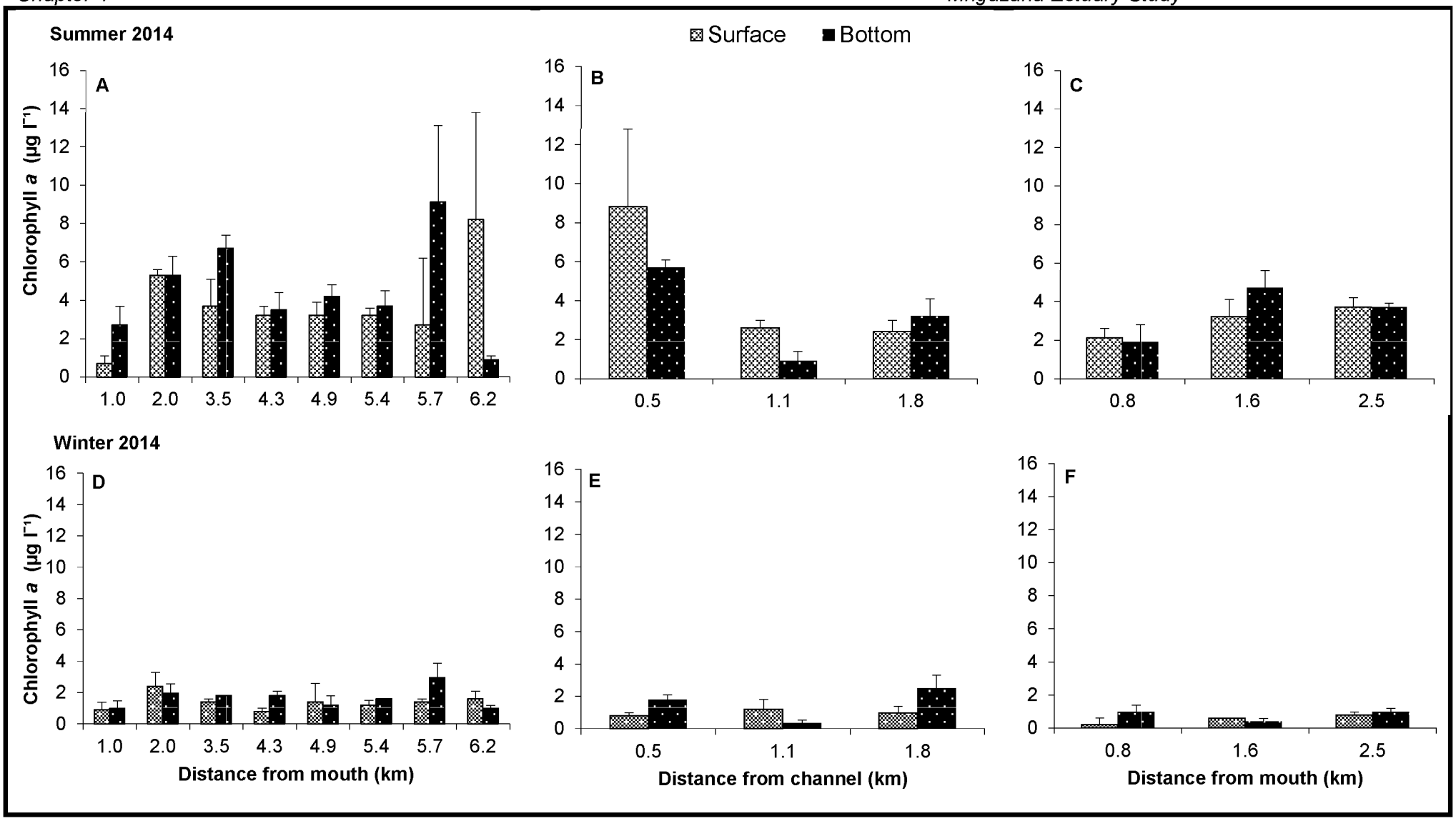

Figure 4.15: Mean phytoplankton biomass (chlorophyll *a*, mean ± SE) recorded for surface and bottom waters along the length of the estuary during summer and winter of 2014 (A & D) Main channel, (B & E) Creek 2 and (C & F) Creek 1.

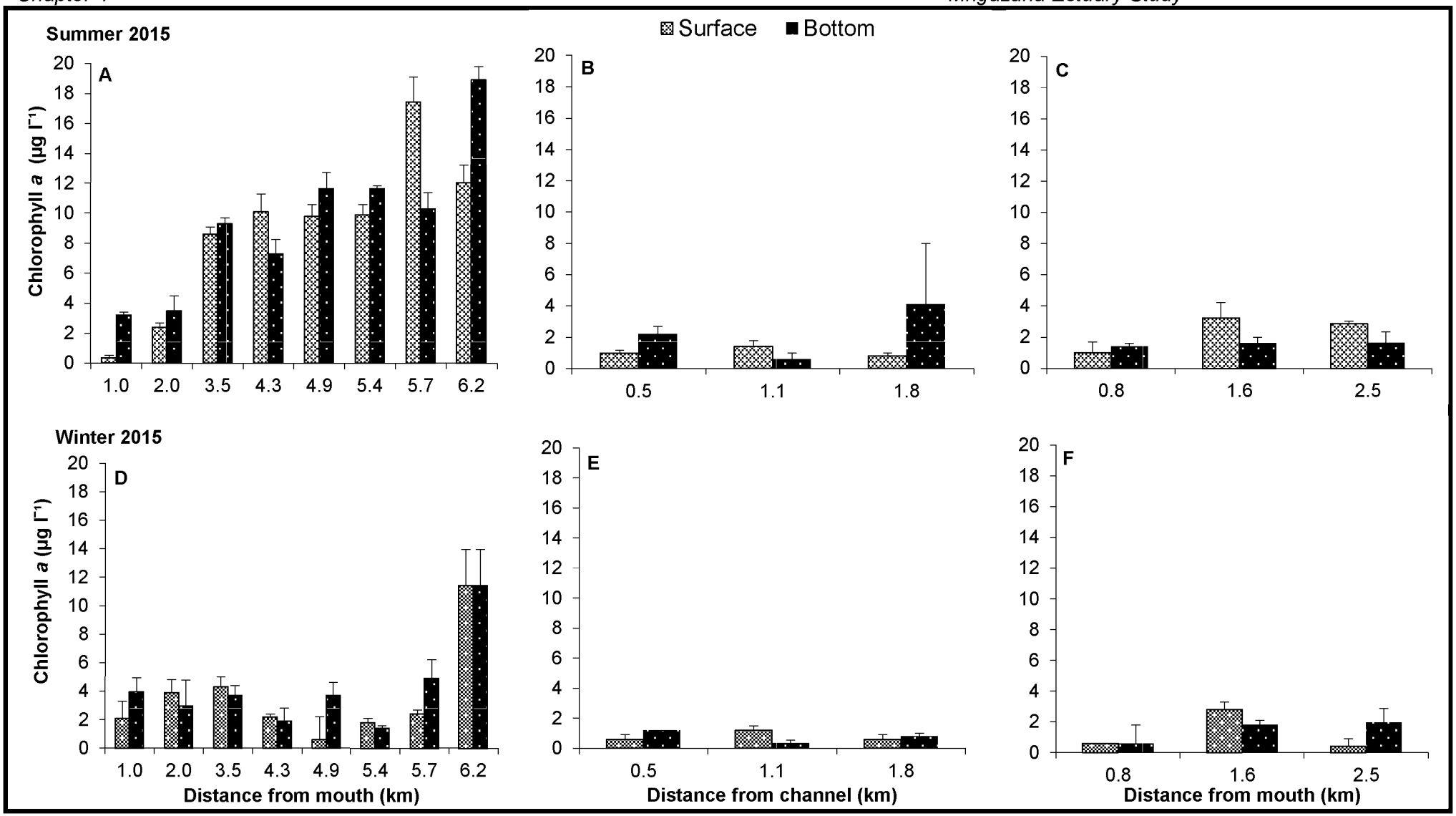

Figure 4.16: Mean phytoplankton biomass (chlorophyll *a*, mean ± SE) recorded for surface and bottom waters along the length of the estuary during summer and winter of 2015 for (A & D) Main channel, (B & E) Creek 2 and (C & F) Creek 1.

4.2.3.1 Phytoplankton community composition

Figures 4.17 – 4.20 (A–C) illustrate the seasonal phytoplankton community composition recorded at Mngazana Estuary. Actual counts are in Table A9 (Appendix). During the study period, the following phytoplankton groups were recorded: flagellates, dinoflagellates, diatoms, blue-green (cyanobacteria) and green (chlorophytes) algae. In summer 2014 (Fig. 4.17), flagellates (> 90 % RA) dominated the lower and middle reaches of the main channel while low diatom abundance (~10 % composition) was recorded across the entire estuary. Blue-green algae were positively correlated with distance from the mouth and dissolved oxygen while negatively correlated with TOxN and SRP (Table A1, Appendix). Winter 2014 (Fig. 4.18), cyanobacteria dominated the upper reaches of the estuary whereas chlorophytes were only recorded on Creek 2. Flagellates were positively correlated with pH while cyanobacteria were negatively correlated with pH (Table A2, Appendix).

During summer 2015 (Fig. 4.119), diatoms were dominant (> 55 %) at 1 km (Site 8) and 2 km (Site 7) from the mouth in the lower reaches along the main channel. There was a strong marine influence indicated by presence of marine diatom species; *Chaetoceros* sp. Ehrenberg; *Asterionellopsis glacialis* (Castracane) Round, *Thalassionema nitzschiodes* (Grunow) Mereschkowsky, *Guirnardia striata* (Stolterforth) Hasle, *Thalassiorisa* sp. Cleve and *Pleurosigma elongatum* W Smith (Fig. B4, Appendix). There was a positive correlation between diatoms and salinity (Table A, Appendix). In the upper reaches of the estuary (4.9 km) flagellates increased to > 70 % RA and were negatively correlated to dissolved oxygen (Table A3, Appendix). In winter 2015 (Fig. 4.20), the main channel was heavily dominated by flagellates.

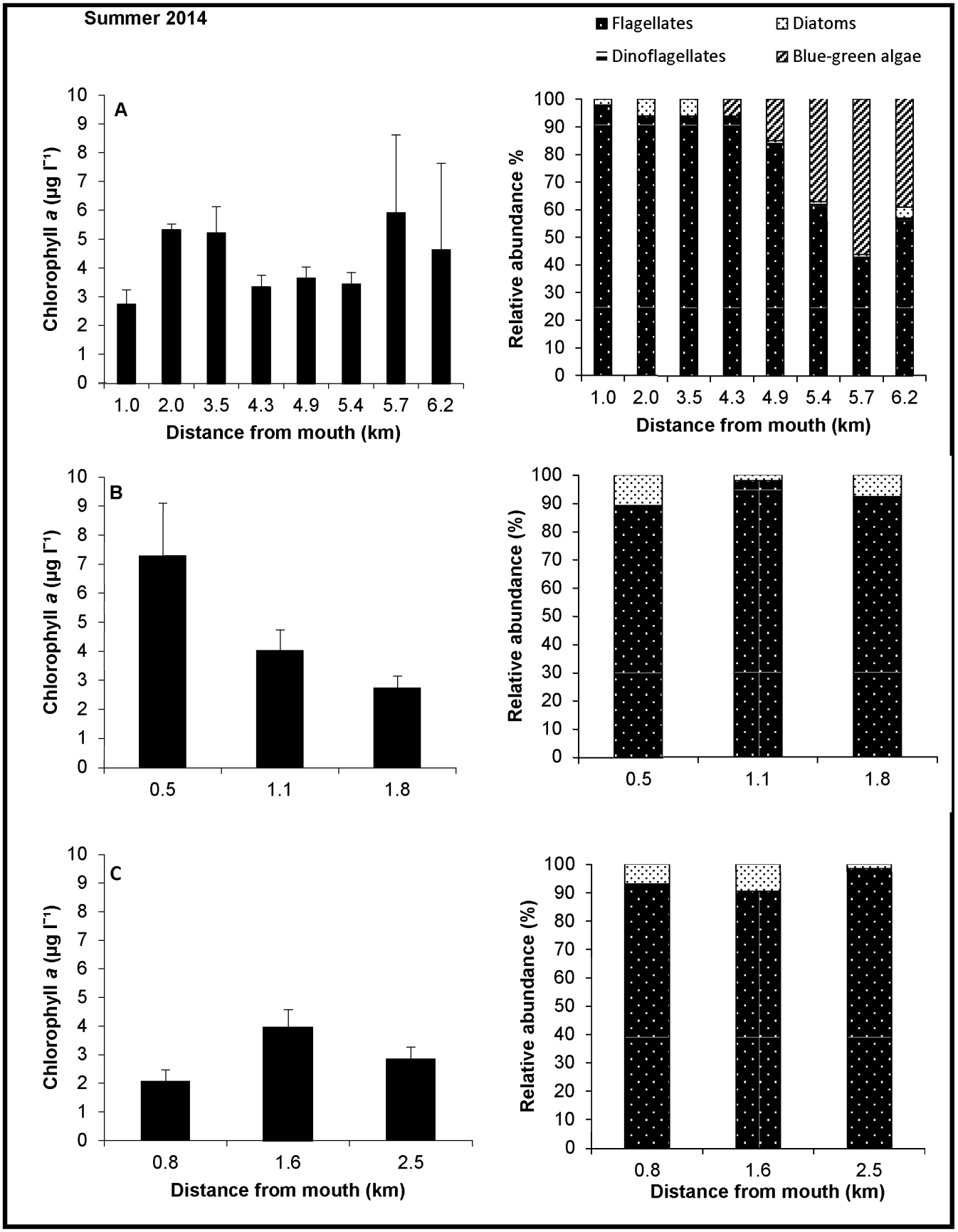

Figure 4.17: Vertically averaged phytoplankton biomass (± SE) and corresponding phytoplankton community composition (RA, as %) per site during summer 2014 for (A) Main channel, (B) Creek 2 and (C) Creek 1.

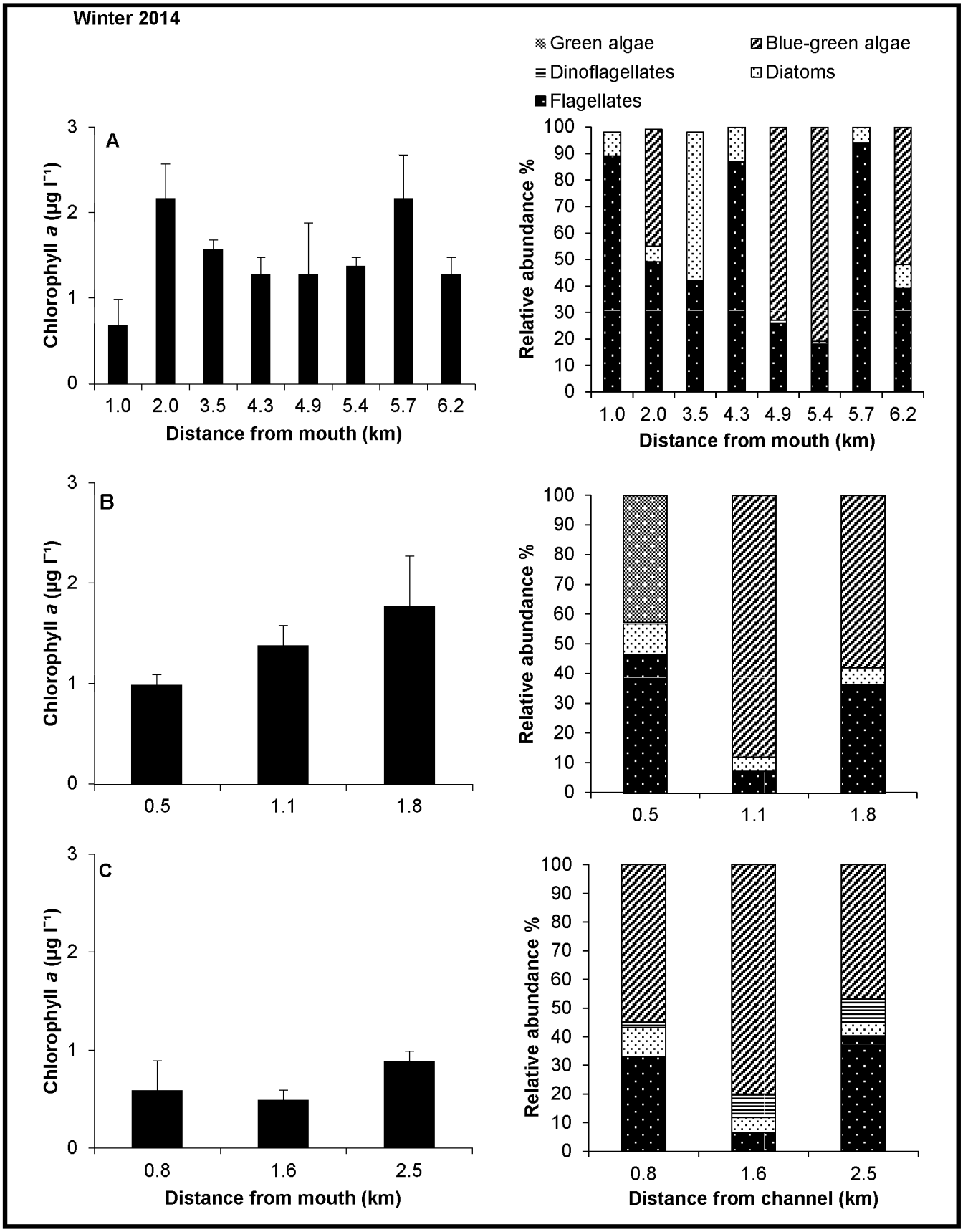

Figure 4.18: Vertically averaged phytoplankton biomass (± SE) and corresponding phytoplankton community composition (RA, as %) per site during winter 2014 for (A) Main channel, (B) Creek 2 and (C) Creek 1.

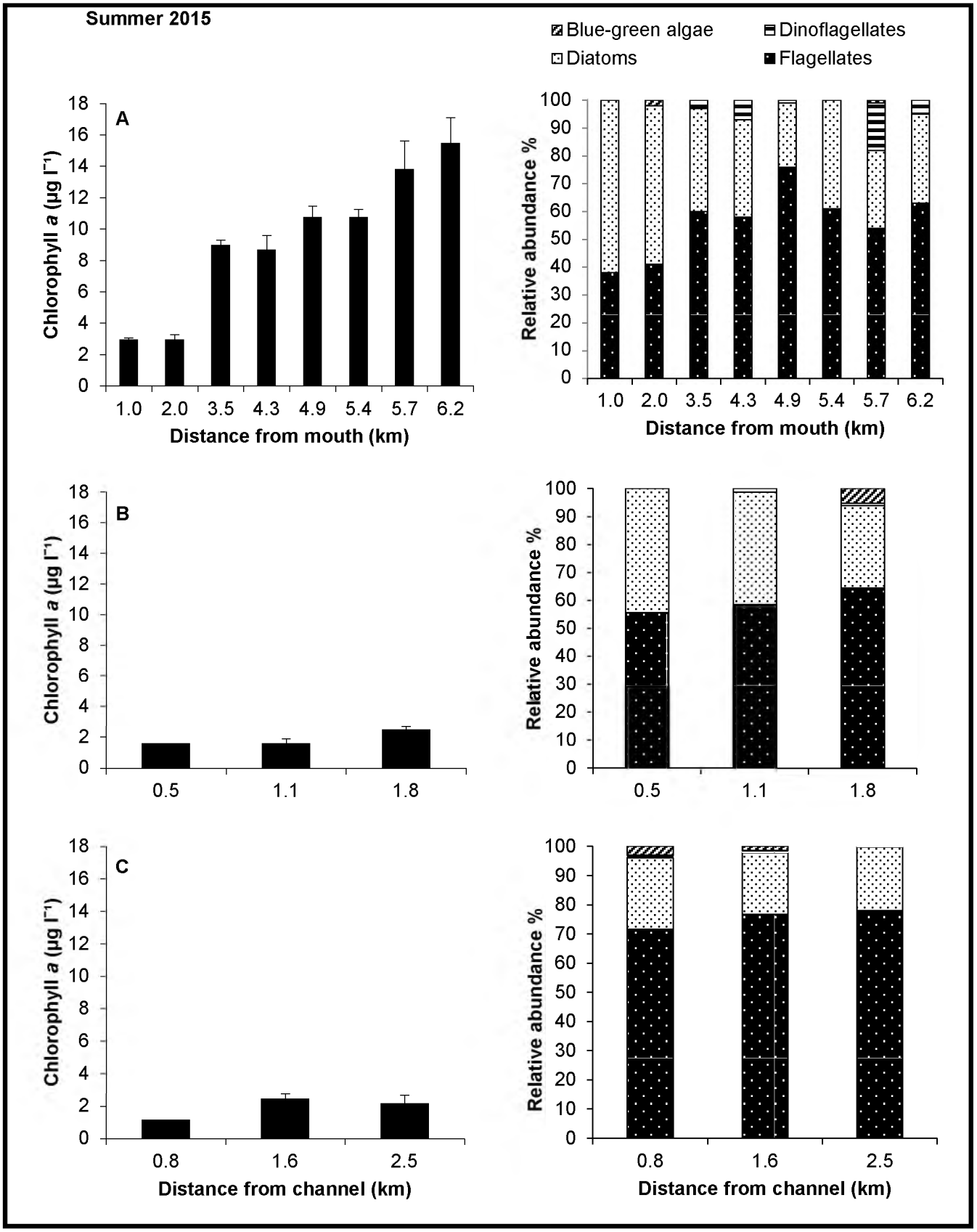

Figure 4.19: Vertically averaged phytoplankton biomass (± SE) and corresponding phytoplankton community composition (RA, as %) per site during summer 2015 for (A) Main channel, (B) Creek 2 and (C) Creek 1.

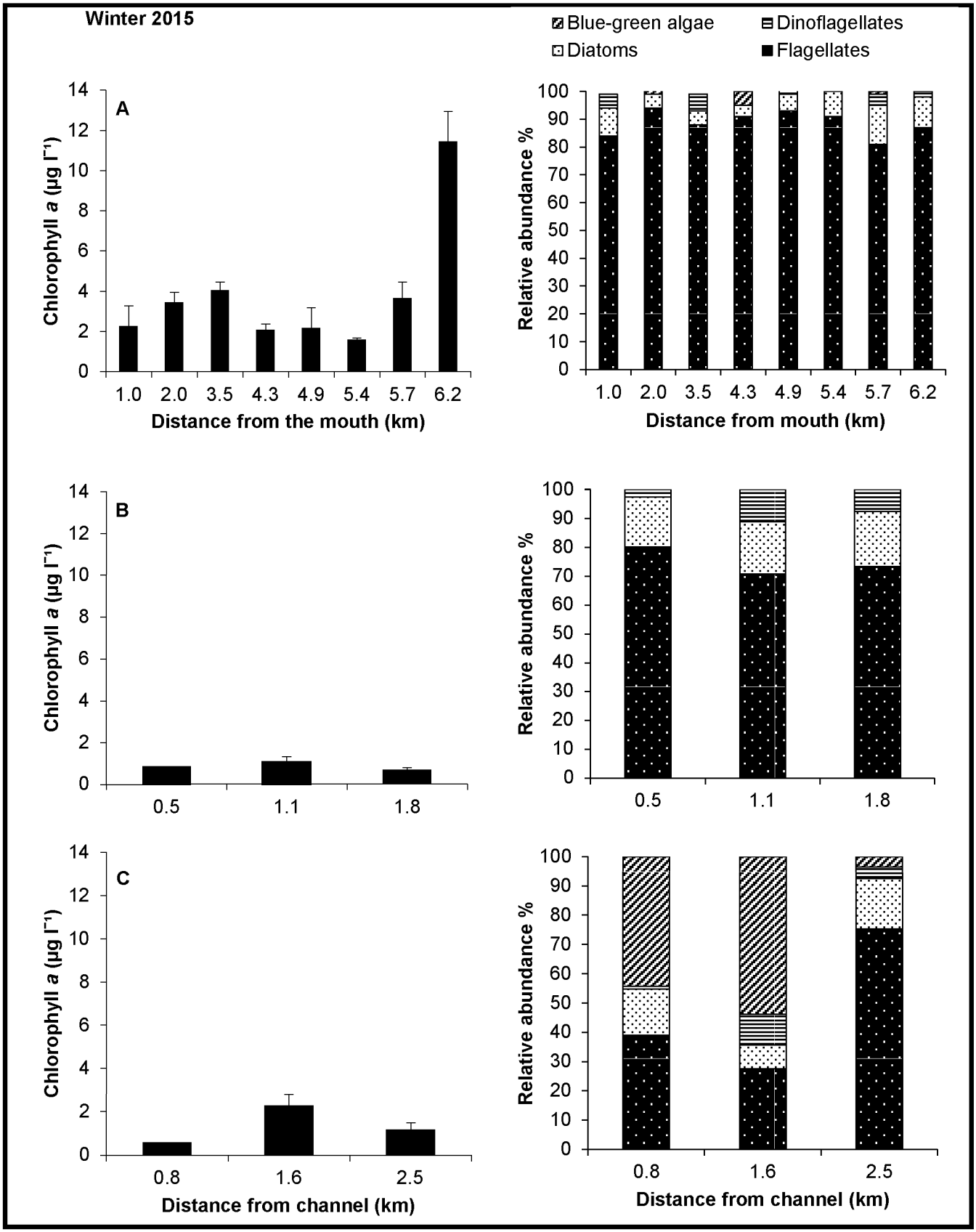

Figure 4.20: Vertically averaged phytoplankton biomass (± SE) and corresponding phytoplankton community composition (RA, as %) per site during winter 2015 for (A) Main channel, (B) Creek 2 and (C) Creek 1.

Canonical Correspondence Analysis (CCA) numerical results for all sampling sessions are displayed in tables below the plots. In summer 2014 (Fig 4.21A), the first axis was positively correlated with dissolved oxygen (0.55) and NH_4^+ (0.36) and negatively correlated with temperature (-0.03), salinity (-0.70), SRP (-0.22) and TOxN (-0.47). The dominant flagellates were closely associated with TOxN and pH. The first canonical axis represented 96.3 % of the variation of the species-environment relation (Table 4.2A).

In summer 2015 (Fig 4.21B) the first axis was negatively correlated with temperature (-0.41), NH_4^+ (-0.10) and SRP (-0.16) while positively correlated with dissolved oxygen (0.61), salinity (0.56), TOxN (0.22) and pH (0.58) respectively. 52.8 % of the variance in species-environment relation was described by the first axis. The dominant flagellates were closely associated with NH_4^+. The second canonical axis represented 47.2 % of the variation of the species-environment relation (Table 4.2B).

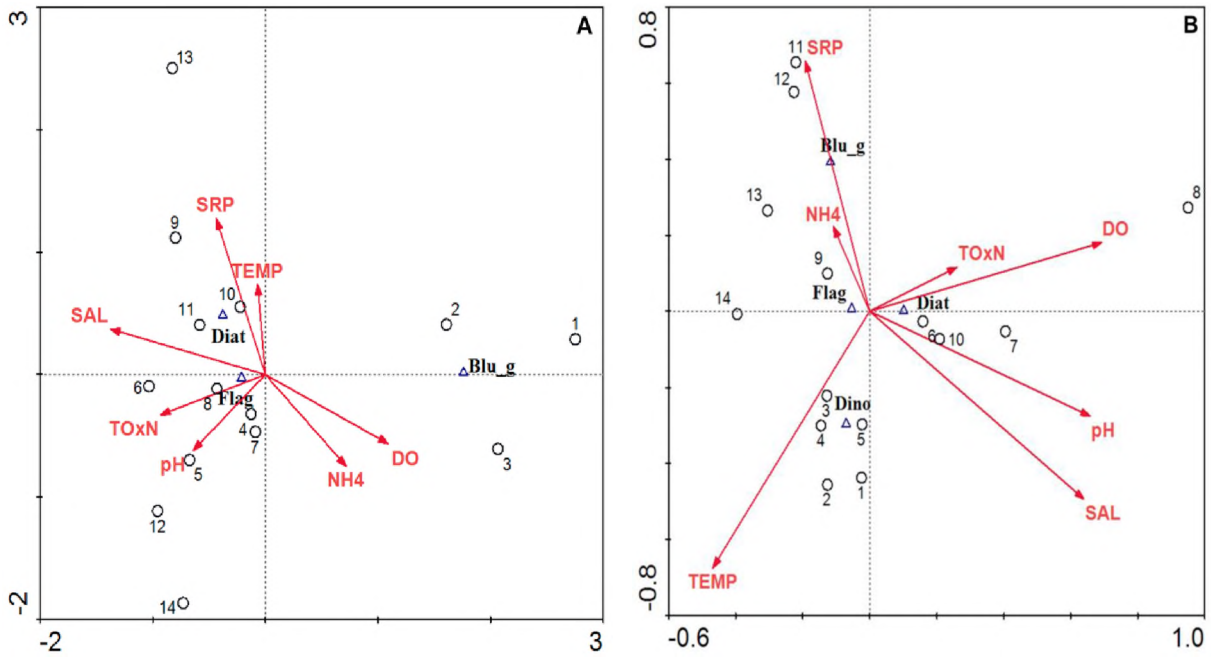

Figure 4.21: CCA ordination plots of phytoplankton groups (relative abundance) with physico-chemical variables (Temperature = TEMP; Salinity = SAL; Dissolved Oxygen = DO; and pH) and nutrient concentrations (NH_4^+, TOxN and SRP) at Mngazana Estuary in summer (A) 2014 and (B) 2015. The arrows represent each physico-chemical factor pointing in the direction of its maximum change. The sites are indicated by numbers (i.e. 1) and phytoplankton groups are abbreviated as: **Flag** = Flagellates; **Diat** = Diatoms; **Dino** = Dinoflagellates; **Blu_g** = Blue-green algae. Numbers represent sites.

Table 4.2: CCA summary results for phytoplankton groups and environmental factors correlations of the first two axes for summer (A) 2014 and (B) 2015.

A	**Axis 1**	**Axis 2**	**Total inertia**
Eigenvalues	0.278	0.011	0.367
Species-environment correlations :	0.902	0.649	
Cumulative percentage variance			
of species data :	75.8	78.7	
of species-environment relation:	96.3	3.7	
Sum of all eigenvalues			0.367
B	**Axis 1**	**Axis 2**	**Total inertia**
Eigenvalues :	0.051	0.034	0.165
Species-environment correlations :	0.884	0.740	
Cumulative percentage variance			
of species data :	30.8	51.4	
of species-environment relation:	52.8	47.2	
Sum of all eigenvalues			0.165

In winter 2014 (Fig 4.22C) first canonical axis represented 76.5% of the variation of the species-environment relation (Table 4.3C) and positively correlated with SRP (0.57), salinity (0.01) and NH_4^+ (-0.15), The axis was negatively correlated with temperature (-0.37), dissolved oxygen (-0.18), pH (-0.20), and TOxN (-0.19). The dominant cyanobacteria were associated with SRP.

However, in winter 2015 (Fig 4.22D) the first canonical axis represented 86.7% of the variation of the species-environment relation with 13.3 % representing the variance in the phytoplankton group composition (species data) (Table 4.3D). The dominant flagellates were closely associated to TOxN. This first axis was positively correlated with salinity (0.18), SRP (0.13) and negatively correlated with temperature (-0.21), pH (-0.41), NH_4^+ (-0.27), and TOxN (-0.17).

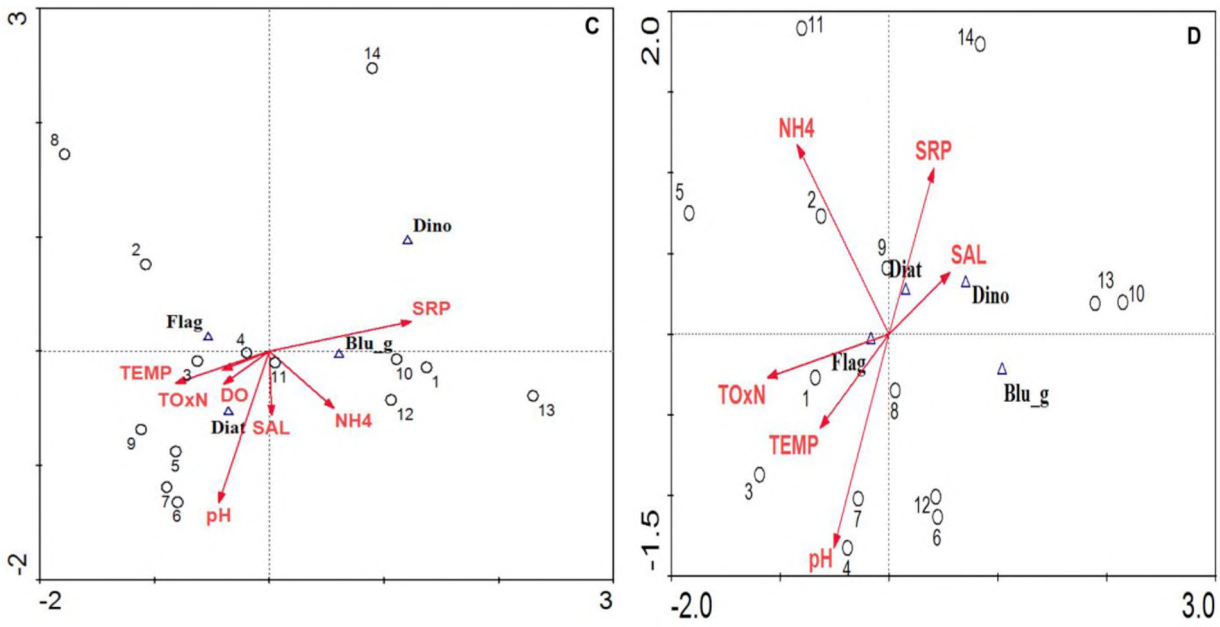

Figure 4.22: CCA ordination plots of phytoplankton groups (relative abundance) with physico-chemical variables (Temperature = TEMP; Salinity = SAL; Dissolved Oxygen = DO and pH) and nutrient concentrations (NH_4^+, TOxN and SRP) at Mngazana Estuary in winter (C) 2014 and (D) 2015. The arrows represent each physico-chemical factor pointing in the direction of its maximum change. The sites are indicated by numbers (i.e. 1) and phytoplankton groups are abbreviated as: **Flag** = Flagellates; **Diat** = Diatoms; **Dino** = Dinoflagellates; **Blu_g** = Blue-green algae. Numbers represent sites.

Table 4.3: CCA summary results for phytoplankton groups and environmental factors correlations of the first two axes for winter (C) 2014 and (D) 2015.

C	Axis 1	Axis 2	Total inertia
Eigenvalues :	0.243	0.050	0.664
Species-environment correlations :	0.743	0.614	
Cumulative percentage variance			
of species data :	36.6	43.8	
of species-environment relation:	76.5	23.5	
Sum of all eigenvalues			0.664
D	**Axis 1**	**Axis 2**	**Total inertia**
Eigenvalues :	0.114	0.017	0.468
Species-environment correlations :	0.563	0.457	
Cumulative percentage variance			
of species data :	24.3	27.8	
of species-environment relation:	86.7	13.3	
Sum of all eigenvalues			0.468

4.2.4 Faecal bacteria

Escherichia coli counts varied mainly along the main channel than the creeks (Fig 4.23A - C). Counts were significantly higher in the upper reaches of the estuary during summer 2014 and 2015 ($U = 162$; $p < 0.05$; $n = 48$) particularly 6.2 km (Site 1) and 5.7 km (Site 2) from the mouth ($H = 18$; $p < 0.05$; $n = 24$, Table 4.4). There were no significant differences in *E. coli* counts between seasons along the creeks ($p > 0.05$). Correlation analyses illustrated significant association of *E. coli* counts ($r = 0.59$; $p < 0.05$ – summer 2014), ($r = 0.74$; $p < 0.05$ – summer 2015) and ($r = 0.60$; $p < 0.05$ – winter 2015) with distance from the mouth of the estuary. There was a strong positively significant correlation between *E. coli* and temperature in summer 2015 (Table A3, Appendix) and a positively significant correlation with salinity during summer of 2014 (Table A1, Appendix). Overall, *Escherichia coli* counts were highest in summer 2014 and lowest during winter 2015.

Table 4.4: Variability in *Escherichia coli* counts measured along the length of the estuary and within the creeks at Mngazana Estuary during sampling period (± SE, N=3).

Distance from mouth/channel (km)	*Escherichia coli* (Counts. 100 ml^{-1})			
	Summer 2014	Summer 2015	Winter 2014	Winter 2015
Main Channel				
1.0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
2.0	**1.6 ± 0.8***	0 ± 0	0 ± 0	0 ± 0
3.5	0.8 ± 0.8	1.6 ± 1.6	0 ± 0	0 ± 0
4.3	6.7 ± 3.0	13.3 ± 8.8	6.7 ± 5.4	8.3 ± 2.2
4.9	16.6 ± 6.8	5.8 ± 4.6	3.3 ± 3.3	0.8 ± 0.8
5.3	10.0 ± 7.6	**25.0 ± 12.8***	0 ± 0	0 ± 0
5.7	**187.5 ± 88***	**49.1 ± 25.4***	15.8 ± 12.2	8.3 ± 6.0
6.2	**95.0 ± 39.2***	**70.0 ± 19.8***	6.7 ± 3.6	10.0 ± 4.3
Creek 2				
0.5	5.8 ± 0.3	1.7 ± 0.8	0.8 ± 0.8	0 ± 0
1.1	3.3 ± 2.22	0 ± 0	7.5 ± 3.8	0 ± 0
1.8	1.7 ± 0.8	1.7 ± 1.6	35.8 ± 18.6	0 ± 0
Creek 1				
0.8	2.5 ± 2.5	1.7 ± 0.8	5.8 ± 3.6	0.8 ± 0.8
1.6	1.7 ± 1.6	3.3 ± 2.2	0 ± 0	0 ± 0
2.5	5.8 ± 0.8	5.8 ± 3.6	9.7 ± 3.6	5.0 ± 5.0

***Average counts which are significantly higher or lower per sampling season per site ($p < 0.05$)**

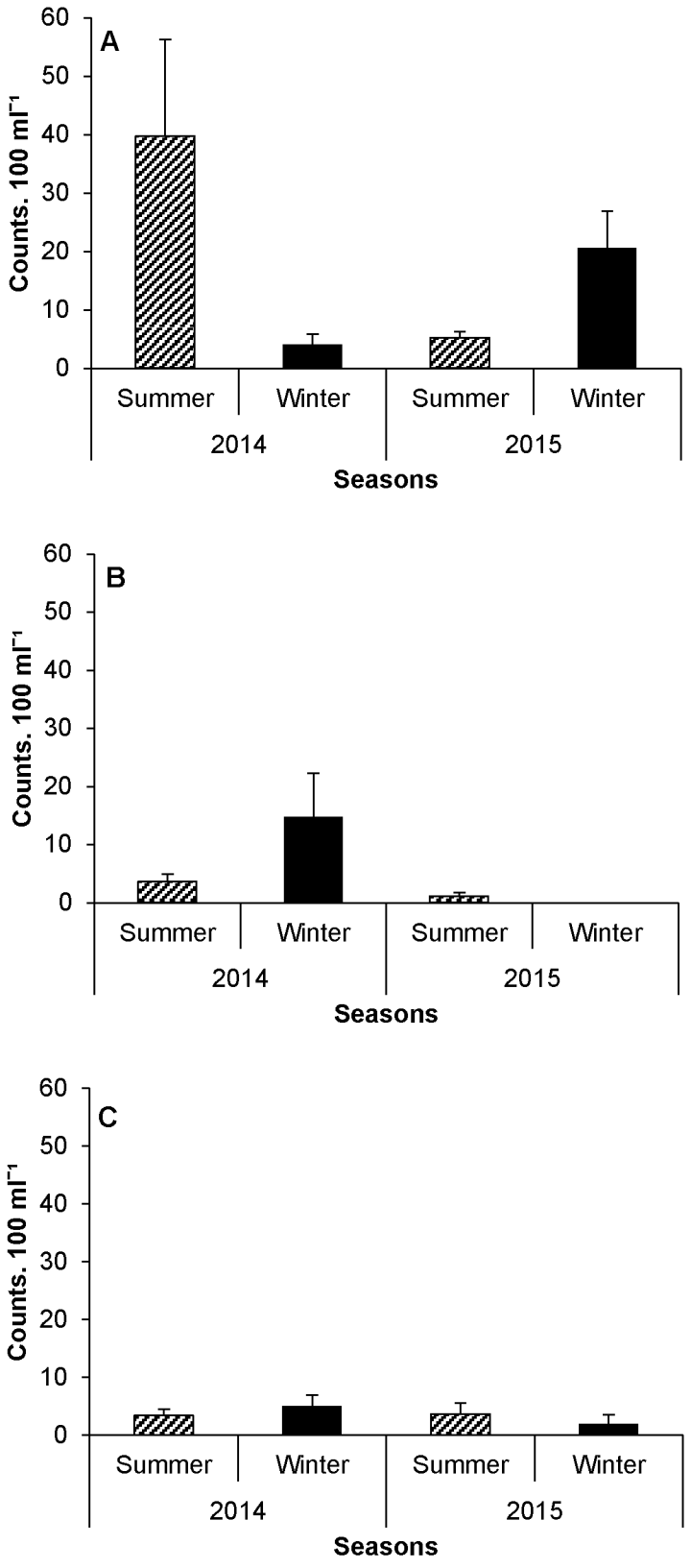

Figure 4.23: Seasonal mean *E. coli* counts recorded during the study period (mean ± SE) for (A) Main channel, (B) Creek 2 and (C) Creek 1.

4.3 Discussion

Freshwater input into estuaries is dependent on multiple factors such as geology, climate (i.e. rainfall), vegetation, land and human use in the catchment area of rivers and variable flow rates that include episodic events such as floods. These events present major perturbations to an estuary (Schumann *et al.* 1999, Rogers & O'Keefe 2003, Snow & Taljaard 2007). The main objective of this study was to evaluate the microalgal response to changes in the physical and chemical parameters at Mngazana Estuary. The results indicate that the estuary is marine dominated with average salinity (~30 ppt) persisting up to the upper reaches of the estuary (4.3 km from the mouth) and within the Creeks. The system is well oxygenated (~8 $mg \cdot l^{-1}$) with high summer temperatures (~27 °C) and lowest temperatures in winter (~17 °C) along the entire estuary (including Creek 1 and 2). According to Snow (2007), phytoplankton biomass in permanently open estuaries is a function of inorganic nutrient concentrations in the river water and favourable water retention time (days to weeks). Due to the current developments at the estuary head (approximately 7 km from the mouth), natural river inflow is low (visual observation) and is likely to affect the physico-chemical variables and nutrient inputs into the system. However, freshwater inflow is currently not been monitored at the head of the estuary therefore the "actual" quantity entering the estuary is currently unknown.

The average phytoplankton chlorophyll *a* biomass during the present study was 4.0 ± 0.2 µg Chl-*a* l^{-1} for the whole system. Seasonal differences were evident with summer biomass higher than winter in both studies (present and Ngesi 2010). Both studies measured a high biomass at 6.2 km from the mouth, recording biomass of up to 15.5 ± 1.7 (present study) and 32.8 ± 1.4 µg Chl-*a* l^{-1} (Ngesi 2010). This site is located in the upper reaches of the estuary and one of the drivers of increased biomass might be nutrient input. The average *E.coli* levels at this site were 95 ± 39 counts per 100 ml^{-1} indicative of a possible source of contamination. Low biomass of less than 10 µg Chl-*a* l^{-1} was found along both the creeks for the duration of the current study while Ngesi (2010) reported peaks of up to 29.3 µg Chl-*a* l^{-1} along Creek 1 during summer 2003. Phytoplankton is strongly influenced by physico-chemical factors (Jiang *et* al. 2015) and the variability in river flow is a dominant driver of the dynamics of phytoplankton biomass in microtidal systems (Paerl *et al.* 2014). This holds true for some South African systems (Adams & Bate 1999).

Snow *et al.* (2000) argued that low freshwater input into an estuary induces a shift from phosphorus to nitrogen limitation. Overall, nutrient concentrations during the present study (i.e. NH_4^+ - 32.3 µM) were lower than those reported by Ngesi (2010) where NH_4^+

concentrations reached a maximum of 92.8 µM but both studies found no significant differences ($p > 0.05$) between surface and bottom concentrations for most sampling sessions indicative of a well-mixed system. Mngazana Estuary, being a mangrove dominated system, may be enriched with nutrients leaching from the sediment. A study by Emmerson (2005) suggested that nutrients in this estuary are autochthonous, mainly from detritus mineralisation and crab frasse production. The differences in nutrient concentrations between the two studies could be attributed to hydrological dynamics and frequent tidal flushing which reduces retention within the system. Kaselowski & Adams (2013) argued that low nutrient concentrations could indicate rapid uptake by primary producers. The present study found elevated concentrations of nutrients along the main channel with the potential source being diffuse release of nutrients from the Creeks. This estuary could be classified as a mesotrophic system. Ngesi (2010) previously classified Mngazana Estuary as an oligotrophic system.

Flagellates were previously documented as the dominant phytoplankton group in most South African estuaries (i.e. Gamtoos, Greak Brak etc., Adams & Bate 1999, Nunes 2012). Snow *et al.* (2000) maintained that flagellates are mostly dominant in nutrient depleted waters. This was also evident in the Kromme and Berg estuaries (Snow & Adams 2006, Snow 2007). Lemley *et al.* (2015) recently found flagellates dominating selected estuaries in the Gouritz water management area, along the southern coast of South Africa. Internationally, Hall *et al.* (2013) reported flagellates as the dominant group that resulted in the blooms recorded in the New River Estuary, Carolina. This study is in agreement with Ngesi (2010) who reported the dominance of flagellates (> 60 % RA) in the Mngazana Estuary during the 2002/03 sampling. Nutrient-rich systems such as the permanently open Sundays Estuary consist of phytoplankton compositions dominated by diatoms and dinoflagellates (Kotsedi *et al.* 2012). The marine tidal influence was indicated by species such as *Guirnardia cylindrus* (Cleve) and *Chaetoceros* sp. Ehrenberg, and there was a positive correlation between diatoms and salinity. Green algae (chlorophytes) were only recorded during winter 2014 throughout the present study.

Jiang *et al.* (2015) found spatial heterogeneity in phytoplankton composition with the dominant community contribution controlled by salinity, temperature, nutrients and turbidity along the Changjiang (Yangtze River) Estuary. Seguro *et al.* (2015) study showed light availability (turbidity) as the main ecological driver of phytoplankton abundance and composition in the tropical estuary along the Gulf of Nicoya, Costa Rica despite noticeable inorganic nutrient inputs along the estuary. The CCA plots illustrated seasonal differences in how biological variables (phytoplankton groups) were influenced by physico-chemical

parameters across the sampling sites during the study. The distribution of the phytoplankton groups was heavily influenced by the interplay between nutrients and the acting physico-chemical parameters (i.e. salinity) across seasons, (i.e. Fig 4.21 showing the increase in blue-green algae as salinity decreases).

High bacteria (*E. coli*) presence in the upper reaches of the estuary is induced by the presence of cattle as expected. Wang *et al*. (2004) mentioned that cow manure releases faecal microbes that get carried by runoff to estuarine waters. The high salinity along the main channel and the Creeks due to a high tidal prism (Branch & Grindley 1979) may be the limiting factor on the survival of *E. coli* in the water column. Rozen & Belkin (2001) reported a 74 % optimal *E. coli* survival in 25 % seawater (~15 ppt) since high salinity induces an osmotic shock to different *E.coli* strains. Anderson *et al*. (1979) reported that decreasing salinity was accompanied by higher survival. However, a seasonal trend was evident (i.e. high counts during summer period). Faecal pollution by faecal indicator bacteria (FIB) impacts the water quality and poses health risks to water users, particularly bathers. *E. coli* studies on estuaries along the South African coast are few to none except for reports mainly on South African rivers. This is a huge oversight and needs addressing so as to continue with the progressive nature of collecting information on the health of estuarine systems of South Africa particularly when users are concerned.

However, Hamilton-Atwell (2007) reported water pollution by *E.coli* and faecal coliforms in the Klein Estuary, Western Cape, (South Africa) where up to 2 419 counts per 100 ml^{-1} were recorded at various stations along the estuary where stormwater drains are present. The distribution and counts along an estuary seem to depend on the input source, influential environmental variables and the adaptive capacity of the bacteria. Microbial cells are irreversibly damaged and die when exposed to unfavourable marine conditions (i.e. high salinity ~35 ppt and nutrient-poor conditions) (Jozic *et al*. 2014). Furthermore, de Brauwere *et al*. (2011) mentions that a rapid die-off of *E. coli* also occurs when the bacteria is exposed to a range (320 – 700 nm wavelength) of solar radiation. The ability of *E. coli* to survive and grow in the environment is likely due to its flexibility in energy acquisition. According to Ishii & Sadowsky (2008), *E. coli* is a heterotrophic bacterium requiring only simple carbon and nitrogen sources. De Brauwere *et al*. (2011) revealed tidal processes play an important role on the distribution of bacteria in the Scheldt Estuary. This suggests evidence that hydrodynamics coupled with high salinity impact the survival of *E. coli* rendering the estuary safe for human contact but the easy access of cattle to the estuary needs to be addressed to minimise the introduction of faecal coliforms into the estuary, particularly in the upper reaches where low salinity conditions may occur from time to time.

4.4 Conclusion

This study was conducted to compare microalgal biomass along the Mngazana Estuary to that measured by Ngesi (2010) and the level of microbial contamination of the estuary waters. The focus on the importance of the Mngazana Estuary has mainly been on the mangroves forests in this system (Colloty *et al.* 2002, Rajkaran & Adams 2007, Rajkaran & Adams 2012). This study illustrated the system is oligotrophic coupled with low microalgal production as a result of reduced freshwater inflow. The quantity and quality of freshwater required for an estuary (the Reserve) is enforced by the National Water Act (Act 36 of 1998) in South Africa and has been evaluated (Adams *et al.* 2002). According to the latest review by Adams (2014) the environmental flow requirements of estuaries have not been at the forefront of management due to the lack of long-term monitoring data. Furthermore, it is emphasised that the implementation of environmental water requirement determinations is recognised as being important to support the intrinsic, ecological, social and economic values of estuaries. Adhering to such implementations would yield a better inflow of freshwater into the Mngazana Estuary as well.

Mngazana Estuary is in a near pristine state (good condition) with low anthropogenic impacts in and around the estuary but the water quality was noted as being in a fair state (Van Niekerk & Turpie 2012). Based on the study by Ngesi (2010), the current study is in agreement that freshwater reduction does not entirely limit the phytoplankton dynamics of this system in terms of nutrient input In addition, the lack of a river-estuary interface (REI) region (< 10 ppt, Bate *et* al. 2002) at Mngazana Estuary adds to the low biomass found during this study since no salinity stratification occurs. Based on the description of the REI zone by Bate *et al.* (2002), the lack of freshwater inflow into the estuary contributed to the lack of this zone since salinity was generally in the region of 20 ppt at the head of this estuary. The study however does not represent the reference state of the estuary in terms of physico-chemical variables, nutrient inputs and microalgal response due to the ongoing construction and freshwater abstraction during the current study. The rapid flushing of this system indicates that the build-up of nutrients to induce algal blooms is highly unlikely. However, the lack of distinct point sources of nutrients along the estuary during this study points to the remineralisation of organic matter in the mangrove mud suggesting Mngazana Estuary to be a benthic-driven system. This stresses the need for further investigation of the microalgal and nutrient dynamics of this estuary upon the completion of the upgrading of the bridge and it can be hypothesised that the state of the water quality will improve if water requirements can be met and enforced.

Chapter 5. Water quality characteristics of an urban located Nahoon Estuary, Eastern Cape.

5.1 Introduction

Estuarine and coastal regions are becoming more susceptible to contamination as a result anthropogenic influences in urbanised and industrialised areas (MEGA 2013, VishnuRadhan *et al.* 2015). Morris (1986) claimed that the most serious threat facing South African estuaries was sociological, meaning urban ecosystems evolve over time and space as the outcome of dynamic interactions between *socio-economic* and *biophysical* processes operating over multiple scales (Alberti & Marzluff 2004). Scharler & Baird (2003) emphasised that rapid urbanisation coupled with poor sewage infrastructure and increasing industrial and agricultural developments contribute to material input into estuaries. Deteriorating infrastructure and rapid growth along coastal cities further worsens human impacts on water resources.

According to Alberti and Marzluff (2004) urbanisation is seen as the process by which humans substitute ecosystem services (i.e. catchment purification) with human services (i.e. wastewater treatment plants). There is a lack of a formal definition of what an "urban estuary" is. However, from the literature it can be gathered that an urban estuary is "*an estuary situated in a developed coastal area with characteristic disturbance gradients that alter natural biochemical cycles and where the water quality and health of the system has been noticeably impacted by land-use activities*" (Kaye *et al.* 2006, Elsdon *et al.* 2009, Carey *et al.* 2013, Pretorius 2014, Greening *et al.* 2014). Estuaries in urban cities are exposed to different disturbances of varying magnitude, thus a simple definition might not capture all urban estuaries. The definition of an estuary needs to incorporate all estuarine types hence defining an urban estuary will need to take into consideration the different urban modifications occurring on estuaries. The deleterious effects of urbanisation include the introduction of biological barriers, unnatural shapes and degrees of connectivity, furthermore, it homogenises natural patterns by changing land use and modifying the natural processes that maintain diversity (Alberti & Marzluff 2004).

There are evident flow regime alterations along some of the estuaries in South Africa. The construction of two dams above the Kromme Estuary (Mpofu Dam 107 x 10^6 m^3 and Churchill Dam 33.3 x 10^6 m^3) resulted in the water becoming saline towards the head of the estuary due to reduced inflow (< 0.2 $m^3 \cdot s^{-1}$). This further affected flood frequency and

magnitude along with the deposition of materials deposit into the estuary (Scharler *et al.* 1997, Snow and Adams 2006). Another example of flow alterations is evident at Sundays Estuary where the natural river flow was artificially augmented to improve the poor water quality to satisfy urban water demands of Port Elizabeth (nearest city) and to meet irrigation demands within the catchment (Kotsedi *et al.* (2012). Jafta (2010) reported that freshwater inflow between 0.010 – 0.016 $m^3 \cdot s^{-1}$ to the Bushmans Estuary decreased over time due to water abstraction by more than 30 weirs and farm dams in the catchment. A study by Viskich *et al.* (2016) revealed that intensive urban development in and around the Diep River Estuary altered biodiversity, flow and salinity regimes and deteriorated the water quality.

Pressures such as dam construction and pollution (i.e. sewage runoff) threaten the health status of numerous South African estuaries and ultimately lead to degraded functionality of these systems (Turpie *et al.* 2002, Kotsedi *et* al. 2012). Stormwater discharges can represent a large source of *Escherichia coli* (Migula 1985), Castellani and Chalmers (1919) to receiving aquatic ecosystems, especially in highly urbanised settings. However, the impacts are dependent on stormwater loads and the flow of receiving water body (Pretorius 2014). The Swartkops Estuary (Eastern Cape, South Africa) is an example of an urban estuary where water quality and health of the system has been noticeably impacted by land-use activities (Grindley 1974, Day 1981, Colloty *et al.* 2000). Pretorius (2014) evaluated the Swartkops Estuary and found salinity to be higher during winter (25.8 ± 3.03 ppt) than summer months (18.2 ± 1.9 ppt) and the system remaining well oxygenated (7.2 ± 0.4 $mg \cdot l^{-1}$). Seasonal differences were found in phytoplankton biomass (summer – 58.3 ± 25.2, winter – 9.2 ± 4.1 µg Chl-*a* l^{-1}). High nutrient concentrations were noted to support phytoplankton biomass of bloom concentrations (>20 µg Chl-*a* l^{-1}; Adams & Bate 1999) from the middle to upper reaches of the estuary. Swartkops is hugely surrounded by development (residential, industries, sewage treatment works, wool washeries and tanneries) (Enviro-Fish Africa 2009).

The Nahoon Estuary is situated within the urban area of East London in the Eastern Cape. Urban development accounts for 3 % of the land cover (of the Buffalo City Municipality) and mainly residential development is associated with East London (MEGA 2013). The Nahoon Dam (22.1 x 10^6 m^3) was built above the Nahoon Estuary and forms part of the Amatole Water Supply System under the Mzimvubu – Keiskamma Water Management Area (WMA) and developments along the Nahoon Estuary commenced around the 1950's This estuary is bounded on both sides by the East London suburbs of Nahoon and Nahoon Valley. Major developments have occurred along the Nahoon Estuary (Table 5.2). Morris (1986) further stated that the accelerated increase in residential development along the estuary lead to an

increase in the recreational usage of the estuary. Human development along the Nahoon River channel included early wooden jetties that were washed away during flood events. Two bridges were constructed downstream of the Abbotsford Bridge and according to Morris (1986), the Abbotsford Bridge forms a barrier to any tidal activity further upriver while the other bridges were built well above the highest recorded flood level and do not pose any restriction to flow.

Permanently open estuaries tend to be dominated by tidal processes, Nahoon Estuary can expected to be highly saline (30 – 35 ppt) with dissolved oxygen above 5 $mg \cdot l^{-1}$ along the estuary length. The minimum temperatures are expected in winter (~5.3 °C) and maximum during summer (~31.4 °C) (MEGA 2013). Seasonal differences can thus be expected due to fluctuation in temperature and nutrient availability. Campbell *et al.* (1991) reported a chlorophyll *a* biomass range of (0 – 6 µg Chl-*a* l^{-1}) along the Nahoon Estuary from a study undertaken in 1984/85, the first phytoplankton biomass study performed in the estuary.

This study was aimed at evaluating the present water characteristics of the Nahoon Estuary and identifying any point sources along the length of the estuary. The objective was to provide an update on the biological (microalgal) response to environmental variables influencing the Nahoon Estuary since the last study by Campbell *et al.* (1991).

5.1.1 Study Hypotheses

Physical variables

- Dissolved oxygen at Nahoon Estuary will be above 6.0 $mg \cdot l^{-1}$ reflecting an oxygenated system.

Chemical variables

- Nutrient levels, soluble reactive phosphorus (SRP), ammonium (NH_4^+) and total oxidised nitrogen (TOxN) will be higher towards the head of the estuary.

Biological variables

- Water column chlorophyll *a* will increase with distance from the mouth due to nutrient introduction into the system.
- Phytoplankton blooms (>20 µg Chl-*a* l^{-1}) will be recorded at sites close to point sources along the estuary.
- Microbial contamination (Faecal bacteria) will be low (< 2000 CFU 100 ml^{-1}) along the length of the estuary.

5.1.2 Study Area

The Nahoon Estuary (32°59' S; 27°56' E) is a permanently open estuary that lies between East London and Beacon Bay, Eastern Cape, South Africa (Fig. 5.1). The Nahoon River is 72 km; the estuary is relatively short, measuring 5 km from the estuary head to the mouth. The catchment area is approximately 564 km^2 with a mean annual runoff (MAR) of 34 x 10^6 m^3 and is located in a warm-temperate with summer-dominant rainfall biogeographic region (Reddering & Esterhuysen 1986). Nahoon Estuary was found to be in a fair condition (Whitfield 2000) and had a conservation importance score of 70.0 (Turpie et al. 2002). This estuary is situated within the residential area of the Nahoon and Beacon Bay suburbs (Sale 2007) and is concealed by steep cliffs that reach up to 105 m in high places thus limiting access to the estuary and some floodplain areas (MEGA 2013). The Nahoon Estuary lies in a wave-dominated coast and has a developed flood-tidal delta. Furthermore, according to Reddering (1988b it is microtidal with an average tidal range of 0.73 m and a coastal spring tide of 1.6 m.

Flood events occur frequently in East London, particularly the Nahoon region, changing the Nahoon Estuary since the 1970s. Other flooding events were recorded in 1985, November 2005, June 2011 and the recent floods in April 2013 (Daily Dispatch 2013), and the annual rainfall of this region is 920.6 mm (MEGA 2013). Data from Weather SA (South African Weather Services) indicates this region experienced a mean annual rainfall of 915 mm over a 66 years period (1950 – 2015). Summer rainfall (average ± SE: 91.0 ± 4.5 mm) dominates this area with less rainfall experienced during the winter period (average ± SE: 48.9 ± 4.5 mm). The average monthly rainfall (± SE) is 76.4 ± 2.0 mm $month^{-1}$. The minimum temperatures range from 5.7 to 20.8 ºC with maximum temperatures that range from 16.5 to 28 ºC for a period of 66 years (1950 – 2015) (Fig 5.2).

The intertidal estuarine vegetation at Nahoon Estuary is a combination of the salt marsh and a mangrove forest dominated by *Avicennia marina*, and a limited number of *Bruguiera gymnorrhiza* and *Rhizophora mucronata* individuals. The dominant salt marsh species at Nahoon include *Bassia diffusia* (Thunb.) Kuntze, *Sarcoconia tegetaria* S.Steffen, L.Mucina & G.Kadereit, *Triglochin striata* Ruiz & Pav., *Stenotaphrum secundatum* (H.Walter) Kuntze, *Sporobolus virginicus* (L.) Kunth, *Juncus kraussii* Hochst subsp. *Kraussii* and *Nasturtium officinale* R. Br. (Geldenhuys 2013, Hoppe-Speer *et al.* (2015).

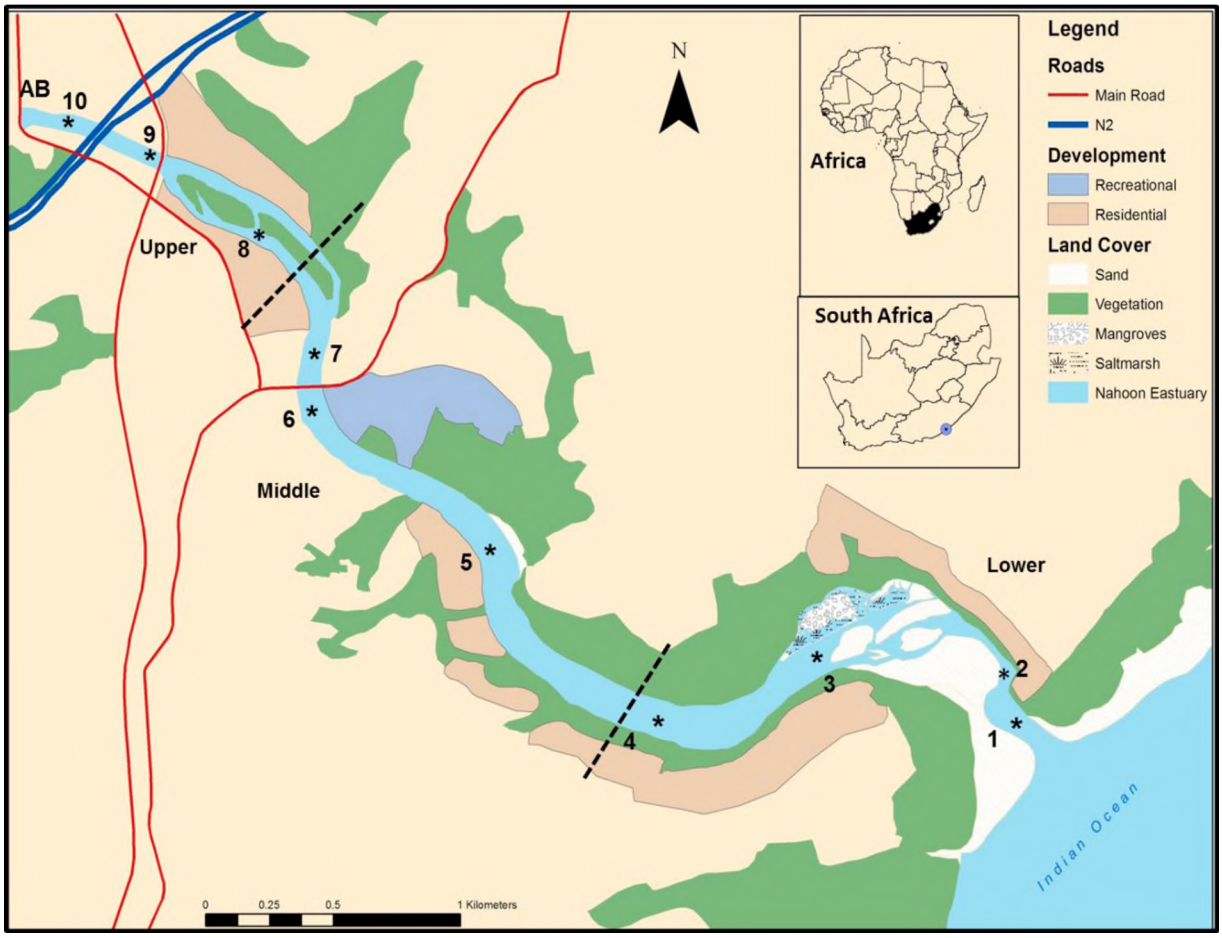

Figure 5.1: Map of the Nahoon Estuary located along the east coast of South Africa. Asterisks (*) indicate sampling sites along the channel. **AB** - indicates location of the Abbotsford Bridge and dashed line (**- - -**) delineates between estuarine reaches.

Reddering & Esterhuysen (1986) noted that the intensive residential development on the banks of the estuary resulted in substantial human interaction with the estuarine environment, resulting in Nahoon being heavily used for recreational purposes compared to the Qgunube and Kwelera (approximately 10 km radius) estuaries. Due to the Nahoon Dam, which has a capacity of 22.1 x 10^6 m^3 (Morant & Quinn 1999), the amount of freshwater that reaches the estuary has decreased over the years (van der Westhuizen 2007). The Abbotsford bridge/causeway near the estuary head constricts natural river water flow downstream and confines the estuarine conditions and tidal influences upstream (Wiseman *et al.* 1993). The most significant modifications in the Nahoon Estuary are related to human activities (Table 5.2) along with natural perturbations. Nahoon Dam, located approximately 27 km upstream from the estuary mouth is the one major development on the Nahoon River catchment. The dam supplements industrial and urban water supplies to the area and certain adjoining townships (Geldenhuys 2013). The houses along the estuary have jetties extending into the channel and two road bridges and an expressway cut across the channel but they have no significant influence on the natural flow of the water (Wiseman *et al.* 1993).

The residential developments at Nahoon have increased over the last 50 years and up to 182 026 permanent residents live within 10 km of the estuary (Sale 2007). The Nahoon Estuary is famous for its recreational services which include fishing, canoeing, swimming and birding (Reddering & Esterhuysen 1986, Sale 2007). According to Bate *et al.* (1986) the estuary mouth lies about 1.5 km east of the Bats Cave sewage outfall and may receive polluted sea water during certain weather conditions. Over time, there has been loss of biodiversity and changes to river hydrology due to developments such as artificial bank stabilisation, jetties (extending ~30 m from the bank) and slipways (MEGA 2013). The varying depths and distance of sampling sites are summarised in Table 5.1

Table 5.1: Characteristics of the sampled sites at Nahoon Estuary.

Sampling station	Depth (m)	Distance from mouth (km)	Observed impacts
Site 1	1.5	0.1	Litter on the adjacent beach.
Site 2	2.0	0.3	Effluent from a nearby restaurant. Recreational dog walking.
Site 3	3.0	0.8	Launch site close by.
Site 4	3.5	1.5	No visible activities.
Site 5	4.0	2.2	Jetties along the banks; recreational fishing.
Site 6	4.0	3.3	Recreation park (liquid waste flows into the estuary); recreational fishing.
Site 7	2.5	3.5	Stormwater drain close by; jetties along the bank; artificial bank stabilisation.
Site 8	1.0	4.0	Jetties along the bank; artificial bank stabilisation.
Site 9	3.0	4.5	Fishing along the banks.
Site 10	1.0	5.0	Abbotsford Bridge.

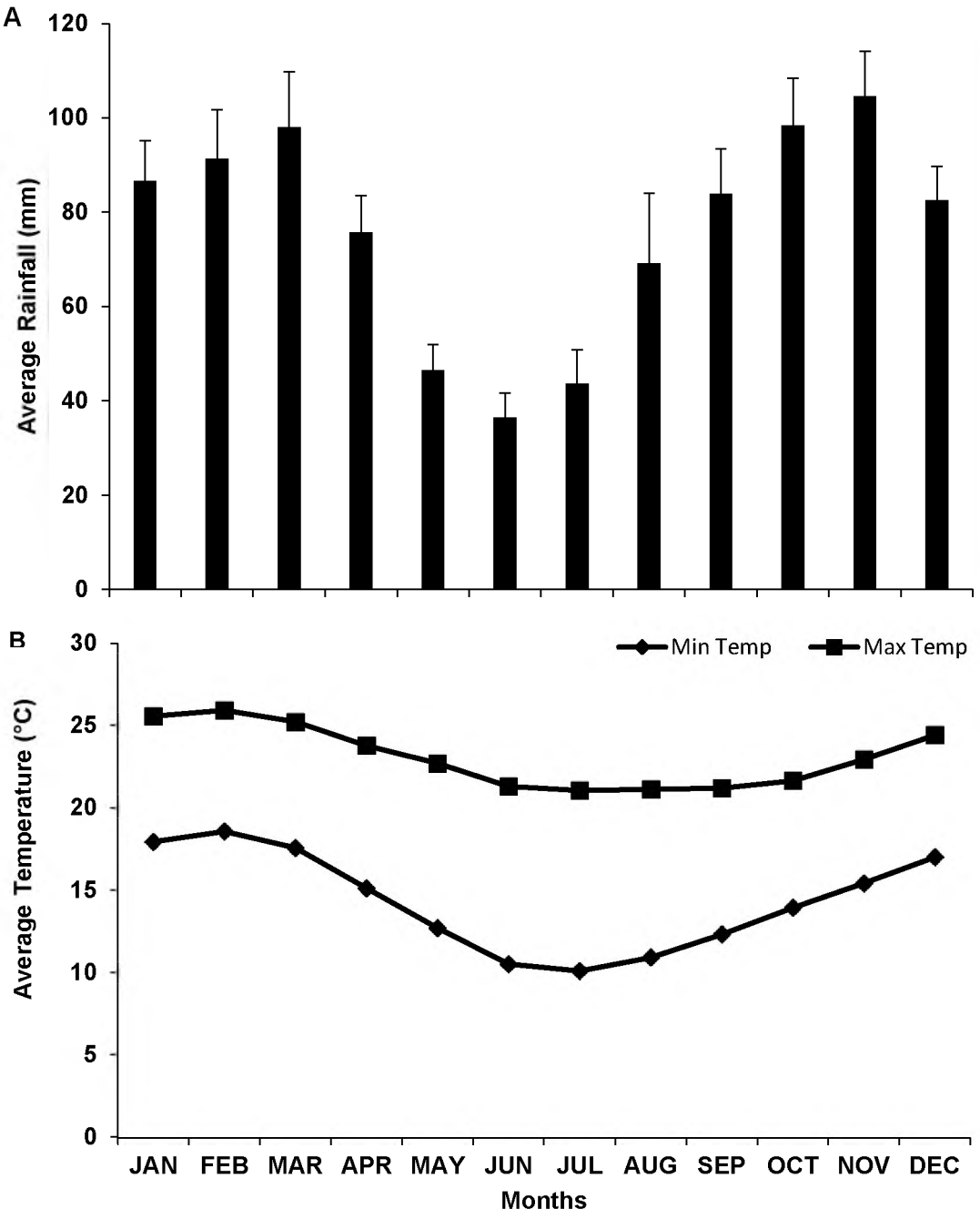

Figure 5.2: Historical (a) rainfall (1950 to 2015) and (b) temperature (1950 to 2015) data collected monthly at East London weather station. (Average ± SE present but in some cases not visible).

Table 5.2: Timeline human actions along the Nahoon Estuary and adjacent beach as well as the occurrences of documented floods (adapted from Geldenhuys 2013) and modified.

Year	Event and description	Reference/Source
August 1878	Abbotsford bridge on the Nahoon River was originally completed	Morris 1986
1950's	Suburban development bordering the Nahoon Estuary commenced as estuarine areas became desirable locations for residential and tourism-related development	MEGA 2013
Early 1960's	Development on the top of the cliffs overlooking the estuary began	Morris 1986
1966	The Nahoon Dam (32°54'13.7" S 27°47'50.7" E) (Fig. 5) was built 27 km upstream from the Nahoon river mouth. The Ngqkana, Kwetyana, Rwantsa and other smaller tributaries enter the Nahoon river upstream of the Nahoon Dam	MEGA 2013
Early 1970's	The development of plots and duplex flats started expanding toward the estuarine marshes	Morris 1986
1970	Flooding Event: Flooding occurred to the extent that the river flow left the Abbotsford Causeway about 6 m under water. The original Jack Batting Bridge which was adjacent to and upriver of the present Beacon Bay Bridge was irreparably damaged by the flood and was removed	Daily Dispatch, 29 August 1970 Morris 1986 MEGA 2013
1973	Establishment of the 'Nahoon Trust' a body established to ensure the preservation of the natural environment of the Nahoon estuary	Morris 1986
1985	Flood Event (Fig. B5, Appendix)	MEGA 2013
March 1998	Legislation relating to Environmental Impact Assessment (EIA) came into effect making the development of further jetties and other structures within and adjacent to the estuary subject to environmental authorisation	MEGA 2013
November 2005	Flood Event	MEGA 2013
June 2011	Flooding Event: Flood occurred and the Nahoon Dam overflowed (Fig. B5, Appendix)	MEGA 2013
April 2013	Nahoon beach closed after a sewage spill	Daily Dispatch 8 April 2013
	Flood event: Flood waters flowing into the estuary (Fig. B5, Appendix)	Daily Dispatch 22 April 2013
2014	Algae invade Nahoon Estuary upper reaches (Fig. B5, Appendix)	Daily Dispatch 2 October 2014
August 2015	Water hyacinth washed into the estuary after heavy rains	Visual observation

5.2 Results

5.2.1 Physico-chemical variables

5.2.1.1 Salinity

Water column salinity in summer 2015 was significantly higher than winter 2015 ($U = 451$; $p < 0.05$; $n = 77$) while summer 2014 did not vary significantly from winter 2014 $p > 0.05$). Conversely, winter 2014 salinity was significantly higher than during winter 2015 ($U = 178$; $p < 0.05$; $n = 58$) and no differences were found between summer sessions ($p > 0.05$). Average salinity at Nahoon was ~30 ppt throughout the study period with ~35 ppt recorded at sites near the mouth. However, a decrease with distance from the mouth was evident during 2015, average salinity of ~17 ppt (summer 2015) and ~20 ppt (winter 2015) were recorded at 5 km (Site 10) from the mouth (Fig. 5.3A - D). Salinity was negatively correlated with distance from the mouth in winter 2014 (Table A6, Appendix) and summer 2015 (Table A7, Appendix).

5.2.1.2 Temperature

Significant seasonal trends in temperature indicated summer temperatures were significantly hotter than winter during both 2014 ($U = 62$; $p < 0.05$; $n = 45$) and 2015 ($U = 0$; $p < 0.05$; $n = 77$). The highest summer temperature was 27 ± 0.3 °C in the upper reaches compared to 21 ± 0.1 °C near the mouth. Winter temperatures ranged from 16 – 20 °C during the study, particularly in winter 2015 (Fig. 5.3H). Temperature was significantly hotter in summer 2015 than recorded in summer 2014 ($U = 257$; $p < 0.05$; $n = 63$) but remained relatively constant along the length of the estuary (Fig. 5.3E - H). A significantly positive correlation between temperature and distance from the mouth along with a negative correlation between temperature and salinity were evident during winter 2014 (Table A6, Appendix) and summer 2015 (Table A7, Appendix) respectively.

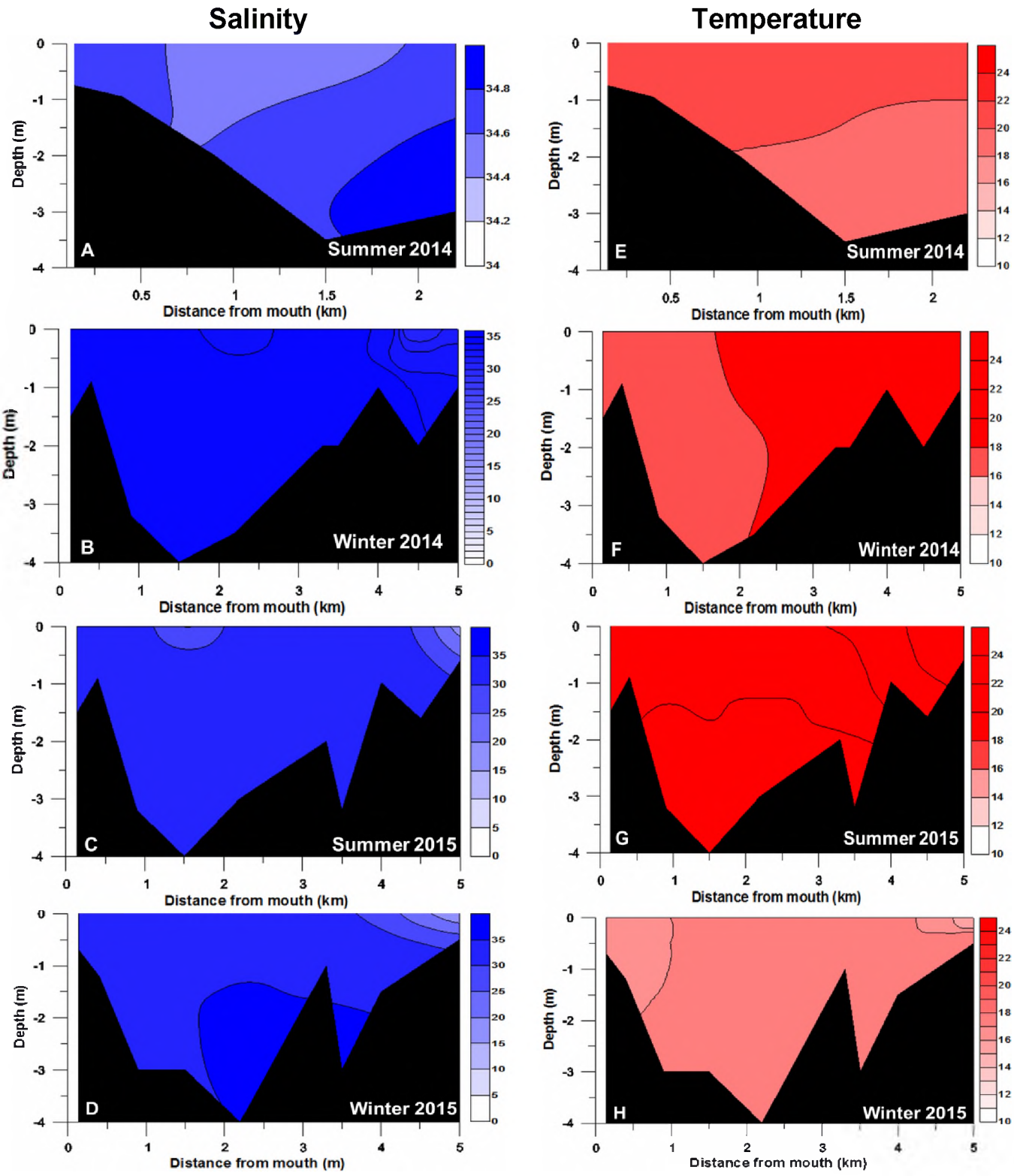

Figure 5.3: Salinity (ppt) (A – D) and temperature (°C) (E – H) profiles along the Nahoon Estuary during the study period. **Summer 2014 sampled up to site 5 (2.2 km) due to weather**.

5.2.1.3 Dissolved Oxygen and pH

The average dissolved oxygen (DO) during winter 2014 was 6.5 ± 0.4 mg·l^{-1} with higher values near the mouth (~8 mg·l^{-1}) decreasing with distance to (~4 mg·l^{-1}) in the upper reaches of the estuary (Fig. 5.4A). The Nahoon water column was more oxygenated in summer 2015 (8.3 ± 0.01 mg·l^{-1}) and increased from ~7 mg·l^{-1} near the mouth to ~10 mg·l^{-1} at the head of the estuary (Fig. 5.4B). Summer 2015 DO values were significantly higher than winter 2014 ($U = 245$; $p < 0.05$; $n = 64$).

Water column pH measurements were significantly alkaline in summer during 2014 ($U = 27$; $p < 0.05$; $n = 45$) and 2015 ($U = 0$; $p < 0.05$; $n = 77$). Summer 2014 measurements were more alkaline compared to summer 2015 ($U = 119$; $p < 0.05$; $n = 63$). The average pH level was ~8 during the entire study and no variation was evident along the length of the estuary during summer sessions. The lowest pH levels were during winter 2014 and 2015 (Fig. 5.5A).

Nahoon recorded low turbidity, Secchi depth readings were consistently high (60 – 105 cm) during the sampling sessions. However, conditions of high turbidity were evident during winter 2015 resulting in low Secchi depth of ~25 cm particularly near the head of the estuary (Fig. 5.5B).

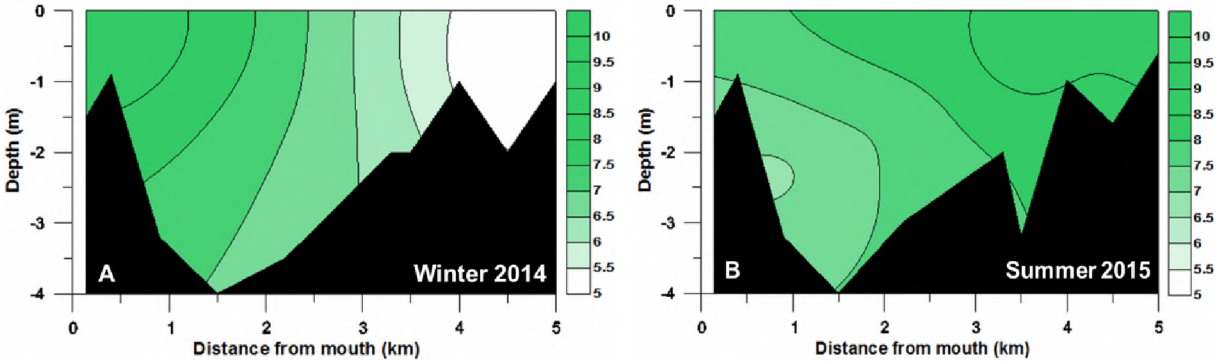

Figure 5.4: Dissolved oxygen (mg·l^{-1}) along the Nahoon Estuary during the sampling period. **No dissolved oxygen data for summer 2014 and winter 2015 due to a faulty meter**.

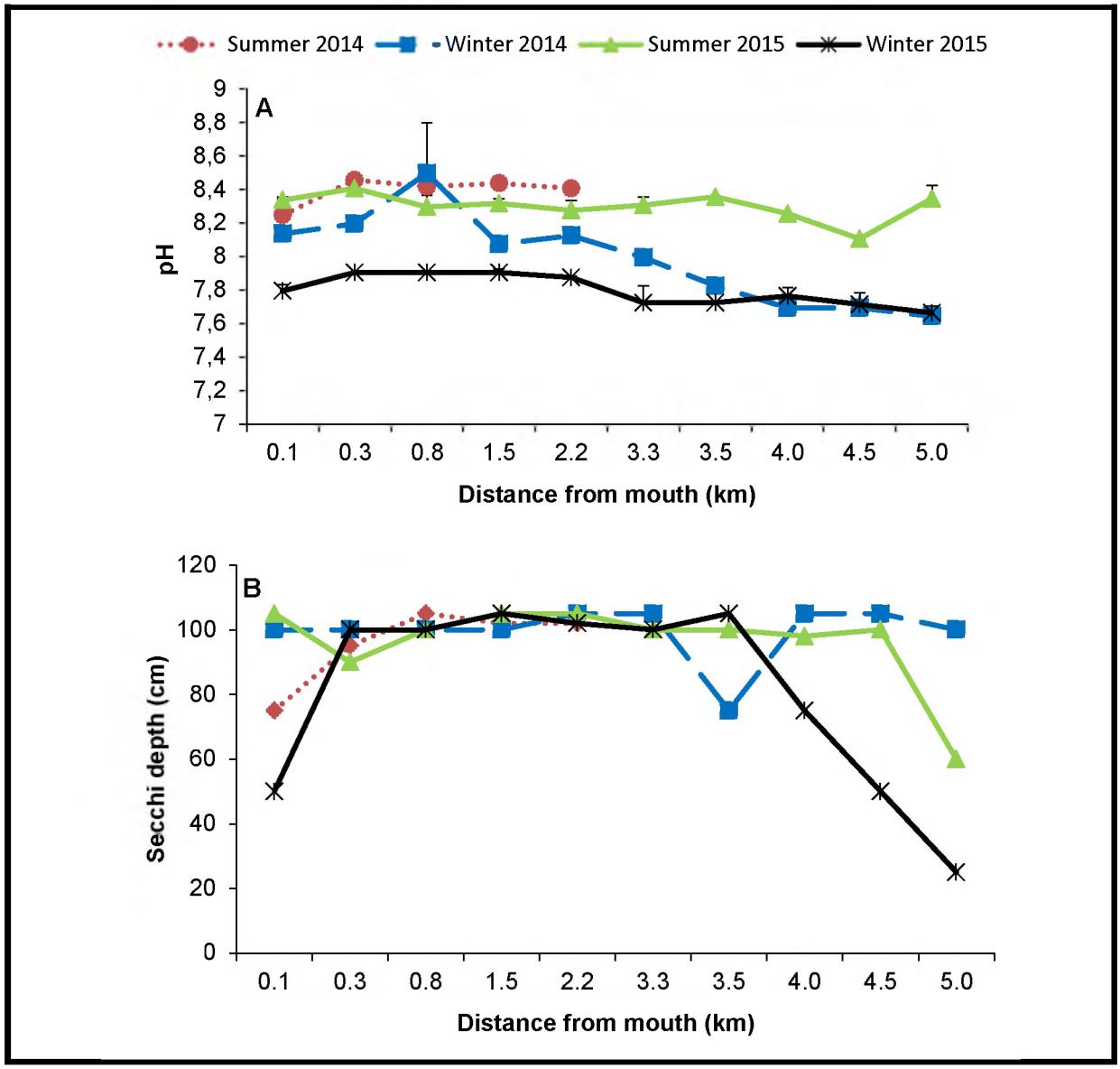

Figure 5.5: Mean pH (± SE present but not visible) (**A**) and Secchi depth (**B**) recorded at Nahoon Estuary during the study period.

5.2.2 Inorganic nutrient concentrations

5.2.2.1 Ammonium (NH_4^+)

Ammonium (NH_4^+) concentrations in summer 2015 ($U = 469$; $p < 0.05$; $n = 82$) and winter 2015 ($U = 57$; $p < 0.05$; $n = 123$) were significantly higher compared to summer and winter 2014. Winter concentrations during 2014 and 2015 were significantly higher than recorded summer 2014 and 2015 concentrations ($U = 535$ and 223; $p < 0.05$; $n = 87$) along the length of the estuary. However, surface values did not differ significantly from bottom values throughout the study period ($p > 0.05$) (Fig. 5.6A & B). Significant correlations with distance from the mouth and temperature were observed during winter 2014 (Table A6, Appendix).

5.2.2.2 Total oxidised nitrogen (TOxN)

Total oxidised nitrogen (TOxN) summer concentrations were similar between 2014 and 2015 ($p > 0.05$) while winter concentrations were significantly higher than summer during both 2014 ($U = 88$; $p < 0.05$; $n = 87$) and 2015 ($U = 716$; $p < 0.05$; $n = 129$) (Fig.5.6C & D). Bottom concentrations were significantly higher than surface concentrations only during summer 2014 ($F = 5.4$; $p < 0.05$; $n = 25$). TOxN was negatively correlated with salinity, dissolved oxygen and pH and positively correlated with distance from the mouth (Table A6, Appendix).

5.2.2.3 Soluble reactive phosphorus (SRP)

No significant soluble reactive phosphorus (SRP) variations were found between summer 2014 and 2015 ($U = 731$; $p > 0.05$; $n = 89$) as well as winter 2014 and 2015 ($U = 1512$; $p > 0.05$; $n = 123$). Nonetheless, winter 2014 ($U = 184$; $p > 0.05$; $n = 87$) and winter 2015 ($U = 842$; $p > 0.05$; $n = 125$) concentrations were significantly higher than summer 2014 and 2015. Bottom SRP concentrations did not significantly differ from surface concentrations ($p > 0.05$) (Fig. 5.6E & F). During winter 2014, SRP concentrations were positively correlated with temperature and distance from the mouth (Table A6, Appendix). In summer 2015, SRP was positively correlated to distance from the mouth and negatively correlated with salinity (Table A7, Appendix).

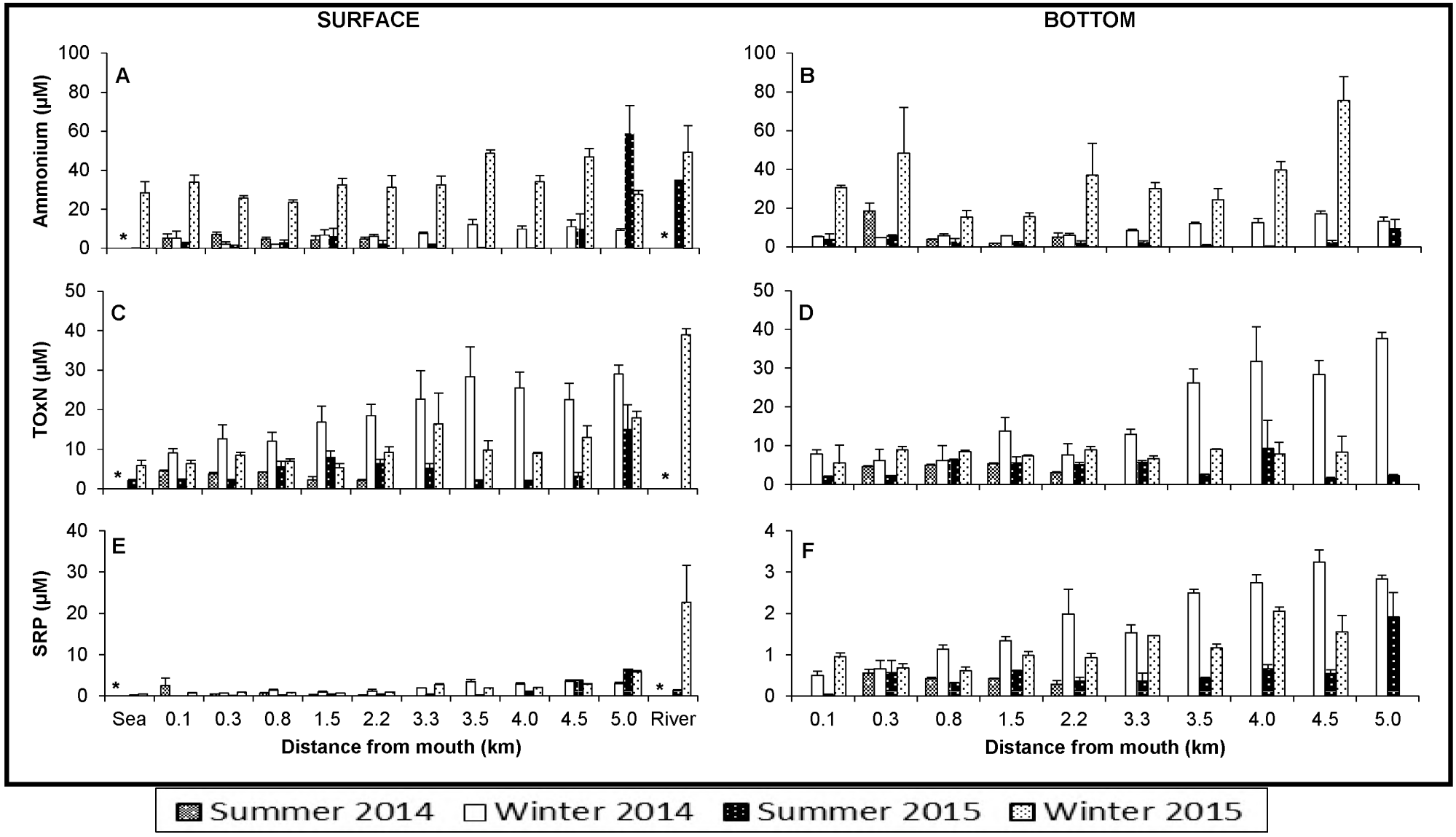

Figure 5.6: Nutrient concentrations of ammonium (A-surface & B-bottom), TOxN (C-surface & D-bottom) and SRP (E-surface & F-bottom) along the main channel of the Nahoon Estuary during the study period. **Summer 2014 sampled up to site 5 (2.2 km) due to weather**. Asterisk (*) indicates no data.

5.2.3 Phytoplankton

Phytoplankton biomass

Water column chlorophyll *a* (Chl *a*) biomass varied significantly between seasons with summer concentrations significantly higher than winter in 2014 ($U = 297$; $p < 0.05$; $n = 90$) and summer 2015 concentrations significantly higher than winter 2015 ($U = 834$; $p < 0.05$; $n = 117$). Summer biomass between 2014 and 2015 did not differ significantly ($p > 0.05$) (Fig. 5.7). The maximum chlorophyll *a* concentration was in winter 2015 (22.8 µg Chl-*a* l^{-1}), this occurred at 3.5 km (Site 7) and was the only bloom condition (≥ 20 µg Chl-*a* l^{-1}) recorded during the study (Fig. 5.8). Summer 2015 concentrations significantly increased with distance from the mouth ($H = 34.2$; $p < 0.05$; $n = 60$) (Fig. 5.8) and were significantly positively correlated with distance from the mouth and negatively to pH (Table A7, Appendix). Both winter 2014 and 2015 concentrations significantly differed along the length of the estuary. The lowest concentration was recorded at 5 km (Site 10) in winter 2015 and was significantly negatively correlated with pH (Table A8, Appendix). Surface and bottom concentrations did not significantly differ throughout the study period ($p > 0.05$).

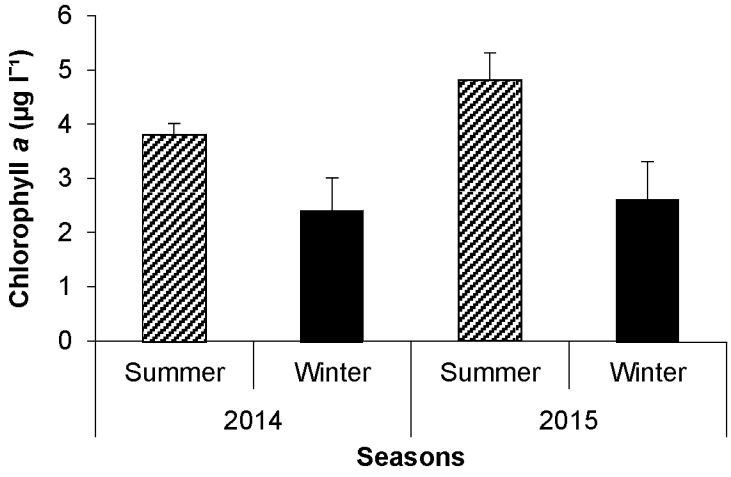

Figure 5.7: Seasonal mean phytoplankton biomass (chlorophyll *a*) recorded during the study period (mean ± SE) along the Nahoon Estuary. **Summer 2014 sampled up to Site 5 (2.2 km) due to weather**.

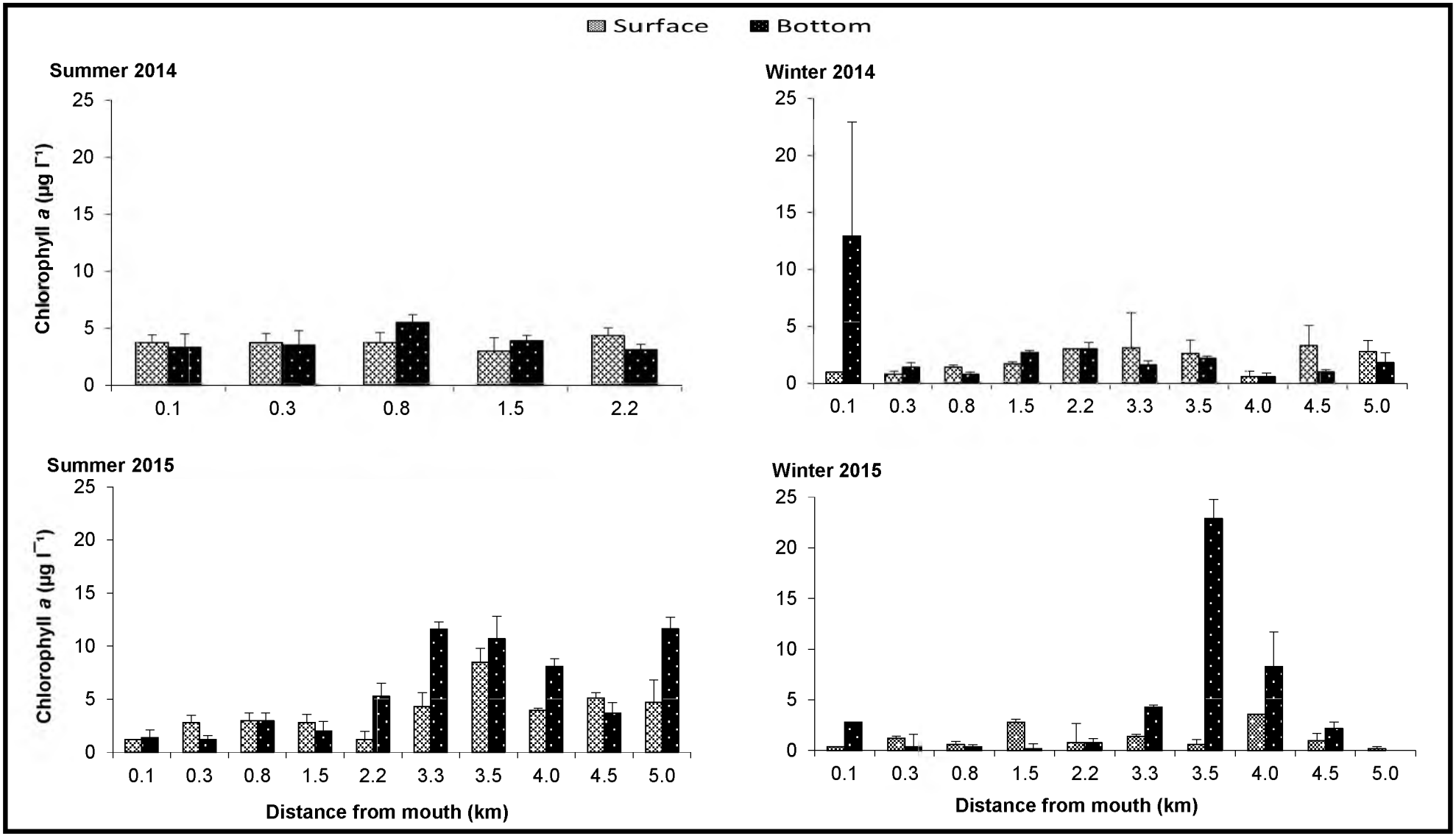

Figure 5.8: Mean phytoplankton biomass (chlorophyll *a*, mean ± SE) recorded for surface and bottom waters along the length of the Nahoon Estuary during summer and winter sampling periods**. Summer 2014 sampled up to site 5 (2.2 km) due to weather**.

5.2.3.1 Phytoplankton community composition

During the present study, the following phytoplankton groups were recorded: flagellates, dinoflagellates, diatoms, blue-green (~cyanobacteria) and green (~chlorophytes) algae. Actual counts are in Table A10 (Appendix).The community structure during summer 2014 (Fig. 5.9) was dominated by flagellates up to 1.5 km from mouth while cyanobacteria were present in low abundance. Flagellates were significantly positively correlated with temperature (Table A5, Appendix). Cyanobacteria dominated the upper reaches during winter 2014 (Fig. 5.9). This phytoplankton group was significantly positively correlated with distance from the mouth, temperature and nutrients (NH_4^+, TOxN and SRP) (Table A6, Appendix). Flagellates were negatively correlated with nutrients (Table A6, Appendix)

During summer 2015 (Fig. 5.10), the composition of diatoms was negatively correlated with chlorophyll *a* and positively with pH. The dominance of flagellates from the middle to the upper reaches of the estuary was significantly positively correlated to temperature (Table A7, Appendix). Winter 2015 was largely dominated by flagellates along the length of the estuary (Fig. 5.10). Chlorophytes were only present during winter 2015 at Site 9. Diatoms were negatively correlated to temperature and positively correlated with pH (Table A8, Appendix).

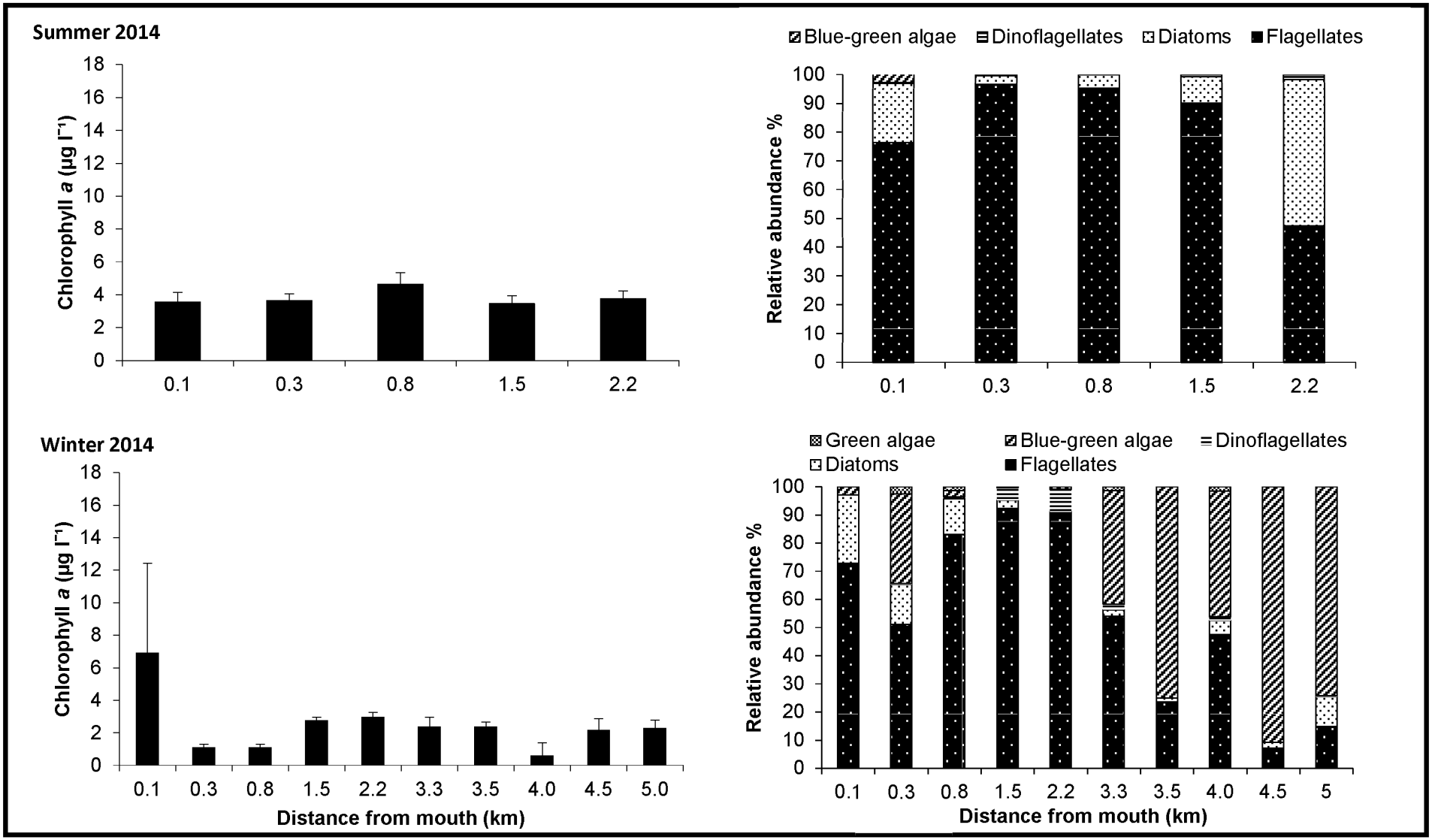

Figure 5.9: Vertically averaged phytoplankton biomass (± SE) and corresponding phytoplankton community composition (RA, as %) per site during summer 2014 and winter 2014. S**ummer 2014 sampled up to site 5 (2.2 km) due to weather.**

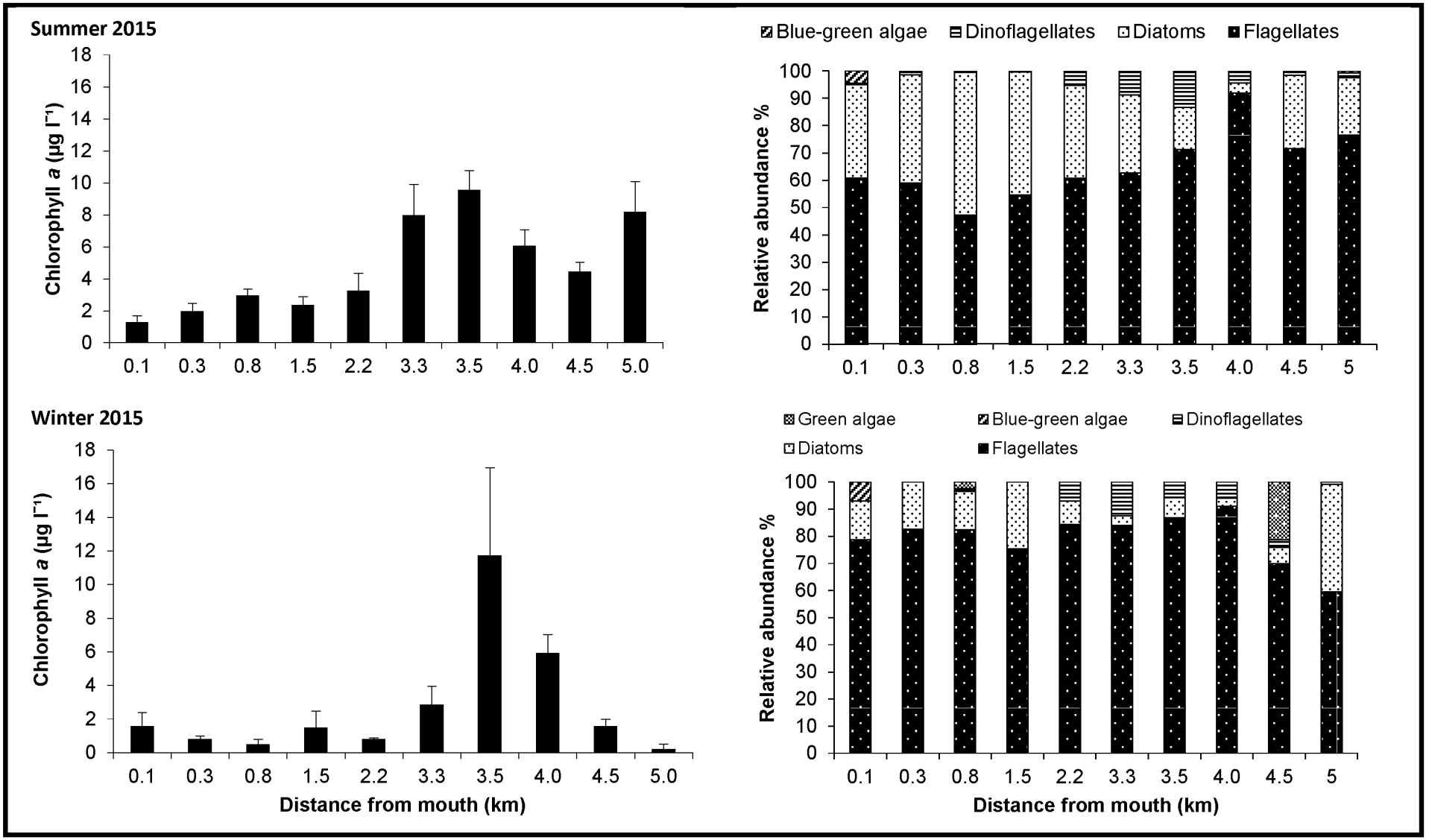

Figure 5.10: Vertically averaged phytoplankton biomass (± SE) and corresponding phytoplankton community composition (RA, as %) per site during summer 2015 and winter 2015.

Summary results for the Canonical Correspondence Analysis (CCA) plots during the sampling sessions are displayed below the plots. The first canonical axis described 89.2 % of the variation of the species-environmental relation during summer 2014 (Table 5.3A). The axis was positively correlated salinity, pH and SRP (0.61, 0.39 and 0.04) respectively while it was negatively correlated with TOxN (-0.83). The negative correlation with NH_4^+ (-0.30) indicated a lower influence on Sites 2 and 3 compared to other variables (Fig. 5.11A). The dominant flagellates were associated with NH_4^+.

During summer 2015 (Fig. 5.11B), the first canonical axis described 83.3 % of the variation of the species-environmental relation (Table 5.3B) with the axis negatively correlated to salinity (-0.44), pH (-0.47) and TOxN (-0.04). The axis was however positively correlated with temperature (0.74), dissolved oxygen (0.52), NH_4^+ (0.39), and SRP (0.64). The dominant flagellates were associated with TOxN.

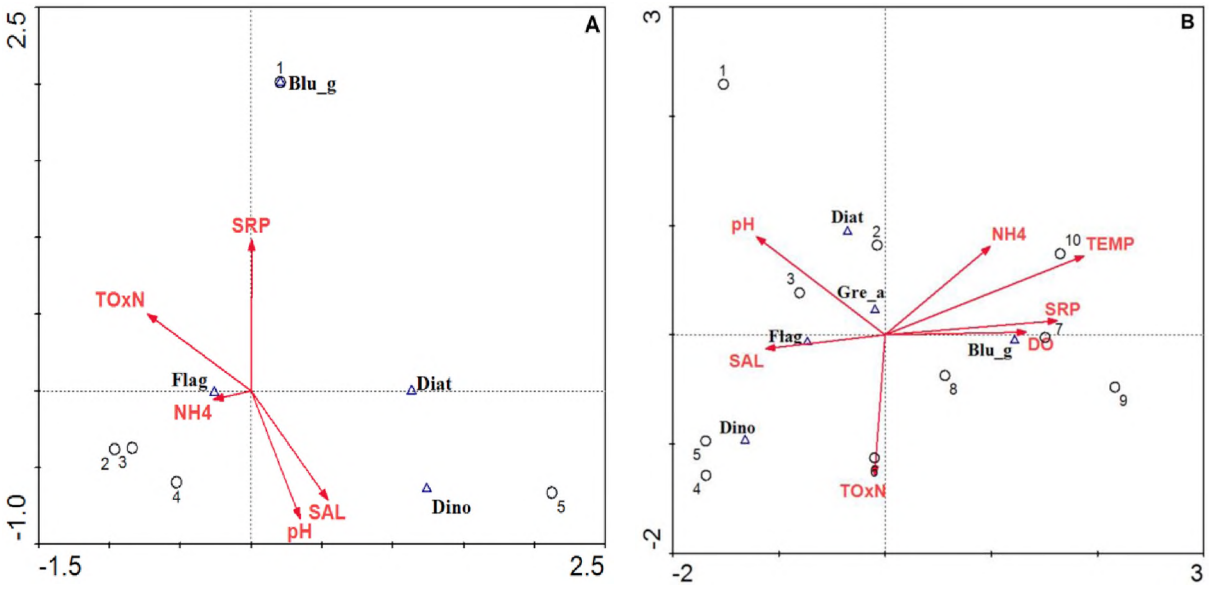

Figure 5.11: CCA ordination plots of phytoplankton groups (relative abundance) with physico-chemical factors (Temperature = TEMP; Salinity = SAL; Dissolved Oxygen = DO and pH) and nutrient concentrations (NH_4^+, TOxN and SRP) at Nahoon Estuary in summer (A) 2014 and (B) 2015. The arrows represent each physico-chemical factor pointing in the direction of its maximum change. The sites are indicated by numbers (i.e. 1) and phytoplankton groups are abbreviated as: **Flag** = Flagellates; **Diat** = Diatoms; **Dino** = Dinoflagellates; **Blu_g** = Blue-green algae. Numbers represent sites.

Table 5.3: CCA summary results for phytoplankton groups and environmental factors correlations of the first two axes for summer (A) 2014 and (B) 2015.

A	**Axis 1**	**Axis 2**	**Total inertia**
Eigenvalues :	0.226	0.026	0.254
Species-environment correlations :	1.000	1.000	
Cumulative percentage variance			
of species data :	89.2	99.5	
of species-environment relation:	89.2	10.8	
Sum of all eigenvalues			0.254
B	Axis 1	Axis 2	Total inertia
Eigenvalues :	0.464	0.080	0.586
Species-environment correlations :	0.986	0.941	
Cumulative percentage variance			
of species data :	79.2	92.8	
of species-environment relation:	83.3	16.7	
Sum of all eigenvalues			0.586

The results during winter 2014 indicated that the first axis was positively correlated with temperature (0.63), NH_4^+ (0.85), TOxN (0.83) and SRP (0.82) while negatively correlated with salinity (-0.71), dissolved oxygen (-0.69), and pH (-0.84) (Fig. 5.12C). The dominant flagellates were closely linked to pH. The species-environment relation was described by 15.3 % along the second canonical axis (Table 5.4C).

In winter 2015 (Fig. 5.12D), this first axis was only positively correlated with SRP (0.53) and NH_4^+ (0.71). The axis was negatively correlated with temperature (-0.05), salinity (-0.15), TOxN (-0.40) and pH (-0.44). The dominant flagellates were associated with temperature. The first canonical axis described 47.7 % of the variation of the species-environmental relation in winter 2015 (Table 5.4D).

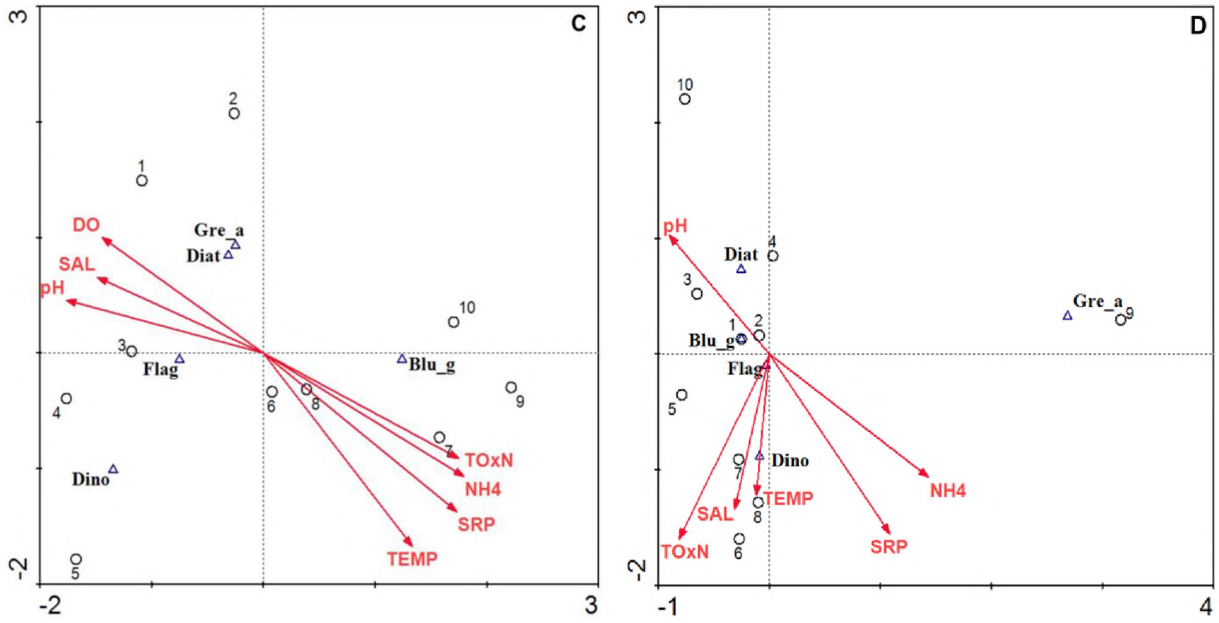

Figure 5.12: CCA ordination plots of phytoplankton groups (relative abundance) with physico-chemical factors (Temperature = TEMP; Salinity = SAL; Dissolved Oxygen = DO and pH) and nutrient concentrations (NH_4^+, TOxN and SRP) at Nahoon Estuary in winter (C) 2014 and (D) 2015. The arrows represent each physico-chemical factor pointing in the direction of its maximum change. The sites are indicated by numbers (i.e. 1) and phytoplankton groups are abbreviated as: **Flag** = Flagellates; **Diat** = Diatoms; **Dino** = Dinoflagellates; **Blu_g** = Blue-green algae; **Gre_a** = Green algae. Numbers represent sites.

Table 5.4: CCA summary results for phytoplankton groups and environmental factors correlations of the first two axes for winter (C) 2014 and (D) 2015.

C	**Axis 1**	**Axis 2**	**Total inertia**
Eigenvalues :	0.474	0.073	0.586
Species-environment correlations :	0.997	0.906	
Cumulative percentage variance			
of species data :	80.9	93.4	
of species-environment relation:	84.7	15.3	
Sum of all eigenvalues			0.586
D	Axis 1	Axis 2	Total inertia
Eigenvalues :	0.155	0.102	0.363
Species-environment correlations :	0.946	0.980	
Cumulative percentage variance			
of species data :	42.7	70.9	
of species-environment relation:	47.7	52.3	
Sum of all eigenvalues			0.363

5.2.4 Faecal bacteria

Bacterial counts along the Nahoon Estuary showed no significant variation between summer 2014 and 2015 ($U = 171$; $p > 0.05$; $n = 43$) while winter 2014 counts were significantly higher than winter 2015 ($U = 271$; $p < 0.05$; $n = 60$). The highest mean bacterial counts (36.2 ± 28 counts. 100 ml^{-1}) were recorded during summer 2014 (Fig. 5.13), particularly at 0.3 km (Site 2) from the mouth. No seasonal differences were observed during 2014 ($U = 172$; $p > 0.05$; $n = 43$) however, summer 2015 bacterial counts were significantly higher than winter 2015 ($U = 278$; $p < 0.05$; $n = 60$). *E. coli* counts did not differ significantly across the sampling sites ($p > 0.05$) except in winter 2014 ($H = 17.9$; $p < 0.05$; $n = 30$) and the lowest counts were during winter 2015 (Table 5.5). *E. coli* was significantly positively correlated with salinity during summer 2014 (Table A5, Appendix), with temperature and chlorophyll *a* during winter 2014 (Table A6, Appendix).

Table 5.5: *Escherichia coli* counts measured along the length of the Nahoon Estuary during sampling period (± SE, N=3).

Distance from mouth (km)	*Escherichia coli* (Counts 100 ml^{-1})			
	Summer 2014*	Summer 2015	Winter 2014	Winter 2015
0.1	4.17 ± 4.1	3.3 ± 3.3	0 ± 0	0 ± 0
0.3	**375** ± 0 TNTC	44.2 ± 16.7	**138.3 ± 56****	3.3 ± 2.2
0.8	12.5 ± 5.8	9.2 ± 7.9	3.3 ± 3.3	0 ± 0
1.5	0 ± 0	12.5 ± 8.0	1.7 ± 1.7	7.5 ± 5.2
2.2	15.0 ± 11.5	7.5 ± 7.5	10 ± 5.2	1.7 ± 0.8
3.3	N.D	6.7 ± 5.5	5.8 ± 3.6	5.8 ± 2.2
3.5	N.D	11.7 ± 6.5	19.2 ± 7.2	2.5 ± 1.4
4.0	N.D	12.5 ± 9.0	11.7 ± 7.2	6.7 ± 3.6
4.5	N.D	7.5 ± 6.3	10 ± 8.7	0.8 ± 0.8
5.0	N.D	19.2 ± 9.6	20 ± 5.2	0.8 ± 0.8

***Summer 2014 sampled up to site 5 (2.2 km) due to weather, N.D indicates No Data**

****Average counts which are significantly higher or lower per sampling season per site ($p < 0.05$)**

TNTC: Too numerous to count. (Only one replicate reported here).

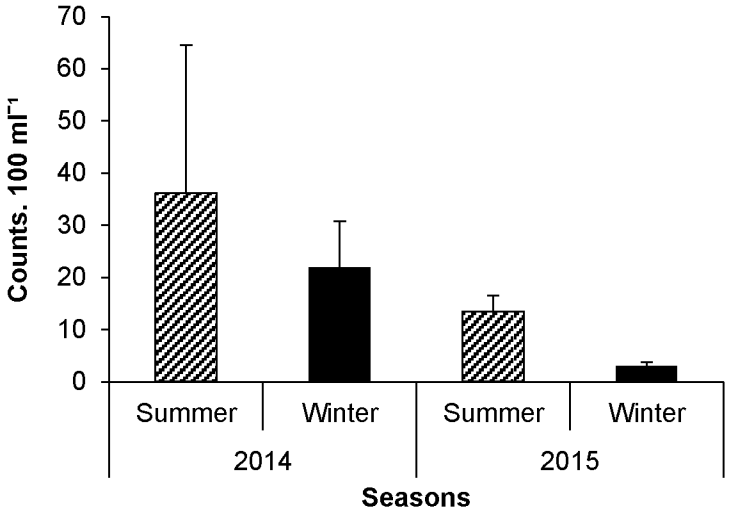

Figure 5.13: Seasonal mean *E. coli* counts recorded along the Nahoon Estuary during the study period (mean ± SE).

5.3 Discussion

The water quality characteristics of estuaries are governed via the integral role played by water circulation patterns, and mostly the quality of the inflowing river water (Snow & Taljaard 2007). Hence, water quality has been an important issue for management of estuaries along the South African coast (Harrison *et al.* 2000). The significance of this study was to determine the current water quality at Nahoon Estuary by evaluating the nutrient status, microalgae dynamics and bacterial contamination along the length of the estuary. The physico-chemical variables at Nahoon reflect the findings by Harrison (2004) where salinity averaged ~33 ppt for the duration of the study, a longitudinal salinity gradient was evident during summer 2015 where salinity decreased from 36 ppt at the mouth to ~17 ppt at the estuary head (current study). Campbell *et al.* (1991) also observed high salinity of ~36 ppt with no change along the estuary length. Average summer temperatures (~21 °C) showed no longitudinal variation while winter (~18 °C) values fluctuated along the estuary length. The waters of the Nahoon Estuary remained well oxygenated (~6 $mg \cdot l^{-1}$), a noticeable increase was evident during this study where the lower reaches had low dissolved oxygen (4 $mg \cdot l^{-1}$) compared to the upper reaches (10 $mg \cdot l^{-1}$). The increase in dissolved oxygen is likely to be due to high river discharge and indicates a high assimilative capacity of the water body (Iriarte *et al.* 2015, VishnuRadhan *et al.* 2015). Harrison (2004) found a similar average dissolved oxygen at Nahoon (~8 $mg \cdot l^{-1}$) and pH remained fairly constant (~8) throughout this study. Based on the salinity regime of this estuary, it is clearly tide dominated as previously noted (Whitfield 1992, Cooper 2001, Harrison 2004). No salinity stratification and thermoclines were evident during the current study, freshwater inflow into the estuary is low and therefore, the dilution effect is lacking.

Intensive residential development on the banks of the Nahoon Estuary have resulted in substantial human interaction with the estuarine environment and nutrient loading into other urbanised estuaries has increased over the last decades with more contaminated stormwater discharges in response to population growth and also upgrading of sewage treatment plants (Campbell *et al.* 1991, Dugdale *et al.* 2012, Van Niekerk & Turpie 2012). The nutrient status of the Nahoon Estuary is similar to the variability reported by Scharler & Baird (2005) where nutrient concentrations were greater in the upper reaches of the Swartkops and Gamtoos estuaries. High nutrient concentrations were mostly found along the upper reaches of this estuary from 3.5 to 5 km from the mouth and a strong seasonal variation was evident with winter higher than summer concentrations. Throughout the study period, concentrations increased with distance from the mouth. The nutrients can be attributed to the low freshwater flowing in and the inlet systems (i.e. stormwater drains)

bearing runoff from the river banks (Campbell *et al.* 1991) or dilution by marine waters. The river section, just above the Abbotsford Bridge has been observed to support water hyacinth (*Eichhornia crassipes* (Mart) Solms) which indicates eutrophic conditions (Pretorius 2014), therefore, the incoming river water brings nutrients to the upper reaches of Nahoon Estuary. Heavy rains induced the presence of dense algae mats in the upper reaches (below Abbotsford Bridge; Site 10) of the Nahoon Estuary during October 2014 and this was indicative of nutrient enrichment, most likely related to sewage and agricultural runoff from upstream (Fig. B5.5 Appendix; Daily Despatch, 2 October 2014).

The concentration of nutrients in the lower reaches indicate that the mangrove forest exports low nutrients into the water column and the nearshore environment as observed at Mngazana Estuary (Emmerson 2005). A study by Geldenhuys (2013) revealed that nutrient concentrations in the Nahoon River were higher (i.e. NH_4^+: 1.2 – 28.2 µM, TOxN: 9 – 34 µM) compared to concentrations from the mangroves and salt marsh communities in the lower reaches (NH_4^+: 0.9 – 1.05 µM, TOxN: 4 µM) (near Site 3). Scharler & Baird (2005) argued that reduced nutrients in the lower reaches of estuaries may affect the system's productivity and diversity, since lack of nutrients can lead to less uptake by primary producers. This can be traced back to reduced freshwater inflow and the associated decreased levels of nutrient input. Nutrient concentrations during this study were significantly higher during winter than summer seasons despite this region being dominated by summer rainfall. The Nahoon Dam and upstream water abstraction play a tremendous role in the low freshwater inflow into the Nahoon Estuary as proven in the Kromme Estuary (Snow & Adams 2006) where freshwater reaches the estuary when overtopping of the dams occurs due to heavy rains (Scharler & Baird 2005). When looking globally, the dissolved inorganic nitrogen and phosphate (~65 %) are derived from anthropogenic sources and David *et al.* (2016) noted nutrient fluxes in the catchment of the Pamba River (India) where NH_4^+ (~50 µM) and SRP (~1.5 µM) increased dramatically to ~153 µM NH_4^+ and ~26 µM SRP respectively, due to anthropogenic use of the system.

The phytoplankton biomass (chlorophyll *a*) of the Nahoon Estuary varied along the estuary length. Chlorophyll *a* biomass ranged from (0 – 34 µg Chl-*a* l^{-1}) during the current study which is higher than the 0 – 6 µg Chl-*a* l^{-1} and 5 – 15 µg Chl-*a* l^{-1} ranges reported for Nahoon and Gqunube estuaries respectively by Campbell *et al.* (1991). The average chlorophyll *a* for the entire system was 3.5 ± 0.3 µg Chl-*a* l^{-1} and the only observed algal bloom condition (≥ 20 µg Chl-*a* l^{-1}) was in the bottom waters 3.5 km (Site 7) from the mouth during winter 2015. The low biomass at Nahoon is due to low freshwater inflow rendering the nutrients being introduced into the system inadequate to support high biomass despite their

accumulation. Snow & Adams (2006) found low chlorophyll *a* biomass of 3.0 µg Chl-*a* l^{-1} at Kromme Estuary due to low freshwater input. The biomass recorded in the heavily urbanised Swartkops Estuary was higher (0 – 248 µg Chl-*a* l^{-1}) than biomass at Nahoon Estuary (Pretorius 2014) while Jafta (2010) found chlorophyll *a* biomass of <2 – 9 µg Chl-*a* l^{-1} along the Bushmans Estuary which also experiences low freshwater inflow which is the source of nutrient input due to farm dams. Nahoon Estuary lies in wave- dominated coast and is prone to tidal flushing, evident with the high salinity (~30 ppt) up to 5 km from the mouth. The biomass recorded at Nahoon is similar to Hart *et al.* (2015) who reported an average chlorophyll *a* biomass of ~7 µg Chl-*a* l^{-1} in the well-flushed, anthropogenically impacted Guana Tolomoto Matanzas (GTM) Estuary, Florida. According to Adams *et al.* (1999), flushing events induced by heavy rainfall or dam release result in a short-term low biomass in estuaries. To note, phytoplankton production dynamics appear to be site-specific and time-varying combinations of all the factors that regulate balance between production, consumption and transport (Cloern *et al.* 2014).

The community composition can illustrate the changes in the water quality of an estuary, the phytoplankton groups and indicator species can therefore provide useful insights regarding responses to specific environmental conditions (Lemley 2015). The Nahoon Estuary was dominated mostly by flagellates along the estuary length during the current study. However, interplay between phytoplankton groups was evident since the estuary was dominated by blue-green algae (cyanobacteria) along the upper reaches in winter 2014. Diatoms showed an abundance that decreased with distance from the mouth during summer 2015. Accordingly, green algae (chlorophytes) appeared in low composition 4.5 km from the mouth only during summer 2015. The dominance of flagellates in South African estuaries has been documented extensively (Adams & Bate 1994, 1999). The Swartkops Estuary was reported to being dominated by flagellates during the study by Pretorius (2014) and this group was said to be associated with elevated nutrient levels along the estuary. The low composition of green algae (chlorophytes) signals the relatively high saline conditions at Nahoon since Kotsedi *et al.* (2012) revealed that a high composition of green algae was associated with low salinity along the upper reaches in the Sundays Estuary. The abundance of cyanobacteria in this estuary was high along the upper reaches signalling an excess of nutrients. Such nutrients could be from runoff from adjacent lawns and impervious surface in the surrounding residential houses, Lemley *et al.* (2015) noted that this phytoplankton group was dominant in the vicinity of point and non-point sources at the Goukou and Goukamma estuaries. Furthermore, *Symplocastrum* sp. was identified as the dominant cyanobacteria species indicating the presence of nutrient-reach waters from a wastewater treatment plant (WWTP). According to an analysis by Carstensen *et al.* (2015), diatoms are the taxonomic

group that are mostly associated with phytoplankton blooms, seconded by dinoflagellates. The only recorded algal bloom (≥20 µg Chl-*a* l^{-1}) during this study was made up of cyanobacteria.

Dalu *et al.* (2014) found nutrients, salinity and hydrological variables (i.e. water depth) to be critical factors that affected the phytoplankton variation along the Kowie system. Salinity was found to be the most important factor modulating phytoplankton composition in the study by Haraguchi *et al.* (2015). Furthermore, the authors illustrated that cyanobacteria was highly associated with SRP while diatoms were associated with high salinity and transparency along the Patos Lagoon Estuary in Brazil. The phytoplankton groups observed during the study were associated with different physico-chemical variables in each season throughout the study period (i.e. temperature in winter 2015) along upper reaches of the estuary. The distributions of the phytoplankton groups were negatively associated with salinity during summer sampling seasons. The low cyanobacteria (~5 %) composition near the mouth during summer 2014 was highly influenced by SRP while the abundance of flagellates was associated with NH_4^+. The winter seasons showed that pH positively influenced diatoms along the lower reaches of the estuary. However, the combination between nutrients and physico-chemical variables overall influences the distribution of the phytoplankton distribution.

As mentioned in Chapter 4, *E. coli* contamination studies on estuaries are few in South Africa. The bacterial counts along the Nahoon Estuary showed a seasonal variation with the highest counts during summer 2014. The inlets (stormwater drains) along the upper reaches of the estuary do not seem to be sources of faecal pollution in this estuary. The high counts observed near the mouth are in line with the statement by Bate *et al.* (1986) that the estuary mouth lies about 1.5 km east of the Bats Cave sewage outfall and may receive polluted seawater during certain weather conditions. Despite the well-developed flood tide delta at Nahoon, no dense flocks of birds were seen during the study which could indicate another contributor to *E. coli* counts. Pretorius (2014) reported high *E. coli* counts along the highly urbanised Swartkops Estuary and attributed the high bacterial contamination to runoff from canals that deposit runoff into the estuary. Furthermore, *E.* coli counts were also attributed to wastewater treatment works that discharge into the river (Pretorius 2014). This illustrates the necessity of monitoring wastewater effluents entering water bodies. Studies on *E.coli* contamination in certain South African rivers under anthropogenic influences have been documented (i.e. Paulse *et al.* 2007) and stormwater drainage pipes were attributed as significant sites of faecal pollution into the river water. Pretorius (2014) proposed that future studies investigate riverine and estuarine sediments as potential sources of *E. coli*. This

would be vital since Malham *et al.* (2014) argues that human pathogens can accumulate within sediments and on suspended sediments in riverine and estuarine waters.

5.4 Conclusion

This study evaluated the present water column conditions at Nahoon Estuary. Based on the NBA 2011 assessment (Van Niekerk & Turpie 2012), the water quality at Nahoon Estuary was classified as poor and the overall ecological category being C [(2)]. The impact of urbanisation is evident around estuaries worldwide and the anthropogenic influence such as Nahoon Dam and water abstraction upstream of Nahoon plays a major role in the decreased freshwater inflow and the introduction of nutrients into the system. Poorly maintained and inadequate wastewater treatment facilities continue to be a major health risk within some South African municipalities. Although no wastewater treatment plants (WWTP) are adjacent to this estuary, urban runoff along impervious surfaces and via stormwater drains needs to be monitored. This study showed the importance of microalgae as indicators of changes in the water column to reflect nutrient status and the presence of cyanobacteria and high phytoplankton biomass in the upper reaches of the estuary confirms the input of excessive nutrient concentrations.

Most studies on the impacts of urbanisation on environmental systems correlate changes in environmental systems with simple aggregated measures of urbanisation (i.e. percent of impervious surfaces) (Alberti & Marzluff 2004). The presence of faecal bacteria at Nahoon during the present study necessitates the implementation of rigorous assessments and monitoring of this estuary to find the major source of faecal contamination. It is recommended that drastic measures be taken to combat pollution upstream of the Nahoon Estuary to limit the possibility of eutrophic conditions along the upper reaches so as to also improve its overall ecological state.

[2] Moderately modified estuary: A loss and change of natural habitat and biota have occurred, but the basic ecosystem functions and processes are still predominantly unchanged.

Chapter 6. General discussion, management recommendations and conclusions.

6.1 General Discussion

The aims of this research were to investigate the microalgal dynamics (biological response) of the Mngazana and Nahoon estuaries in response to physico-chemical variables and nutrients; to determine the presence and distribution of bacterial contamination and to observe any visible anthropogenic impacts along the two estuaries. The results of this study will add to the available information on South African estuaries and the current estuarine management initiatives guided by the Integrated Coastal Management (ICM) Act (No. 24 of 2008). Estuarine hydrodynamics are fundamental in influencing the spatial and temporal variability in physico-chemical drivers (Snow & Taljaard 2007) and this determines the biological response of particular biota. The water quality in estuaries changes continuously due to factors such as hydrology, climatic conditions and impacts from human activities. James *et al.* (2013) argues that due to the dynamic nature of estuaries, change occurs over a scale of hours to years and the effects of climate change will alter freshwater delivery to estuaries thus affecting their dynamic nature (i.e. stratification and mixing) (Liu & Chan 2016).

Baird (1999) stated that estuaries are complex ecosystems and it is unlikely that all the components and interrelationships will ever be fully analysed and understood. This sparks the need to explore more realistic scenarios of change that reflect how human activities are altering ecosystems and biodiversity (Cardinale *et al.* 2012). In the South African context, the key pressures on estuaries were identified as flow modification, pollution and exploitation of living resources, habitat destruction and climate change (Van Niekerk & Turpie 2012). According to Alberti & Marzluff (2004), urban ecosystems provide unique opportunities to test hypotheses about the interactions between human and ecological processes. Furthermore, human requirements in and around urban areas such as water supply, waste disposal and recreation depend on ecosystems for natural resources and their productivity over the long term. However, these activities lead to modification of nutrient flows in rivers and the overall morphology of estuaries themselves, particularly via reclamation and this has altered the biogeochemical functioning of estuarine systems and their associated provision of ecosystem services (Jickells *et al.* 2014). It can be deduced that by virtue of location, estuaries experience different pressures. Yang *et al.* (2015) argues that the integrity of

estuarine ecosystems and the livelihood of the urban residents are threatened by the twin forces of climate change and human development.

The two estuaries investigated during this study lie in different biogeographical regions along the South African coastline and the magnitude of anthropogenic impacts exerted on them differ with Mngazana being a rural estuary and Nahoon situated in an urban area. Mngazana Estuary is surrounded by mostly mangroves (Rajkaran & Adams 2004) and a largely natural catchment (CCA 2009), and the only modification with reference to flow dynamics is the bridge currently being upgraded at the head of the estuary (Fig. B1 & 3 Appendix). The present study (Chapter 4) revealed that the microalgae biomass and community distribution at Mngazana were similar to what Ngesi (2010) reported illustrating the long-term issue of the lack of freshwater inflow but also low levels of anthropogenic disturbance. Masefield *et al.* (2014) stated that the natural vegetation within catchments and riparian areas plays a crucial role in maintaining aquatic ecosystem integrity and any deviation away from natural conditions could provide insight into the deterioration of estuary health. Estuaries along the former Transkei are known to be undeveloped (Colloty *et al.* 2002) compared to estuaries surrounded by urban developments (i.e. Nahoon Estuary – see Fig. B7 Appendix). The present study indicated that the Nahoon Estuary experiences moderate levels of impacts compared to other urbanised estuaries (i.e. Diep Estuary – Viskich *et al.* 2016; Swartkops Estuary – Adams *et al.* 2014) but experiences similar pressure in terms of low freshwater inflow (66.7 % MAR reduction) like Kromme Estuary (100 % MAR reduction) due to dam development upstream (Snow & Adams 2006). Signs of excess nutrients along the upper reaches of the Nahoon Estuary were evident during this study, with high river nutrient concentrations recorded compared to the rest of the estuary. Eutrophication is seen as one of the primary water quality challenges facing estuaries (Greening *et al.* 2014). The magnitude of the impacts in urban located estuaries (via industrial waste, housing etc.) is greater than that experienced by rural located estuaries, but Elsdon *et al.* (2009) revealed that rural estuaries have stronger patterns of change than urban estuaries suggesting that rural estuaries are more dynamic in environmental parameters and nutrients. The low diversity in planktonic communities in estuaries can thus be explained via the intermediate disturbance hypothesis as described by Flöder & Sommer (1999).

Deleterious water quality affects the biological functioning of estuaries and subsequently produces complications such as odour, aesthetics, human pathogens and associated public health risk, and with climate change such deleterious effects may be exacerbated (Liu & Chan 2016, Wetz *et al.* 2016, Whitfield *et al.* 2016). Therefore, water quality models are useful in assisting with the understanding of biological processes and the assessment of the

influences of climate change on water quality conditions in aquatic systems despite the associated limitations (Liu & Chan 2016). According to Borja *et al.* (2011), it is essential to distinguish whether degradation results from factors such as flow modification, pollutant stress, habitat degradation. in order to direct appropriate remedial actions and such actions should encompass long-term monitoring procedures of aquatic systems.

The use of microalgal groups as indicators of change in estuarine waters is vital since the change in composition can easily illustrate changes in water chemistry (i.e. low nutrients: flagellates; high nutrients: cyanobacteria) (Table 2.1 see Chapter 2). A step further is to identify species that make up the different phytoplankton compositions allowing for the monitoring of 'problematic' species during phytoplankton blooms (Carstensen *et al.* 2015). However, some of the difficulties in evaluating the community composition include the bio-assessment process that requires the standardisation of the sampling and analytical procedures (Garmendia *et al.* 2013) and expertise in phytoplankton identification. The importance of selecting relevant indicators of change in estuarine waters has been evaluated (Lemley *et al.* 2015), Borja *et al.* (2008, 2009) stressed the development of reliable methods to integrate multiple physico-chemical and biological elements into a single evaluation of aquatic system condition. Furthermore, the ecological integrity of an aquatic system should be evaluated using all information available. This simply emphasises the need for long-term monitoring that allows for the development of indices. The present study, the change in microalgal groups successfully indicated the changes in nutrient dynamics in the two estuaries particularly using the presence of cyanobacteria.

Anthropogenic activities have increased the load of faecal bacteria and pathogenic viruses in estuaries and coastal areas through point and diffuse sources (i.e. sewerage discharges and agricultural runoff) (Malham *et al.* 2014, Monteiro *et al.* 2016). The monitoring of microbial contamination in water bodies provides a link between ecosystem health and human health and tracing the source of contamination is a vital step towards mitigation and disease prevention (Henry *et al.* 2016a). The concentration levels of *E. coli* are significantly influenced by various nonpoint sources such as surface runoff, septic tanks, recreational activities and animal faeces (Kim *et al.* 2007, Malham *et al.* 2014). The presence and survival of human pathogenic microorganisms present a great concern to human health in estuaries hence the implementation of evaluation methodologies such as microbial source tracking (MST) are becoming increasingly important (Harwood *et al.* 2014, Henry *et al.* 2016a). This research also investigated the presence and distribution of bacterial contamination along the Mngazana and Nahoon estuaries and the results revealed the variability of bacterial counts along the length of each estuary. The evaluation of *E. coli* in

these estuaries revealed the need for more microbial contamination assessments around other South African estuaries to combat any threats to human health (i.e. for bathers) and the overall threat to estuarine health. The effects of physico-chemical drivers on the survival of bacteria are yet to be fully studied and understood around South African estuaries.

The methodology for microbial contamination assessment is however not globally standardised, in Europe, recreational waters are protected under the EU Water Framework Directive (WFD) (2000/60/EC). In terms of the South African context, water quality guidelines for recreational use are used to monitor possible microbial contamination. These revised guidelines recommend the use of long-term datasets (RSA DEA 2012). According to the Department of Environmental Affairs (DEA), intestinal enterococci (*Enterococcus faecalis*) counts should be used in microbial assessments in South Africa using the 'simple sample target value[3] approach. If the enterococci measurements exceed 240 counts 100 ml^{-1}, management actions are then required. The current state of the investigated estuaries in terms of bacterial contamination invites for further studies seeking long-term datasets in understanding the dynamics of microbial activity in estuaries. Despite different guidelines and frameworks, robust microbial contamination detection methodology is needed. According to Henry *et al*. (2016b) detection of pathogen concentrations is complicated by a range of environmental factors (i.e. turbidity) and experimental processing limitations. Therefore, there is a need to undertake independent site surveys to identify potential, site specific, point-sources that may significantly bias water quality assessments of the entire system since a single sample may not be representative of prevailing microbial levels, either spatially or temporally (Henry *et al*. 2016b & references therein). Harmel *et al*. (2016) warns that even though monitoring *E. coli* levels is critical to evaluate current water quality conditions, determine restoration effectiveness and inform management processes; the uncertainty in *E. coli* data needs to be considered carefully. The work by Harmel *et al*. (2016) suggests that sample collection procedures produce the highest amount of uncertainty since it is affected by the timing and location of sample collection.

Estuarine monitoring tends to be costly, project specific and discontinuous, thus impeding the desired long-term comparison of estuarine environments (Taljaard *et al*. 2003). Hence for effective monitoring, a multi-metric approach that classifies estuaries in terms of trophic status should be implemented. Lemley *et al*. (2014) noted that eutrophication is a highly complex and subtle issue to detect and address. Nutrient loading via runoff in urban estuaries (i.e. Nahoon Estuary) coupled with autochthonous nutrients from the surrounding

[3] Single sample target value: allows for a timeous response and implementation of appropriate management actions to any do-to-day situation that could pose potential risk to human health (RSA DEA 2012).

mangroves (i.e. Mngazana Estuary) can synergistically induce a negative impact on the water quality of an estuary (Monteiro *et al.* 2016). Water quality studies utilising a coupled hydrodynamic and water quality model tend to contain some limitations and assumption (Liu & Chan 2016), conversely, the work by Lemley *et al.* (2015) showed the effectiveness of using multiple indicators in classifying eutrophic conditions in selected Western Cape estuaries (South Africa).

Lemley *et al.* (2015) used the dominant classification (≥ 50 %) of multiple indicators that enabled the rating of trophic status in that estuaries could be classified as either oligotrophic ('Good'), mesotrophic ('Fair') or eutrophic ('Poor'). The assessment method used the following indicators; inorganic nutrients (DIN and DIP), dissolved oxygen, phytoplankton, and microphytobenthos biomass. This study illustrated the importance of incorporating multiple indicators into eutrophic condition scoring and highlighted the importance of using hydro-morphological characteristics (i.e. bathymetry, mouth condition) for an accurate classification of South African systems (Lemley *et al.* 2015). The flexibility and adaptive potential of such an assessment is highly encouraged to provide space for existing knowledge when assessing estuaries (Lemley *et al.* (2015). The present study on the current conditions at both Mngazana and Nahoon Estuaries did not employ the abovementioned assessment but does allow for a continued assessment of these estuaries and the future incorporation of the multi-metric approach to classify their trophic status.

6.2 Management Recommendations

Estuaries in South Africa must be managed in a coordinated and efficient way, in accordance with the National Estuarine Management Protocol under the ICM Act (No. 24 of 2008). According to Morant and Quinn (1999), for successful management of estuaries two components need to be considered; (1) reference framework, and (2) predictive tools. These two components help guide and focus management upon issues of concern. Van Niekerk (2007) argued that the major constraint hindering the effective management of South Africa's estuaries is the fragmentation of and overlap in legislation. It was further suggested that estuarine management should be undertaken within a framework that emphasises the principles of sustainable development, biodiversity conservation, the precautionary principle, integrated management, self-regulation and sensitivity to local circumstances (Van Niekerk 2007). The success of estuaries management relies on the human capital to implement effective management that includes regular monitoring and permanent flow gauges in South African estuaries. This alerts monitoring bodies of any changes in freshwater inflow into estuaries.

Mngazana Estuary

The current management plan (dated March 2015) for the Mngazana Estuary noted that some of the main constraints to the management of the estuaries along the Wild Coast include limited knowledge and understanding of these systems, lack of governance and management capacity, absence of coherent planning framework leading to lack of catchment management. This region is governed by two forms of governance, namely; constitutional and traditional with traditional being the "dominant regulator of behaviour in the area" (UKZN 2015). However, this current management plan does not provide detailed information on the environmental description of this estuary such as physical (i.e. physico-chemical drivers), biodiversity characteristics (i.e. invertebrates, phytoplankton and other biota) and published scientific studies on Mngazana. This is a major oversight given the importance of this estuary in South Africa (Colloty *et al*. 2002) and revision of this plan is therefore hugely recommended. It does, however, mention an Adaptive Management Process model that is being followed at Mngazana Estuary (Fig. 6.1) but largely focuses on the utilisation of the mangrove forests by the community.

Nahoon Estuary

The Nahoon Estuary has a detailed management plan (dated October 2013) and it focuses on all aspects of the estuarine environment, its uses and anthropogenic impacts exerted on the estuarine area but it was stressed that there is insufficient scientific information to enable a comprehensive evaluation of the ecological functioning of this estuary (MEGA 2013). Despite this, the management plan of the Nahoon Estuary follows the legislative procedures outlined for estuarine management in South Africa (ICM Act No. 24 of 2008).

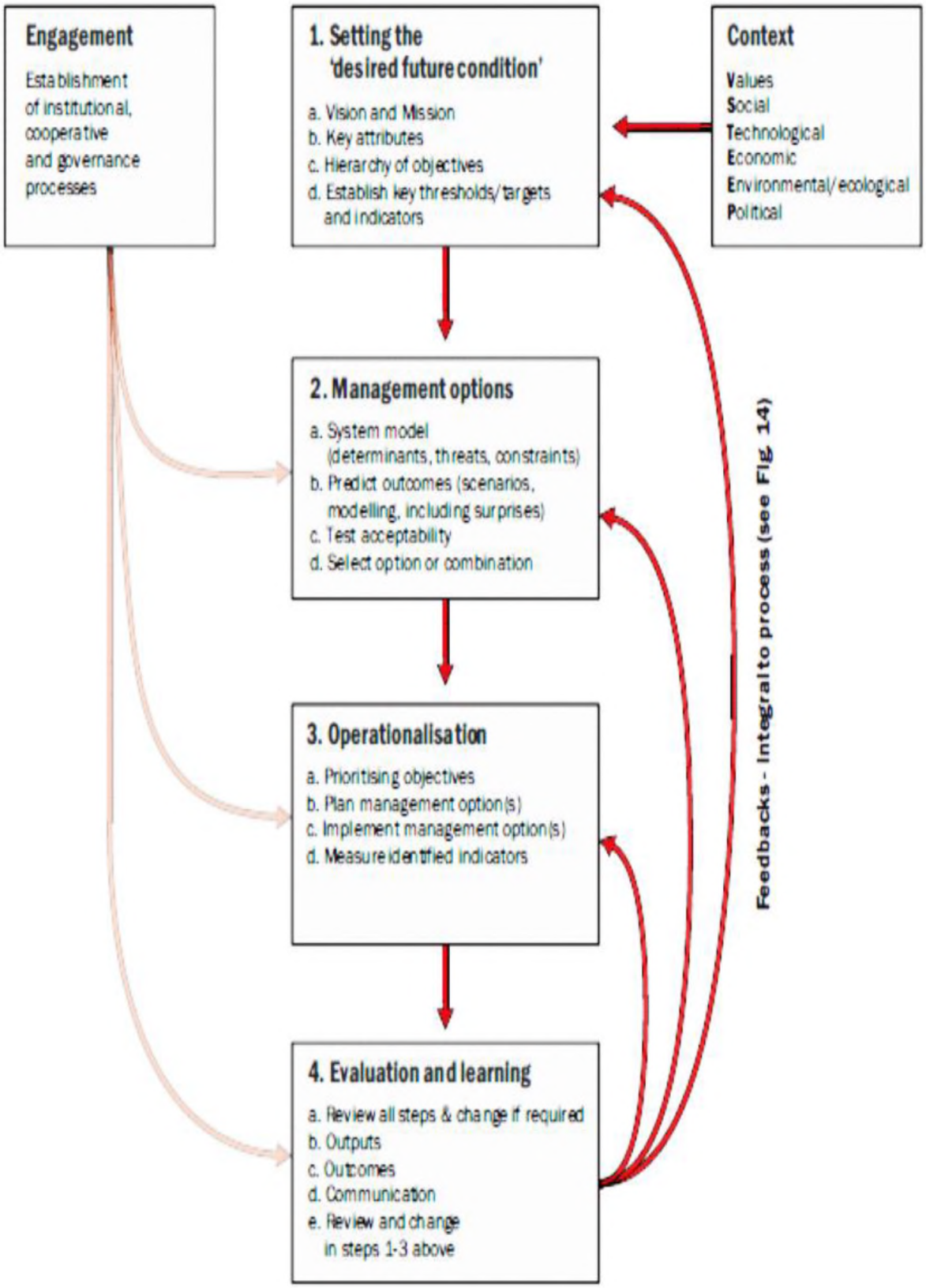

Figure 6.1: Adaptive Management Process adopted at Mngazana Estuary (UKZN 2015).

Current study

The present study adopted the DPSIR (Driver, Pressure, State, Impact, Response) framework as a useful tool for management (Fig. 6.2). This framework allows for the analysis of the environmental problems focusing on identification of links between socio-economic drivers, exerted pressures on the environment, the resulting state of the environment, felt impacts and societal responses addressing the identified areas of concern (OECD 1993). This framework was applied to the results of this study focussing individually on each estuary.

Even though the investigated estuaries differ in their locations, the identified *drivers* of change are similar between both estuaries. Human-induced change has been noted, with water abstraction, nutrient input from mismanagement of the catchments, stormwater drains due to urban development etc. requires the implementation of strict management of inputs from the adjacent catchments of these estuaries which will address the *pressure* of excess nutrients input in urban estuaries. The installation of water supply infrastructure will potentially allow for the monitoring of water use in rural areas surrounding estuaries while the upgrading thereof in urban towns will combat unregulated runoff such as sewage outfalls into watercourses. The two estuaries share a similar *state* in terms of lack of dilution due to minimum freshwater introduction and microbial contamination despite the results of this study showing minimum levels of microbial contamination in both estuaries. Stricter monitoring measures should be taken by the presiding municipalities (Government structure) in trying to mitigate future introductions of microbial contaminants into these estuaries. For example, implementation of borders for livestock owners might decrease livestock presence near the estuarine waters of Mngazana Estuary while regular maintenance of urban wastewater treatment plants might combat the issue in urban settings. The overall *impact* is the resulting low water quality which affects users and the resident organisms (i.e. fish). According to Monteiro *et al.* (2016) the *response* measures to mitigate identified impacts will be related to regulation, which leads to the establishment of some limits and restrictions for water use depending on the current water quality. The constant evaluation of estuarine waters using the multi-metric approach (Lemley *et al.* 2015) would therefore enable effective monitoring and management. Furthermore, any regulation will need to consider the specific characteristics of an estuary individually (i.e. nutrient sources and chlorophyll *a*) and the fluctuations in freshwater inflows and the associated seasonal rainfall patterns. Management interventions of estuaries regardless of location should then aim for the preservation of the ecological functioning of these water systems in the face of climate change and the conservation of estuary biodiversity.

Shortcomings of this study and future research

The study at Mngazana Estuary was undertaken during the upgrading of the bridge at the head of the estuary which may have affected the freshwater inflow into the estuary. It is highly recommended that a similar study be carried out upon completion of the bridge to compare changes in microalgal dynamics of the estuary. The Nahoon Estuary study lacked information on the number of wastewater treatment works (WWTW) close to the Nahoon Estuary and the quantity of effluents deposited. Also, there is a need to conduct surveys to determine if there are septic tanks and use of agrochemicals on the lawns of the residential developments to quantify the human impacts along this estuary. Future sampling of the two estuaries with regards to phytoplankton biomass should be conducted in the 0.5 m intervals since the peak biomass typically occurs from 0.5 to 1.0 m deep.

Overall, this research did not assess benthic microalgae (microphytobenthos) in both estuaries during the study period and the reasoning is given in Chapter 2. The inclusion of microphytobenthos adds substantially when looking at the total production of the entire estuary. In terms of bacterial contamination, this study focused on *E. coli* counts,/ and intestinal enterococci counts are recommended by the Department of Environmental Affairs (RSA DEA 2012). Robust methodology is hugely needed when dealing with sampling and laboratory analyses of *E.coli* since getting the samples to the lab under six hours is not always possible (Harmel *et al*. 2016). Therefore, the *E. coli* values recorded during this study are an underestimate of the actual microbial contamination within the two estuaries. It is also the recommendation from this research that microbial contamination assessments be conducted on South African rivers to note the introduction of faecal contaminants into estuaries and work by Paulse *et al*. (2007, 2009, 2012) on selected Western Cape rivers can be used as a baseline.

Future studies on these two estuaries need to include the assessment of epiphyte and microphytobenthos biomass to be in line with the multi-metric eutrophic classification approach as proposed by Lemley *et al*. (2015). In terms of phytoplankton community structure, the identification of individual species based on: (1) high abundance, (2) existing literature that identifies a species as being potentially harmful or toxic and/or having high nutrient affinity (Lemley *et al.* 2015) is recommended. Furthermore, other research techniques that seek to evaluate the estuarine health of South African systems are also encouraged (i.e. Masefield *et al*. 2014). Research on estuaries in their respective Water Management Areas (i.e. Lemley *et al*. 2014, 2015) along the South African coast serves as a

great tool of quantifying and comparing the functioning and impacts on estuaries within a known boundary (i.e. river catchment). This study was conducted over two successive years and it is recommended that long-term data be collected to make further conclusions about these dynamic estuarine environments.

6.3 Conclusion

The present study compared a rural versus an urban estuary and found similarities in term of low microalgal biomass due to low freshwater inflow. However, there are differences such as the surrounding land use, sources of nutrients and anthropogenic influences. This provides a good foundation for similar studies to try and link human pressures on our environment and the response of the environment to such pressures for management purposes. Such comparisons are meaningful in trying to understand the dynamics of a near-pristine estuary (Mngazana Estuary) and the urban influenced Nahoon Estuary during the same time period despite the different biogeographical regions they are located in. The microalgal dynamics of the two estuaries reflect the water quality of the two estuaries and reflect activities from the surrounding catchment. The Eastern Cape estuaries provide an ideal opportunity to determine whether climate change or anthropogenic factors are the greatest threat to these coastal environments (Rajkaran 2011). The work on zooplankton by Deyzel (2012) left the author to conclude that morphologically, Mngazana Estuary, given the mouth dynamics, delta formation, strong tidal forcing, catchment characteristics and non-linearity of water flow (main channel versus Creek 1 and 2) could be best classified as a structural complex. It is only through the integration of the defensible scientific, proactive management and strict legal initiatives that South African estuaries can be effectively managed (Van Niekerk 2007).

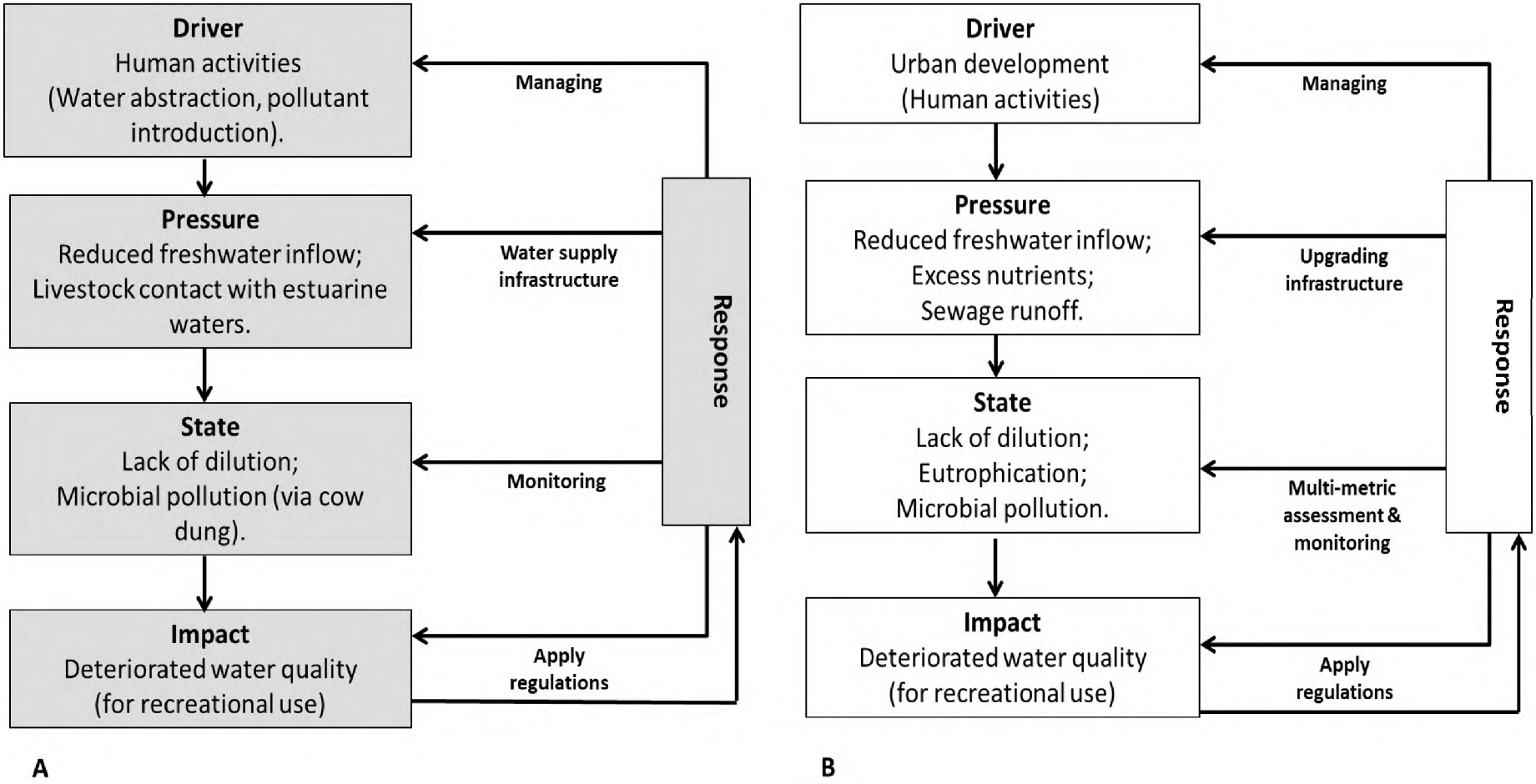

Figure 6.2: The schematic example of the DPSIR framework as applied to (A) Mngazana Estuary and (B) Nahoon Estuary.

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APPENDICES

APPENDIX A:

Table A.1: Mngazana Estuary Spearman rank correlation (*r*) between physico-chemical variable, nutrients, phytoplankton biomass and *E. coli* during Summer 2014. Abbreviations: DFM = Distance from mouth; TEMP = Temperature; DO = Dissolved Oxygen; COND = Conductivity; NH_4^+ = Ammonium; TOxN = Total oxidised nitrogen, SRP = Soluble reactive phosphorus; Chl-a = Chlorophyll a*;* Flag = Flagellates; Blu_g = Blue-green algae. Significant values are bold ($p < 0.05$). N = 28; (N = 14 for *E. coli*).

Variable	DFM	Salinity	TEMP	DO	pH	COND	NH_4^+	TOxN	SRP	Chl-a	Flag	Diatoms	Blue_g
Salinity	-0,300												
TEMP	-0,215	-0,277											
DO	**0,515**	-0,346	0,050										
pH	-0,079	**0,437**	0,167	**0,420**									
COND	-0,315	**0,925**	-0,102	-0,258	**0,489**								
NH_4^+	0,099	-0,282	0,103	0,231	-0,048	-0,399							
TOxN	**-0,637**	0,163	0,229	**-0,456**	-0,019	0,111	0,112						
SRP	**-0,714**	0,142	-0,070	**-0,568**	-0,129	0,102	-0,072	**0,767**					
Chl-a	0,213	0,124	-0,011	-0,068	-0,026	0,116	0,005	**-0,445**	**-0,454**				
Flag	**-0,497**	**0,453**	-0,166	-0,371	0,188	**0,423**	-0,157	0,261	0,260	-0,183			
Diatoms	**-0,561**	0,116	0,155	-0,243	0,022	0,092	0,286	**0,661**	**0,596**	0,045	-0,007		
Blue_g	**0,829**	**-0,459**	-0,024	**0,464**	-0,208	**-0,441**	0,077	**-0,575**	**-0,610**	0,159	**-0,554**	**-0,581**	
E. coli	**0,586**	**-0,675**	-0,055	0,433	-0,400	**-0,597**	0,044	**-0,628**	**-0,604**	0,250	**-0,550**	**-0,556**	**0,858**

Table A.2: Mngazana Estuary Spearman rank correlation (*r*) between physico-chemical variable, nutrients, phytoplankton biomass and *E. coli* during Winter 2014. Abbreviations: DFM = Distance from mouth; TEMP = Temperature; DO = Dissolved Oxygen; COND = Conductivity; NH_4^+ = Ammonium; TOxN = Total oxidised nitrogen, SRP = Soluble reactive phosphorus; Chl-a = Chlorophyll a; Flag = Flagellates; Dinoflag = Dinoflagellates; Blu_g = Blue-green algae; Green_Al = Green algae. Significant values are bold ($p < 0.05$). N = 28; (N = 14 for *E. coli*).

Variable	DFM	Salinity	TEMP	DO	pH	COND	NH_4^+	TOxN	SRP	Chl-a	Flag	Dinoflag	Diatoms	Blue_g	Green_Al
Salinity	**-0,820**														
TEMP	-0,185	0,001													
DO	**0,522**	**-0,840**	0,024												
pH	**0,463**	**-0,577**	**0,420**	**0,410**											
COND	**-0,743**	**0,840**	0,189	**-0,643**	-0,331										
NH_4^+	0,199	-0,345	-0,199	**0,419**	0,048	-0,296									
TOxN	**0,552**	-0,335	-0,141	0,177	0,305	-0,296	0,169								
SRP	0,023	0,357	**-0,505**	**-0,413**	**-0,499**	0,216	-0,218	0,080							
Chl-a	**0,488**	**-0,437**	0,126	0,201	**0,513**	**-0,519**	-0,208	0,324	-0,289						
Flag	0,158	-0,086	-0,076	0,089	**0,387**	0,078	-0,062	0,219	-0,129	0,141					
Dinoflag	**-0,478**	**0,594**	-0,057	**-0,455**	**-0,499**	**0,443**	-0,132	**-0,515**	**0,426**	**-0,622**	-0,145				
Diatoms	-0,342	**0,399**	-0,006	-0,372	-0,067	**0,435**	-0,225	-0,098	0,170	-0,102	0,239	0,195			
Blue_g	-0,070	0,075	-0,322	-0,034	**-0,458**	-0,156	0,114	-0,250	0,232	-0,207	**-0,685**	0,208	**-0,554**		
Green_Al	-0,345	0,117	0,304	-0,111	0,039	0,134	0,075	-0,227	**-0,376**	-0,056	0,054	-0,026	0,298	**-0,375**	
E. coli	-0,007	0,034	**-0,662**	-0,079	-0,351	0,032	-0,241	-0,041	0,210	-0,045	0,238	0,047	0,059	0,051	-0,299

Table A.3: Mngazana Estuary Spearman rank correlation (*r*) between physico-chemical variable, nutrients, phytoplankton biomass and *E. coli* during Summer 2015. Abbreviations: DFM = Distance from mouth; TEMP = Temperature; DO = Dissolved Oxygen; COND = Conductivity; NH_4^+ = Ammonium; TOxN = Total oxidised nitrogen, SRP = Soluble reactive phosphorus; Chl-a = Chlorophyll a; Flag = Flagellates; Dinoflag = Dinoflagellates; Blu_g = Blue-green algae. Significant values are bold ($p < 0.05$). N = 28; (N = 14 for *E. coli*).

Variable	DFM	Salinity	TEMP	DO	pH	COND	NH_4^+	TOxN	SRP	Chl-a	Flag	Dinoflag	Diatoms	Blue_g
Salinity	0,158													
TEMP	**0,660**	-0,206												
DO	0,035	0,000	-0,039											
pH	**0,397**	**0,544**	0,116	**0,462**										
COND	0,318	**0,918**	-0,004	-0,112	**0,591**									
NH_4^+	-0,288	**-0,579**	-0,048	0,260	-0,363	**-0,681**								
TOxN	-0,049	-0,153	-0,151	-0,072	-0,268	-0,235	0,201							
SRP	**-0,776**	-0,224	**-0,595**	-0,118	**-0,455**	-0,353	**0,394**	0,106						
Chl-a	**0,886**	0,086	**0,752**	-0,054	0,313	0,271	-0,206	-0,183	**-0,773**					
Flag	-0,032	-0,333	-0,005	**-0,554**	-0,317	-0,186	-0,196	-0,285	0,014	-0,018				
Dinoflag	0,350	-0,050	0,365	0,054	0,298	0,069	-0,095	-0,335	-0,371	0,329	0,286			
Diatoms	-0,084	**0,629**	-0,227	0,370	**0,398**	**0,481**	-0,169	0,077	-0,049	-0,132	**-0,649**	**-0,392**		
Blue_g	-0,268	-0,013	-0,241	-0,062	0,029	-0,089	0,077	-0,146	0,257	-0,258	-0,009	0,125	-0,315	
E. coli	**0,741**	-0,065	**0,708**	-0,272	0,018	0,000	-0,074	-0,237	**-0,562**	**0,814**	0,362	0,419	-0,525	-0,210

Table A.4: Mngazana Estuary Spearman rank correlation (*r*) between physico-chemical variable, nutrients, phytoplankton biomass and *E. coli* during Winter 2015. Abbreviations: DFM = Distance from mouth; TEMP = Temperature; DO = Dissolved Oxygen; COND = Conductivity; NH_4^+ = Ammonium; TOxN = Total oxidised nitrogen, SRP = Soluble reactive phosphorus; Chl-a = Chlorophyll a; Flag = Flagellates; Dinoflag = Dinoflagellates; Blu_g = Blue-green algae; Green_Al = Green algae. Significant values are bold ($p < 0.05$). N = 28; (N = 14 for *E. coli*).

Variable	DFM	Salinity	TEMP	pH	COND	NH_4^+	TOxN	SRP	Chl-a	Flag	Dinoflag	Diatoms	Blue_g
Salinity	**-0,805**												
TEMP	-0,004	-0,215											
pH	0,053	-0,163	**0,420**										
COND	**-0,853**	**0,937**	-0,001	0,047									
NH_4^+	0,372	-0,113	-0,171	-0,063	-0,199								
TOxN	**0,649**	**-0,455**	0,032	-0,208	**-0,461**	0,181							
SRP	-0,180	0,283	**-0,487**	**-0,531**	0,129	0,006	-0,016						
Chl-a	**0,638**	**-0,647**	0,204	0,326	**-0,623**	0,040	0,149	-0,252					
Flag	**0,392**	-0,344	0,346	0,267	-0,261	0,129	0,336	-0,334	0,289				
Dinoflag	-0,278	0,297	**-0,474**	-0,147	0,188	-0,133	**-0,441**	0,357	-0,054	**-0,484**			
Diatoms	-0,360	0,304	**-0,387**	-0,264	0,295	0,067	-0,372	**0,465**	-0,361	**-0,551**	**0,555**		
Blue_g	-0,173	0,054	-0,145	-0,205	-0,038	-0,128	-0,198	0,054	-0,044	**-0,473**	0,011	-0,026	
E. coli	**0,605**	-0,392	-0,003	0,397	-0,392	0,284	0,135	-0,210	0,352	0,450	0,149	-0,319	0,070

Table A.5: Nahoon Estuary Spearman rank correlation (*r*) between physico-chemical variable, nutrients, phytoplankton biomass and *E. coli* during Summer 2014. Abbreviations: DFM = Distance from mouth; TEMP = Temperature; COND = Conductivity; NH_4^+ = Ammonium; TOxN = Total oxidised nitrogen, SRP = Soluble reactive phosphorus; Chl-a = Chlorophyll a; Flag = Flagellates; Dinoflag = Dinoflagellates. Significant values are bold ($p < 0.05$). N = 20; (N = 10 for *E. coli*).

Variables	DFM	Salinity	TEMP	pH	COND	NH_4^+	TOxN	SRP	Chl-*a*	Flag	Dinoflag	Diatoms
Salinity	-0,074											
TEMP	0,124	-0,472										
pH	-0,074	-0,396	0,255									
COND	**-0,815**	0,159	0,061	0,262								
NH_4^+	-0,492	-0,075	-0,160	0,209	0,176							
TOxN	-0,466	0,184	-0,538	-0,017	0,285	-0,233						
SRP	**-0,902**	-0,311	-0,198	0,050	0,521	0,343	0,527					
Chl-*a*	0,075	0,222	0,161	-0,074	-0,074	-0,390	0,373	-0,034				
Flag	0,038	-0,245	**0,668**	**0,736**	0,176	0,139	-0,357	-0,223	0,273			
Dinoflag	0,125	-0,203	0,550	-0,003	-0,052	0,165	-0,661	-0,221	0,062	0,479		
Diatoms	0,173	-0,405	0,255	-0,384	-0,302	0,343	-0,636	-0,084	-0,566	-0,242	0,382	
E. coli	0,000	**0,900**	-0,300	0,103	0,100	0,700	-0,200	-0,200	0,671	0,410	-0,154	-0,872

Table A.6: Nahoon Estuary Spearman rank correlation (*r*) between physico-chemical variable, nutrients, phytoplankton biomass and *E. coli* during Winter 2014. Abbreviations: DFM = Distance from mouth; TEMP = Temperature; DO = Dissolved Oxygen; COND = Conductivity; NH_4^+ = Ammonium; TOxN = Total oxidised nitrogen, SRP = Soluble reactive phosphorus; Chl-a = Chlorophyll a; Flag = Flagellates; Dinoflag = Dinoflagellates; Blue_g = Blue-green algae; Green_Al = Green Algae. Significant values are bold ($p < 0.05$). N = 20; (N = 10 for *E. coli*).

Variable	DFM	Salinity	TEMP	DO	pH	COND	NH_4^+	TOxN	SRP	Chl-a	Flag	Dinoflag	Diatoms	Blue_g	Green_Al
Salinity	**-0,674**														
TEMP	**0,671**	**-0,798**													
DO	**-0,864**	**0,594**	**-0,590**												
pH	-0,374	**0,444**	**-0,599**	0,307											
COND	0,339	-0,134	0,229	-0,359	0,135										
NH_4^+	**0,691**	**-0,647**	**0,540**	**-0,729**	**-0,526**	0,353									
TOxN	**0,701**	**-0,697**	**0,718**	**-0,653**	**-0,730**	0,102	**0,864**								
SRP	**0,743**	**-0,646**	**0,594**	**-0,686**	**-0,537**	0,250	**0,824**	**0,789**							
Chl-a	0,051	-0,051	0,015	0,042	0,221	0,163	0,003	0,024	0,096						
Flag	**-0,453**	0,358	-0,376	**0,475**	**0,658**	-0,119	**-0,677**	**-0,703**	**-0,667**	0,030					
Dinoflag	0,286	-0,044	-0,073	-0,252	**0,448**	0,171	-0,026	-0,153	-0,057	0,010	**0,545**				
Diatoms	-0,222	0,248	-0,160	0,282	-0,123	-0,267	-0,306	-0,225	-0,411	-0,427	-0,121	**-0,476**			
Blue_g	**0,494**	-0,408	**0,522**	**-0,515**	**-0,769**	0,141	**0,685**	**0,770**	**0,768**	0,056	**-0,926**	**-0,456**	-0,110		
Green_Al	-0,129	0,261	-0,106	0,251	0,198	0,032	-0,337	-0,252	-0,226	-0,410	**0,190**	0,186	0,141	-0,214	
E. coli	0,255	-0,152	-0,021	-0,213	-0,137	-0,444	0,340	0,596	0,407	-0,055	-0,596	0,035	-0,186	0,569	0,156

Table A.7: Nahoon Estuary Spearman rank correlation (*r*) between physico-chemical variable, nutrients, phytoplankton biomass and *E. coli* during Summer 2015. Abbreviations: DFM = Distance from mouth; TEMP = Temperature; DO = Dissolved Oxygen; COND = Conductivity; NH_4^+ = Ammonium; TOxN = Total oxidised nitrogen, SRP = Soluble reactive phosphorus; Chl-a = Chlorophyll a; Flag = Flagellates; Dinoflag = Dinoflagellates; Blue_g = Blue-green algae. Significant values are bold ($p < 0.05$). N = 20; (N = 10 for *E. coli*).

Variable	DFM	Salinity	TEMP	DO	pH	COND	NH_4^+	TOxN	SRP	Chl-a	Flag	Dinoflag	Diatoms	Blue_g
Salinity	**-0,731**													
TEMP	**0,599**	**-0,705**												
DO	0,308	**-0,554**	**0,679**											
pH	-0,218	0,088	0,038	0,289										
COND	-0,399	**0,657**	-0,178	-0,307	0,136									
NH_4^+	0,142	-0,314	0,185	0,095	0,335	-0,364								
TOxN	0,281	-0,325	-0,139	0,120	-0,109	**-0,514**	0,224							
SRP	**0,791**	**-0,606**	**0,545**	0,321	-0,056	-0,178	0,227	0,237						
Chl-a	**0,539**	-0,216	0,188	0,059	**-0,531**	-0,284	-0,222	0,109	0,282					
Flag	0,372	-0,242	**0,567**	0,152	-0,300	0,053	-0,274	-0,237	0,354	0,400				
Dinoflag	0,258	0,114	0,009	-0,120	**-0,494**	0,150	**-0,574**	-0,146	0,104	**0,749**	0,239			
Diatoms	-0,408	0,154	-0,405	-0,038	**0,534**	-0,111	0,439	0,215	-0,265	**-0,722**	**-0,792**	**-0,696**		
Blue_g	-0,262	-0,004	0,186	-0,036	0,206	0,073	0,385	-0,035	-0,251	-0,328	-0,087	-0,177	0,131	
E. coli	0,128	-0,055	-0,006	0,573	-0,021	0,012	-0,061	-0,067	0,239	0,160	-0,250	0,237	0,122	-0,157

Table A.8: Nahoon Estuary Spearman rank correlation (*r*) between physico-chemical variable, nutrients, phytoplankton biomass and *E. coli* during Winter 2015. Abbreviations: DFM = Distance from mouth; TEMP = Temperature; NH_4^+ = Ammonium; TOxN = Total oxidised nitrogen, SRP = Soluble reactive phosphorus; Chl-a = Chlorophyll a; Flag = Flagellates; Dinoflag = Dinoflagellates; Blue_g = Blue-green algae. Significant values are bold ($p < 0.05$). N = 20; (N = 10 for *E. coli*).

Variable	DFM	Salinity	TEMP	pH	NH_4^+	TOxN	SRP	Chl-a	Flag	Dinoflag	Diatoms	Blue_g
Salinity	-0,248											
TEMP	0,305	0,343										
pH	-0,027	0,179	-0,184									
NH_4^+	0,247	-0,205	0,108	**-0,513**								
TOxN	0,397	**-0,534**	0,019	0,053	0,216							
SRP	**0,697**	-0,339	0,153	-0,252	0,317	**0,574**						
Chl-a	0,170	0,229	0,433	**-0,537**	0,162	-0,172	0,257					
Flag	-0,083	0,235	0,447	0,071	0,093	-0,147	-0,152	0,060				
Dinoflag	**0,483**	-0,038	**0,698**	-0,160	0,100	**0,464**	**0,483**	0,418	0,292			
Diatoms	-0,336	-0,248	**-0,475**	**0,633**	-0,384	-0,070	-0,369	**-0,644**	-0,237	**-0,561**		
Blue_g	-0,367	-0,129	-0,281	-0,388	0,086	-0,301	-0,258	-0,259	0,086	-0,246	-0,086	
E. coli	0,140	-0,030	**0,642**	0,043	0,189	-0,061	-0,030	**0,847**	-0,190	0,333	0,062	-0,467

Table A.9: Total number of actual phytoplankton cells recorded at Mngazana Estuary per sampling season (Equation described in Chapter 3)

Distance from mouth/channel (km)	Summer 2014	Summer 2015	Winter 2014	Winter 2015
Main Channel				
1.05	287	1138	222	568
2.0	201	1904	429	356
3.55	201	800	867	516
4.33	381	827	756	597
4.91	289	662	1375	363
5.37	498	943	1094	325
5.70	444	1157	456	443
6.22	114	832	757	794
Creek 2				
0.57	267	371	852	276
1.13	264	263	951	322
1.79	311	295	739	221
Creek 1				
0.83	222	213	310	486
1.56	206	241	497	1169
2.45	351	194	240	266

Table A.10: Total number of actual phytoplankton cells recorded at Nahoon Estuary per sampling season (Equation described in Chapter 3)

Distance from mouth (km)	Summer 2014*	Summer 2015	Winter 2014	Winter 2015
0.14	316	848	407	326
0.38	**571**	1156	**665**	278
0.86	415	4631	433	252
1.45	311	2241	513	306
2.19	410	1978	506	269
3.30		1923	849	294
3.55		1486	1176	444
4.0		1452	817	711
4.55		1057	5339	280
5.0		1690	2224	213

***Summer 2014 sampled up to site 5 (2.2 km) due to weather.**

APPENDIX B:

Figure B1: Infrastructure upgrading of the road bridge upstream of the Mngazana Estuary. **1**: Old Bridge; **2**: Two pipes being used to allow river water to flow into the estuary; **3**: During construction, freshwater is also being pumped from the river section into the estuary via the pump. (Images: Cotiyane 2015).

Figure B2: Man-made developments around the estuary. **1**: Flood Bridge located near Site 5 (4.3 km); **2**: Water abstraction for subsistence farming occurring near Site 5; **3**: Sparsely populated cottages located near the mouth of the Mngazana Estuary and **4**: Boat garages along the lower reaches of the Mngazana Estuary (Images: Cotiyane 2014).

Figure B3: Construction progress on the infrastructure upgrading of the road bridge upstream of the Mngazana Estuary (March 2016). **1 & 2**: New Bridge is near completion; **3**: Freshwater inflow increased marginally from June 2015 to March 2016; **4**: and substantially after a rainfall event which may affect the turbidity of the estuary (Images: Dr Rajkaran 2016).

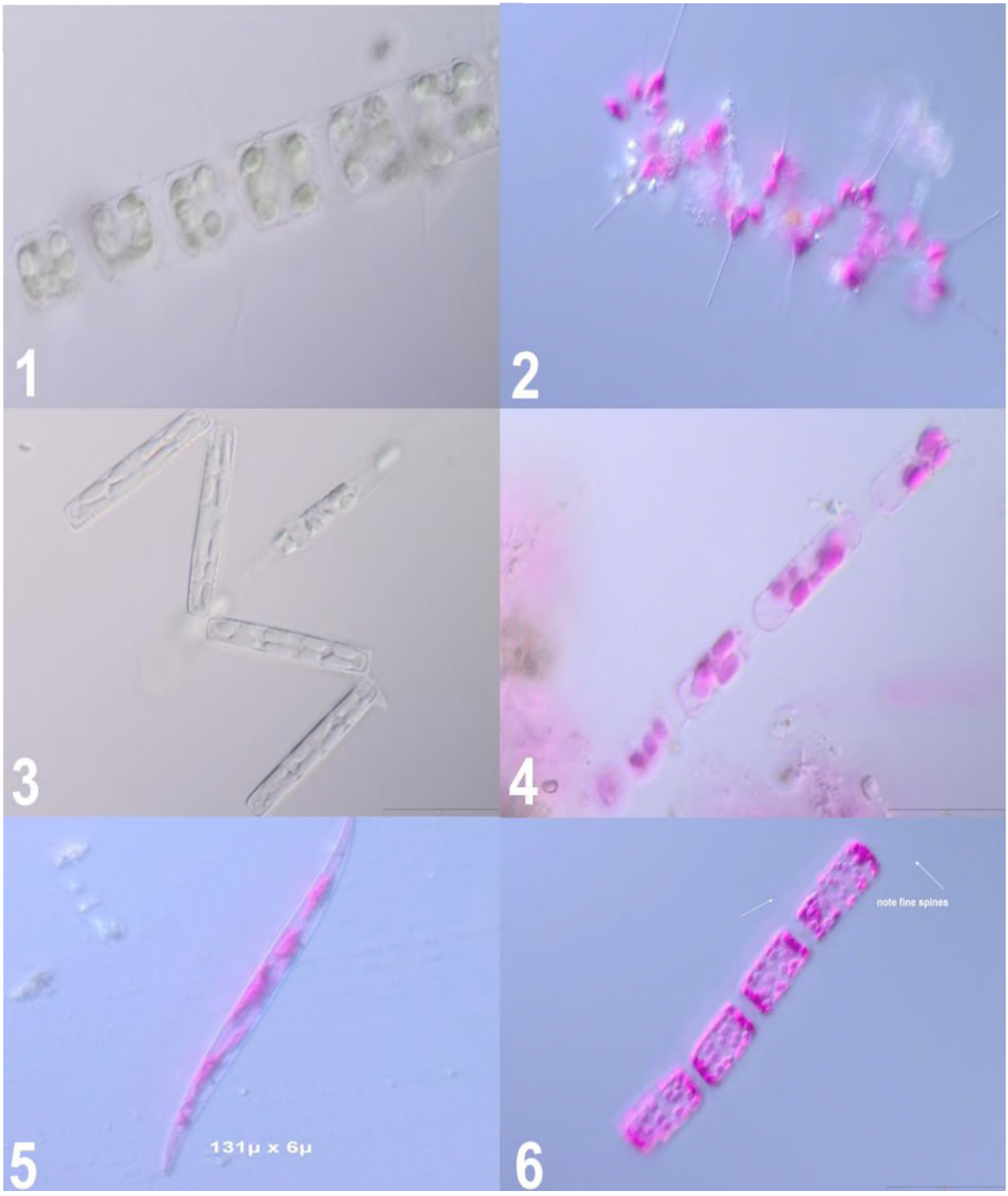

Figure B4: Diatoms species indicating marine influence found 1 km (Site 8) from the mouth at Mngazana Estuary. **1**: *Chaetoceros sp.* Ehrenberg; **2**: *Asterionellopsis glacialis* (Castracane) Round; **3**: *Thalassionema nitzschioides* (Grunow) Mereschkowsky; **4**: *Guirnardia cylindrus* (Cleve) Hasle; **5**: *Pleurosigma elongatum* W Smith and **6**: *Thalassiorisa sp*. Cleve (Images: P Smailes, NMMU).

Figure B5: Lower reaches of the Nahoon Estuary. **1**: 1985 flood event; **2**: Abbotsford Bridge shown with no river water inflow; **3:** 2011 floods along the Nahoon Estuary; **4**: High velocity flood waters flowing into the estuary during 2013; **5** & **6:** Dense algal presence 5 km from the mouth at Nahoon in October 2014. (Images: Mbikwana 2008, MEGA 2013, Daily Dispatch 2013, 2014).

Figure B6: Modification along the lower reaches of the Nahoon Estuary. **1**: Jetties along the bank; **2**: One of the stormwater drains along the estuary; **3:** Example of the town house occupying the river banks with artificial bank stabilisation; **4**: Recreation boats floating throughout the estuary (Use is high during peak holiday times) (Images: MEGA 2013, Cotiyane 2014, 2015).

Figure B7: Present day images of the two estuaries illustrating the difference in land-use around them (Images: Google Earth 2016).