CONTRIBUTIONS TO THE USE OF MICROALGAE IN ESTUARINE FRESHWATER RESERVE DETERMINATIONS

Submitted in fulfillment of the requirements for the degree of

DOCTOR OF PHILOSOPHY

(SCIENCE)

of

Nelson Mandela Metropolitan University

by

GAVIN CHARLES SNOW

December 2007

Estuaries are spiritual and physical meeting places between man and nature.

Their health reflects our health and they too have moods.

This picture of Knysna Estuary[1] was taken shortly after the August 2006 flood.

[1] Photo taken by Ms Anusha Rajkaran (NMMU).

Promoter:

Prof. J.B. Adams — Botany Department, PO Box 77000, Nelson Mandela Metropolitan University, South Africa, 6031.

Co-promoter:

Prof. G.J.C. Underwood — Department of Biological Sciences, University of Essex, Wivenhoe Park, Colchester, Essex, CO4 3SQ, England.

The research described in this thesis was conducted at the Botany Department, Nelson Mandela Metropolitan University, Port Elizabeth.

Acknowledgements

Many people and organizations have provided advice, finance and support throughout the period of study and I am indebted to all of you. A big thank you to my promoters Prof. Janine Adams and Prof. Graham Underwood, as well as to Prof. Guy Bate, who have continually gone the extra mile for me; I have and will continue to learn so much from you.

Research, bursary and travel funds were provided by UPE / NMMU, National Research Foundation (NRF), Water Research Commission (WRC) and British Council (Association of Commonwealth Universities).

Thanks to all of the staff and students in the Botany Department at UPE / NMMU and the Biological Sciences Department at University of Essex who assisted me in the laboratory and in the field; I would still be stuck in the mud without you. To Ms Pat Smailes, thank you for the assistance and an endless source of enthusiasm with regards to counting and identifying microalgal cells.

Thanks to Dr Angus Paterson (formerly of CES) and Dr Steve Mitchell (WRC) for arranging finance, on such short notice, for the post-flood study of Knysna and Swartvlei estuaries and to the South African National Parks for the assistance.

To my family and friends, thank you for all of the encouragement you have given me that has helped me to reach this point.

To my wife Berny, I really appreciate the sacrifices made during my studies and the continuous support you have provided, I could not have wished for a more devoted partner. To Payton and Heather, my special daughters born during this study, this is just the start of an exciting journey together.

Contents

Abbreviations

AFDW	-	Ash-Free Dry Weight
ANOVA	-	Analysis of Variance
BOD	-	Biological Oxygen Demand
Chl a	-	Chlorophyll a
CSIR	-	Council for Scientific and Industrial Research
DIN	-	Dissolved Inorganic Nitrogen (nitrate + nitrite + ammonium)
DIP	-	Dissolved Inorganic Phosphorus (SRP)
DO	-	Dissolved Oxygen
DSi	-	Dissolved Silicate
DWAF	-	Department of Water Affairs and Forestry
EC	-	Electrical Conductivity
EPS	-	Extracellular Polymeric Substances (exopolymers)
HPLC	-	High Performance Liquid Chromatography
ICOLLs	-	Intermittently Closed and Open Lakes and Lagoons
LMW	-	Low Molecular Weight Carbohydrates
MPB	-	Microphytobenthos (benthic microalgae)
MSL	-	Mean Sea Level
NTU	-	Nephelometric Turbidity Units
ORP	-	Redox Potential
OM	-	Organic Matter
PCA	-	Principal Components Analysis
POE	-	Permanently Open Estuaries
ppt	-	Parts per thousand (measure of salinity, often reported as ‰)
REI	-	River-Estuary Interface
RDM	-	Resource Directed Measures
SEM	-	Standard Error of the Mean (or SE)
SRP	-	Soluble Reactive Phosphorus
TDS	-	Total Dissolved Solids
TOCE	-	Temporarily Open/Closed Estuaries
TOxN	-	Total Oxidised Nitrogen (nitrate + nitrite)
TSS	-	Total Suspended Solids

List of tables

List of figures

CHAPTER 1

General introduction

The ecologist Garrett Hardin (1968) introduced a useful concept called the tragedy of the commons, which describes how ecological resources become threatened or lost. The term “commons” is based on the commons of old English villages and is symbolic of a resource that is shared by a group of people. If every person were to use each resource in a sustainable fashion it would be available in perpetuity. However, if people use more than their share they would only increase their personal wealth to the detriment of others. In addition, an increase in the population would mean that the size of each share would have to decrease to accommodate the larger number of people. As a result, resources are threatened by personal greed and uncontrolled population growth.

Freshwater is an example of a common resource that is under threat in South Africa where the average annual rainfall is less than 60% of the global average (Mukheibir & Sparks 2006). The increasing demands for freshwater as well as its eutrophication are major concerns with regards to estuarine health, environmental resource management and human health. The correct management of water is necessary to ensure that it is utilised in a sustainable manner. The National Water Act (No. 36 of 1998) has provided the rights to water for basic human needs and for sustainable ecological function; the Basic Human Needs Reserve and Ecological Reserve are both provided as a right in law.

The amount of water necessary for an estuary to retain an acceptable ecological status, known as the Estuarine Ecological Reserve, is determined through the implementation of procedures (rapid, intermediate or comprehensive) compiled by the Department of Water Affairs and Forestry (1999) in its Resource Directed Measures (RDM) for the Protection of Water Resources. The impact of restricted flow on estuaries can be reduced by manipulating the water released from impoundments, the regulation of water abstractions within the river catchment or both (Hirji *et al*. 2002). The reserve assessment method is designed to evaluate ecosystem requirements by employing groups of specialists from different disciplines. In South Africa, this includes hydrologists, sedimentologists, water chemists and biologists (including microalgae specialists). The use of microalgae in ecological assessments has largely been based on research that was initiated at the Nelson Mandela Metropolitan University (formerly University of Port Elizabeth) and subsequently at Rhodes University (Grahamstown) and the University of KwaZulu-

Natal (Durban). The microalgal research can be divided into two main focus areas; phytoplankton and benthic microalgae.

Phytoplankton

The spatial distribution of phytoplankton, which consists of microalgal cells adapted to life spent suspended in the water-column, is almost totally dependent on water motion. Local studies (Hilmer & Bate 1990; 1991; Allanson & Read 1995; Grange & Allanson 1995) have found a strong relationship between phytoplankton biomass, measured using chlorophyll *a* (chl a) as an index, and freshwater inflow. Factors such as circulation patterns, nutrient concentration and turbidity were shown to have an effect on the spatial distribution, growth rates and species composition of phytoplankton communities in South African estuaries (Adams *et al*. 1999). In addition, research by Hilmer and Bate (1991) found that vertical and longitudinal salinity gradients, an indication of consistent freshwater input, resulted in phytoplankton dominance in the Sundays Estuary but this did not extend to estuaries further west along the south Cape coast, largely due to lower nutrient concentrations in the latter systems. Drainage from the nutrient-poor Table Mountain quartzite in the Cape fold mountains results in most southern Cape rivers and estuaries being oligotrophic in contrast to the Sundays and Gamtoos estuaries, which are enriched with fertilizer nutrients from agricultural return flow. The maximum phytoplankton biomass in the Sundays and Gamtoos estuaries was dependent on the flow rate and a retention time of 3 spring tidal cycles (~42 days) was optimal (Hilmer 1990; Snow *et al.* 2000a). The maximum phytoplankton biomass generally occurred at a vertically averaged salinity of less than 10 ppt and was termed the river-estuary interface zone (REI). However, further research is required to determine whether an REI can exist in permanently open estuaries starved of freshwater, in other types of estuaries or in other biogeographical zones.

Estuaries have been classified into permanently open (POE), temporarily open/closed (TOCE), estuarine lakes, estuarine bays and river mouths (Whitfield 1992). Although the Whitfield (1992) classification included river mouths, the definition of an estuary as being one where either there is a tidal influence from the sea or a measurable salinity gradient, precludes them from this discussion. Estuarine lakes and bays are the least common estuary types with only 8 and 3 examples respectively in the country. In addition, the climate along the South African coast

ranges from cold temperate on the west coast, warm temperate along the south and south-east coasts and sub-tropical along the east coast, creating a complex diversity of estuaries. The research presented in this thesis addresses some of the relationships between microalgal biomass and abiotic factors in different types of estuaries.

Benthic microalgae

A large proportion of research on estuarine benthic microalgae, or microphytobenthos (MPB), has found a poor relationship between microphytobenthic (MPB) biomass and the chemistry of the overlying water (Snow 2000a). As a result, further research was necessary to determine whether spatial patterns of MPB could be related to water chemistry and hydrology or whether the distribution was determined by biogeochemical parameters in the sediment.

Recent research of European estuaries has described the stabilising effect that benthic microalgae have on soft-sediment habitats in intertidal and shallow subtidal marine ecosystems (Paterson & Black 1999; Underwood & Paterson 2003; Stal 2003). This is largely the result of extracellular carbohydrates produced by motile benthic diatoms, creating a matrix of cells, sediment particles and extracellular polymeric substances (EPS) (Underwood & Paterson 2003). Du Preez (1996) looked at EPS production by *Anaulus australis,* a neritic diatom that is both planktonic and benthic depending on conditions in the surf-zone. It is known that the nature of the sediment and the nutrient loading of the overlying water does affect both MPB biomass and biofilm properties (Underwood 2002; Underwood & Paterson 2003). However, no work has previously been conducted in South African estuaries, hence research investigating the spatial patterns of carbohydrate fractions and the potential sediment stabilising effects in different South African systems was initiated.

Objectives

The basic questions underlying the research described in this thesis were:

1. What factors determine the spatial patterns of microalgal biomass (phytoplankton and MPB) in South African estuaries?

2. How is MPB biomass related to sediment carbohydrates and sediment stability?
3. How can microalgae be used to determine the freshwater inflow requirements of estuaries?

Chapters 2, 3 and 4 focus on microalgae in permanently open estuaries. The factors that determine the spatial patterns of benthic microalgae and their extracellular carbohydrates in estuaries along the southern and Eastern Cape coasts are dealt with in Chapter 2. Chapter 3 is a comprehensive freshwater reserve study of microalgae in the freshwater starved Kromme Estuary and Chapter 4 describes the spatial patterns of microalgae in the strongly seasonal Berg Estuary in the cool temperate biogeographic zone.

A flood in August 2006 along the southern Cape coast provided an opportunity to study the post flood response of phytoplankton in two rare types of estuaries; Knysna estuarine bay and Swartvlei estuarine lake (Appendix Fig.s A.5-A.9). The water chemistry and phytoplankton results of the studies that are described in Chapter 5 contribute to the information used by researchers to determine the freshwater inflow requirements (or freshwater reserves) for those two systems.

Allanson (2001) discussed the diversity of estuarine types and the individual responses of these systems to physical and chemical determinants in this highly dynamic environment. In addition, he highlighted the need for techniques with which to integrate these features into conceptual and numerical models. Chapter 6 provides a conceptual model describing the water quality and characteristics of TOCE's in South Africa. Thereafter, available literature on estuaries in the warm- and cool-temperate biogeographic regions of South Africa is reviewed and assessed against this model. An understanding of the processes was a necessary step to better understand the spatial patterns of microalgae in TOCE's, particularly in relation to mouth condition. Research conducted by Perissinotto (University of KwaZulu-Natal), Froneman (Rhodes University) and Gama (Nelson Mandela Metropolitan Univesity) have described the spatial patterns of microalgae in TOCE's along the KwaZulu-Natal and Eastern Cape coasts respectively. A comprehensive study of microalgae in the Mngazi Estuary, related to the mouth phases is presented in Chapter 7. The Mngazi Estuary is located in the transition zone between warm temperate and sub-tropical biogeographic zones and the anthropogenic influence on the quality and

quantity of the river water is unusually low for South Africa; potentially making the Mngazi a suitable example of an estuary in its reference state (Appendix Fig. A.2).

CHAPTER 2

Physico-chemical factors determining the distribution of benthic microalgae and carbohydrates in permanently open South African estuaries

Abstract

Flocculation is an important process by which suspended sediment, organic matter, nutrients and microalgal cells are deposited from the water-column to the benthos in estuaries. The rate and site of deposition are largely functions of the morphology and flow rates within an estuary. Intertidal sediment from six South African estuaries was sampled for benthic chlorophyll a (chl a) and carbohydrates with the aim to establish which environmental variables determine the spatial distribution of benthic microalgal biomass and associated carbohydrates. Chl a content, based on mass (μg g^{-1}) (n = 320), and concentration, based on area (mg m^{-2}) (n = 130), were significantly correlated (r = 0.33, P < 0.01 and r = 0.16, P < 0.01 respectively) to total oxidised nitrogen (TOxN) in the overlying water. However, the correlation coefficients were relatively low. The strongest associations were with sediment related variables; ash-free dry weight (AFDW) (r = 0.53, P < 0.001), % moisture (r = 0.62, P < 0.001), very fine sand (63-125 μm) (r = 0.31, P < 0.001) and the silt-clay fraction (< 63 μm) (r = 0.23, P < 0.001). The lowest chl a content was below the detectable limit in the coarse sand (oligotrophic) at the mouth region of the Keurbooms Estuary and the highest, 104.8 μg g^{-1}, was measured in the middle reaches of the Mngazana Estuary. Chl a content was significantly correlated to chl a concentration in 130 samples that had a broad range of organic and mud contents (r = 0.87, P < 0.001). The largest deviation from the linear regression line of these two measures were the muddy sites in the Gamtoos and Sundays estuaries, probably as a result of higher pore space and lower bulk density of these sediments.

Exopolymers were strongly associated with nutrients in the Swartkops Estuary, particularly soluble reactive phosphorus (SRP) (r = 0.357; P < 0.001), where a strong gradient in SRP was present (ranging from 6.9 μM at the mouth to 30.4 μM near to the head of the estuary). A PCA of environmental variables separated sampling sites and estuaries along gradients in sediment type and nutrients (PC1) and sediment type and organic content (PC2). Chl a and the carbohydrate fractions were most strongly correlated with PC2. These results suggest that sites with a high organic content (> 3%) and fine sediment content (< 125 μm sediment contributes more than 20% of the sediment) are most likely to support a high microalgal biomass. Colloidal carbohydrates are also likely to be high at these sites providing a significant energy source to bacteria and potentially increasing the stability of the sediment.

Introduction

There are five main types of estuarine systems in southern Africa: Permanently open, temporarily open/closed (TOCE), river mouths, estuarine lakes and estuarine bays. Permanently open estuaries, the focus of this study, generally have a steady discharge of riverwater maintaining a longitudinal salinity gradient between the head and mouth of the estuary (Whitfield 1992). Rivers that meander to the sea form vast intertidal flats with soft or semi-soft sandy or muddy substrates. These intertidal flats are ideal shallow water habitats, which become colonised by a wide variety of fauna and flora. Sediments accumulate and the rich nutrient influx and shallow warm waters results in a highly productive ecosystem (Lubke & de Moor 1998). Many South African estuaries such as the Gamtoos Estuary (Snow 2000a) have channel-like morphologies. As a result, the intertidal zones are steep and narrow restricting the overall area available for benthic microalgal production and the transfer of autochthonous energy to higher trophic levels.

The process of flocculation is important as this leads to the sedimentation of organic matter, fine sediment, microalgal cells and nutrients. The process provides an allochthonous supply of energy for resident micro- and macrobenthic fauna and has been the focus of a number of recent studies; van der Lee (2000), Winterwerp (2002), Jiang *et al.* (2004), Garcia (2005) and Manning *et al.* (2006). In general, the microtidal (< 2 m tidal variance), wave-dominated estuaries along the southern coast of South Africa have three distinct geomorphological zones (Cooper *et al.* 1999); a sandy barrier in which a constricted tidal inlet is formed and landward of which is an extensive flood-tidal delta. The flood-tidal delta is deposited as a result of the flood dominance of tidal currents in constricted inlets. Landward of these barrier-associated environments is a deeper water area typified by fine sediment deposition, in many instances enhanced by the flocculation of suspended clays in the saline estuarine water. At their upstream limit such estuaries typically exhibit a fluvial delta where bottom sediments are coarse and water depths are shallow.

Day (1981) described the distribution of sediments within estuaries and emphasised that sediments of both marine and terrestrial origin are present in most estuaries. Normally in an estuary there is a preponderance of fine silt and clay of fluvial origin at the head of the estuary, grading to medium or coarse sand of marine origin at the mouth. Both Day (1981) and Dyer (1979) described the process of flocculation, which occurs in estuaries at a salinity of 1-4 ppt for very fine sediments

(< 2 µm) and over the full salinity range for other fine sediment types (e.g. montmorillonite), and is the cause of the turbidity maximum found in many estuaries. The turbidity maximum is the zone where suspended particulate matter concentrations is higher than further upstream or downstream in the estuary and is usually located near to the point of the saline intrusion (Dyer 1994). Dyer (1979) did mention that sand and gravel-type sediment moves downstream to the tip of the saline intrusion in the river-dominated reaches of an estuary. When temperatures exceed 25 ºC, as frequently occurs during summer in sub-tropical regions, the phenomenon of flocculation is likely to accelerate resulting in the deposition of large amounts of mud particles (Jiang *et al.* 2004).

Benthic microalgal biomass (chlorophyll a)

Microalgae, being primary producers, are important components of estuarine ecosystems. They influence the exchange of nutrients between the water-column and the sediment (Rysgaard *et al.* 1995; Nedwell *et al.* 1999), provide a food source for deposit feeding fauna (Davis & Lee 1983; Hillebrand & Kahlert 2002), contribute to sediment stability (Sullivan 1999; Underwood 2000) and both stimulate and compete with various bacterial sediment processes (Haynes *et al*. 2007). Chlorophytes, euglenoids, cyanobacteria and diatoms, collectively termed microphytobenthos (MPB), inhabit the top few centimetres of intertidal sediment in estuarine and coastal sediments worldwide (Cahoon *et al.* 1999; Underwood & Kromkamp 1999). The photosynthetic pigment chlorophyll a (chl a) is frequently used as an measure of MPB biomass and has been used extensively in a number of studies describing the distribution of benthic microalgae in aquatic ecosystems (Thornton *et al.* 2002; Cartaxana *et al.* 2006; Jesus *et al.* 2006; Snow & Adams 2006).

Large standard deviations in chl a measurements sampled over a small distance are common phenomena in intertidal sediments. MacIntyre *et al.* (1996) reported on small-scale variation in the distribution of MPB biomass and recommended that at least five cores were needed to reduce the coefficient of variance to 45%. Recent studies of the Gamtoos (Snow *et al.* 2000b) and Kromme estuaries (Snow *et al.* 2000a) in South Africa described a relationship between average benthic chl a and river flow. However, no apparent relationship was evident between benthic chl a and distance from the mouth of the estuaries, despite the

presence of a strong TOxN gradient along the length of the Gamtoos Estuary. Similar results have been found in other estuaries and coastal waters (Underwood *et al.* 1998; Perissinotto *et al.* 2002; Welker *et al.* 2002; Mundree *et al.* 2003), suggesting that the quality of overlying water at the time of measurement was not significantly related to benthic microalgal chl a. Instead, there was a much stronger correlation between the microphytobenthos and the sediment, a possible source of nutrients. However, similar studies have found a closer association between water-column nutrients and benthic chl a (Sündback 1996) suggesting that the relationship is not simple and hence further research is required to improve our understanding of this relationship.

Recent publications (Perkins *et al.* 2003; Tolhurst *et al.* 2005) have highlighted a problem encountered with regards to presenting chl a data as content (mass per unit mass; e.g. µg Chl a g^{-1}) or concentration (mass per unit volume; e.g. mg Chl a m^{-3}). Natural soft marine sediments consist of six components; (1) non-cohesive mineral grains (sand particles), (2) cohesive particles (fine silt and clay), (3) water, (4) gas, (5) biota and (6) other matter (detritus, extracellular polymeric substances, heavy metals and salt) (Tolhurst *et al*. 2005). The first five are interdependent but the densities can vary (e.g. sand has a higher density and usually has a lower water content than fine sediment), which means that a change in one will affect at least one of the other four. By reporting chl a as a content, chl a is being expressed as a fraction relative to the remaining components. In contrast, chl a expressed as a concentration is expressing chl a as an exact amount of chl a in a fixed volume of sediment. As such, trends in the distribution of chl a can be incorrectly interpreted, particularly when comparing muddy and sandy sites. Muddy sites tend to have higher chl a contents than sandy sediments (Tolhurst *et al*. 2005).

Carbohydrates

The MPB inhabiting fine intertidal sediment is usually dominated by epipelic diatoms, which move through the sediment by excreting extracellular polymeric substances (EPS) from the raphe slit present in each of the silica cell walls (valves) that make up the cell (Stal 2003; Underwood & Paterson 2003). In coarser sediments, the episammic microphytobenthos is generally dominated by diatoms that either attach themselves to sand particles by a pad or short stalk of EPS or are capable of movement by excreting EPS. Cyanobacteria commonly found in fine sandy

sediments are also capable of excreting a polysaccharide sheath, which allows for gliding mobility. Through the remineralisation of organic matter they enrich the sediment with nutrients, helping to create a suitable environment for diatoms (Stal 2003).

The extracellular carbohydrate exudates of microphytobenthos, or exopolymers, can be broadly grouped into low molecular weight (LMW), small sugar units and glycollates, and larger, increasingly polymeric molecules (EPS). Collectively, this material is termed "colloidal carbohydrate", and high contents of colloidal carbohydrates can be present on mudflats (typically between 50 and 5000 μg g^{-1}). The production of colloidal carbohydrates is usually closely related to the rate of photosynthesis and can also increase significantly when nutrients are limiting, referred to as the overflow hypothesis (Stal 2003; Underwood & Paterson 2003). The amounts of colloidal carbohydrate and EPS generally increase over a tidal emersion period (Hanlon *et al.* 2006). Although other organisms, such as bacteria, produce extracellular carbohydrates, colloidal carbohydrates present in microphytobenthic biofilms are closely related to microalgal biomass and photosynthetic activity (Smith & Underwood 2000). In diatom-rich biofilms, between 20% and 40% of the extracellular carbohydrate is polymeric (i.e. EPS). The remaining LMW carbohydrates are an important food source for bacteria and may play an important role in the ecology of fine sediments. Bacterial production is usually closely associated with LMW compounds, resulting in rapid decreases in LMW material in the absence of photosynthesis, particularly in sandy sediments (van Duyl *et al.* 1999; Underwood 2002). Microalgal-bacterial coupling is generally strongest in sandy sediments with low organic content compared to fine mud with a high organic content (Köster *et al.* 2005). Carbohydrate content is more closely associated to epipelic diatom biomass than episammic biomass.

Underwood & Smith (1998b) investigated the chl <u>a</u>: colloidal carbohydrate distribution for a range of European mudflats and published a model describing the relationship; Log [colloidal carbohydrate (μg g^{-1}) + 1] = 1.40 + [log chl <u>a</u> (μg g^{-1}) + 1]. This relationship was only found to fit for sediments dominated by fine clay particles and at sites where epipelic diatoms dominated (> 50%) the microphytobenthic assemblage. Assemblages dominated by green algae and cyanobacteria did not show a significant colloidal carbohydrate: chl <u>a</u> relationship. Similar trends have been

found in more recent studies (Blanchard *et al.* 2000; Thornton *et al.* 2002; Bellinger *et al.* 2005).

To date, no published reports of carbohydrates or of their stabilising effects in the intertidal sediment of South African estuaries have been produced. Du Preez (1996), however, has presented a considerable amount of information on the EPS of the diatom *Anaulus australis* in the surf-zone. This diatom also spends time on/in the sediment behind the breaker-line in calm weather.

Research aims

The aims of the study were:

1. To determine the relative importance of the water-column chemistry and sediment characteristics in determining the spatial distribution of intertidal benthic microalgae, using chl a as an index of biomass, in six permanently open estuaries along the south-eastern coast of South Africa.
2. To describe the spatial pattern of carbohydrate fractions (colloidal carbohydrates in particular) in six South African estuaries and relate these to physical and chemical variables in the estuary and benthic microalgal biomass (chl a).

Study location

Six estuaries (Table 2.1) were selected for the study and all except for the Keurbooms (Western Province) occur in the Eastern Cape Province of South Africa (Fig. 2.1). All six estuaries are examples of drowned river valley estuaries, being typically confined within bedrock valleys and influenced, to varying degrees, by aeolian sediment deposition as many dunes along the coast are unvegetated (Cooper *et al.* 1999). There is a shift from a warm temperate climate in the west to subtropical in the east. Intertidal benthic samples were collected with the result that only permanently open estuaries could be considered in this study except for the Mngazi, which closes temporarily and was open for more than a month prior to sampling. The Swartkops was sampled four times, the Sundays, Mngazana and

Gamtoos twice and the Keurbooms and Mngazi estuaries only once because of floods during March 2003 and mouth closure in June 2003 respectively.

Table 2.1

Sampling dates, estuary coordinates and number of sites sampled in each estuary (ordered from west to east).

Estuary name	Coordinates	Date sampled	Number of sites
Keurbooms	34°02'S; 23°23'E	25/08/2002	5
Gamtoos	35°58'S; 25°01'E	21/02/2003	5
		07/08/2002	5
Swartkops	33°52'S; 25°38'E	12/02/2002	6
		05/12/2002	6
		15/08/2003	6
		30/10/2001	6
Sundays	33°43'S; 25°51'E	25/07/2002	5
		19/02/2003	5
Mngazana	31°42'S; 29°25'E	22/06/2003	5
		25/01/2003	5
Mngazi	31°41'S; 29°27E	26/01/2003	5

Thirty-one sites were sampled ensuring that a broad range of benthic chl a and associated environmental variables were collected. Two of the six estuaries had distinct point-source discharges of high nutrient water. An agricultural return flow pipe enters the Gamtoos near to the head of the estuary and a polluted stormwater canal enters from the nearby Motherwell Township into the mid to lower reaches of the Swartkops Estuary. Water samples were collected from these sources during sampling trips and analysed for quality. There were six sampling sites in the Swartkops and five sites in each of the remaining estuaries. Sites were fairly evenly spaced along the length of the estuaries to capture the salinity gradient in the water-column and changes in sediment type along their longitudinal axes.

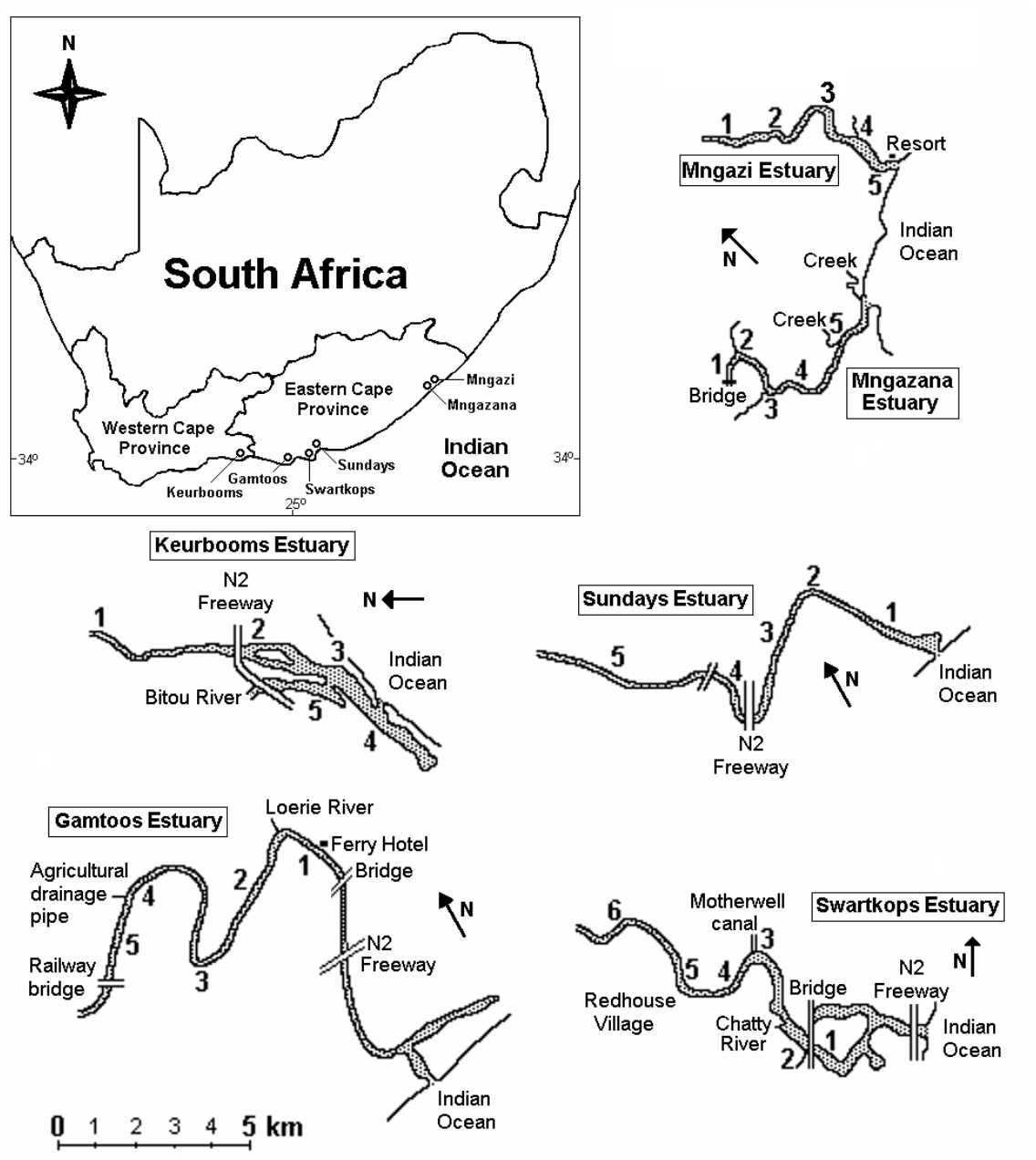

Figure 2.1. Locality map of the Keurbooms, Gamtoos, Swartkops, Sundays, Mngazana and Mngazi estuaries indicating the approximate locations of sampling stations in the study.

Materials and Methods

Microalgal biomass

Five replicate samples of benthic chl a were collected from just above the water line within a square metre of each other from each site by scraping a known area of surface sediment (< 2 mm depth) during spring low tide. Scrapes of known areas of intertidal sediment were necessary to convert chl a content (μg g^{-1}) to chl a concentration (mg m^{-2}). The samples were freeze-dried, approximately 0.1 g was added to 4 ml of 95% ethanol and then stored for 24 hrs at 0 ^{o}C. Once the chl a was extracted the samples were whirli-mixed then filtered through glass-fibre filters (Whatman GF/C). The extract was analysed on a high performance liquid chromatograph (HPLC) attached to a Waters Lambda-Max 481 LC spectrophotometer and Waters LM-45 solvent delivery system. A 30% methanol / 70% acetone mixture was used as a carrier. The system was calibrated using the chl a of red seaweed (*Plocamium corallorhiza*) because it contains no chlorophyll b which interferes with the chl a reading at 665 nm (du Preez *pers. comm.*). Chl a was expressed on a mass per unit mass basis (micrograms of chl a per gram of freeze-dried sediment (μg g^{-1}) and 130 samples were also measured on a mass per unit area basis (mg m^{-2}) to determine the difference between the two.

Fractionation of carbohydrates

Sediments remaining from the freeze-dried replicate samples were analysed for carbohydrate. The method used was based on the phenol-sulfuric acid assay (Dubois *et al.* 1956). This assay is used to measure total carbohydrate in the sediment (i.e. intracellular, extracellular and particle-bound) by hydrolyzing more complex carbohydrates into simple carbohydrates using acid and heat. Phenol is then added to the sample, which binds to the sugars to form fluorogen. The yellow product is measured using a spectrophotometer at an absorbance of 485 nm. Colloidal carbohydrates were extracted from the sediment using saline water (Underwood *et al.* 1995) and include low molecular weight (LMW) carbohydrates and extracellular polymeric substances (EPS). The EPS is extracted from the colloidal carbohydrate solution using ethanol (70% final concentration) followed by centrifuging (Underwood *et al.* 1995). EPS are long-chain molecules produced and excreted by microbial metabolism. The absorbance of a standard series of anhydrous

glucose solutions was used to calibrate the analysis. Carbohydrate content (m/m) was expressed in micrograms of glucose equivalents per gram of freeze-dried sediment (μg g^{-1}).

Overlying water nutrients and salinity

Salinity was recorded at each site using a YSI 30 CTD with a 10 ft cable and probe. Four replicate water samples for nutrient analysis were collected from just below the water surface near each intertidal site, filtered through Whatman (GF/C) filter paper and preserved using $HgCl_2$ (20 μl of 5% solution added to 30 ml sample) and frozen at –20 ^{o}C. Total oxidised nitrogen (TOxN) was determined using the reduced copper-cadmium method described by Bate and Heelas (1975). Dissolved inorganic phosphorus (DIP) otherwise referred to as soluble reactive phosphorus (SRP), ammonium and dissolved silicate (DSi) were determined manually using standard colorimetric methods (Parsons *et al.* 1984).

Sediment particle size

A scrape of sediment to a depth of approximately 1 cm was collected from directly below the area where each chl a replicate was collected on all occasions (excl. Swartkops Estuary, October 2001). In the laboratory each sediment sample was dried at 105 ºC to constant mass. Samples were then disaggregated using a mortar and pestle before being shaken through a series of steel mesh sieves (mesh apertures of 500, 250, 125, and 63 μm). The dry sieve method could underestimate the proportion of cohesive sediments (< 63 μm) but it was decided that the error would be small because South African estuary sediment is generally sandier than the U.K. and European counterparts (personal observation). Each fractionated sediment size-class was weighed and the dry mass of each fraction was expressed as a percentage of the total. Sediments were allocated to five different classes; sand (mud content < 10%), muddy sand (mud content 10-25%), sandy mud (mud content 25-50%), mud (mud content 50-85%), and clay-mud (mud content > 85%) based on the sediment's mud content (proportion of < 125 μm sediment), according to Figge *et al*. (1980) cited in Riethmüller *et al.* (2000).

Ash-free dry weight (AFDW) and moisture content

A subsample of sediment collected for sediment particle size analysis was placed into predried and weighed crucibles. Once the wet mass was recorded, the samples were dried in an oven at 105 ºC for 24 hours, reweighed (dry mass) and then placed into an ashing-oven at 550 ºC for 1 hr. The cool ashed sediments were weighed and the data analysed.

Data analysis

Averages of the four replicate water sample nutrient concentrations and five benthos-related variables were used when analyzing the data. Before testing for significant differences, the Kolmogorov-Smirnov Test was used to check if the data were parametric. If the data were parametric, then a Students t-test was used to compare two sets of data for significant differences and the Tukey's test was used if there were more than two sets. However, if the data were non-parametric, then a Mann-Whitney Rank Sum test was used to compare two sets of data and the Kruskal-Wallis Anova on Ranks test was used if more than two sets were compared. Pearsons Product Moment Correlation was used to test the strength of association between variables. All tests were performed using the statistical software package Statistica (Version 7). Principal Component Analysis (PCA) using Minitab (Version 13) was used to examine the entire physical and chemistry data sets for variability. Environmental variables and microalgal biomass (chl a) are presented as box-whisker plots using Grapher 6.1.21 (Golden Software, Inc.) and sediment composition is presented using Excel 2000 (Microsoft®). Mean values are expressed as mean ± standard error of the mean.

Results

Overlying water and sediment characteristics

A high variance of salinity and nutrient concentrations was measured in the estuarine water during the study (Fig. 2.2). Low salinity (< 5 ppt) was recorded in the upper reaches of the Keurbooms, Gamtoos, Mngazana and Mngazi estuaries, and high salinity (> 30 ppt) in all estuaries other than the Keurbooms and Gamtoos. Nutrient concentrations measured in the overlying water of the Swartkops Estuary were

relatively high compared to the other five estuaries ($TOxN_{max}$ = 32.0 µM; $NH_4^+{}_{max}$ = 13.4 µM; DIP_{max} = 53.9 µM; DSi_{max} = 128.3 µM). By contrast, nutrients in the overlying water, excluding silicate, were low in the Mngazana and Mngazi estuaries ($TOxN_{max}$ = 2.2 µM; $NH_4^+{}_{max}$ = 4.2 µM; DIP_{max} = 0.3 µM). There was a general increase in average DSi from westerly estuaries to those in easterly areas. Intertidal sediments in the Mngazi and Mngazana estuaries had the highest organic contents; up to 9.9% and 7.5% respectively, and the highest moisture contents (> 35%).

Salinity in the Keurbooms Estuary in August 2002 was relatively low throughout, ranging from less than 1 ppt to 19.5 ppt. A relatively high concentration of TOxN was measured at the head of the estuary (23.2 µM). Ammonium was below detectable limits throughout the estuary.

An agricultural drainage pipe emptying into the upper reaches of the Gamtoos Estuary (Fig. 2.1) was an important source of nutrients. TOxN concentration measured in water flowing out of the pipe was 489 µM in February 2003 and 267 µM in August 2002. In February 2003, TOxN in the estuary decreased from 21.5 µM at the head of the estuary to below detectable limits just 5 km downstream (site 3; Fig. 2.1). The TOxN concentration in the overlying water was significantly higher in August 2002 compared to February 2003 (n = 20; t = 14.5; P < 0.001). The higher average TOxN concentration could have been the result of increased river flow, which was evident from the decrease in salinity 8.1 km from the mouth from 18 ppt in February to 11 ppt in August. Sediment was coarsest at site 3 where the proportion of > 125 µm sediment was 93% and was finest at site 2 where < 125 µm sediment was > 52% (Fig. 2.3). The organic content of the sediment in the upper estuary was generally low (< 1%) and only exceeded 1% at sites 1 and 2.

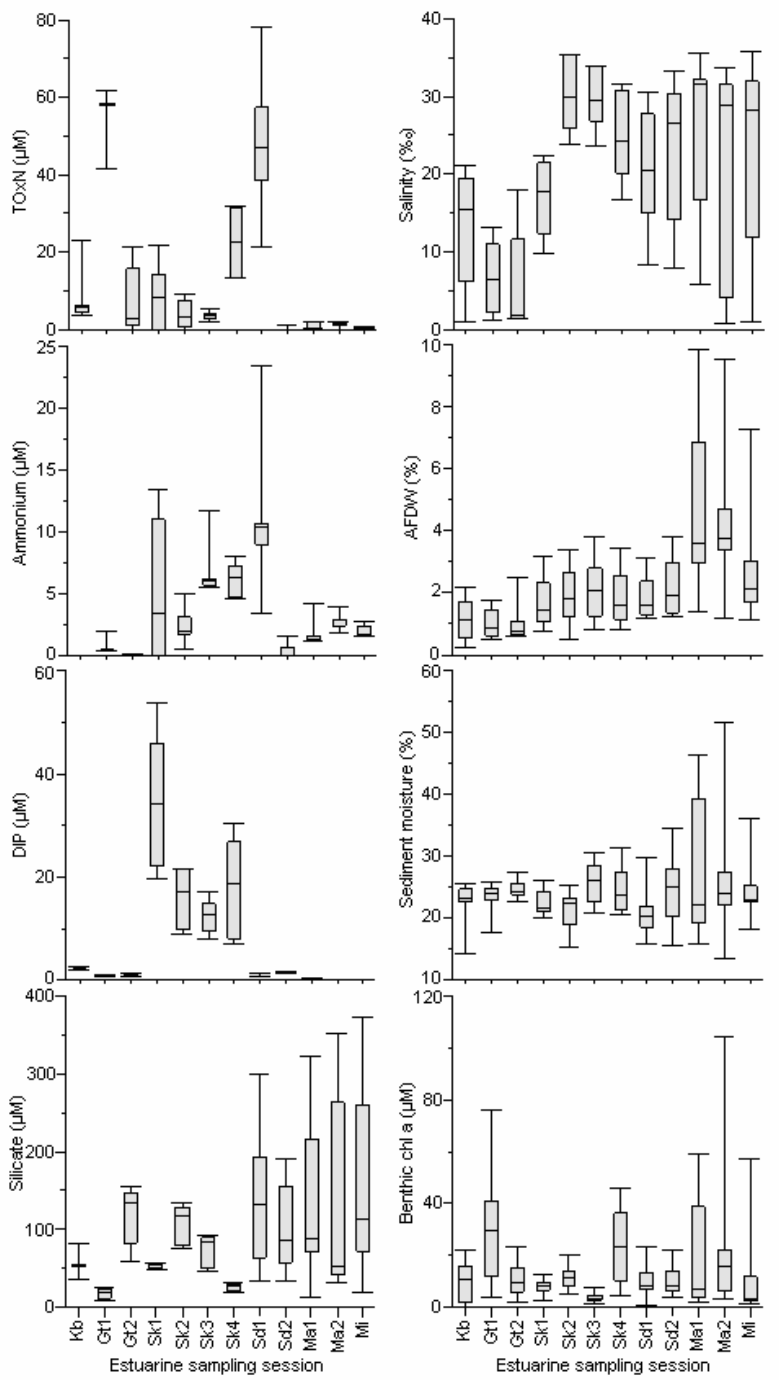

Figure 2.2. Box-whisker plots of water-column nutrients, ash-free dry weight (AFDW), sediment moisture content and benthic chl <u>a</u> in the Keurbooms (Kb = 25/08/2002), Gamtoos (Gt1 = 21/02/2003; Gt2 = 07/08/2002), Swartkops (Sk1 = 30/10/2001; Sk2 = 12/02/2002; Sk3 = 05/12/2002; Sk4 = 15/08/2003), Sundays (Sd1 = 25/07/2002; Sd2 = 19/02/2003), Mngazana (Ma1 = 25/01/2003; Ma2 = 22/06/2003) and Mngazi (Mi = 26/01/2003) estuaries in the study. The line within the box, the boundaries of the box and the whiskers represent the median, 25th and 75th percentiles and the 10th and 90th percentiles respectively.

The Swartkops Estuary had two distinct sources of nutrients, the Swartkops River, which flows through industrialised areas, and a stormwater canal draining the large informal Motherwell Township. Swartkops riverwater was consistently high in SRP and the Motherwell canal was high in DIN and DSi (maximum concentrations of 239 µM and 1107 µM respectively). However, in August 2003, the DIN concentration was higher (31.4 µM) at the site nearest to the head of the estuary than water near the Motherwell canal. As a result, DIP was generally highest at the head of the estuary and TOxN highest in the lower reaches. This was not the case in August 2002 when both DIN and DIP, 39.4 µM and 30.4 µM respectively, were highest in the upper reaches of the estuary. In October 2001, DIN followed a similar pattern to that of February 2002, decreasing upstream and being below detectable limits in the upper reaches.

The seepage of fertilizers from citrus agriculture in the catchment of the Sundays River is a major source of nutrients into the Sundays Estuary. In July 2002 the DIN: DIP ratio was > 44, which indicates possible P-limited microalgal growth (assuming a threshold ratio of 16:1). The sediment at site 3, 5.1 km from the mouth was sandy mud, being a mix of sediment dominated by 125-250 µm sediment particles and derived from fringing coastal dunes and fluvial sediment. The intertidal zone at this site was sloped gently in a broad stretch of the estuary, favouring the settling of suspended sediments and organic matter. Highest water-column nutrient concentrations and the finest sediment were located nearest to the head of the estuary but the intertidal zone in this region was steep, the sediment compacted and the estuary channel narrow. This favours the resuspension of settled particles during pulses in flow, which does not favour the accumulation of microalgal biomass.

The organic contents in the Mngazana and Mngazi estuaries had maxima of 9.9% (site 4; 3.4 km from the mouth) and 7.3% (site 3; 1.7 km from the mouth) respectively. The sediment at site 4 in the Mngazana Estuary was mud and at site 3 in Mngazi was clay-mud, having much higher proportions of fine sediment particles than other sites (Fig. 2.3). The nutrient concentration in the overlying water was low by comparison with the other estuaries in the study, with TOxN generally being < 2.0 µM in January and June, and DIP generally being < 0.2 µM in January. Ammonium concentration was elevated throughout both estuaries, ranging from 1.1 to 4.2 µM.

In January 2003, TOxN and DIP were low in the overlying water of the Mngazana Estuary (< 2.2 µM and 0.3 µM respectively). Ammonium was 4.2 µM at

the head of the estuary where surface salinity was 5.7 ppt and herds of cattle frequent. A second maximum of 1.6 µM was measured at site 4 where the benthic sediment was finest and organic content highest. Site 3 in the Mngazi Estuary followed a similar pattern to that in the Mngazana. The highest contents of organic matter, fine sediment (< 125 µm) and moisture occurred at this site in the middle reaches of the estuary (Fig. 2.3). TOxN and DIP were less than 2 µM and 0.2 µM respectively throughout the estuary and ammonium was highest in the middle and lower reaches of the estuary, ranging from 1.5 µM to 2.7 µM.

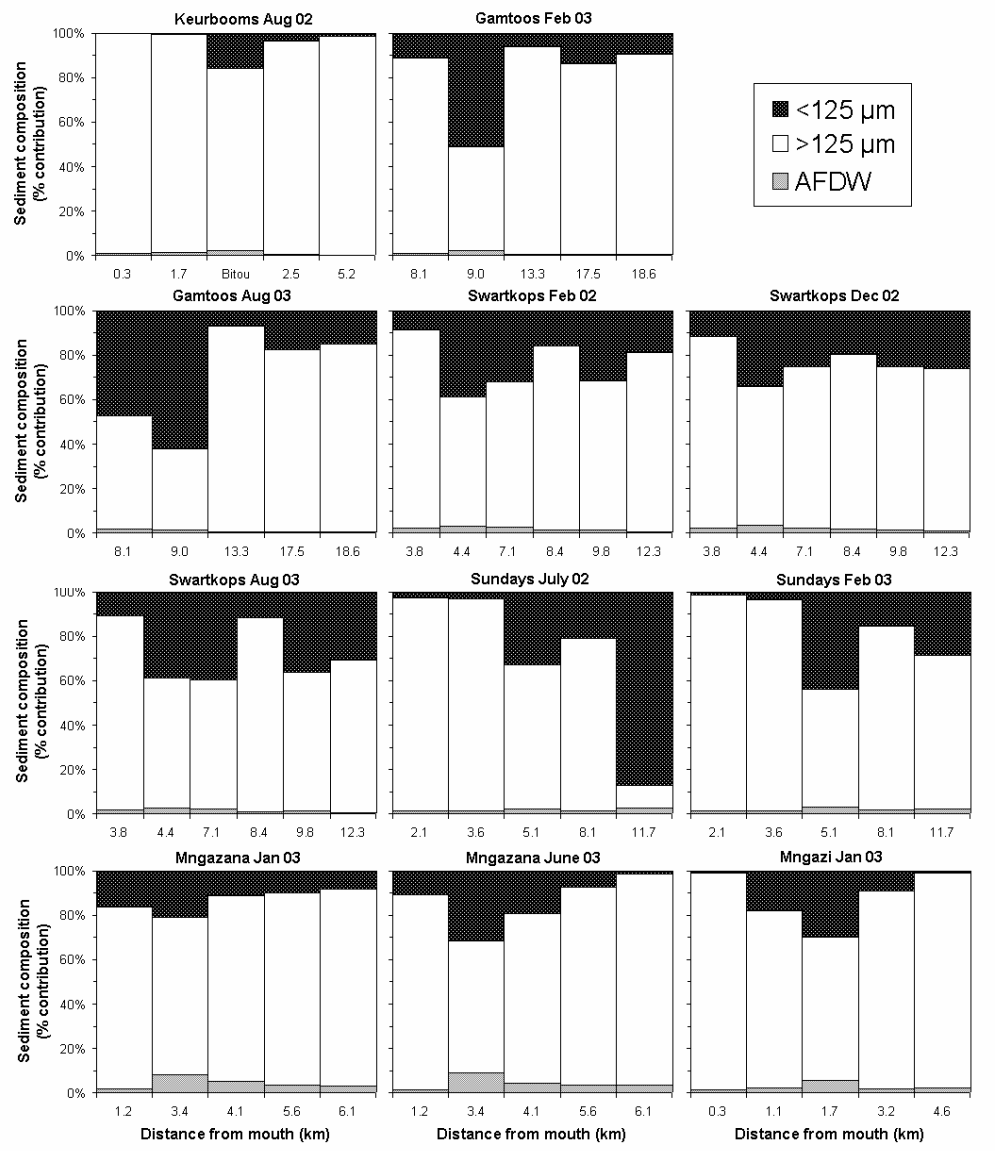

Figure 2.3. Stacked columns comparing the relative contributions of sediment particle size (> 125 µm and < 125 µm grain sizes) and organic content in the intertidal sediments of six open South African estuaries.

Variability of environmental variables

In the PCA, 45.7% of the total variability that was in the physical and chemistry data was described by the first two axes, PC's 1 and 2 (Fig. 2.4), and a further 26.5% was described by PC's 3 and 4. The PC loadings of the first axis showed that the sampling stations in the Gamtoos, Swartkops and Keurbooms estuaries were largely separated by the type of sediment and nutrient concentrations in the overlying water. The sediment in the Keurbooms Estuary was predominantly medium grained sand (250-500 μm) and the water was low in nutrients. In contrast, nutrient concentrations were much higher in the Gamtoos and Swartkops estuaries and there was a full gradient in sediment particle sizes; ranging from sites high in fines (< 125 μm) to sites with a high coarse sand content (> 125 μm).

Sampling sites along PC2 were separated by gradients in the organic matter, moisture and fine particulate sediment contents. This was most evident in the Mngazana, Mngazi and Sundays estuaries. Sites located near to the mouth of the Sundays Estuary or near to the head of the Mngazana and Mngazi estuaries were dominated by sand and gravel, whereas the sediment in the middle reaches of the estuaries had a high content of organic matter and fines.

Correlations between benthic microalgal biomass and environmental variables

The PCA highlighted high variation in the physical variables and chemistry of the six estuaries. This variation was used to determine the strength of association, through the use of correlation analyses, between benthic microalgal biomass and any single or group, using axes scores, of environmental variables (Table 2.2).

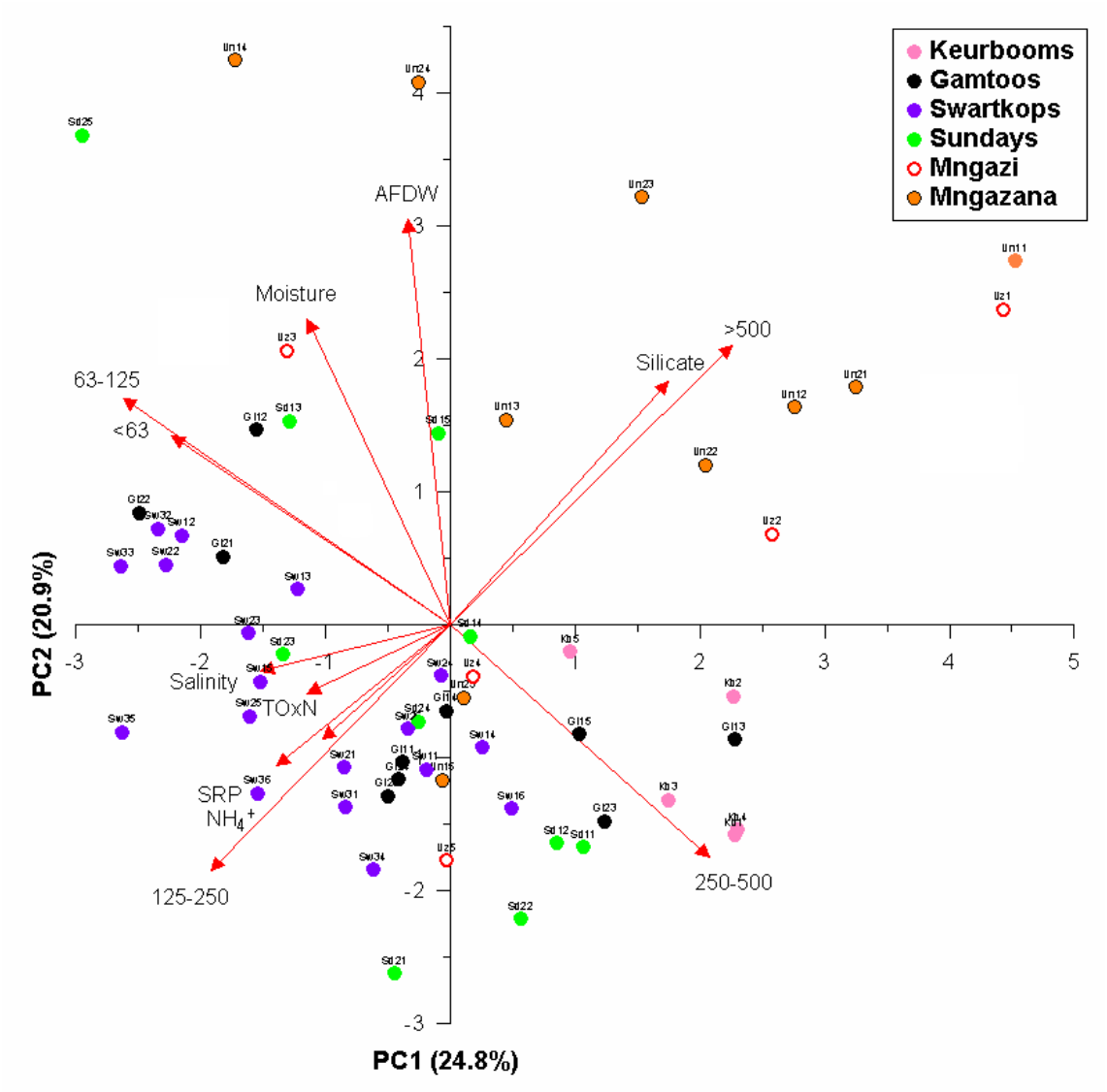

Figure 2.4. Scatter plot of average sample scores (n = 5) on principal components 1 and 2, with variability scores, from a PCA of the physical and chemistry variables of the sediment and overlying water. Estuaries are colour coded and environmental vectors included as arrows.

Benthic chl a was positively correlated to TOxN, AFDW, moisture content and to sediment particle sizes of less than 125 µm. The strongest of these correlations were with AFDW and moisture contents. DSi was the only variable that chl a was negatively correlated to.

It is unlikely that any single environmental variable was responsible for the distribution of microalgal biomass in the estuaries and this was confirmed by the significant correlations between the PC axis scores and chl a (Table 2.2). Sites with high PC1 scores; sites at the head of the Mngazi and Mngazana estuaries and site 2 in the Keurbooms Estuary, had low concentrations of nutrients in the overlying water and the sediment had a high content of coarse particles (> 250 µm). In contrast, sites with low PC1 scores; sites 3 in the Swartkops and Sundays estuaries, had relatively high concentrations of DIN (> 9 µM), DIP (> 1 µM) and DSi (> 20 µm) and had a high proportion of fine sediment (< 250 µm). PC1 was significantly correlated to all environmental variables excluding AFDW.

PC2 was significantly correlated to all environmental variables except for water-column salinity and TOxN. There was a slightly stronger correlation between the PC2 scores and chl a (r = 0.379), the result of strong associations with AFDW (r = 0.534) and the moisture content of the sediment (r = 0.622). The highest chl a concentrations of the study (38.9 to 104.8 µg g^{-1}) were measured in the middle reaches of the Mngazana Estuary (site 4) where the intertidal sediment had a high proportion of fine sediment (> 30%) (Fig. 2.3), AFDW (approximately 8%) and moisture content (approximately 40%). In contrast, sites closest to the mouths of the Mngazi (appendix figure A.2), Mngazana (appendix figure A.3) and Sundays estuaries were dominated by coarse marine sediment that contained very little organic matter and was easily drained.

Table 2.2 (continued to following page)

Pearson's correlation coefficients relating sediment associated variables and water chemistry to benthic chl a and carbohydrates in six permanently open estuaries (N = 290). Coefficients that are significantly correlated ($p < 0.01$) are in bold.

	Water chemistry variables					Sediment associated variables						
	Salinity	**TOxN**	**NH4**	**SRP**	**DSi**	**AFDW**	**Moisture**	**> 500**	**250-500**	**125-250**	**63-125**	**< 63**
TOxN	**-0.37**											
NH4	**0.35**	**0.20**										
SRP	**0.27**	-0.03	**0.26**									
DSi	**-0.48**	-0.13	-0.12	**-0.20**								
AFDW	**0.32**	**-0.29**	0.01	**-0.23**	0.14							
Moisture	**0.16**	-0.12	-0.12	-0.11	**-0.16**	**0.73**						
> 500	**-0.23**	**-0.28**	-0.12	**-0.25**	**0.60**	**0.37**	-0.04					
250-500	-0.12	-0.11	-0.08	-0.10	-0.10	**-0.32**	**-0.23**	-0.06				
125-250	**0.29**	0.05	**0.18**	**0.16**	**-0.46**	**-0.25**	-0.03	**-0.63**	**-0.38**			
63-125	0.08	**0.32**	0.02	**0.17**	-0.06	**0.25**	**0.36**	**-0.30**	**-0.66**	0.08		
< 63	0.03	**0.39**	0.03	**0.21**	0.05	0.15	**0.21**	**-0.27**	**-0.46**	-0.08	**0.70**	
PC1	**-0.46**	**-0.32**	**-0.31**	**-0.41**	**0.53**	-0.09	**-0.32**	**0.67**	**0.58**	**-0.57**	**-0.73**	**-0.61**
PC2	0.18	0.13	**0.22**	**0.27**	**-0.47**	**-0.81**	**-0.62**	**-0.53**	**0.48**	**0.47**	**-0.47**	**-0.39**
PC3	0.00	**-0.72**	0.12	0.03	**-0.36**	**0.48**	**0.37**	0.05	0.13	**0.19**	**-0.31**	**-0.46**
PC4	0.00	-0.11	**0.63**	**0.53**	**0.36**	-0.01	**-0.40**	**0.36**	**-0.20**	**-0.16**	-0.02	0.09
Chl a	0.16	**0.15**	-0.03	0.00	**-0.27**	**0.53**	**0.62**	-0.09	-0.14	-0.05	**0.31**	**0.23**
Total	0.00	**-0.18**	-0.06	-0.11	-0.08	**0.86**	**0.81**	0.13	**-0.25**	-0.14	**0.31**	**0.19**
Colloidal	0.00	-0.02	-0.01	0.01	**-0.25**	**0.64**	**0.67**	0.02	**-0.20**	-0.06	**0.28**	**0.17**
EPS	**0.16**	0.08	0.06	**0.24**	**-0.30**	**0.40**	**0.51**	-0.13	**-0.27**	0.08	**0.35**	**0.28**

Table 2.2 continued

	Physico-chemical PCA scores				Biological variables		
	PC1	**PC2**	**PC3**	**PC4**	**Chl a**	**Total**	**Colloidal**
TOxN							
NH4							
SRP							
DSi							
AFDW							
Moisture							
> 500							
250-500							
125-250							
63-125							
< 63							
PC1							
PC2	0.00						
PC3	0.00	0.00					
PC4	0.00	0.00	0.00				
Chl a	**-0.32**	**-0.38**	**0.15**	**-0.28**			
Total	**-0.24**	**-0.67**	**0.43**	**-0.20**	**0.77**		
Colloidal	**-0.29**	**-0.45**	**0.32**	**-0.19**	**0.89**	**0.84**	
EPS	**-0.43**	**-0.28**	0.15	-0.09	**0.81**	**0.65**	**0.85**

Comparison of chl a content and concentration

A total of 130 chl a samples, measured on a m/m (content) or m/a (concentration) basis were collected from the Gamtoos (08/2002), Sundays (06/2002 and 02/2003), Swartkops (12/2002) and Keurbooms (08/2002) estuaries. The linear regression of chl a content (x) compared to concentration (y) had a good fit ($y = 1.67x$; $R^2 = 0.69$), and the two variables were significantly correlated ($r = 0.87$; $P < 0.001$) (Fig. 2.5). Samples with chl a contents or concentrations that were off the regression line included site 3 in the Sundays Estuary (July '02) and sites 1 and 2 in the Gamtoos Estuary (August '02). Chl a content at sites 1 and 2 in the Gamtoos Estuary averaged 40.3 ± 5.3 μg g^{-1} and the sediment was muddy (proportion of sediment <125 μm; 55.5%). In the Sundays Estuary, chl a was 20.6 ± 0.9 μg g^{-1} at site 3, and the sediment had a high sand content (proportion of sediment <125 μm; 33.6%). The larger amount of pore space in the Gamtoos mud, which has a lower bulk density, probably contained a greater number of microalgal cells than the same mass of Sundays muddy sand resulting in the uncoupling of these sites from the regression line. It is likely that other muddy sites, with a high chl a content, would have a lower chl a m/a than the average.

Chl a content in organic matter

Organic matter can include living (e.g. microalgal cells) or non-living material (e.g. detritus) whereas mineral particles only consist of inorganic material. By reporting chl a as a proportion of organic matter (μg Chl a g^{-1} AFDW), referred to here as OM chl a, the effect of sediment particle size was removed. The ratio, chl a: AFDW, is the inverse of the Autotrophic Index (Collins & Weber 1978), which was developed to distinguish the effects of inorganic nutrients and organic enrichment. The OM chl a followed similar trends to chl a content in freeze-dried sediment (μg Chl a g^{-1} sediment) in the Sundays, Mngazana and Mngazi estuaries but there were distinct differences in the other three estuaries (Fig. 2.6). This indicates areas of an estuary that have been recently recolonised (pioneer stage) or areas that have reached a more mature stage, i.e. if sediment chl a was high at a site with high organic content, then it is likely that the area has not been eroded recently and there has been a build up of detritus with time resulting in a low OM chl a (mature stage).

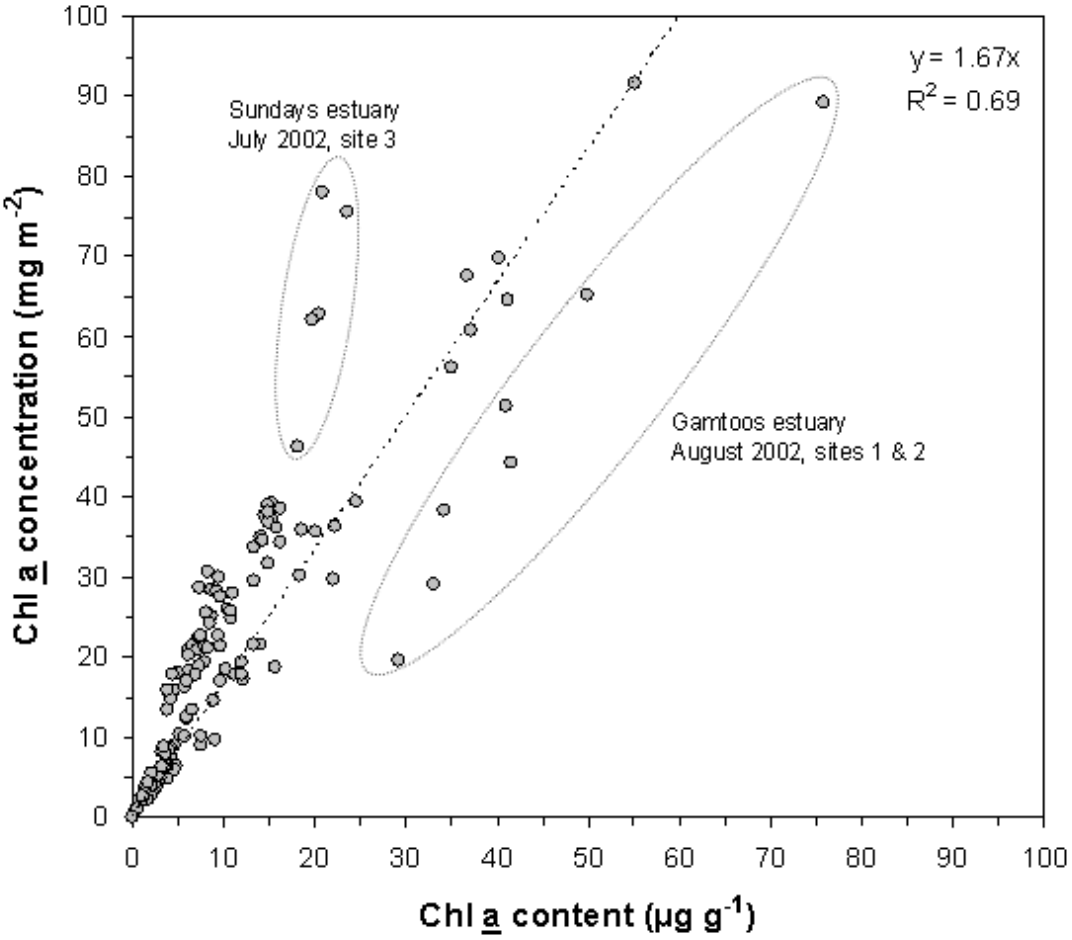

Figure 2.5. Benthic chl a content (µg g^{-1}) in relation to chl a concentration (mg m^{-2}) in the Gamtoos, Sundays, Swartkops and Keurbooms estuaries (N = 130). A linear regression with associated goodness of fit (R^2) included.

OM chl a was low in relation to sediment chl a in the Bitou tributary of the Keurbooms, middle reaches of the Gamtoos and mid-lower reaches of the Swartkops estuaries suggesting that these habitats were stable and there had been an accumulation of organic matter with elevated microalgal biomass at these sites. By contrast, sites near to the head of the Keurbooms and Swartkops, as well as in the mouth region of the Mngazana estuaries had high OM chl a relative to sediment chl a. This could indicate areas of estuaries that have been recently eroded, or the accumulation of organic matter was very slow, and where the microalgae have successfully recolonised. It is likely that areas with an accumulation of detritus, which have low OM chl a's, are areas with a high biomass of heterotrophs and an elevated biological oxygen demand.

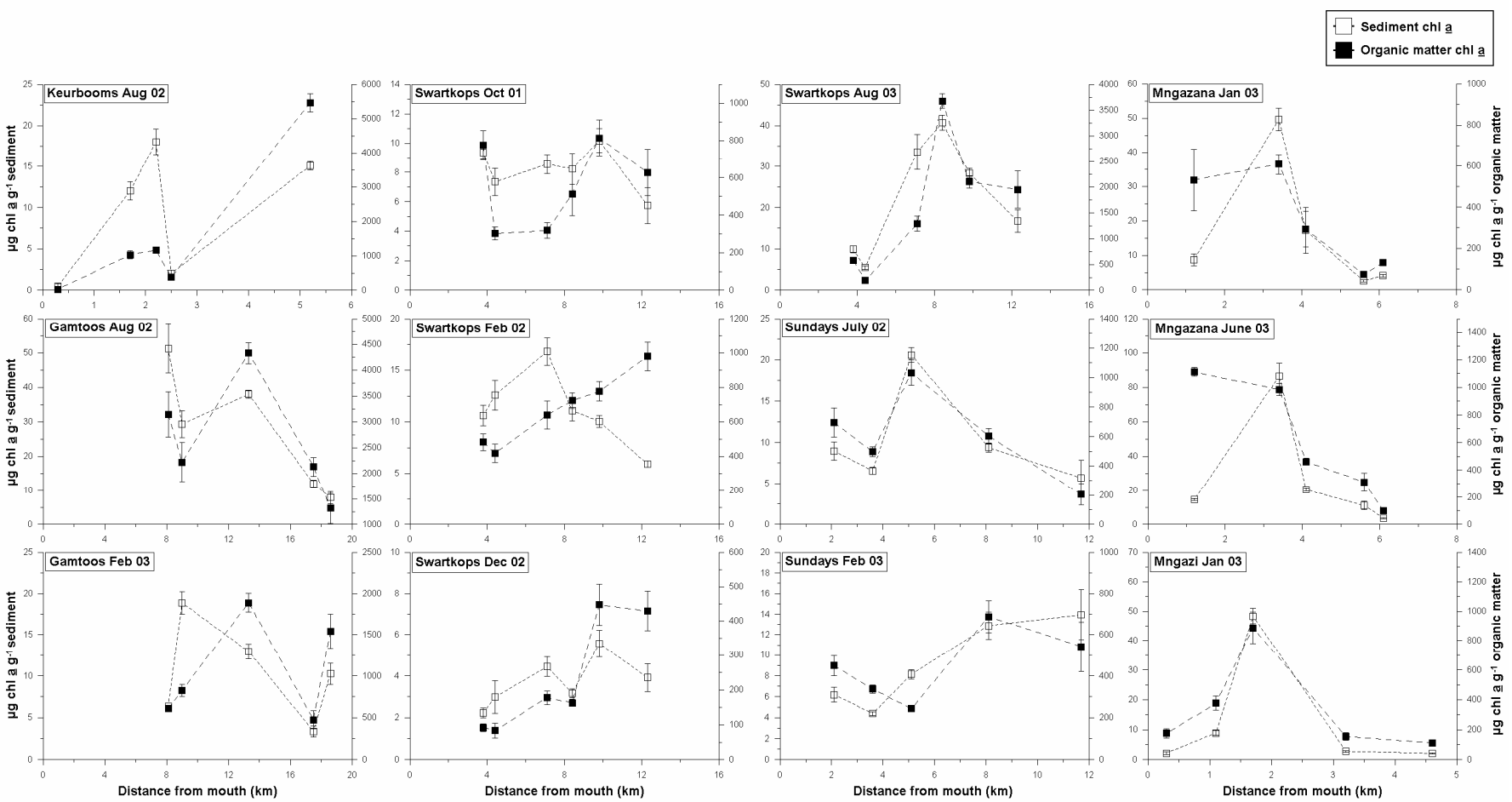

Figure 2.6. Benthic chl a content, expressed as µg Chl a g^{-1} freeze-dried sediment (sediment + organic content) and µg Chl a g^{-1} organic matter content (organic content only), in relation to distance from the estuary mouths.

Spatial patterns of carbohydrate

There were a number of weak, yet significant (P < 0.01), correlations between the carbohydrate fractions and water chemistry. Total carbohydrate was negatively correlated with TOxN as a result of the high carbohydrate content, more than 10000 µg g^{-1}, in the middle reaches of the Mngazana and Mngazi estuaries where water-column TOxN was low (< 1.8 µM). Total carbohydrate at all other sites, including sites with high TOxN concentrations, was much lower (< 5000 µg g^{-1}). In addition, EPS was positively correlated with salinity and negatively correlated with DSi. The highest EPS contents (> 400 µg g^{-1}) were measured in the middle reaches of the Mngazi, Mngazana and Swartkops estuaries at sites where water-column salinity and DSi were greater than 22 ppt and 500 µM respectively. Site 1 in the Gamtoos Estuary was the one exception where salinity was 11 ppt.

There was a significant correlation (r = 0.36; P < 0.01) between EPS and SRP (Table 2.3). SRP concentrations in all estuaries, excluding the Swartkops, ranged from 0 to 2.6 µM and was negatively correlated with EPS (r = -0.204; P = 0.004). This association was also the result of the close association between chl a and carbohydrate. In contrast, there was a strong correlation between EPS and SRP in the Swartkops Estuary (r = 0.357; P < 0.001) where SRP ranged from 6.9 µM at the mouth to 30.4 µM at the site nearest to the head of the estuary. There was a stronger association between benthic chl a, colloidal carbohydrate and EPS in this estuary with water chemistry than sediment associated variables, i.e. they were significantly correlated to salinity, TOxN, SRP and DSi and there was only a weak correlation with silt content (< 63 µm sediment).

Total carbohydrate is a measure of the organic matter present in sediment that is digestable using the Dubois assay and hence it is not unexpected that AFDW and total carbohydrate were so strongly correlated (r = 0.86). The water-soluble carbohydrate fractions (colloidal and EPS) were also strongly correlated with organic (r = 0.64 and 0.40 respectively) and moisture contents (r = 0.67 and 0.51 respectively).

Table 2.3

Pearson's correlation coefficients (*r*) relating sediment associated variables and water chemistry to benthic chl a and carbohydrates in the Swartkops Estuary (N = 90). Coefficients that are significantly correlated ($P < 0.01$) are in bold.

	Water chemistry					Sediment associated variables							Biological variables		
	Salinity	**TOxN**	**NH4**	**SRP**	**DSi**	**AFDW**	**Moisture**	**> 500**	**250-500**	**125-250**	**63-125**	**< 63**	**Chl a**	**Total**	**Colloidal**
TOxN	**-0.56**														
NH4	-0.09	**0.42**													
SRP	**-0.91**	**0.50**	-0.02												
DSi	0.09	**-0.75**	**-0.65**	0.13											
AFDW	**0.75**	-0.17	0.24	**-0.71**	-0.24										
Moisture	0.12	0.07	**0.44**	**-0.27**	**-0.33**	**0.58**									
> 500	0.11	0.06	0.02	-0.08	-0.10	-0.17	**-0.57**								
250-500	-0.24	-0.15	-0.22	**0.30**	**0.35**	**-0.47**	**-0.40**	**0.31**							
125-250	**0.38**	0.01	0.15	**-0.42**	**-0.29**	**0.27**	0.11	-0.14	**-0.77**						
63-125	-0.24	0.14	0.25	0.14	-0.11	0.21	**0.61**	**-0.53**	**-0.57**	0.03					
< 63	0.08	0.18	-0.12	0.00	-0.07	**0.48**	**0.29**	**-0.32**	**-0.34**	-0.17	**0.38**				
Chl a	**-0.45**	**0.71**	0.09	**0.47**	**-0.39**	-0.16	-0.01	-0.01	-0.06	-0.04	-0.02	**0.32**			
Total	0.01	**0.27**	0.00	-0.02	-0.20	**0.42**	**0.47**	**-0.41**	**-0.36**	-0.05	**0.46**	**0.71**	**0.51**		
Colloidal	**-0.49**	**0.77**	0.20	**0.39**	**-0.59**	-0.13	0.13	-0.07	-0.141	0.01	0.09	**0.30**	**0.91**	**0.52**	
EPS	**-0.49**	**0.74**	0.21	**0.36**	**-0.57**	-0.13	0.16	-0.07	-0.16	0.00	0.13	**0.30**	**0.90**	**0.54**	**0.97**

Underwood and Smith (1998b) model

Benthic chl a and colloidal carbohydrate were significantly correlated in this study and the results were compared to the model published by Underwood and Smith (1998b), which described the relationship using data from European estuaries (Fig. 2.7). The *β* regression parameter of chl a and colloidal carbohydrates obtained in this study (*β* = 1.066) was similar to that (*β* = 1.108) reported for the model and more than 95% of the samples analysed fell within the 95% prediction limits. A *β* regression parameter of > 1 implies that there was a gradual gain of colloidal carbohydrates, relative to benthic microalgal biomass, in the estuaries studied.

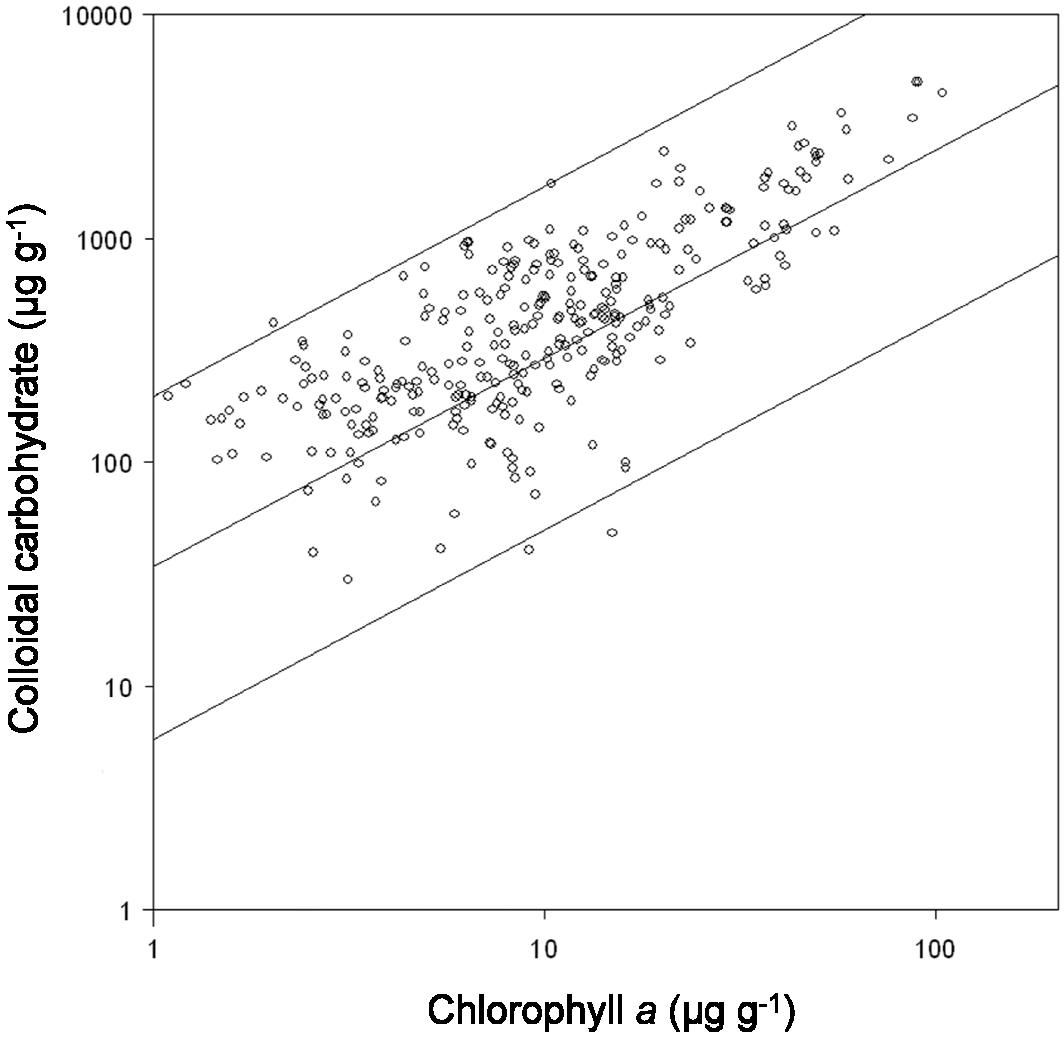

Figure 2.7. Sediment chl a and colloidal carbohydrate content in the Keurbooms, Gamtoos, Swartkops, Sundays, Mngazana and Mngazi estuaries compared to the model (regression line with 95% limits) of Underwood & Smith (1998b).

Discussion

Benthic microalgae are important contributors to primary production in shallow aquatic ecosystems, particularly those with large intertidal areas. This study described the spatial patterns of benthic microalgal biomass and associated benthic carbohydrates, in relation to physical and chemical variables, in the intertidal areas of six permanently open estuaries along the warm temperate and sub-tropical coast of South Africa. Nutrient concentrations in the Keurbooms, Mngazi and Mngazana estuaries were relatively low, with DIN and DIP concentrations less than 2.0 µM and 0.2 µM in the Mngazana and Mngazi estuaries. In the Gamtoos, Swartkops and Sundays estuaries the concentrations of nutrients in the water-column were highest during the winter months (July and August) when rainfall is generally highest. TOxN concentrations at the sites nearest to the heads of the Gamtoos, Swartkops and Sundays estuaries were 61.9, 31.4 and 78.4 µM respectively. SRP was generally less than 2.5 µM in all of the estuaries except for Swartkops Estuary where concentrations ranged from 17.1 to 30.4 µM in the upper reaches during the study. The catchment areas of these estuaries are either heavily industrialised (Swartkops) or are impacted by fertilizers in agricultural return flow (Gamtoos and Sundays estuaries).

All of the estuaries have the three geomorphological zones that are typical of micro-tidal, wave dominated estuaries along the southern and south-eastern coast of South Africa (Cooper *et al.* 1999); sand-dominated flood-tidal deltas near to the mouths, deeper and mud-dominated middle reaches and sand-dominated fluvial deltas near to the heads of the estuaries. The highest proportion of fine particle sediment (< 125 µm) in the intertidal zones occurred where salinity in the overlying water ranged from 8.4 ppt in the Sundays to 32.2 ppt in the Mngazana estuary. Tidal flow and the high variability of river flow frequently resuspend sediment in the lower and upper reaches of estuaries, not favouring the accumulation of fine particulate matter or the colonisation of microalgae (Adams *et al.* 1999).

Fine particle sediment (< 125 µm), organic matter and sediment water content were closely associated in the estuaries studied. This was especially noticeable in the Mngazana and Mngazi estuaries. The high content of organic-rich muds in the middle reaches of these two estuaries was probably the result of the geology of the catchment areas (allochthonous supply) and an accumulation of organic matter generated in the estuary itself (autochthonous supply). The catchment areas are

dominated by shale, which easily erode to form clay (Blyth & de Freitas 1984), and the water temperature frequently exceeded 25 ºC which has been found to accelerate the rate of flocculation and sedimentation of fine particulate materials (Jiang *et al.* 2003). A strong covariance between fine particle sediments, organic matter and the water content in intertidal estuarine sediment has been found in previous studies (Lucas *et al.* 2003; Perkins *et al.* 2003).

Microphytobenthic biomass

The average chl a content during the study was 13.9 ± 0.9 SE µg g^{-1}, ranging from below detectable levels found in marine sand at the mouth of the Keurbooms Estuary to 104.8 µg g^{-1} in organic-rich sediment in the middle reaches of the Mngazana Estuary. These chl a contents, measured in the surface two millimetres of sediment, were generally lower but comparable to contents measured in English, Scottish and Portuguese estuaries (Table 2.4).

Table 2.4.

Microphytobenthic chl a ranges from intertidal sediments in different estuaries.

Estuary	Sample depth (mm)	Chl a range (µg g^{-1})	References
Southampton Water Estuary, England	< 1	21-168	Friend *et al.* (2003)
Eden Estuary, Scotland	2	30-238	Perkins *et al.* (2003)
Arlesford Creek, England	2	< 0.1-460	Perkins *et al.* (2003)
Blackwater Estuary, England	2	88-210	Snow (unpublished data)
Colne Estuary, England	2	6.7-272	Snow (unpublished data)
Stour Estuary, England	2	21-52	Snow (unpublished data)
Colne Estuary, England	2	22-42	Hanlon *et al.* (2005)
Tagus Estuary, Portugal	2	21^{s}-77^{m}	Cartaxana *et al.* (2006)
Ria Formosa, Portugal	2	1-202	Friend *et al.* (2003)

^{s} = average in sand and ^{m} = average in mud

Chl a measured in this study was significantly correlated to TOxN in the water-column and moisture, organic matter and the fine particled sediment contents in the intertidal sediments. This close association between chl a content and sediment associated variables has been found in a number of other studies (Friend *et al.* 2003; Cartaxana *et al.* 2006; Jesus *et al.* 2006). However, reports by Perkins *et al.* (2003) and Tolhurst *et al.* (2005) have described the interdependence of chl a content, a mass ratio, with four other biogeochemical parameters in sediments. As the content of one changes, this affects at least one other parameter, e.g. an increase in fine cohesive sediment results in an increase in pore space, moisture content, chl a content and a reduction in total density. This study found that chl a content (μg g^{-1}) was significantly correlated to chl a concentration but variance did occur at muddy sites with an elevated organic content. In addition, there was a significant, positive correlation between chl a concentration and DIN in the water-column. However, the correlation coefficients of chl a content (0.16) and concentration (0.33) were relatively low so this association should be regarded with caution. The strength of association between microalgal biomass and nutrients was much stronger in the Swartkops Estuary, in relation to the other estuaries, and may be the result of the consistently high nutrient concentrations entering the system from industry or stormwater runoff.

When the effects of sediment content were removed, by adjusting chl a content to the organic content, then sites that had high chl a in relation to organic content could be easily identified. This could be a useful tool to identify sites that have recently been eroded and have been successfully recolonised by benthic microalgae. In contrast, sites with high organic matter and chl a content were more likely to be in a mature state. Factors that may affect the accumulation of fine sediment and organic matter at a site include the intervals between floods, water temperatures in excess of 25 ºC (Jiang *et al.* 2003), geohydrology, accelerated erosion in the estuary's catchment area and the growth rates of microalgae, macroalgae and macrophytes in rivers that flow into the estuary as well as in the estuary itself. Eutrophication can accelerate the growth rates of flora, accelerating the accumulation of decaying plant material.

Carbohydrates

All carbohydrate fractions measured during this study were significantly correlated to chl a, the proportion of fine sediment (< 125 µm) and the organic and water contents. Similar associations have been found in a number of other estuaries (de Brouwer *et al.* 2003; Friend *et al.* 2003; Lucas 2003) and colloidal carbohydrate content has been related to sediment stability (Underwood 2000; Tolhurst *et al.* 2003). This indicates a high carbohydrate content, and more stable sediment, in the middle reaches of permanently open estuaries in the Eastern Cape, South Africa, based on the tripartite geomorphology described by Cooper *et al.* (1999). In general, open estuaries along the coast are micro-tidal (tidal variation is less than two metres), wave-dominated and have three distinct geomorphological zones; a sandy barrier in the mouth region with an associated flood-tidal delta. Sandy sediments generally form wide intertidal flats. Further upstream of the flood-tidal delta there is a deeper area where the deposition of fine particulate matter (clays, silt and organic matter) is favoured, usually enhanced by flocculation. At the upstream limit, the water is shallower and a fluvial delta dominated by coarse sediment is present. Results from this study found the same general pattern in geomorphology and the carbohydrate and microalgal contents were highest in the middle reaches of the estuaries. It is expected that a reduction in river flow, either as a result of water abstraction or the construction of impoundments in the catchment area, and the subsequent reduction in the frequency and intensity of floods will allow the flood-tidal delta to penetrate further upstream. This is likely to result in a larger area of the lower reaches of the affected estuary becoming shallower, broader and the sediment less stable.

De Brouwer *et al.* (2003) attributed the correlation between median grain size and carbohydrate contents (all fractions) to direct and indirect influences of sediment grain size on carbohydrate content. A direct relationship exists between the amount of organic matter adsorbed to sediment grains and sediment grain size. This is because finer sediment particles have a larger surface area to volume ratio than coarse sediment particles providing more adsorption sites. Sediment grain size has an indirect effect on extracellular carbohydrate contents because diatoms maintain higher growth rates on silt-dominated sediments. In turn, the particles at the sediment surface become bound together through the secretion of extracellular mucopolysaccharides, a process termed “biostabilisation”. Over an extended period

of stable conditions, the benthic environment becomes more mature, leading to increased levels of extracellular carbohydrates.

Yallop *et al.* (2000) also found multicollinearity between biological and biochemical variables in the Severn Estuary (U.K.). Once collinear variables were eliminated, only three variables were used in a model to determine sediment stability; water content, EPS and chl a. The model provided evidence that the relationship between these variables could change from “pioneer” to “mature” biofilms. Benthic chl a generally increased as the water content increased but sediment immersion or an erosion event could reset the sediment to an early colonisation phase. An example of an early pioneer phase during the current study would be in the sediment in the mouth area of the Keurbooms Estuary. Tidal resuspension keeps this sediment in a continual pioneer state resulting in low chl a, colloidal carbohydrate and organic contents. The finer sediments in the middle reaches of the Mngazi, Mngazana and Gamtoos estuaries occur in more stable environments allowing microalgal biofilms to reach maturity and an accumulation of organic matter in the cohesive sediment to occur. Yallop *et al.* (2000) described a high bacterial cell density during this phase, which is also capable of contributing to the colloidal carbohydrate content in the sediment.

The foregoing suggests that biogeochemical parameters play an important role in determining the carbohydrate content in intertidal mudflats, supporting a simple model developed by Underwood & Smith (1998b). This model describes a strong relationship between colloidal carbohydrate and chl a and is valid for intertidal mudflats where epipelic diatoms constitute > 50% of the microphytobenthic assemblage. Microalgal biomass and colloidal carbohydrate were strongly correlated in the study reported here and almost all samples fell within the 95% limits of the Underwood and Smith (1998b) model. Other studies of mudflats in Western Europe (de Brouwer *et al.* 2003; Lucas *et al.* 2003) and England (Bellinger *et al.* 2005) have shown similar relationships.

Conclusions

Conditions that favour the accumulation of fine sediment and organic material in estuarine sediments are likely to support a high biomass of benthic microalgae, which will increase the stability of the sediment through the production of colloidal carbohydrates. These conditions include reduced river flow through increased

abstraction and the impoundment of rivers, which decrease the frequency and intensity of floods. Loads of suspended particulate material such as organic matter, fine sediment particles, microalgal cells and absorbed nutrients (DIP in particular) are then more likely to flocculate and settle out of the water-column as a result of these more stable conditions. The process of eutrophication, in which there is a gradual accumulation of organic matter as a result of elevated nutrient concentrations, can also provide a suitable environment for high benthic microalgal biomass. Extremely high benthic chl a concentrations (in excess of 300 mg m^{-2}) were measured in the eutrophic Mdloti and Mhlanga estuaries on the KwaZulu-Natal coast (Perissinotto *et al*. 2004). These estuaries are temporarily open-closed estuaries (TOCE), which makes them more susceptible to the effects of elevated nutrients.

Benthic microalgal biomass in the intertidal sediment of permanently open estuaries was associated with TOxN in the water-column but the association was much stronger with biogeochemical parameters in the sediment (organic and moisture contents and the proportion of mud). This could be the result of, in part at least, measuring chl a as a content and not as a concentration. However, chl a content was significantly correlated to the concentration, and a similar association between microalgal biomass and sediment type was found in the Swartkops Estuary in a previous study using chl a concentration (Rodriguez 1993). The highest chl a contents measured during this study were generally in the intertidal sediments bordering the deeper middle reaches of the estuaries, where deposition typically exceeds the resuspension of sediment. Sites in these reaches of the estuaries had the highest proportion of fine sediment and organic matter, and it is likely that the remineralisation of nutrients from the sediment, NH_4^+ in particular, was an important source of nutrients during the warmer, drier summer months when nutrient concentration was lowest. However, there was a high level of variation in the microalgal biomass and nutrient concentration in the water-column between sampling sites, estuaries and sampling dates. From this it does not seem possible to generate a generic model of factors that determine microalgal biomass in all six estuaries. Instead, this study emphasises the need to conduct more extensive investigations of each estuary to get a better understanding of processes affecting the distribution of the microphytobenthos.

CHAPTER 3

Response of microalgae in the Kromme Estuary to managed freshwater inputs

Snow, G.C. & Adams, J.B. 2006. Response of micro-algae in the Kromme Estuary to managed freshwater inputs. *Water SA*. **32** (1), 71-80.

Abstract

The Kromme is a permanently open estuary that receives little freshwater input because the capacity of the dams is equivalent to the mean annual run-off of the catchment. The estuary is marine dominated and phytoplankton chlorophyll a (chl a) is low because of reduced freshwater pulses that introduce nutrient-rich freshwater. Water released (2 x 10^6 m^3) from the Mpofu Dam in 1998 showed little biological effect on the estuary. This study addresses other run-off scenarios to determine which would be beneficial in stimulating microalgal production. Recent surveys together with past research were used to describe the present state and reference condition of the estuary. Average intertidal chl a was 12.9 ± 2.5 µg g^{-1} and 4.9 ± 0.4 µg g^{-1} during November 2003 and July 2004. These concentrations are low but comparable to those found in intertidal sediments in other South African estuaries which could indicate that intertidal microalgal biomass is not severely limited by low freshwater inputs. Average water-column chl a concentrations have ranged from 0.6 ± 0.1 µg l^{-1} to 5.6 ± 0.3 µg l^{-1}.

Present state conditions can thus be described as that where water-column chl a seldom exceeds 5 µg l^{-1} and small flagellates dominate the phytoplankton. The diatoms introduced via freshwater have been lost. Under reference conditions baseflow would have been greater than 1 m^3 s^{-1} for approximately 8 months of the year. The flocculation of fine particles associated with the mixing of fresh and saline water would have resulted in phytoplankton peaks (chl a >10 µg l^{-1}) in the middle reaches of the estuary. A more suitable habitat would also have been present for the epipelic (motile microalgae that inhabit muddy sediments) microalgae. An assessment of the possible future run-off scenarios indicated that the most beneficial for the microalgae would be a flow release from the Mpofu Dam of 5 x 10^6 m^3 in October and January. This would lead to a 25-33% increase in phytoplankton chl a and a 50% increase in intertidal benthic chl a for approximately 2 months after the releases.

Introduction

The Kromme Estuary is located in the Eastern Cape Province, 80 km west of Port Elizabeth. The estuary is relatively narrow with a mean width of approximately 80 m, and extends for 14 km from a permanently open mouth to a rocky sill that forms the tidal head of the estuary. Its major tributary is the Geelhoutboom River which enters 8 km from the mouth. The catchment size and length of the Kromme River are approximately 1000 km^2 and 100 km respectively. Rainfall shows a bimodal pattern with maxima in autumn and spring. January and February are the driest seasons (Bickerton & Pierce 1988).

There are two dams upstream of the estuary and their combined holding capacity (Mpofu Dam 107 x 10^6 m^3, appendix figure A.1, and the Churchill Dam 33.3 x 10^6 m^3) exceeds the mean annual runoff (MAR) of the Kromme River (estimated to be in excess of 105 x 10^6 m^3) (Reddering 1988). This has resulted in increased salinity towards the head of the estuary. In addition to reduced river flow, the dams have also affected the frequency and magnitude of flood events, and the availability of riverine material replenishing the estuarine nutrient pool (Scharler *et al.* 1997). A release policy, which provides 2 x 10^6 m^3 per annum was proposed to account for the evaporative loss of the estuary (Jezewski & Roberts 1986). However, flow records and personal communication with the Mpofu Dam managers indicate that very few or no releases have been executed for 2002-2005. The construction of farm dams and a weir, together with abstraction of water for agriculture, have significantly reduced the flow of water input from the Geelhoutboom River.

A freshwater release study in 1998 (Bate & Adams 2000; Snow *et al.* 2000a) indicated that a release of 2 x 10^6 m^3 had little beneficial effect on the estuary and that a consistent baseflow was probably necessary to maintain water-column production. The results reported in this study were a component of a Department of Water Affairs and Forestry comprehensive ecological reserve (freshwater requirement) study on the Kromme Estuary. The response of the microalgae to different run-off scenarios was investigated to determine optimal conditions for microalgal production.

The present state (distribution and biomass) of the microalgae was documented based on two recent surveys and past research results (Scharler *et al.* 1997; Bate & Adams 2000; Snow *et al.* 2000a). The reference condition (before anthropogenic

influences) was predicted as well as the response of the microalgae to different freshwater inflow scenarios. These scenarios were:

i) 5 x 10^6 m^3 release from the Mpofu Dam during November,

ii) 5 x 10^6 m^3 release during November and another in January,

iii) Maintain present flow in the Kromme River but increase flow in the Geelhoutboom tributary

iv) 5 x 10^6 m^3 release during November and increased flow from the Geelhoutboom tributary

v) 7.5 x 10^6 m^3 release over a two-month period (October-November).

Materials & methods

Study site

The Kromme Estuary lies in a relatively undisturbed and pristine area. It meanders through rural land and there is little urban, industrial and agricultural development (Baird & Heymans 1996). Recently, there has been a lot of clearing along the banks of the estuary associated with recreational / residential developments. A road bridge has been constructed across the estuary, approximately 3 km from the mouth, and a marina development consisting of a number of canals with waterfront housing and mooring facilities, is located on the west bank near to the mouth (Emmerson *et al.* 1982) (Fig. 3.1). The substrate at the mouth area varies between medium and coarse sand with some silt. In the lower and middle reaches, mud and silt increase whereas the upper reaches consist of coarse silty sand, stones and rock.

The estuary was sampled for microalgae in November 2003 and in July 2004. These surveys focussed on the Geelhoutboom tributary where few data are available. During November 2003, two sampling sites were located below the confluence of the Kromme Estuary and the Geelhoutboom tributary, 1.7 and 8.1 km from the mouth, and two sites were located within the Geelhoutboom, 9.9 and 11.1 km from the mouth. An additional site was located near to the head of the estuary but only physico-chemical measurements were taken there. On 30 July 2004 there were 10 sites in total, all matching the locations of Scharler *et al.* (1997). Sites at 1.7, 5.1, 6.6, 8.1, 10.2, 11.8 and 13.2 km from the mouth closely match the sites sampled during the 1998 freshwater release study (Snow *et al.* 2000a).

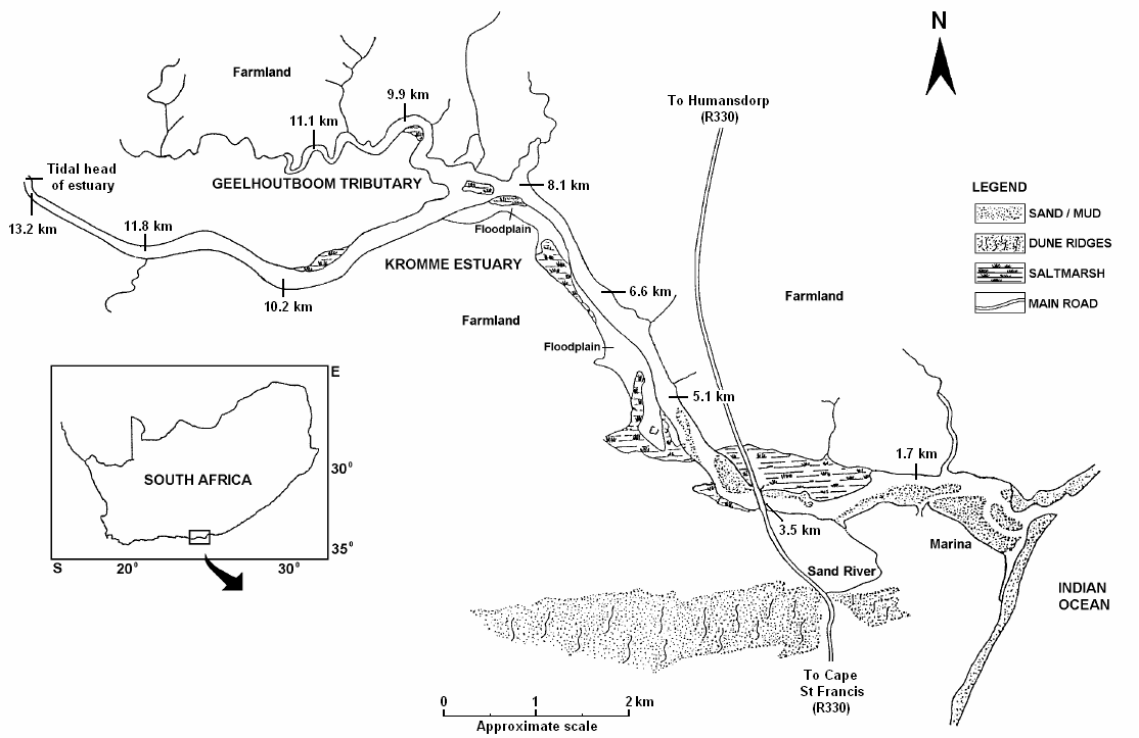

Figure 3.1. Map of the Kromme Estuary and Geelhoutboom Tributary, showing distances of the sampling stations (km from the mouth) for the 2004 surveys (modified from Bickerton & Pierce 1988).

Physico-chemical factors

Water-column conductivity, salinity, dissolved oxygen (DO) content, total dissolved solids and pH were measured using a YSI multiprobe. Measurements were taken every 50 cm from the surface at each site. Water transparency was measured with a Secchi disc.

Phytoplankton biomass

Water-column samples were collected from the surface and bottom and then gravity filtered through plastic Millipore towers using Whatman (GF/C) glass fibre filters. The samples were collected using a 500 ml weighted pop-bottle. Chl a was extracted by placing the filters into glass vials containing 10 ml of 95% ethanol (Merck 4111). The samples were extracted overnight at 1-2 ºC and filtered. Absorbances were read at 665 nm, before and after acidification, using a UV/Vis spectrophotometer. Chl a concentration was calculated according to Hilmer (1990).

Microphytobenthos biomass

Microphytobenthos biomass was estimated using benthic chl a (µg Chl a g^{-1} freeze-dried sediment; expressed as µg g^{-1}). Four 1 cm deep intertidal and subtidal sediment cores were collected from each site, frozen and kept in the dark before being freeze-dried in the Secfroid Lausanne Suisse freeze-drier. The process of freeze-drying removes interstitial water that improves chlorophyll extraction. Once freeze-dried, 4 ml of 95% ethanol were added to approximately 100 mg of sample and pigments were extracted in a fridge for 24 hours.

After extraction the samples were well mixed using a whirlmixer (WM/250/SC/P) and the extract injected into a high performance liquid chromatograph (HPLC) attached to Waters-Lambda-Max 481 LC spectrophotometer and Waters LM-45 solvent delivery system for chl a analysis. A 30% methanol and 70% acetone mixture was used as a carrier. The system was calibrated using the chl a of red seaweed (*Plocamium collorhiza*) because it contains no chlorophyll *b* that might interfere with the chl a reading. Chl a absorbance was measured at 665 nm and the concentration determined using the modified equation of Nusch (1980) (Snow *et al.* 2000a).

Phytoplankton identification

Surface and bottom water (500 ml) were collected from each site and preserved with 1 ml of 25% Glutaraldehyde solution for phytoplankton identification. The filtrate from phytoplankton chl a was preserved using mercuric chloride and frozen for nutrient analysis.

Two drops of Rose Bengal were added to 60 ml of the preserved water samples and poured into a 26.5 mm internal diameter settling chamber and allowed to stand for 24 hours before identification with a Zeiss IM 35 inverted microscope at 630X magnification. A minimum of 200 cells was counted in each sample and the cells were classified as small flagellates (3.5 µm x 2.8 µm), big flagellates (6.7 µm x 4.2 µm), diatoms, dinoflagellates, cyanophytes (blue-green algae), chlorophytes (green algae), euglenoids and coccolithophorids. Data were only available for the November 2003 survey.

The numbers of cells were calculated in 1 ml using the formula:

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

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 (ml)

Nutrients

Total oxidised nitrogen (TOxN; Nitrate + nitrite) concentration was determined using the reduced copper-cadmium method (Bate & Heelas 1975). TOxN was collected from the surface and the bottom at each site using a weighted pop-bottle. The nitrate in the filtered (Whatman GF/C filters) water samples was reduced to nitrite then determined as NO_2^--N plus NO_3^--N. Nitrite was quantitatively incorporated into a diazo-couple (red/purple) compound and then the concentration determined at 540 nm using a GBC UV-VIS spectrophotometer. All results were expressed in µMolar values as these units obviate any problems of interpretation between N and other oxidised nitrogen (NOx) compounds when using mass based units (mg ℓ^{-1}).

Ash-free dry weight (AFDW) and moisture content

Three 1 cm sediment samples were taken from the intertidal and subtidal zones. In the laboratory the wet sediments were weighed and placed into pre-dried (24 hours at 105 ºC) and pre-weighed crucibles. The sediments were dried in the oven for 24 hours at 105 ºC and were weighed again (dry weight). The crucibles were placed into a furnace at 550 ºC for an hour and were weighed again (ash-free dry weight). Moisture content is the difference between the wet weight and dry weight, and the organic content is the difference between the dry weight and ash weight.

Statistical analyses

Before testing for significant differences, the Kolmogorov-Smirnov Test was used to check if the data were parametric. If the data were parametric, then a Students t-test was used to compare two sets of data for significant differences and the Tukey's test was used if there were more than two sets. However, if the data were non-

parametric, then a Mann-Whitney Rank Sum test was used to compare two sets of data and the Kruskal-Wallis ANOVA on Ranks test was used if more than two sets were compared. If the data sets were of unequal sizes, then the Dunn's All Pairwise Multiple Comparison Procedures method was used (ANOVA on ranks). Pearsons Product Moment Correlation was used to test the strength of association between variables. All tests were performed using the statistical software package Statistica (Version 7).

Results and Discussion

Microalgal survey: 30 July 2004

Both water quality and microalgal chl a remained relatively constant throughout the Kromme Estuary and Geelhoutboom tributary. There was a gradual decrease in temperature, electrical conductivity (EC) and redox potential (ORP) from the mouth to the head of the Kromme Estuary (Fig. 3.2), suggesting that there was an insignificant freshwater input at the head of the estuary and in the Geelhoutboom tributary. Temperature and EC were relatively low in the Geelhoutboom tributary, 11.1 km from the mouth (13.2 ºC and 41.1 mS cm^{-1} respectively). Factors such as salinity, total dissolved solids and turbidity remained relatively constant throughout the estuary with just a slight decrease at the site nearest to the head of the estuary. Salinity, TDS and pH were lowest in the Geelhoutboom tributary, 11.1 km from the mouth (35 ppt, 34.5 g l^{-1} and 8.15 respectively). In contrast, turbidity and light attenuation through the water-column were highest in the Geelhoutboom, with maxima of 100.7 nephelometric turbidity units (NTU) and 3.04 m^{-1} respectively. Pearsons Correlation analyses found significant decrease in temperature ($r = -0.823$; $P < 0.01$; $n = 10$) and electrical conductivity ($r = -0.657$; $P < 0.05$; $n = 10$) with increasing distance from the mouth of the estuary. Salinity was significantly correlated to total dissolved solids (TDS) ($r = 0.999$; $P < 0.01$; $n = 10$) and pH ($r = 0.846$; $P < 0.01$; $n = 10$), which suggests that the majority of TDS was made up of salt and this determined the pH of the water in the estuary.

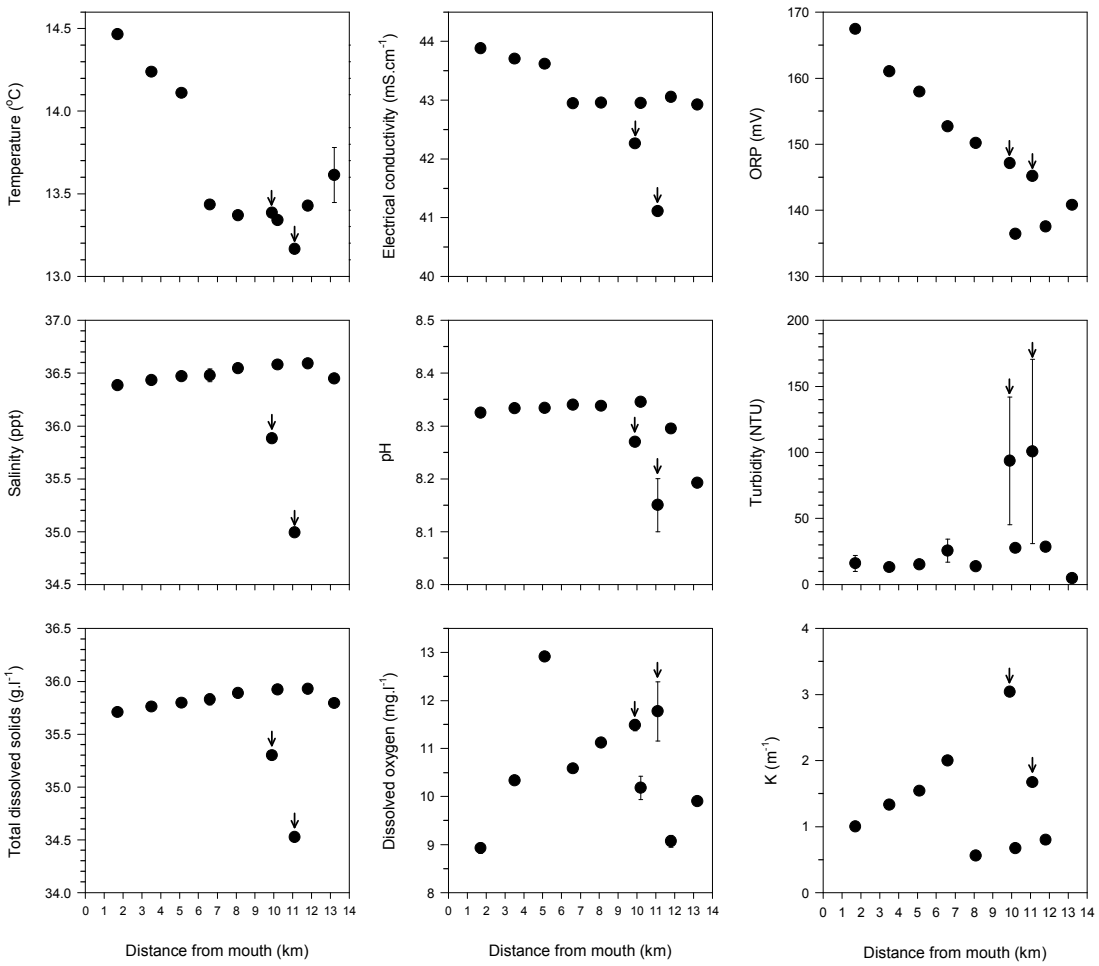

Figure 3.2. Physico-chemical variables measured in the Kromme Estuary (30 July 2004) in relation to distance from the mouth. Arrows indicate measurements recorded in the Geelhoutboom Tributary. Vertical bars represent ± SE mean.

Phytoplankton chl a remained relatively constant throughout the estuary with only a slight decrease at the site nearest to the head of the estuary. The highest average phytoplankton chl a was 5.0 µg l^{-1}, 9.9 km from the mouth in the Geelhoutboom tributary (Fig. 3.3), but there were no significant differences in phytoplankton chl a between all sites ($P > 0.05$). There was a gradual decrease in phytoplankton chl a with increasing distance from the mouth of the estuary and was significantly correlated with light attenuation through the water-column. This suggests that phytoplankton cells contributed significantly to the attenuation of light and/or were closely associated with suspended particles in the water-column.

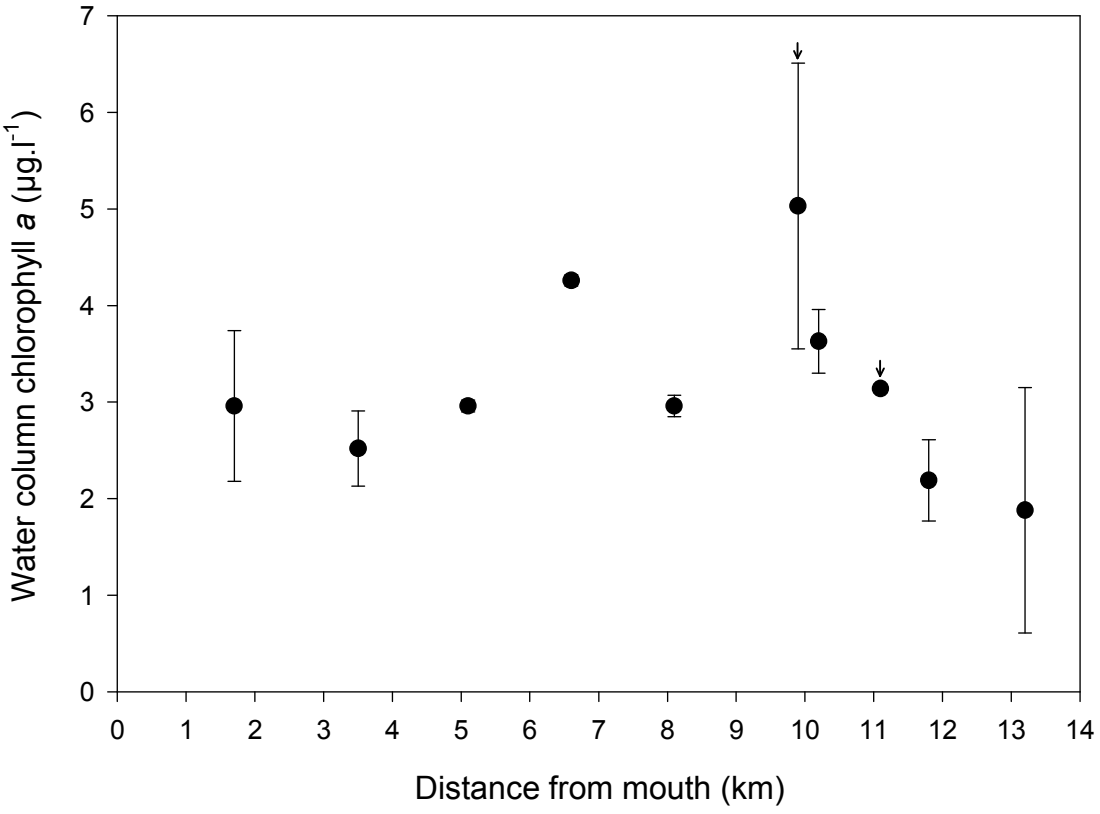

Figure 3.3. Water-column chl a in the Kromme Estuary (30 July 2004). Arrows indicate measurements recorded in the Geelhoutboom Tributary. Vertical bars represent ± SE mean.

Intertidal benthic chl a ranged from 1.8 ± 0.2 µg g^{-1} to 12.4 ± 0.7 µg g^{-1}, showing a gradual increase from near the mouth to sediment in the Geelhoutboom tributary, 11.1 km from the mouth (Fig. 3.4). Subtidal benthic chl a was significantly higher than intertidal chl a ($t = -2.805$; $P = 0.007$; $n = 28$), ranging from 3.6 ± 0.7 µg g^{-1} (1.7 km from the mouth) to 12.7 ± 0.8 µg g^{-1} (5.1 km from the mouth) (Fig. 3.4). There was no significant correlation between subtidal benthic chl a and any physico-chemical factor but intertidal chl a was inversely correlated to salinity ($r = -0.837$; $P < 0.05$; $n = 7$) and its covariables (TDS and pH). As a result intertidal chl a increased from the mouth of the estuary to 11.1 km from the mouth in the Geelhoutboom tributary and was highest where overlying water had the lowest electrical conductivity, salinity, pH and temperature. This indicates a strong response to freshwater entering the Geelhoutboom tributary.

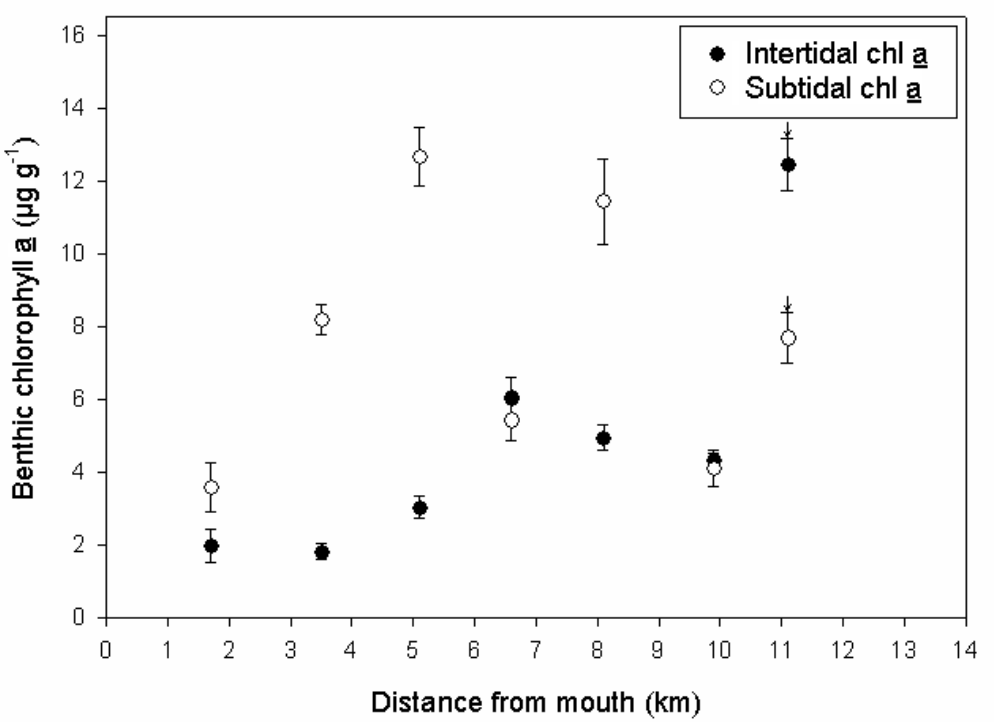

Figure 3.4. Intertidal and subtidal benthic chl a in the Kromme Estuary (30 July 2004). Arrows indicate measurements recorded in the Geelhoutboom Tributary. Vertical bars represent ± SE mean.

Microalgal survey: 24 November 2003

There was a weak longitudinal salinity gradient in the estuary (Fig. 3.5). Average water-column salinity at the mouth of the estuary was 36.2 ppt, which was significantly higher than 9.9 km from the mouth of the estuary (33.9 ± 0.1 ppt) ($Q = 3.65$; $P < 0.05$; $n_{Kromme} = 3$ & $n_{Geelhoutboom} = 5$) in the Geelhoutboom tributary. Average salinity at the head of the Kromme Estuary was 34.4 ± 0.0 ppt. However, both the longitudinal and vertical salinity gradients (< 2 ppt and < 1 ppt respectively) were small (Fig. 3.5). TDS and conductivity followed a similar pattern to salinity, with ranges of 33.5 to 35.5 g l^{-1} and 51.6 to 54.6 mS cm^{-1} respectively.

Light attenuation was highest (K = 2.83 m^{-1}) close to the mouth due to the high turbidity. Lower values were found at the sites in the Geelhoutboom tributary, 9.9 km from the mouth (K = 2.3 m^{-1}) and at 11.1 km from the mouth (K = 2.1 m^{-1}). No significant correlation was observed between light attenuation and water-column depth ($P > 0.05$) (Fig. 3.5).

The highest temperature was measured in the Geelhoutboom tributary (24.5 ± 0.3 °C), 11.1 km from the mouth, and the lowest temperature was 1.7 km from the mouth (20.5 ± 0.3°C) (Fig. 3.5).

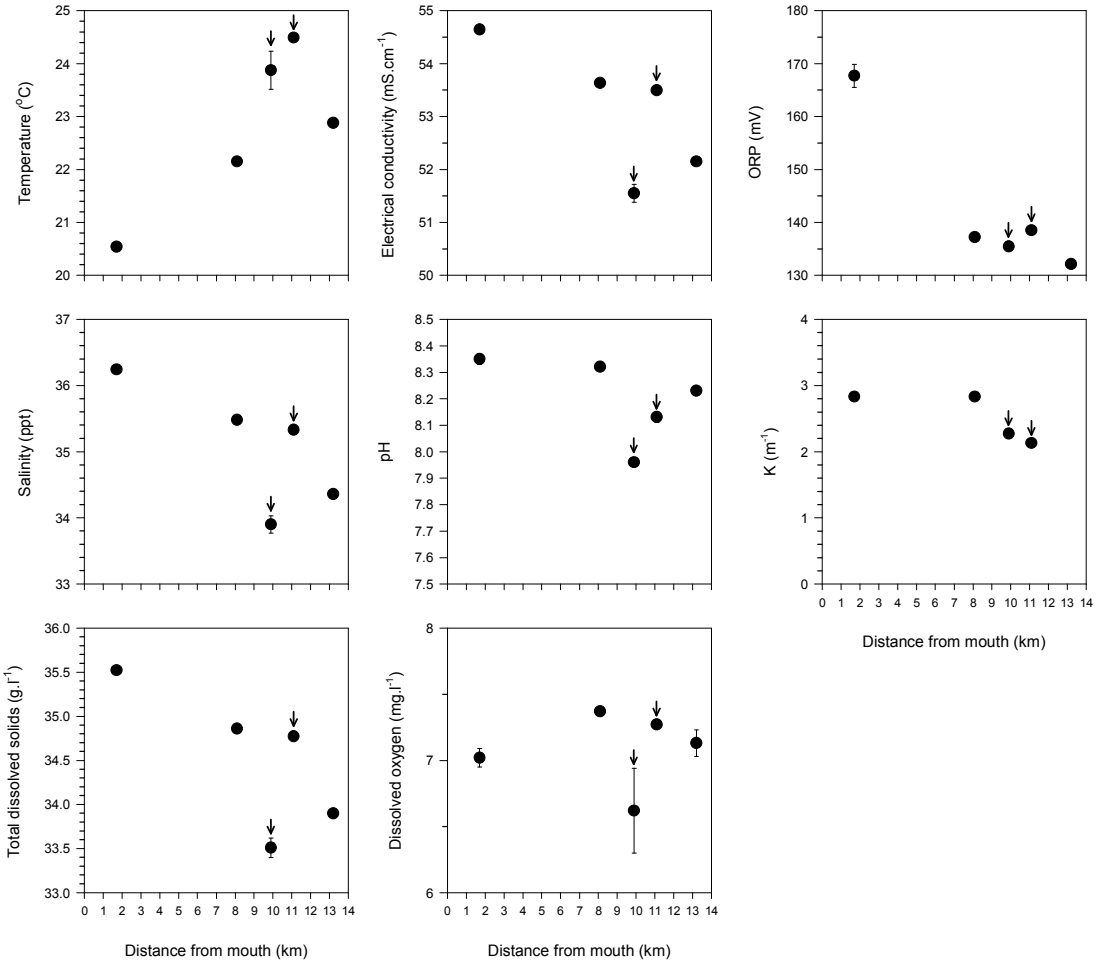

Figure 3.5. Physico-chemical variables measured in the Kromme Estuary (24 November 2003) in relation to distance from the mouth. Arrows indicate measurements recorded in the Geelhoutboom Tributary. Vertical bars represent ± SE mean.

Mean phytoplankton chl a was particularly low during this sampling session (< 1.0 µg l^{-1}) but significantly higher in the surface water compared to the bottom water (T = 320; *P* < 0.036; n = 16) (Fig. 3.6). Highest chl a concentration was observed at the surface in the Geelhoutboom tributary (0.9 ± 0.2 µg l^{-1}), 9.9 km from the mouth of the estuary and TOxN concentration was non-detectable.

Recently built farm dams, abstraction and a weir appear to have reduced flow entering the estuary from the Geelhoutboom tributary. During this study, salinity in the tributary was slightly lower and turbidity slightly higher than that of the Kromme Estuary. This suggests freshwater input from the Geelhoutboom River might have contributed to the elevated chl a in the water-column of the Geelhoutboom tributary (Fig. 3.7).

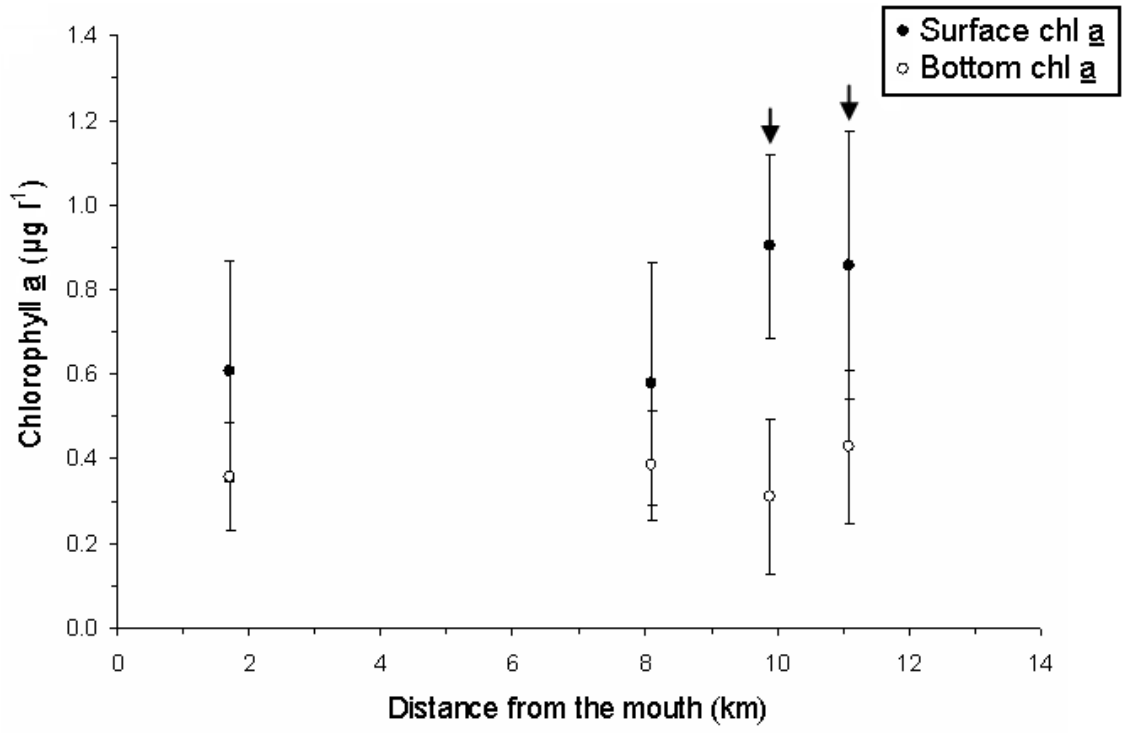

Figure 3.6. Water-column chl a in the Kromme Estuary (24 November 2003). Arrows indicate measurements recorded in the Geelhoutboom Tributary. Vertical bars represent ± SE mean.

Mean intertidal chl a (12.9 ± 2.5 µg g^{-1}) was significantly higher (T = 359; P < 0.001; 16) than subtidal chl a (5.2 ± 1.5 µg g^{-1}) and was particularly high close to the mouth of the estuary (27.1 ± 7.0 µg g^{-1}) (Fig. 3.7). This could be the result of the sites close proximity to the entrance of the St Francis marina canals.

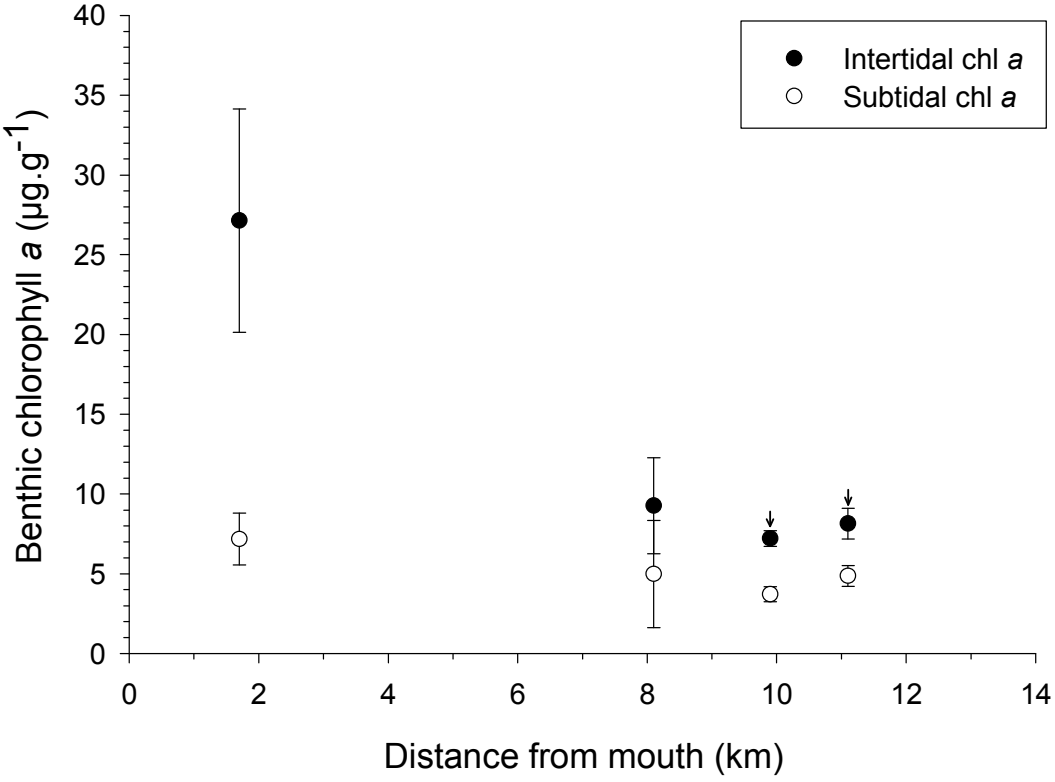

Figure 3.7. Intertidal and subtidal benthic chl a in the Kromme Estuary (24 November 2003). Arrows indicate measurements recorded in the Geelhoutboom Tributary. Vertical bars represent ± SE mean.

Microalgal community composition

On 24 November 2003 flagellates dominated the phytoplankton groups. Small flagellates (3.5 x 2.8 µm) in particular had the highest relative abundance, exceeding 60% at the surface and the bottom throughout the estuary and Geelhoutboom tributary (Fig. 3.8). Diatoms, dinoflagellates, cyanophytes, euglenophytes, chlorophytes and coccolithophorids showed no distinct patterns in the estuary but all groups were represented in water near to the mouth of the estuary.

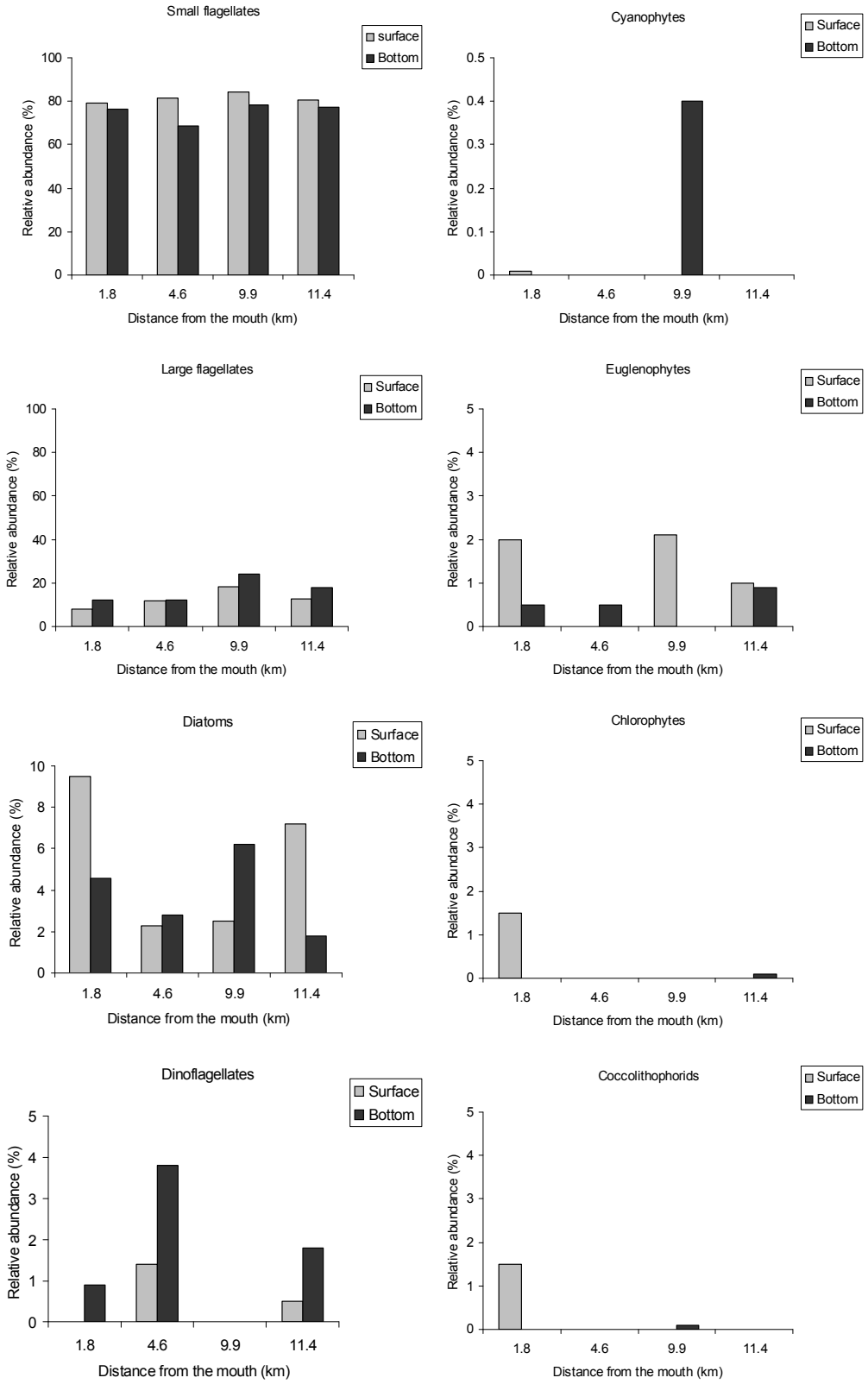

Figure 3.8. Relative abundances of Protista groups and large cyanobacteria in surface and bottom water in the Kromme Estuary (1.8 and 4.6 km from mouth) and Geelhoutboom tributary (9.9 and 11.4 km from the mouth) on 24 November 2003.

Phytoplankton in the Mpofu Dam, collected prior to the release of 2 x 10^6 m^3 freshwater in November 1998 was dominated by diatoms (> 1500 cells ml^{-1}) and flagellates (> 500 cells ml^{-1}). Chlorophytes were present throughout the water-column (> 100 cells ml^{-1}) whereas dinoflagellates were totally absent (Fig. 3.9).

Phytoplankton in the Kromme Estuary during the freshwater release study of 1998/1999 (Snow *et al.* 2000a) was dominated by flagellates prior to and just after the freshwater release. Up to 80% of cells were flagellates and the bulk of the remaining cells were diatoms. By the 17th day after the release blooms of *Extubocellulus* and *Leptocylindrus* spp. had developed 11.8 km from the mouth of the estuary. Diatoms then became the dominant microalgal group with cell densities of more than 11000 cells ml^{-1}. The bloom was short-lived and flagellates dominated the phytoplankton by the 31st day again.

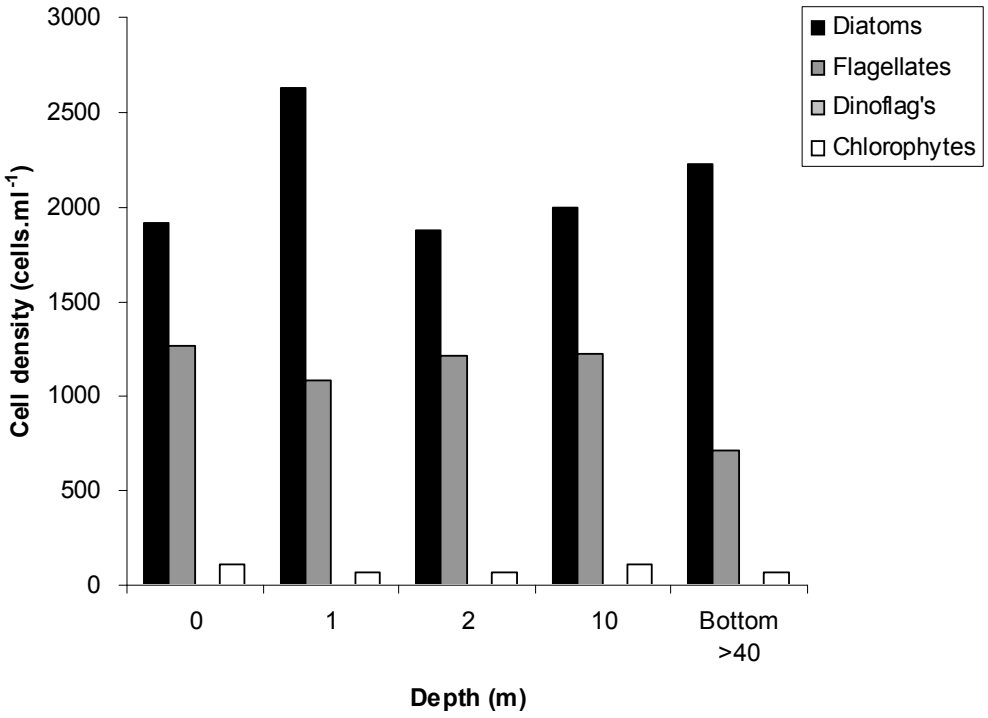

Figure 3.9. Cell densities of diatoms, flagellates, dinoflagellates and chlorophytes in the Mpofu Dam prior to the 2 x 10^6 m^3 release of water (16 November 1998) (Snow *et al.*, 2000a).

Comparison with other studies

Mean phytoplankton chl a in the Kromme Estuary was lower than in the nearby eutrophic Gamtoos estuary (Table 3.1). Mean concentrations in the Kromme ranged

from 0.6 ± 0.1 to 5.6 ± 0.3 µg l^{-1}. Mean salinity during this study (November 2003 and July 2004) was generally similar or higher than seawater and low standard error values indicate weak salinity gradients throughout the estuary. During these extended periods of low freshwater input, mean phytoplankton chl a was particularly low (3.0 µg l^{-1}). After a 2 x 10^6 m^3 pulse of freshwater in the 1998/1999 study (Snow *et al.* 2000a), average chl a concentration increased to a maximum of 5.6 ± 0.3 µg l^{-1}, almost a month after the release.

Average intertidal chl a was 12.9 ± 2.5 µg g^{-1} and 4.9 ± 0.4 µg g^{-1} in the Kromme Estuary during November 2003 and July 2004 of this study respectively. These concentrations are relatively low but comparable to those found in intertidal sediments in other South African estuaries (Table 3.2). The latter indicates that intertidal microalgal biomass is not limited by freshwater inputs.

Table 3.1.

Average salinity and phytoplankton chl a (± standard error) during this study (italics) and previous studies (Scharler *et al.* 1997; Snow 2000; Snow *et al.* 2000a & b) in the Kromme and nearby Gamtoos Estuary.

Estuary	Sampling date	Salinity (ppt)	Phytoplankton chl a (µg l^{-1})
Kromme	*24 November 2003*	*35.0 ± 0.4*	*0.6 ± 0.1 (n = 20)*
	30 July 2004	*36.3 ± 0.2*	*3.0 ± 0.2 (n = 36)*
	16 November 1998	34.2 ± 0.1	4.2 ± 1.1 (n = 30)
	18 November 1998	21.5 ± 2.1	1.7 ± 0.2 (n = 35)
	20 November 1998	23.7 ± 1.3	2.1 ± 0.2 (n = 37)
	22 November 1998	29.4 ± 0.6	4.4 ± 0.5 (n = 27)
	3 December 1998	31.9 ± 0.4	4.3 ± 0.3 (n = 26)
	17 December 1998	34.1 ± 0.2	5.6 ± 0.3 (n = 25)
	5 January 1999	35.4 ± 0.1	5.2 ± 0.7 (n = 25)
	June 1993 – June 1994	28.7 ± 0.5	4.2 ± 3.3
Gamtoos	4 November 1996	12.5 ± 2.1	17.2 ± 1.6
	11 February 1997	9.8 ± 1.9	44.8 ± 6.1
	12 November 1996	10.8 ± 2.1	7.4 ± 0.5

Table 3.2.

Average intertidal chl a during this study (italics) and studies of other South African estuaries (Snow, unpublished data).

Estuary	Sampling date	Intertidal chl a ($\mu g\ g^{-1}$)
Kromme	*24 November 2003*	*12.9 ± 2.5 (n = 20)*
	30 July 2004	*4.9 ± 0.4 (n = 28)*
Gamtoos	7 August 2002	27.7 ± 3.0 (n = 25)
	21 February 2003	10.4 ± 0.9 (n = 25)
Keurbooms	25 August 2002	9.5 ± 0.8 (n = 25)
Mngazana	22 June 2003	27.2 ± 2.3 (n = 25)
	25 January 2003	16.4 ± 2.5 (n = 25)
Mngazi	26 January 2003	12.9 ± 0.9 (n = 25)
Sundays	19 February 2003	9.1 ± 1.0 (n = 25)
	25 July 2002	10.2 ± 1.0 (n = 25)
Swartkops	5 December 2002	3.7 ± 0.6 (n = 30)
	12 February 2002	11.2 ± 0.9 (n = 30)
	15 August 2003	22.5 ± 1.9 (n = 30)
	28 November 2001	5.9 ± 0.9 (n = 30)
	30 October 2001	8.2 ± 0.8 (n = 30)

Present state

The Mpofu and Churchill dams have severely reduced river flow into the Kromme Estuary. Flows of < 0.2 $m^3\ s^{-1}$ are often observed over a number of months resulting in hypersaline conditions in the upper reaches (CSIR 2005). During these conditions, regarded as present state, chl a in the water-column seldom exceeds 5 $\mu g\ l^{-1}$ and small flagellates dominate the phytoplankton.

The Kromme Estuary is marine dominated and can be regarded as oligotrophic. Phosphate occurs in oxic sediments as the essentially insoluble Fe (III) species, but in anoxic environments iron is reduced biologically and phosphate may become solubilized. The mineralization of organic matter into ammonium and the subsequent nitrification in oxic sediment generally results in ammonium concentrations in

sediment pore waters decreasing towards the sediment surface. Where there is a significant benthic oxic layer, it acts not only as a redox barrier for phosphate and iron, but also as a potential nitrification barrier for ammonium (Nedwell *et al.* 1999). The reduction in the frequency and intensity of freshwater pulses has reduced the number of scouring events that are required for the regeneration of 'trapped' nutrients in fine sediment within the Kromme Estuary. Lower nutrient loads reach the estuary, reducing nutrient concentrations within the estuary, which could limit benthic microalgal biomass. Based on a 25% increase in phytoplankton chl a following a 2 x 10^6 m^3 release of water from the Mpofu Dam, it is likely that mean phytoplankton chl a in the estuary has decreased by ~33%. The decrease could be greater but nutrient and TSS levels in the Kromme River are considered to be naturally low (Emmerson *et al.* 1982) and a high biomass similar to the nearby eutrophic Gamtoos Estuary (approximately 45 µg l^{-1}) is unlikely.

Table Mountain Quartzite and Bokkeveld shale occur in the Kromme River catchment (Reddering & Esterhuysen 1983) and therefore inputs of sand and mud are naturally low. However, the Geelhoutboom River is still a source of sediment and this resulted in the higher turbidity measured in the Geelhoutboom tributary during this study. The flocculation of TSS and nutrients during normal flow provides a suitable habitat for epipelic (mud-associated) microalgae. These microalgae are dependent on the continual input and sedimentation of fine sediment in estuaries. Diatoms generally dominate epipelic communities and stabilise the sediment through their carbohydrate exudates used for migration within the surface layers of the sediment (Pinckney & Zingmark 1993; Miller *et al.* 1996; Austen *et al.* 1999). Coarser sand sediments are colonised by episammic microalgae that attach themselves to the sand particles to resist resuspension during periods of high energy (e.g. tidal currents). Fine cohesive sediments usually have high organic matter content, with high rates of bacterial mineralization and high porewater concentrations of dissolved nutrients, while sandflats are more oligotrophic (Underwood & Kromkamp 1999). Chl a or biomass of microphytobenthos in sheltered, muddy habitats is higher than that in exposed sandy habitats (MacIntyre *et al.* 1996) and accounts for some of the higher benthic chl a values recorded in the Geelhoutboom tributary. Prolonged stable conditions and reduced sediment input would favour the dominance of only a few benthic species thus reducing microalgal diversity.

Reference state

River flow prior to the construction of dams in the catchment would have been in excess of 1 m^3 s^{-1} for about 8 months of the year (CSIR 2005). Dams would not have been present so the higher concentration of suspended solids would have flocculated and settled out of suspension once the fresh riverwater and saline estuarine water mixed. The flocculation of fine particles would result in a peak in phytoplankton chl a (> 10 µg l^{-1}) particularly in the middle reaches of the estuary. The latter has also been shown by Hilmer (1990) for the Sundays Estuary. Because of this, there is a suitable environment for epipelic microphytobenthos as well as a source of nutrients for subtidal benthic microalgae. A month after a 2 x 10^6 m^3 release of water from the Mpofu Dam, benthic chl a was > 100 mg m^{-2} at sites near to the mouth, both subtidal and intertidal, and > 60 mg m^{-2} in the intertidal sediment at sites located >5 km from the estuary mouth (Snow *et al*. 2000a). A strong halocline would have existed in the middle and upper reaches (> 5 km from the mouth) so high benthic microalgal biomass, in response to nutrients in the fresher water, would only occur in the intertidal microalgae (commonly > 60 mg m^{-2}). The turbulent flow of water near to the mouth would result in high intertidal and subtidal benthic chl a (> 100 mg m^{-2}).

High phytoplankton biomass (> 20 µg l^{-1}) would have been limited by the naturally low nutrient concentrations of riverwater and residence time. In addition, a pulse of freshwater has been shown to result in a rapid increase in mysids and copepods, which would exert grazing pressure on the phytoplankton community (Jerling & Wooldridge 1994). The CSIR (2005) has shown that a persistent flow of 4-8 m^3 s^{-1} would scour saline waters from deeper areas in the upper reaches but residence time would only be 3-7 days. This residence time would be insufficient for a high phytoplankton biomass to develop.

During a recent study of the nearby Gamtoos Estuary (Snow 2000; Snow *et al.* 2000b), the river-estuary interface zone (REI) was defined as the distance from the mouth of the estuary where a maximum phytoplankton chl a occurred. This generally occurred where vertically averaged salinity was less than 10 ppt. The REI zone is a concept developed in estuaries with a classical “cone-shaped” bathymetry. The upper reaches are narrow and shallow and become deeper and wider towards the lower reaches of the estuary. This means that as freshwater enters an estuary, a longitudinal salinity gradient develops from near zero at the head to approximately 35 ppt near the mouth. This bathymetry allows the ‘fresh’ water to flow over the denser

saline water with gradual mixing. Chl a concentration in the REI of the Gamtoos Estuary was particularly high at a flow of 1 $m^3 s^{-1}$ or residence time of 3 spring-neap tidal cycles (42 days). In the case of the Kromme Estuary, the bathymetry is reversed (i.e. shallow at the mouth and deepest at the head of the estuary). The large volume of water in the upper reaches of the estuary probably limits the effectiveness of the freshwater input in reducing salinity, unless the input is maintained over an extended period. If it were possible for a REI zone to develop in the Kromme Estuary, phytoplankton biomass would still be limited by the naturally low concentration of nutrients and suspended particles.

Microalgal diversity would be high as the phytoplankton community structure would change from being diatom-dominated near the head of the estuary and flagellate-dominated close to the mouth.

Dinoflagellates would also occur but only in saline water near the mouth of the estuary. Snow *et al.* (2000a) showed that freshwater entering the estuary from the Mpofu Dam is dominated by diatoms (> 60% of phytoplankton), flagellates (~30%) and also contains chlorophytes (~5%). As flow has decreased from reference to present state the community structure has changed to one dominated by small flagellates throughout the estuary.

Table 3.3.
Proposed future run-off scenarios indicating flow releases from the Mpofu Dam and the annual volume of river inflow.

Kromme Estuary	***Geelhoutboom***	***Volume*** (10^6 m^3 $annum^{-1}$)
Reference	Reference	97
Present Flows (2 x 10^6 m^3 release)	Present	35
1. Flow release of 5 x 10^6 m^3 (Nov)	Present	38
2. Flow release of 10 x 10^6 m^3 (Oct and Jan)	Present	43
3. Present Flows (2 x 10^6 m^3 release)	Flow from Geelhoutboom River	35
4. Flow release of 5 x 10^6 m^3 (Nov)	Flow from Geelhoutboom River	38
5. Flow release of 7.5 x 10^6 m^3 (Oct - Nov)	Present	41

Comparison of proposed future run-off scenarios

A number of future run-off scenarios (Table 3.3) were considered in the comprehensive assessment of the ecological reserve of the Kromme Estuary (DWAF 2005). Scenario 1 would consist of a flow release of 5 x 10^6 m^3 from the Mpofu Dam in November (Table 3.3). November was chosen for the release as the establishment of a longitudinal salinity gradient will act as a cue for fish migration into the estuary which mainly occurs in late spring and summer (Whitfield 1999). However for 11 months of the year, the flow will be similar to the present state, i.e. no or very little flow. As a result, there will be no or very limited mixing of freshwater with estuarine water and microalgal response would be limited. During the flow release month microalgal biomass would increase due to the introduction of some nutrients and TSS into the estuary. Based on the increases in phytoplankton and benthic microalgal biomasses shortly after a 2 x 10^6 m^3 release of water from the Mpofu Dam (Snow *et al*. 2000b), this is likely to stimulate a 25-33% increase in phytoplankton chl a and a doubling in intertidal benthic chl a for a period of two months after the release. A strong halocline would develop in the upper and middle reaches of the estuary so subtidal benthic chl a is unlikely to change significantly. From about one week after the release, diatoms would dominate the phytoplankton (> 50%) but this would not last for longer than 2-3 weeks before flagellates become dominant. The effects of the freshwater on the microalgae will only persist for approximately 2 months. The rest of the year would be similar to the present state.

Scenario 2, a flow release of 5 x 10^6 m^3 from the Mpofu Dam in October and another in January would introduce nutrients and TSS into the estuary. The microalgal response would be similar to that described for Scenario 1, although biomass would increase on two occasions and there would therefore be a longer lasting effect in the estuary. There would be a 25-33% increase in phytoplankton chl a and a doubling in intertidal benthic chl a for a period of two months after both releases. The rest of the year would be similar to the present state.

For Scenario 3, the Kromme will be in its present state (2 x 10^6 m^3 release) and the Geelhoutboom will have a flow > 0.08 m^3 s^{-1} for two months per year. The Geelhoutboom tributary is shallow in its upper reaches and deepens towards the confluence with the Kromme. This bathymetry is most likely to support the formation of a REI zone and a longitudinal salinity gradient. Based on phytoplankton chl a measured in the Geelhoutboom (November 2003 and July 2004), which ranged from

0.9 µg l^{-1} to 5.0 µg l^{-1}, a continuous base flow is likely to support a higher biomass, ~6-10 µg l^{-1}. The gradual increase in organic matter, fine sediment and nutrient content in the sediment of the Geelhoutboom tributary would lead to an increase in benthic microalgal biomass. However, freshwater input is limited to two months and overall contribution to the Kromme Estuary would not be significant as the volume of the tributary is small relative to the Kromme.

Scenario 4 (flow release of 5 x 10^6 m^3 into the Geelhoutboom in November), is similar to Scenario 1 as the freshwater release will create a salinity gradient in the Kromme Estuary in November. However conditions are improved in the Geelhoutboom tributary after the establishment of a salinity gradient in September, October and November; a combination of natural flow and released freshwater. The responses would be similar to those described for Scenario 3.

Based on hydrological information, there is little difference between Scenario 5, flow release of 7.5 x 10^6 m^3 in October and November, and Scenario 2. The only difference is that the release will occur over two consecutive months and will have a flow of 1-2 m^3 s^{-1}. This is similar to the slightly stronger two-month flow but separated by two months as described for Scenario 2.

A comparison of the different scenarios indicates that freshwater inflow to the Geelhoutboom tributary would be beneficial to the tributary but would have little overall effect on the Kromme Estuary. The best management option for improving microalgal biomass and diversity is to increase flow to the Kromme Estuary via releases from the Mpofu Dam. Scenario 2 with flow releases in both October and January would be most beneficial.

CHAPTER 4

Response of microalgae in the Berg Estuary to reduced freshwater input

Abstract

The Berg River Estuary is a permanently open estuary in the Mediterranean-type climate of the Western Cape Province of South Africa. Construction of the Berg River Dam was completed in 2007 and it was expected to influence the quantity and quality of river water entering the estuary. This study determined the distribution of phytoplanktonic and benthic microalgal communities throughout the Berg Estuary, and then used the results to predict changes in the microalgal biomass in response to reduced flow. Salinity profiles measured in August and November 2005 indicated distinct differences in flow between winter and summer. Riverwater was the greatest source of nutrients in August and previous research has shown that the source switches to marine water during periods of low flow. Strong river and tidal flows appeared to be the major factors preventing phytoplankton biomass from exceeding 8 µg l^{-1}. Benthic chlorophyll a concentration was significantly higher in August compared to November and the pattern of distribution was dissimilar to that observed in other South African estuaries. Flagellates were the dominant group of phytoplankton at most times during the study. Particularly high densities of phytoplankton were present 10.1 km from the mouth in August, dominated by flagellates (77% of total cells) and 28 km from the mouth in November, dominated by diatoms (86% of total cells). Predicted responses of microalgae to reduced river inputs are discussed in detail. Two sampling sessions, coinciding with low and high flow conditions as outlined in the intermediate RDM procedure, were insufficient to accurately determine changes in microalgal biomass and community structure. Instead, predictions had to be made based on results available for other South African estuaries.

Introduction

Since the National Water Act (No. 36 of 1998), which provided the rights to water for basic human needs and sustainable ecological function, the amount of water needed for an estuary to retain its ecological status (estuarine ecological reserve) has been determined for a number of estuaries. There are four levels of assessments for estuaries: desktop, rapid, intermediate and comprehensive. Due to budget constraints, the intermediate determination of Resource Directed Measures (RDM) is the most frequently implemented method. The data requirements for the abiotic and biotic components (including the microalgal component) specify that only two sampling trips, coinciding with typically low and high flows, be undertaken. An example of an intermediate study of the Berg Estuary is described here and the RDM methods analysed.

Research on estuarine microalgal ecology has been neglected in South Africa, with the main effort centering on fish and invertebrate ecology. The result is that our understanding of the microalgal component is small and rather fractured. Recently, the importance of phytoplankton and benthic microalgae as important contributors to primary production has been realised with the result that there has been an increased research effort in estuarine microalgae in the last ten years. Comprehensive freshwater requirement studies relating microalgal biomass to freshwater flow in permanently open estuaries are limited to a few Eastern Cape estuaries and there has been no such study in the Western Cape, other than an intermediate study of the Olifants Estuary. As a result, data from short-term studies will be included for comparative purposes but relating biomass to flow in the Berg Estuary will be based on predictions only.

Research by Hilmer & Bate (1990) showed that the highest phytoplankton biomass occurred when flow rate into an estuary was equivalent to a "residence time" of 3 spring tidal cycles or 42 days. From the point of view of microalgal productivity and biomass in estuarine ecology, a flow rate of this magnitude is considered as the "optimum flow rate". The mean flow rate entering an estuary, however, is normally either above or below this optimum. Where the mean freshwater flow is above the "optimum flow rate" the estuary is effectively river-dominated and when the mean flow rate is below, it is effectively marine dominated. Residence time of an estuary is defined as the time required for a specific flow to replace the storage volume of water in an estuary. In the Gamtoos Estuary, Snow *et al*. (2000b) studied the high

phytoplankton chlorophyll a (chl a) that is found at the interface between fresh and saline water. This zone, called the "river-estuarine interface" or REI, is regarded as the region in an estuary where phytoplankton chl a is highest (Snow *et al*. 2000b). In the Gamtoos Estuary, this was the zone where the vertically averaged salinity was less than 10 ppt. The relationship between chl a and flow rate is shown in Table 4.1. As the flow rate increased in the Gamtoos Estuary, the position of the REI moved down the estuary towards the mouth and the vertically averaged chl a decreased. The data in Table 4.1 indicate that above and below the optimum flow rate, 0.8 to 1.2 $m^3 s^{-1}$, there are lower phytoplankton biomasses.

Table 4.1.

The influence of flow rate on chl a concentration in the Gamtoos Estuary (after Snow 2000b).

Flow ($m^3 s^{-1}$)	Chl a ($\mu g l^{-1}$)
0.3	26.7
0.8	47.6
1	35.7
1.2	49.9
1.25	26.7
2.3	13.5
9.7	15.4
30.5	6.4

There were no data in South Africa to indicate whether there is a diatom species seasonal effect in estuaries, however, Bate *et al.* (2002b) showed that there was no seasonal effect in the Swartkops River over a 13-month period. This applied especially to the upper reaches at higher altitudes where the quality of the water had the lowest TDS but where temperatures were more varied. By implication there is good reason to suspect that in South African estuaries, diatom species changes are more likely to be influenced by water quality variables such as salinity and mineral nutrients than by temperature.

The Berg River Dam is expected to affect the quantity and quality of riverwater entering the estuary. To understand the effects of these changes on microalgae, this study determined the distribution of phytoplankton and benthic microalgal

communities in the Berg Estuary. In particular, to determine which phytoplankton groups were numerically dominant and which diatoms were numerically dominant in the benthos. The purpose of examining the dominant diatom taxa was to relate them to the water flow and quality conditions in the estuary and to accumulate data for the purpose of estimating optimum flow and quality conditions in this and other estuaries. In addition, the study investigated the distribution of microalgae in the estuary by using chl a as an index of microalgal biomass. The strength of association between microalgal biomass and environmental variables was tested and the following hypothesis was proposed to test for a significant difference in average biomass between a summer and winter sampling session: "Average phytoplankton chl a concentration is highest in winter compared to summer due to higher nutrient availability in the freshwater during winter flow". The results of the study are used to predict the changes in biomass and community composition in response to reduced river input.

Materials and methods

Study site

The Berg is one of only three permanently open estuaries (POE's) on the west coast of South Africa. It is a river-dominated estuary with tidal influence measurable up to ~70 km from the mouth (Slinger & Taljaard 1994). In 1966 a new estuary mouth was cut through the sand dunes about 1 km north of the original mouth, which was stabilised between concrete walls (Slinger & Taljaard 1994). The original mouth has silted up and the old channel forms a blind arm running parallel to the coast. The lower 4 km of the estuary is frequently dredged to a depth of at least 4 m to allow for boat navigation. The result of the stabilised mouth and deepened channel is a strong tidal current, particularly in the lower and middle reaches of the estuary. Sediment in the lower reaches (sites 1 to 4, Fig. 4.1) was extremely soft, which is usually indicative of a high percentage of fine sediment particles (greater than 30% of < 125 µm sediment), high organic content (> 3% ash-free dry weight) and a high moisture holding capacity. Above the R27 Bridge the intertidal sediment was generally compacted and appeared to have a high fine sand content (125 to 250 µm sediment particle sizes) and high detritus content. The upper reaches (> 28 km) had a thick fringe of the common reed (*Phragmites australis*) with very little to no exposed intertidal area.

River flow into the estuary was strong in August 2005 and, as a result, sampling was limited to the middle and lower reaches of the estuary (sites 1 to 9, Fig. 4.1). The flow was much weaker in November 2005, hence more sampling effort was placed on the upper reaches of the estuary with an additional two sites 16.5 km (site 8) and 43.2 km from the mouth (site 10) (Fig. 4.1).

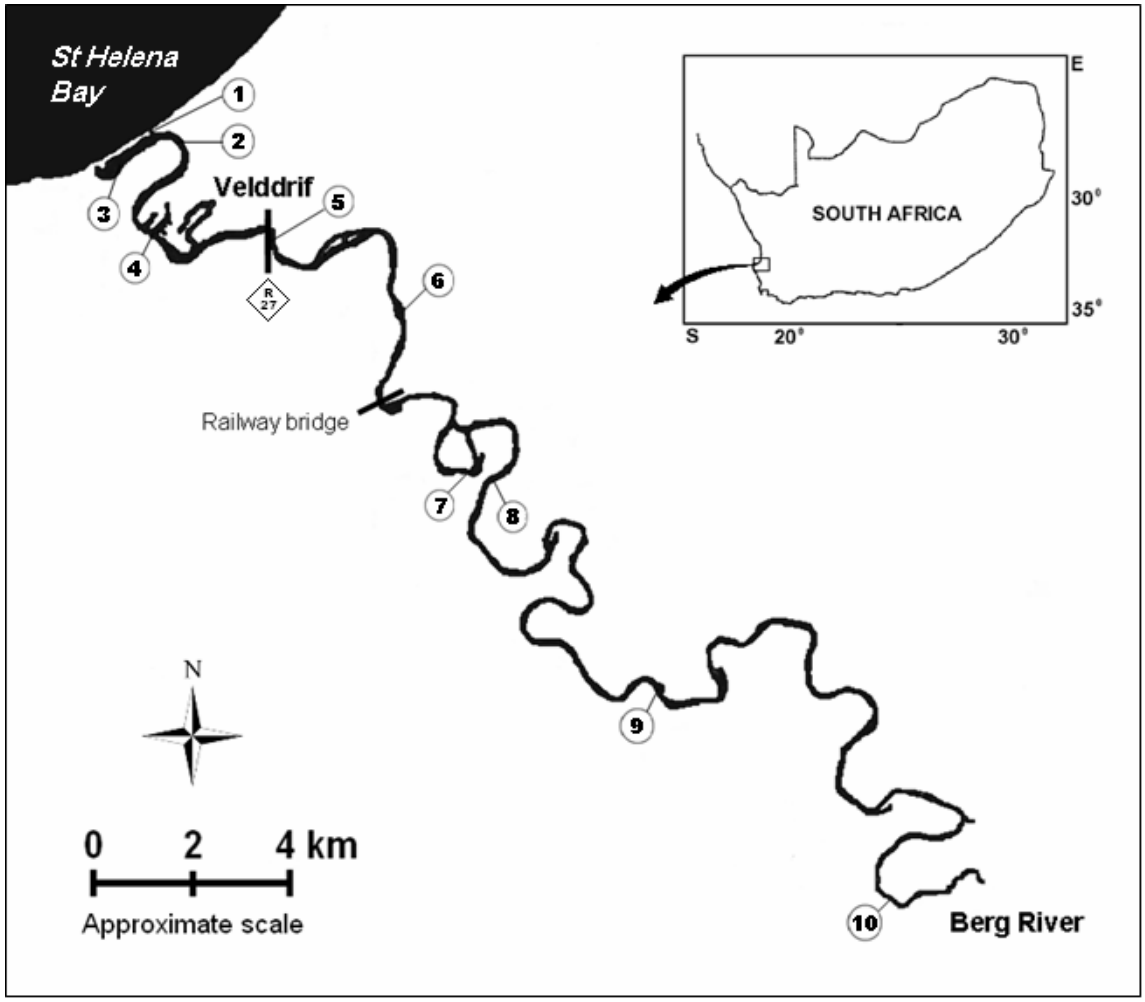

Figure 4.1. Map of the Berg River Estuary indicating locations of the sampling sites.

Water quality

Water quality variables were recorded at each site using a YSI 30 CTD (conductivity, temperature and depth gauge), a YSI pH100 pH meter and a WTW Oxi330i oxygen meter. A Secchi disc was used to determine light attenuation. The vertical attenuation coefficient was determined as described by Cole & Cloern (1987): K (m^{-1}) = 0.4 + (1.09/secchi depth). Filtered water samples (Whatman GF/C) collected in August 2005 were analysed for total oxidised nitrogen (ToxN; nitrate + nitrite) using the reduced copper cadmium method as described by Bate & Heelas (1975). Ammonium (NH_4^+) and soluble reactive phosphorus (SRP) were analysed using standard methods (Parsons *et al.*1984).

Phytoplankton chl a

Water samples (500 ml) were gravity filtered through Whatman GF/C filters, which were then stored in the dark of a cooler box until they could be frozen. The chl a was extracted by placing the frozen filters into 10 ml of 95% ethanol (Merck 4111). After extraction for 24 hours, spectrophotometric determinations of chl a were performed according to Nusch (1980). Absorbance was measured before and after acidification of the extracts with 0.1 N HCl.

Phytoplankton identification

Water samples for phytoplankton enumeration were collected at the surface, 0.5 m, 1.0 m and then at 1.0 m intervals to the bottom. The water samples were fixed with 1.5 ml of 1% (v/v) glutaraldehyde solution. Glutaraldehyde was preferred to a 10% neutral formalin solution as formalin can cause flagellates to lose their flagella making identification difficult (Lund *et al*. 1958; Boney 1989). Samples were then placed in 60 ml settling chambers and allowed to settle for 24 hrs then counted following the Utermöhl method of cell enumeration as modified by Snow *et al.* (2000). Functional and dominant groups were categorised into flagellates, dinoflagellates, greens, blue-greens, diatoms, and euglenoids. It is important to note that all flagellates and the cyanobacteria (blue-greens) were included as phytoplankton in this study. Many flagellates do not contain chloroplasts and are more correctly classified as protozoans. Unfortunately, no distinction between heterotrophic and

autotrophic flagellates can be determined using the available standard microscope techniques described here.

Benthic chl a

Four replicate intertidal benthic samples were collected from premarked locations (20 mm internal diameter circles) at low tide from each site by scraping a known area of surface sediment (< 2 mm depth) just above the estuarine water level. Four subtidal samples were collected from each site using a 20 mm internal diameter corer attached to an extension pole and the surface sediment was scraped from the core. Both intertidal and subtidal samples were stored in the dark of a cooler box until they could be frozen. The samples were freeze-dried, approximately 0.1 g was added to 4 ml of 95% ethanol (Merck 4111) and then stored for 24 hours at 0 ºC. Once the chl a had been extracted the samples were whirlimixed, filtered through Whatman GF/C filters and the extract was analysed on a Waters M-45 high performance liquid chromatograph (HPLC). Due to technical reasons, samples collected in November 2005 were analysed using a spectrophotometer according to Nusch (1980) before and after acidification of the extracts with 0.1 N HCl. Rodriguez (1993) compared results obtained from samples analysed simultaneously using an HPLC and spectrophotometer and found that the spectrophotometer overestimated chl a by a mean value of 20% for the top 10 mm of all intertidal sediment. This was considered when the results were interpreted.

Benthic diatom collection and identification

The epipelon was sampled based on the method described by Round (1981) and the details described in Bate *et al.* (2004). Samples were taken using a length of aluminium piping (~5 mm internal diameter) that was drawn across the sediment and allowed to fill with a mixture of surface sediment and water. This process was repeated up to five times in different positions in order to get a sample that was representative of the different micro-habitats. The mixture was stored in a plastic sample container (250 ml). In a field laboratory, some of the settled material was placed in a Petri-dish in the light and five clean degreased coverslips (covering approximately 40% of the sediment surface) were placed on top of the wet sediment. On the same day (approximately 1 to 2 hours later) the coverslips were carefully

removed with as little sediment as possible. In this way only living cells that had attached to the coverslips were sampled. The five coverslips from each sample were placed in glass bottles and transported to the laboratory. There is no time limit at this stage to process the diatoms further. To each glass bottle containing the coverslips, 2 ml of saturated $KMnO_4$ and 2 ml of 10 M HCl were added. This mixture was heated on a hot plate at approximately 60 °C until the solution cleared and became straw coloured. All acid cleaned samples were washed with distilled water using five consecutive spins (2000 rpm for 10 minutes). Permanent light microscopy slides were made with 1-2 drops of diatom 'digest', placed onto an acid-washed coverslip (previously stored in methanol) and allowed to dry in air. Coverslips treated in this manner allow the drop of sample to spread evenly. Once completely dry, a small amount of Naphrax® mounting medium (Northern Biological Supplies, U.K.) was dotted onto a glass microscopy slide and the coverslip placed over it. Air trapped under the slide and the Naphrax were dispersed by heating the slide on a hot plate (approximately 60 ºC). The Naphrax was allowed to dry for 2-3 days. The slides and the balance of the digested sample were logged and stored to form a permanent record.

Diatom frustules were examined under a Zeiss Axioplan light microscope with Differential Interference Contract (DIC) optics. Using a television camera (JVC KY-F3), images of the dominant taxa were visualised using the AnalySIS image analysis programme (©1999, Soft Imaging System GmbH). Diatom valves were counted in each sample using 1000x magnification until the obvious dominant was established. At least one digital image of every taxon was captured. Dominant species were those species that were clearly present in the greatest number and the sub-dominants were those that had a frequency > 10% but were not dominant. All the images were then printed and used in the counting procedure. This achieves three important aspects: (1) which of the diatoms were dominant and which were sub-dominant, (2) a digital image of each taxon and (3) a count of the total number of taxa. In previous work we have noticed that the number of taxa at different sites in the same estuary is quite different but this does not appear to be related to environmental variables. Diatoms attached to rocks (termed epilithic) have been successfully used as an index of eutrophication in South African rivers (Harding *et al*. 2005) but to date no such index has been developed for estuaries. Diatoms are frequently used as indicators of ecosystem health because they occur in all types of aquatic ecosystems, have a short regeneration time (2 to 3 weeks) and have species specific optima and

tolerances for nutrients such as phosphorus and nitrogen (de la Rey *et al*. 2004). Based on these ortima and tolerances, indicator values and pollution sensitivity scores can be determined for each taxa, which can then be applied in a diatom index such as the Specific Pollution Sensitivity Index (SPI) (de la Rey *et al*. 2004).

Tidal exchange and the soft sediments typical of estuaries introduce a number of environmental factors not encountered by epilithic diatoms in rivers, making the development of an index more difficult. By accumulating diatom and associated environmental data collected during RDM studies, it is likely that an index based on estuarine diatoms will one day be developed. The nomenclature of Krammer & Lange-Bertalot (1986-91) and Krammer (2000) was used with a few exceptions associated with some taxonomic revisions suggested by Round *et al.* (1990). Other taxonomic works consulted included Archibald (1983), Hustedt (1976), Lange-Bertalot & Krammer (1989), Simonsen (1987) and various articles by R.E.M. Archibald, B.J. Cholnoky and F.R. Schoeman (e.g. Schoeman & Archibald 1976).

Data analyses

Before testing for significant differences, the Kolmogorov-Smirnov Test was used to check if the data were parametric. If the data were parametric, then a Students t-test was used to compare two sets of data for significant differences and the Tukey's test was used if there were more than two sets. However, if the data were non-parametric, then a Mann-Whitney Rank Sum test was used to compare two sets of data and the Kruskal-Wallis Anova on Ranks test was used if more than two sets were compared. Pearsons Product Moment Correlation was used to test the strength of association between variables. All tests were performed using the statistical software package Statistica (Version 7). Grapher 6.1.21 (Golden Software, Inc.) was used to create filled contour plots.

Results

Water quality variables

Salinity profiles measured along the length of the estuary varied considerably from August 2005 to November 2005 (Fig. 4.2 and 4.3). During August, vertically averaged salinity ranged from 0.1 ppt (28 km from the mouth) to 2.9 ± 0.1 ppt at the mouth of the estuary. The turbulent flow resulted in the water-column being well

mixed and the vertical salinity gradient never exceeded 0.5 ppt. In November, there was a strong longitudinal salinity gradient but the water-column remained well mixed. Vertically averaged salinity ranged from 0.4 ppt at the head of the estuary (43 km from the mouth) to 32.9 ppt at the mouth of the estuary.

Turbidity, expressed as light attenuation (K), was highest at sites nearest to the head of the estuary in August and November (Fig's 4.2 and 4.3 respectively) where vertically averaged salinity was less than 10 ppt. Average K in August (5.58 ± 0.50 m^{-1}) was significantly higher than in November (2.56 ± 0.28 m^{-1}) ($t = 5.36$; $P < 0.001$; $n = 10$). Light attenuation was negatively correlated to salinity in August ($r = -0.79$; $P < 0.05$; $n = 9$). A similar trend, although not significant, was found in November.

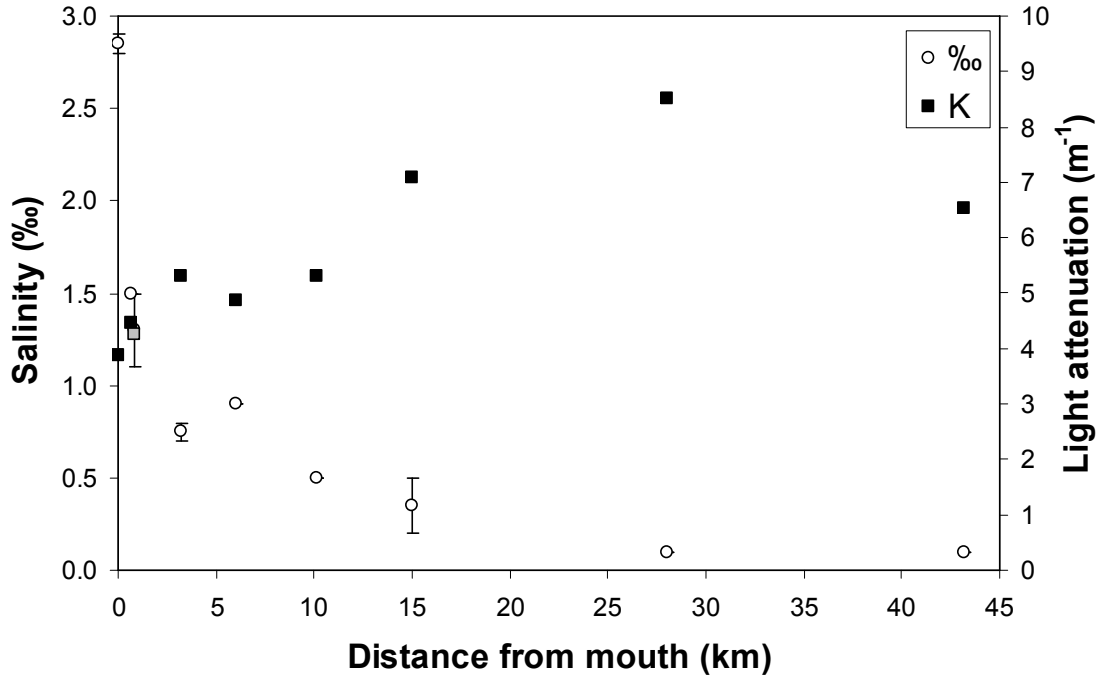

Figure 4.2. Vertically averaged salinity (‰; equivalent to ppt) and light attenuation (K) measured along the longitudinal axis in the Berg Estuary, August 2005. Vertical bars represent standard error of the means. Symbols shaded grey represent samples collected in the blind arm, 0.8 km from the mouth of the estuary.

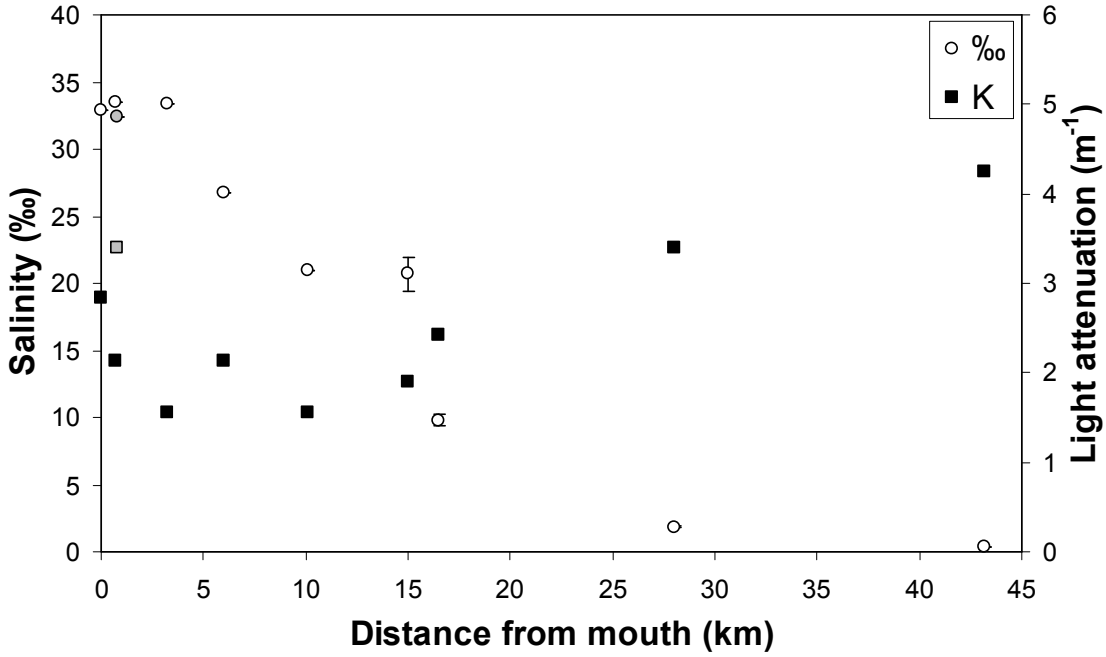

Figure 4.3. Vertically averaged salinity (‰; equivalent to ppt) and light attenuation (K) measured along the longitudinal axis in the Berg Estuary, November 2005. Vertical bars represent standard error of the means. Symbols shaded grey represent samples collected in the blind arm, 0.8 km from the mouth of the estuary.

Temperature in the estuary was generally uniform above 5 km from the mouth, with a slight decrease near to the mouth of the estuary (Fig. 4.4). Average temperature in August (15.3 ± 0.3 ºC) was significantly lower than in November (20.2 ± 0.8 ºC) (t = -5.28; P < 0.001; n = 10). Vertically averaged temperature ranged from 13.7 ± 0.1 ºC at the mouth of the estuary to 16.5 ± 0.1 ºC (6 km from the mouth) in August, and from 16.3 ± 0.0 ºC 0.7 km from the mouth of the estuary to 22.4 ± 0.0 ºC (10.1 km from the mouth) in November.

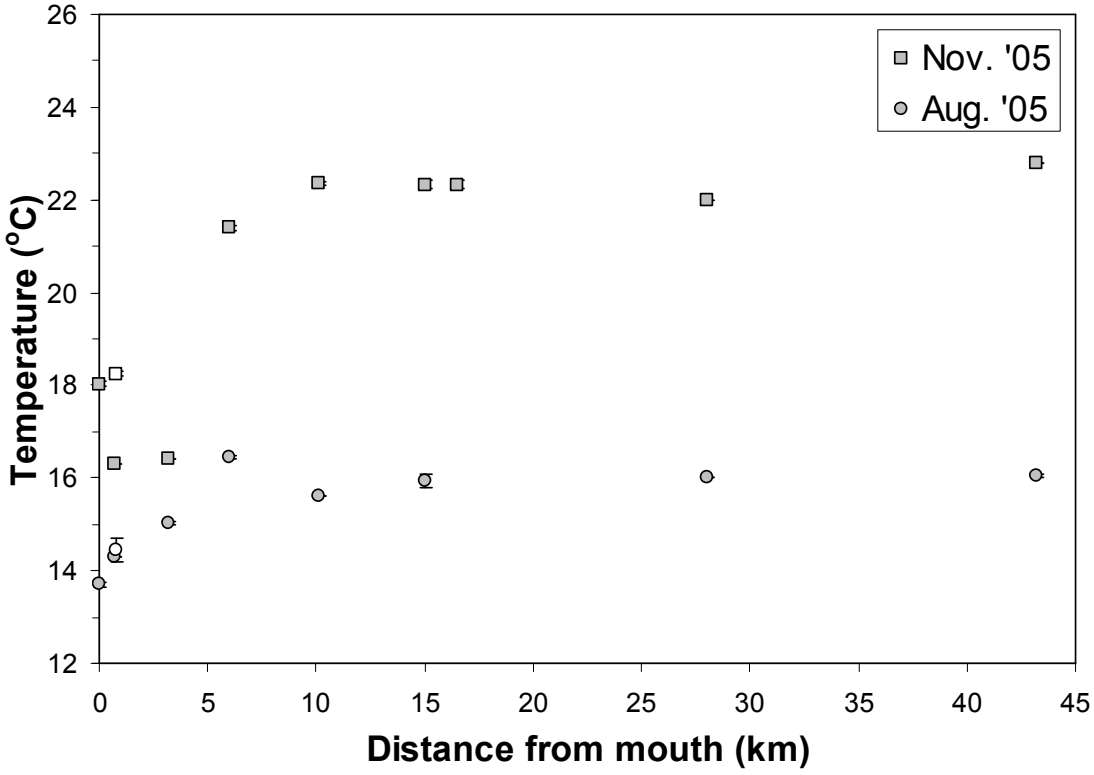

Figure 4.4. Vertically averaged temperature measured along the longitudinal axis in the Berg Estuary, August and November 2005. Vertical bars represent standard error of the means. The unshaded symbols represent measurements in the blind arm, 0.8 km from the mouth of the estuary.

TOxN and SRP concentrations gradually increased with distance from the mouth (Fig. 4.5), ranging from 36.9 to 63.5 µM and 0.1 to 1.2 µM respectively. Ammonium was highest at the mouth (4.8 µM) and closest to the head of the estuary (3.8 µM), and was lowest (1.0 µM) 15 km from the mouth (Fig. 4.5).

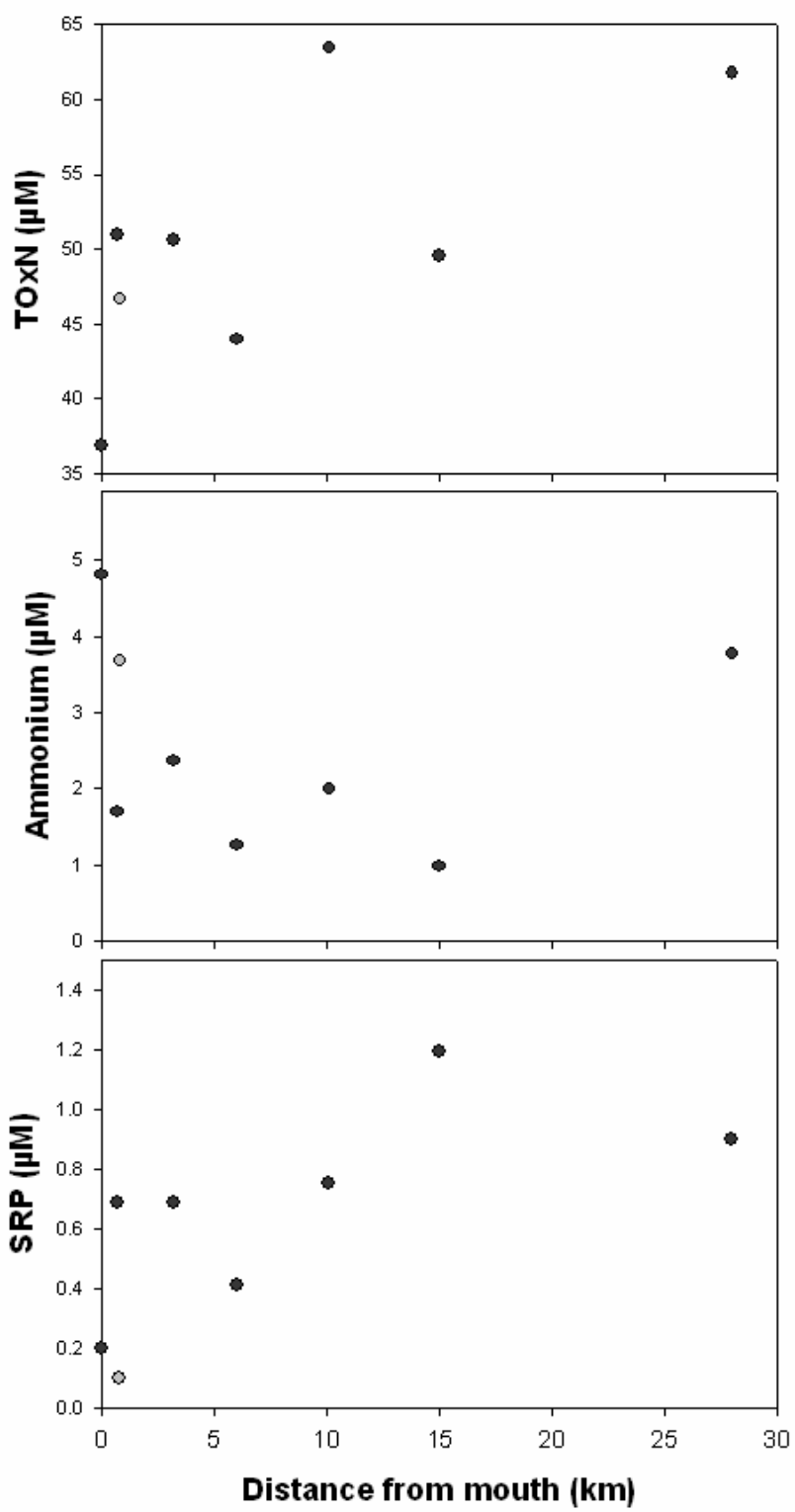

Figure 4.5. Total oxidised nitrogen (TOxN), ammonium and soluble reactive phosphorus (SRP) concentrations measured along the longitudinal axis in the Berg Estuary, August 2005. Concentrations are the average of 0.5 m and bottom samples. The symbols shaded light grey represents measurements in the blind arm, 0.8 km from the mouth of the estuary.

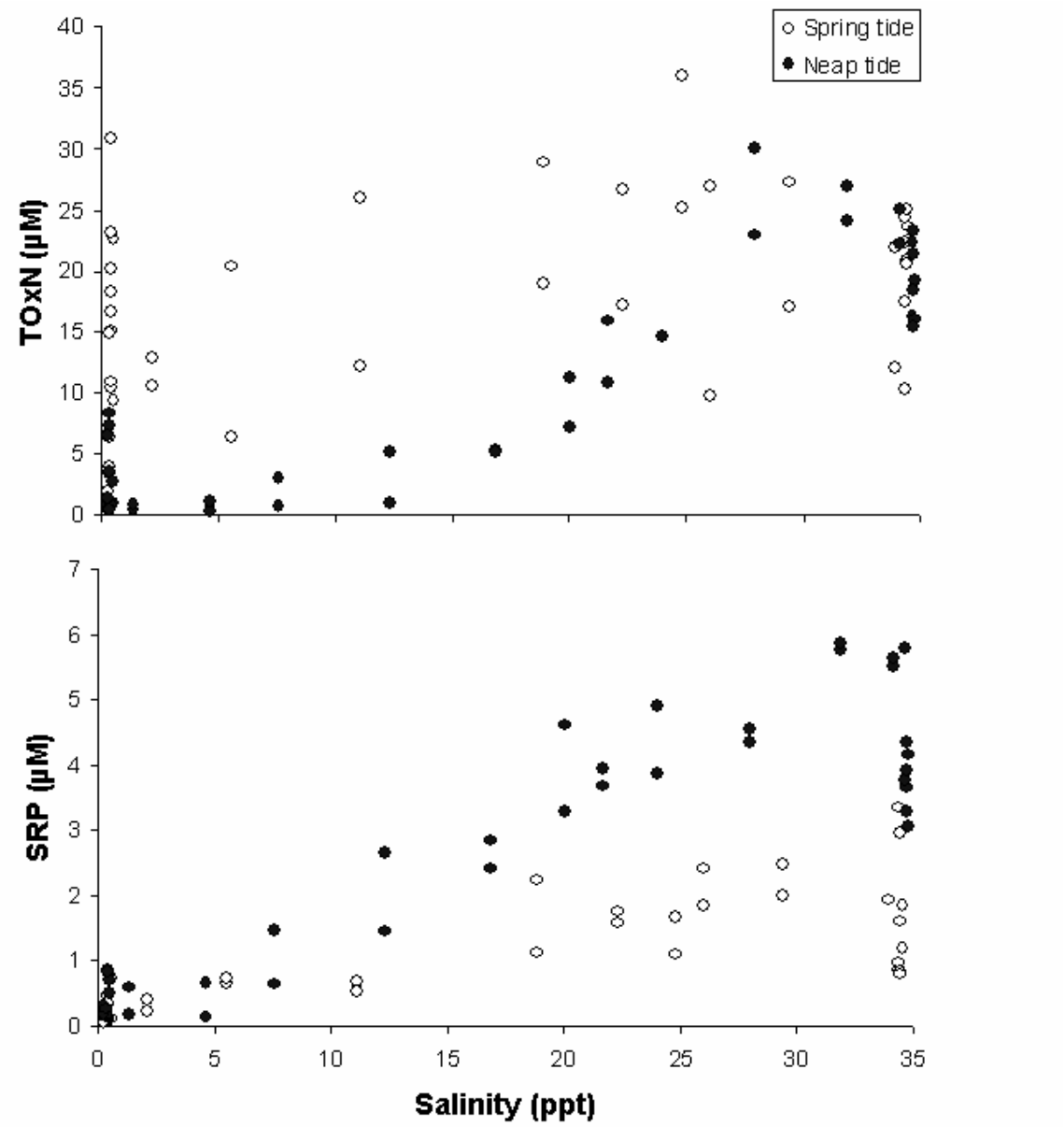

Figure 4.6. Total oxidised nitrogen (TOxN) and soluble reactive phosphorus (SRP) concentrations in relation to salinity in the Berg Estuary, November 2005 (Anchor Environmental Consultants, unpublished data).

Biotic variables

Phytoplankton chl a distribution differed considerably between August and November (Fig. 4.7). The average concentration was significantly higher in August ($t = -7.88$; $n_{(Nov)} = 38$; $n_{(Aug)} = 78$; $P < 0.001$), particularly in the middle and lower reaches of the estuary. Chl a concentration ranged from 4.13 ± 0.25 µg l^{-1}, 15 km from the mouth, to 6.09 ± 0.77 µg l^{-1} at the mouth. There was a strong Pearson's correlation between chl a and ammonium ($r = 0.71$; $P < 0.01$; $n = 16$) and a negative correlation with TOxN ($r = -0.52$; $P < 0.05$; $n = 16$).

In November there was a distinct increase in phytoplankton chl a with distance from the estuary mouth, ranging from 0.26 ± 0.04 µg l^{-1} at the mouth to 6.63 ± 1.01 µg l^{-1}, 43 km from the mouth. Dissolved inorganic nitrogen (DIN; TOxN + ammonium) and SRP increased with salinity, which was the inverse of the phytoplankton chl a. Vertically averaged phytoplankton chl a measured in November was significantly correlated to light attenuation ($r = 0.72$; $P < 0.05$; $n = 10$).

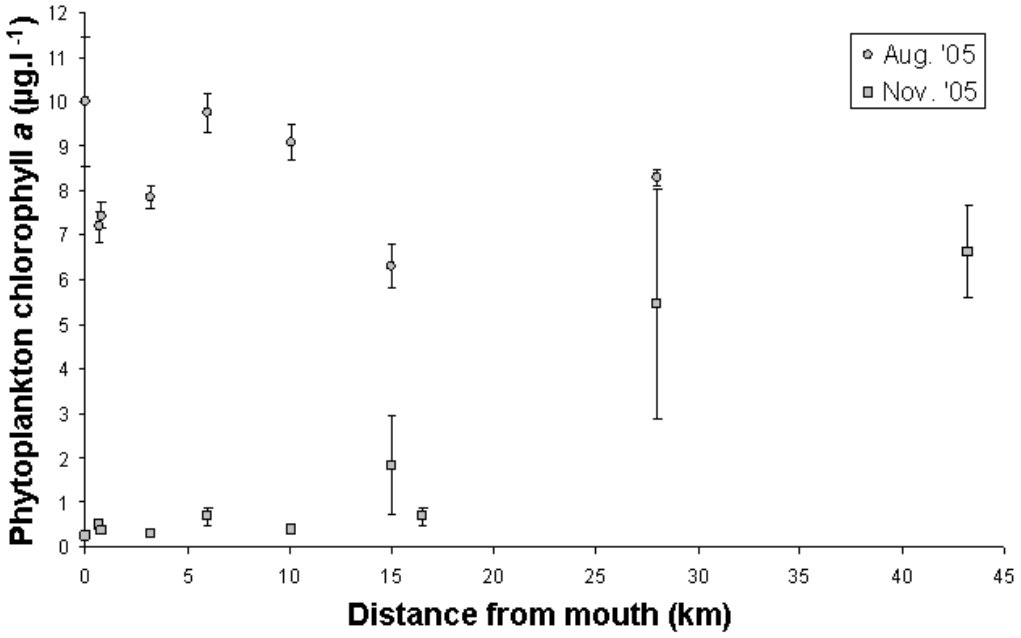

Figure 4.7. Vertically averaged water-column chl a measured along the longitudinal axis in the Berg Estuary, August and November 2005. Vertical bars represent standard error of the means.

In August 2005, phytoplankton was dominated by flagellates (80.9%) and diatoms (17.8%); distribution patterns were similar and ranged from 5901 ± 1081 cells ml^{-1} (28 km from the mouth) to 60527 ± 8934 cells ml^{-1} (10.1 km from the mouth) and 3728 ± 674 cells ml^{-1} (28 km from the mouth) to 16554 ± 4402 cells ml^{-1} (10.1 km from the mouth) respectively (Fig. 4.8). The remaining groups contributed less than 2% of the phytoplankton cell density. Using an all pairwise multiple comparison procedure (Tukey's Test) it was found that the density of flagellates at the two sites nearest to the head of the estuary (15 and 28 km) were significantly lower than at the next two sites closer to the mouth (6 and 10.1 km). A Tukey's Test on diatom density found no significant differences between the sampling sites. However, the distribution pattern is similar to that of the flagellates.

Total phytoplankton cell density was significantly correlated to phytoplankton chl a in the estuary (r = 0.34; P < 0.05; n = 39), which showed a slight increase in the lower-middle reaches of the estuary and at the mouth of the estuary (Fig. 4.9). Only the diatom group was significantly correlated to chl a (r = 0.44; P < 0.01; n = 39).

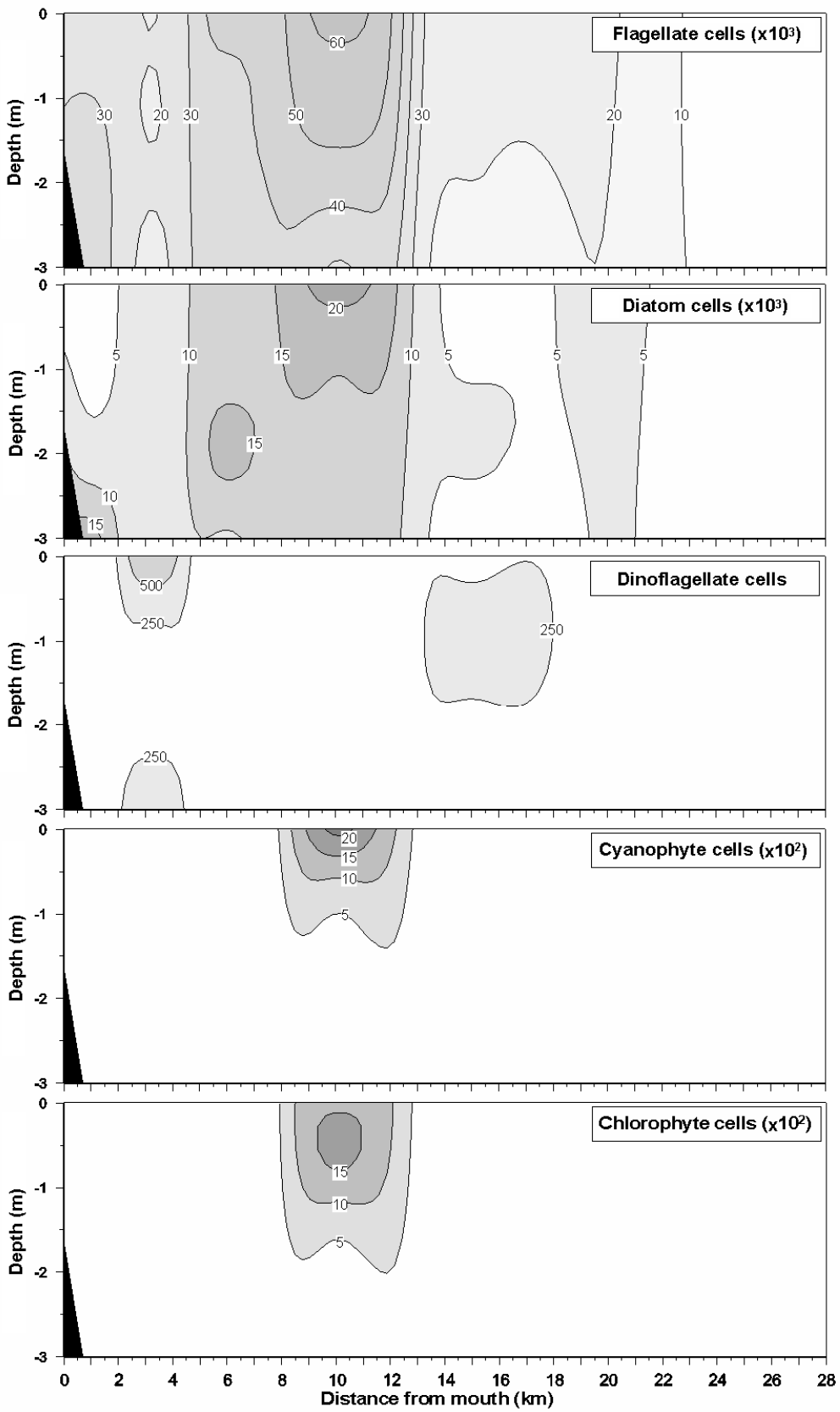

Figure 4.8. Filled contour diagrams of phytoplankton group cell density (cell ml^{-1}) in the Berg Estuary, August 2005. Samples were collected at 0, 0.5, 1, 2 and 3 m depths except at 2 m deep in the case of the mouth site where 3m was not possible.

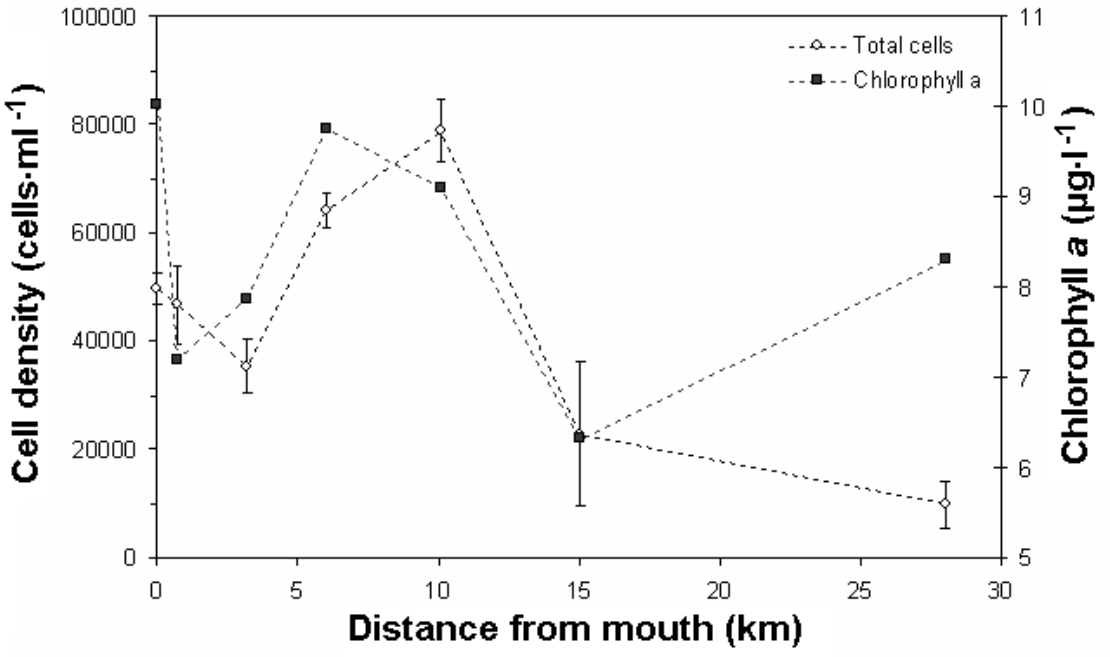

Figure 4.9. Vertically averaged total phytoplankton cell density (cell ml^{-1}) and chl a (µg l^{-1}) measured in August 2005 along the longitudinal axis of the Berg Estuary. Vertical bars represent standard error of the means.

In November 2005, phytoplankton at the water surface was dominated by flagellates (79.2%) and diatoms (19.5%). This high number of flagellates introduced to us the concept of a flagellate: diatom ratio. The ratio ranged from 3 in the lower reaches to 175 middle reaches and < 1.0 in the upper estuary. High densities (25094 to 76142 cells ml^{-1}) of flagellates were present throughout the estuary (Fig. 4.10) and were largely dominated by a 'small' flagellate (Fig. 4.11C). In addition to the 'small' flagellates, a high density of 'large' flagellates (Fig. 4.11A) was present in the estuary from 6.0 to 16.5 km from the mouth (3728 to 17714 cells ml^{-1}), which contributed to a high flagellate: diatom ratio. A bloom of 'small' diatoms (Fig. 4.11B) in the middle and upper reaches of the estuary (28 and 43.2 km), with densities in excess of 300 000 cells ml^{-1} (Fig. 4.10), resulted in a low flagellate: diatom ratio at these sites.

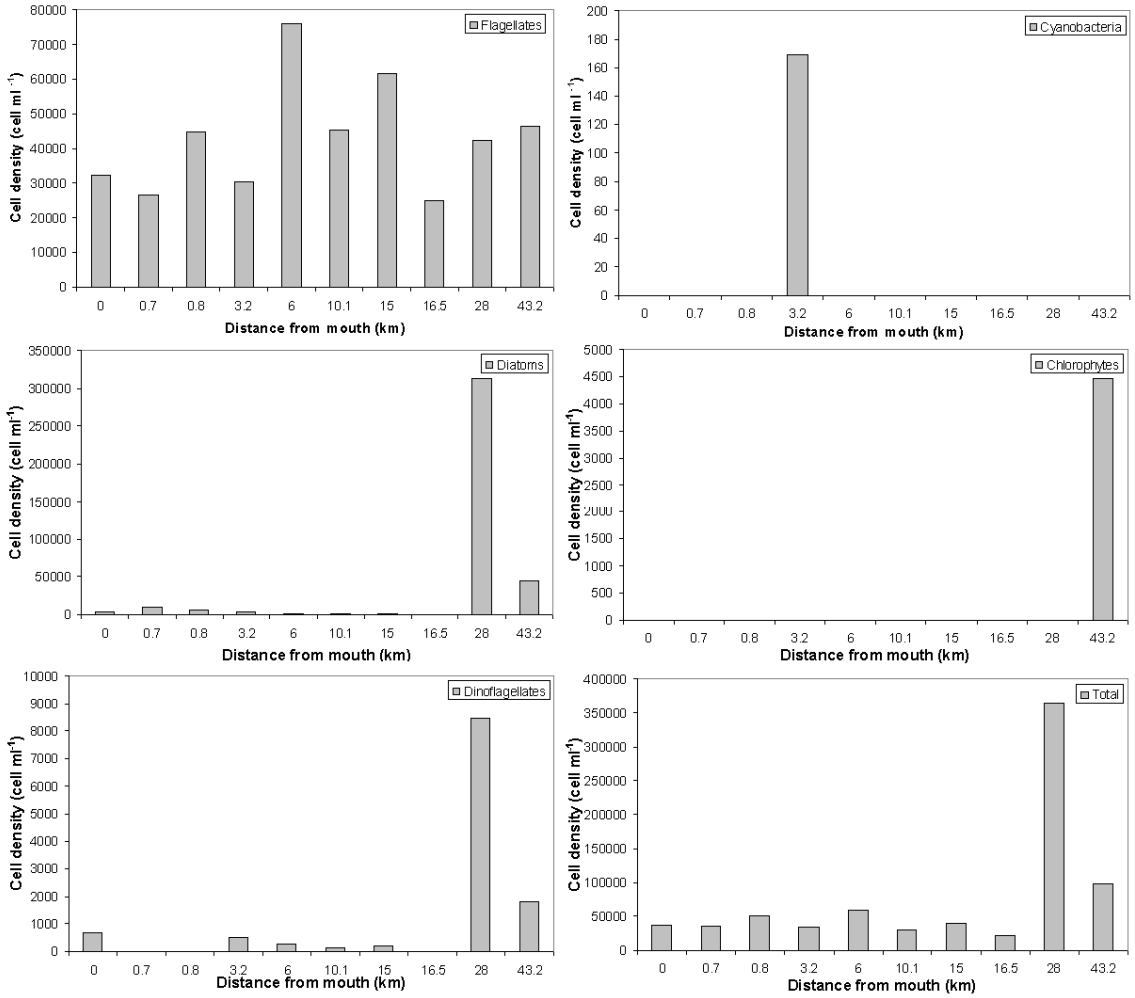

Figure 4.10. Surface phytoplankton cell density (cell ml^{-1}) measured in November 2005 along the longitudinal axis of the Berg Estuary.

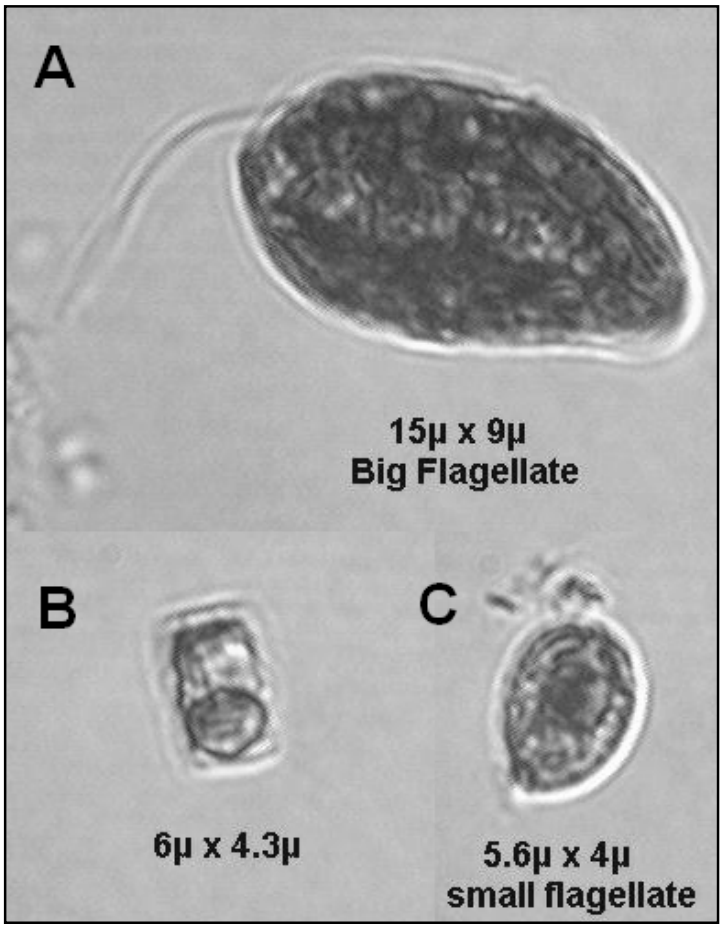

Figure 4.11. Dominant phytoplankton cells collected in November 2005 (A = large flagellate, B = small diatom and C = small flagellate).

Microalgal biomass was highest in the middle and upper reaches of the estuary where chl a concentrations were 5.47 µg l^{-1} (28 km) and 6.09 ± 0.67 µg l^{-1} (43.2 km) respectively (Fig. 4.12). The estuary was shallow (< 1 m) 28 km from the mouth, which possibly reduced tidal flow and provided a more stable habitat in the upper reaches of the estuary. There was a strong association between phytoplankton chl a and the diatoms (r = 0.85; P < 0.01; n = 10) and dinoflagellates (r = 0.87; P < 0.01; n = 10). As was the case in August, there was a weak correlation between the flagellates and chl a (r = 0.08; P = 0.84; n = 10).

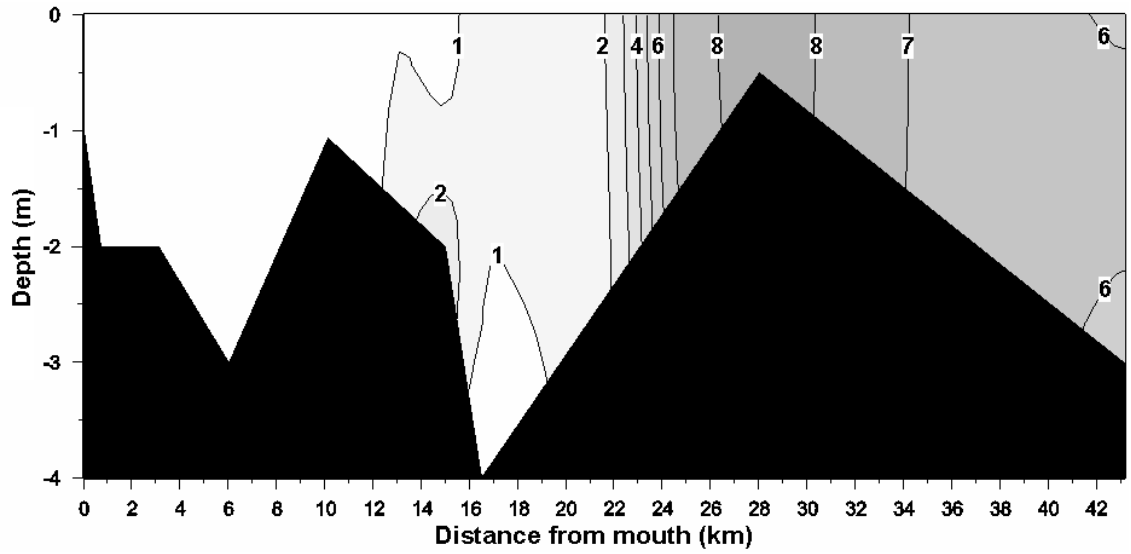

Figure 4.12. Filled contour diagram of phytoplankton chl <u>a</u> (µg l^{-1}) in the Berg Estuary, November 2005.

Benthic chl <u>a</u> content (m/m) measured in August ranged from 2.6 to 31.5 µg g^{-1} in the intertidal zone and 2.1 to 12.6 µg g^{-1} in the subtidal zone (Fig. 4.13). Highest contents were measured in the soft sediments in the blind arm near to the mouth of the estuary. In November the concentration ranged from 1.5 to 3.9 µg g^{-1} in the intertidal zone and 0.8 to 14.9 µg g^{-1} in the sub-tidal zone. Intertidal chl <u>a</u> was lowest at the two sites nearest to the mouth of the estuary and subtidal chl <u>a</u> increased significantly with distance from the mouth (r = 0.84; P < 0.01; n = 10).

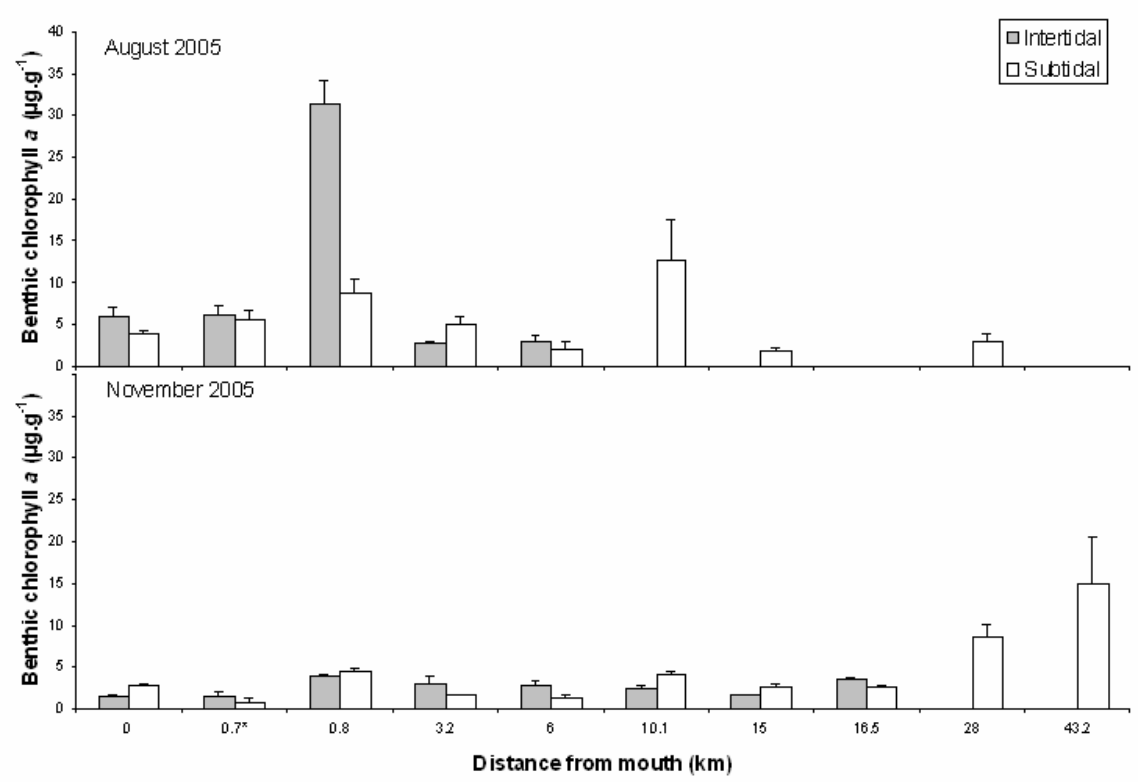

Figure 4.13. Intertidal and subtidal benthic chl a along the longitudinal axis of the Berg Estuary, August and November 2005.

There were 18 dominant benthic diatom taxa in the samples collected in August (Table 4.2) and 13 in November (Table 4.3). *Opephora minuta* was dominant at sites nearest to the mouth of the estuary in August and 10.1 km from the mouth in November. *Bacillaria paxillifer* var. *paxillifer* was present in the middle reaches of the estuary during both sampling sessions and always occurred at sites greater than 6 km from the mouth. *Hippodonta* sp. was only dominant in the estuary in November at sites in the lower reaches of the estuary (from the mouth to 10.1 km). *Denticula sundaysensis* and *Planothidium delicatula* were examples of taxa dominant exclusively in sediment at sites nearest to the head of the estuary in November and August respectively.

Table 4.2.

List of benthic diatom taxa collected in August 2005. The relative abundances of dominant species (dominance) and total number of species (No. taxa) at each site are included.

Distance (km)	Zone	Taxon	Dominance (%)	No. taxa
0	Intertidal	*Opephora minuta* Cleve-Euler	45	23
		Catenula adhaerens (Mereschk.) Mereschk.	16	
		Odontella aurita (Lyngbye) Agardh	15	
	Subtidal	*Fallacia sp. 03* D.G. Mann	38	33
		Catenula adhaerens (Mereschk.) Mereschk.	19	
		Opephora minuta Cleve-Euler	17	
0.7	Intertidal	*Navicula gregaria* (Donkin)	80	28
	Subtidal	None		10
0.8	Intertidal	*Navicula salinicola* Hustedt	34	35
		Cocconeis sp. 02 Ehrenberg	13	
		Navicula gregaria (Donkin)	11	
	Subtidal	*Opephora minuta* Cleve-Euler	23	18
		Catenula adhaerens (Mereschk.) Mereschk.	18	
3.2	Intertidal	*Navicula gregaria* (Donkin)	70	32
		Nitzschia aff perindistincta Cholnoky	17	
		Amphora subacutiuscula Schoeman	10	
	Subtidal	None		11
6	Intertidal	*Amphora sp. 04*	28	12
		Navicula gregaria (Donkin)	24	
		Amphora cf. copulata (Kütz.) Schoe. & Arch.	16	
		Fallacia sp. 03 D.G. Mann	12	
	Subtidal	*Bacillaria paxillifer* var. *paxillifer* (O.F. Muller) Hendey	35	24
		Amphora ovalis var. affinis (Kützing) V Heurck	13	
10.1	Intertidal	*Rhopalodia brebissonii* Krammer	15	40
		Fallacia sp. 03 D.G. Mann	10	
15	Intertidal	*Navicula salinarum* Grunow	22	30
		Fallacia sp. 03 D.G. Mann	15	
		Tryblionella hungarica (Grunow) D.G. Mann	15	
28	Intertidal	*Hippodonta hungarica* (Grun.) Lange-Bertalot *et al*.	32	23
		Bacillaria paxillifer var. *paxillifer* (O.F. Muller) Hendey	22	
		Planothidium delicatula (Kütz) Round & Buktiyarova	14	

Table 4.3.

List of benthic diatom taxa collected in November 2005. The relative abundances of dominant species (dominance) and total number of species (No. taxa) at each site are included.

Distance (km)	Zone	Taxon	Dominance (%)	No. taxa
0	Intertidal	*Hippodonta sp.* Lange-Bertalot *et al*.	20	16
	Subtidal	*Amphora coffeaeformis* (Agardh) Kützing	60	14
0.7	Intertidal	None		
	Subtidal	None		
0.8	Intertidal	*Cocconeis scutellum var. scutellum* Ehrenberg	28	25
		Hippodonta sp. Lange-Bertalot *et al*.	22	
	Subtidal	*Amphora acutiscula* Kützing	85	29
3.2	Intertidal	*Hippodonta sp.* Lange-Bertalot *et al*.	80	25
	Subtidal	*Hippodonta sp.* Lange-Bertalot *et al*.	35	36
		Navicula sp. Bory	15	
		Navicula paeninsulae Cholnoky	10	
6	Intertidal	*Navicula sp.* Bory	60	28
		Nitzschia aremonica Archibald	20	
	Subtidal	*Hippodonta sp.* Lange-Bertalot *et al*.	33	20
		Bacillaria paxillifer var. *paxillifer* (O.F. Muller) Hendey	15	
10.1	Intertidal	*Opephora minuta* Cleve-Euler	85	19
	Subtidal	*Bacillaria paxillifer* var. *paxillifer* (O.F. Muller) Hendey	70	
		Hippodonta sp. Lange-Bertalot *et al*.	10	14
15	Intertidal	None		
	Subtidal	None		
16.5	Intertidal	*Nitzschia sigma* (Kützing) W.Smith	29	25
		Navicula salinicola Hustedt	23	
		Bacillaria paxillifer var. *paxillifer* (O.F. Muller) Hendey	20	
	Subtidal	*Navicula salinicola* Hustedt	90	19
28	Subtidal	*Fragilaria elliptica* Schumann	70	12
		Bacillaria paxillifer var. *paxillifer* (O.F. Muller) Hendey	23	
43.2	Subtidal	*Fragilaria elliptica* Schumann	75	25
		Denticula sundaysensis Archibald	15	

Discussion

Based on historical data (1975 to 1996), Taljaard (2004) described the Berg River Estuary as having a strongly seasonal hydrological regime. During winter, strong freshwater flushing generally limited saline intrusion during the flood tide up to the R27 Bridge (Fig. 4.1). During summer the river inflow to the estuary was low and the system became marine-dominated. Characteristically, estuarine water (salinity between 5 and 30 ppt) occurred between Kliphoek (site 7 in Fig. 4.1) and 40 km from the mouth of the estuary.

The Berg Estuary was well mixed in August and November with distinct changes in environmental variables between the sampling sessions. Temperature followed local climatic conditions with highest temperatures recorded in November (~20°C) and lowest temperatures in August (~15°C). The water temperature in the lower reaches of the estuary was lower, by up to 6°C, than in the upper and middle reaches of the estuary because of seawater intrusion.

An important morphological feature in the Berg Estuary is the depth of the channel 28 km from the mouth. The estuary broadens in this area but is very shallow (0.5 m at low tide), which causes a slight bottle-neck in flow. In November the salinity was low at this site (< 2 ppt) and increased to 10 ppt at the next site downstream (16.5 km). In addition, the attenuation of light through the water-column was highest (>3 m^{-1}) at this site and further upstream.

Nutrient input into the estuary is moderate to high as a result of agricultural inputs. Taljaard (2004) stated that nitrogen in the estuary is mainly in the form of nitrate (NO_3), as opposed to nitrite (NO_2), which is typical of a well-oxygenated system. Turpie & Clark (2005) estimated that freshwater contains 60 to 90 µM dissolved inorganic nitrogen in winter as opposed to 20 to 40 µM in summer. Under low flow conditions, nitrogen concentration has been highest in the lower reaches of the estuary, decreasing upstream and in winter the concentration was highest in the upper reaches. Results of this study have confirmed this finding with a high concentration of TOxN (> 60 µM) in August at the head, decreasing to less than 40 µM at the mouth of the estuary. Soluble reactive phosphorus was generally low in the estuary (< 1.2 µM) and the molar DIN: DIP ratio (i.e. [NO_3 + NO_2 + NH_4]: [PO_4]) was above 40 throughout the estuary and was > 500 in the blind arm near to the mouth. This could be indicative of P-limited microalgal growth. However, at high river flows

the residence time of water in the estuary and turbulent currents may be more important limiting factors than nutrient supply.

Phytoplankton chl a measured in the Berg Estuary during this study was low, generally less than 8 µg l^{-1}, which compares well to other Western Cape estuaries (Table 4.4). However, the method outlined in the intermediate determination of RDM for estuarine ecosystems restricts sampling to two sampling sessions; during typical high and low flow periods, and does not include the transition phases between the two flows. The transition phases would be a period of medium flow and would be most likely to occur during spring and autumn, seasons when phytoplankton blooms are most likely to occur. In addition, the microalgal response to an intrusion of nutrient-rich ocean water, from upwelling events, was not captured by the study.

Table 4.4.

Phytoplankton chl a ranges published for South African estuaries (modified from Adams *et al*. 1999). Western Cape estuaries are marked with an asterisk.

	Chlorophyll *a* (µg.l^{-1})		
Estuary	Minimum	Maximum	Reference
Olifants*	1.7	10.3	Bate 2006
Palmiet*	2	8	Branch & Day 1984
Bot*	0	6	Bally *et al*. 1985
Gamtoos	1.6	115.2	Snow 2000
Sundays	12	23	Hilmer & Bate 1991
Sundays	-	>100 (bloom)	Hilmer & Bate 1990
Kariega	1	8	Allanson & Read 1995
Great Fish	0	52	Allanson & Read 1995
Great Fish	-	>100 (bloom)	Lucas 1986
Keiskamma	0	19	Allanson & Read 1995
Nahoon	1	6	Campbell *et al*. 1991
Gqunube	5	15	Campbell *et al*. 1991
Kwelera	0	10	Campbell *et al*. 1991
Mpenjati	0.14	15.4	Perissinotto *et al*. 2002
Mpenjati	0.5	11.0	Perissinotto *et al*. 2003
Nyara	0.007	4.1	Perissinotto *et al*. 2003
Mdloti	0.09	8.6	Perissinotto *et al*. 2003
Mdloti	0.87	111	Perissinotto *et al*. 2004
Mhlanga	0.73	303	Perissinotto *et al*. 2004
St. Lucia	0	16	Fielding *et al*. 1991

As part of a RDM study, the Olifants Estuary, a permanently open estuary approximately 125 km north of the Berg, was sampled in March and August 2004. Results showed that the system functions as a permanently open estuary and has a well developed, but not eutrophic, REI region (Bate 2006). The pattern of water-column chl a was similar to the Berg Estuary, decreasing from the head of the estuary to the mouth. Maximum chl a concentrations were low, generally <10 μg g^{-1}, and were similar in March and August. This pattern of microalgal biomass appeared to be a function of macro-nutrient concentration and grazing pressure (Bate 2006). In March 2004 in this study, flagellates were the most dominant plankton group and, together with dinoflagellates, peaked in the middle reaches of the estuary. Diatoms increased in density with decreasing salinity, reaching a maximum 20.5 km from the mouth. In August, the slightly higher salinity in the estuary indicated a slightly lower riverwater input. Flagellates were still the dominant group in the estuary but the density was much lower and, together with the diatoms, were evenly spread throughout the estuary.

Unfortunately, there is at present no funded research on heterotrophic flagellates in South African estuaries. Because the groups of microalgae in estuaries are very variable, we have introduced the concept of a flagellate: diatom ratio. The flagellate numbers in this ratio includes all flagellates and not just autotrophic flagellates. The use of diatoms in the ratio is because in our experience diatoms are usually the most dominant group after flagellates and sometimes are more dominant than flagellates. At present, we have no data on the interpretation surrounding the proportions of auto- and heterotrophic flagellates but are aware that the proportions of each as well as the ratio of heterotrophic flagellates to diatoms may in the future enable a clearer understanding of the ecology of estuaries in relation to mineral nutrients and micro-consumers.

There is evidence that changes in freshwater flow can have an effect on phytoplankton community structure as shown in the inner Neva Estuary, Gulf of Finland (Nikulina 2003), when a reduction in flow after dam construction and increased nutrient loads from anthropogenic inputs caused an increased occurrence and biomass of cyanobacterial blooms. Stoermer & Smol (1999) described a decrease in diatoms in estuaries in response to a decrease in dissolved silicate (DSi) in riverwater. This can occur as a result of diatom uptake in lentic inland water, such as impoundments.

Phytoplankton biomass in permanently open estuaries is a function of inorganic nutrient concentration in the riverwater and a favourable water retention time (days to weeks). A study of the Gamtoos Estuary (Snow *et al*. 2000b) found that a flow of approximately 1 m^3 s^{-1} provided the residence time that resulted in the highest vertically averaged chl a concentration (115 µg l^{-1}), or REI, in the upper reaches of the estuary. As river flow increased, the position of the REI moved further downstream and chl a decreased to 10 µg l^{-1} at a flow of 30.5 m^3 s^{-1}. In this study, phytoplankton chl a in the Berg was highest in the lower reaches of the estuary in August. The highest vertically averaged chl a (10 µg l^{-1}) was located at the mouth of the estuary. In November the highest phytoplankton chl a was in the upper reaches of the estuary. There was a distinct decrease in concentration in the middle and lower reaches of the estuary below the shallow 28 km site. Hence, the situation in the Berg Estuary appears to be similar to that of the Gamtoos Estuary in that during winter with high freshwater flow, the chl a maximum was further towards the mouth while in summer low flow conditions the maximum was near the head of the estuary. However, a more comprehensive study is required to confirm this.

The Swan River Estuary, Western Australia, is similar to the Berg Estuary and could provide a valuable glimpse into the effects of reduced river input on the phytoplankton community composition in the Berg Estuary. The Swan Estuary is also in a Mediterranean climate, is micro-tidal and there are moderate to high nutrient loads from urban and rural catchments (Chan *et al*. 2002). The estuary is split into two distinct regions; the lower estuary (the mouth at Fremantle to the narrows near Perth) and the upper estuary (the narrows to the tidal limit). Salinity penetration is generally restricted because of the narrows with the result that the upper estuary is much fresher than the lower estuary. The upper estuary also has a series of deep pockets and these are reported to have higher concentrations of ammonium and phosphate (Horner Rosser & Thompson 2001). A recognised pattern of bloom succession has been established within the upper reaches (Horner Rosser & Thompson 2001; Chan *et al*. 2002). Chlorophyte-dominated blooms occurred in early spring as river flow decreased. In mid summer the salinity in the upper estuary reached ~30 ppt and estuarine diatoms became dominant giving way later to dinophyte- and cryptophyte-dominated flagellate blooms that lasted through the summer and autumn periods. As flow increased and salinity decreased in the upper estuary, freshwater diatoms became dominant. Nutrients in stream flow, and from benthic regeneration under stratification-generated anoxia, appear to be the most

important factors influencing phytoplankton primary productivity in the presence of hydrological changes that have taken place in the Swan River estuary (Chan *et al*. 2002). The successional pattern of phytoplankton species occurring in the upper reaches of the Swan Estuary appeared to be related to changes in the available nitrogen sources; i.e. NO_3-N during periods of high river flow and NH_4-N during the drier summer and autumn period (Horner Rosser & Thompson 2001). Ammonium can be generated in the water-column through a number of processes, such as nitrate reduction, ammonification and excretion, and is available at higher concentrations than nitrate, particularly at times when no external nitrogen inputs are occurring from the catchment.

Flagellates were dominant throughout most of the Berg Estuary in August and November during periods of high and low river inputs respectively. In August, the estuary was near-fresh throughout (< 3 ppt) and a phytoplankton community of high cell density was present 10.1 km from the mouth, which consisted of flagellates (77%), diatoms (21%), cyanobacteria (1%) and chlorophytes (1%). In November a phytoplankton community dominated by a diatom bloom (86%) occurred 28 km from the mouth.

Recent studies of the Keurbooms, Gamtoos, Swartkops, Sundays, Mngazana and Mngazi estuaries found an intertidal (benthic) chl a range of between 1.6 ± 0.9 mg m^{-2} and 272.3 ± 22.9 mg m^{-2} (Snow & Adams 2006). Benthic microalgal biomass followed a pattern based on the distribution of organic matter and fine sediment (< 125 µm). Sites closest to the mouth and head of the estuaries were generally dominated by coarse sediment, were low in organic matter (< 3% ash-free dry weight) and supported a low microalgal biomass. The process of flocculation, a process well described by Day (1981), leads to the settlement of organic matter, fine sediment particles, nutrients such as phosphorus and microalgal cells as floc in the REI zone. This results in sediment in the middle reaches of the estuaries becoming high in organic matter, dominated by fine sediment and supporting a high benthic microalgal biomass. However, results from the Berg Estuary did not follow this generalised pattern (Fig. 4.12) and this could be due to the turbulent tidal currents under high flow conditions in winter.

The highest benthic biomass was measured in the blind arm during high flow in August, probably because this site was protected from the turbulent currents in the main channel (river water flow and tidal exchange). In November, benthic biomass

was highest in the upper reaches of the estuary and decreased towards the mouth. Perhaps the upper reaches of the estuary are less turbulent than near to the mouth of the estuary, favouring the sedimentation of suspended microalgal cells or floccules containing microalgal cells.

Responses to reduced flow

Salinity profiles measured during this study compared well to profiles reported by Schumann (2004). In August 2005, salinity was 2.9 ppt at the mouth and the 1 ppt isohaline was close to site 4 (Fig. 4.1). This is similar to the August 2003 spring low salinity profile (Schumann 2004), where flow was estimated to be 127 m^3 s^{-1}. The salinity profile during the spring low in November 2005 corresponded well with the profile measured during the spring low in November 2003 where flow was less than 0.2 m^3 s^{-1}.

Data supplied on the Berg Estuary showed that its volume is 12 million m^3. The actual annual average flow for the period 15 Jan 1995 to 1 November 2003 was 17.9 m^3 s^{-1}. The maximum was 893 m^3 s^{-1} while the minimum was 0.8 m^3 s^{-1}. Applying the 42-day "Optimum flow rate" rule, the flow rate that would give the maximum biomass ± 10% is 3.3 m^3 s^{-1}. The actual flow data show that the optimum flow rate is exceeded 84% of the time and only within the optimum range for 6% of the time. For 10% of the time the flow rate is below the optimum.

The implication of this is that during periods of high river inflows, the estuary is freshwater dominated and the "REI" would be towards the sea rather than at a position where primary productivity would be maximal. Studies of freshwater requirements are usually undertaken when there is a necessity to decrease the flow in rivers in order to send it elsewhere. The data provided in this report might easily be interpreted to imply that because the average flow rate is far above the optimum for primary productivity, that by decreasing the flow, the estuary might increase its productivity and therefore improve. Assumptions of this nature are incorrect because there would be a shift from the natural condition and the holistic ecology will be altered in a manner that cannot be predicted without a complete understanding of how it is functioning.

Phytoplankton biomass; a reduction in flow during winter is not likely to lead to a change in the phytoplankton biomass peak but the peak is expected to move slightly upstream of the mouth. During summer, a reduction in flow could lead to hypoxic conditions in deeper areas of the estuary, causing the release of PO_4 and NH_4 from the sediment. This could favour the increased occurrence of phytoplankton blooms, similar to those experienced in the Swan-Canning Estuary.

Phytoplankton community structure; an estuarine diatom-dominated bloom occurred during the study, hence a reduced flow during summer is expected to increase the occurrence of these blooms. Chlorophytes were present in the river water and it might therefore be expected that they could bloom in spring as river flow begins to decrease. There has been no evidence of a dinoflagellate bloom but it is likely that this could occur during stable conditions in late summer.

Benthic biomass; It is expected that there will be a slight increase in benthic chl a (~2 μg g^{-1}) in response to a reduction in river flow in winter due to decreased turbulent flow. Reduced flow in summer is likely to increase subtidal chl a in the upper reaches of the estuary but turbulent tide-driven currents will continue to limit benthic microalgal biomass in the lower reaches of the estuary.

Benthic community structure; there were insufficient data to predict the exact change in taxa in the estuary but the increased penetration of high salinity water and the more stable conditions of the water-column is likely to support more marine species in the lower reaches and species that are better adapted to fine sediment and increased organic content in the upper reaches of the estuary.

CHAPTER 5

Post-flood recoveries in an estuarine embayment and estuarine lake, South Africa

Abstract

This study showed that a flood, estimated to be a 1-in-30 year, had a significant influence on the water-column chemistry and physical parameters of a marine dominated estuarine bay, Knysna Estuary, and a temporarily open/closed brackish estuarine lake, Swartvlei. The flood, which occurred in early August 2006, flushed out the upper reaches of the Knysna Estuary and diluted the brackish Swartvlei Lake. Results from three sampling sessions following the flood of each estuary showed that the flood replaced low oxygen water with oxygenated water. However large inputs of organic matter resulted in hypoxic conditions seven weeks later at a number of sites in the Knysna Estuary and anoxic conditions three weeks later in deep Swartvlei water. This decrease in average DO was most likely the result of the reduced river flow, an increase in the mineralization of humic substances that had settled out of the water-column following the flood and the ingress of saline water through the estuary mouth.

The pulse of riverwater introduced low temperature water, which was super-saturated with oxygen, was slightly acidic owing to the high concentration of humic acids, and had elevated concentrations of TOxN and silicate. This resulted in a phytoplankton bloom that persisted from 20 days after the flood in Swartvlei; maximum vertically averaged chlorophyll a of 21.5 ± 10.1 µg l^{-1}. However a similar response was not recorded in Knysna possibly because of a lack of water retention in this large estuarine bay. It is likely that the flocculation of humic material, phosphorus and microalgal cells caused the chlorophyll a maxima at Swartvlei sites where riverwater mixed with brackish lake water. The chlorophyll a maxima were persistent due to the continuous river input and optimal residence time of water in the lake.

Keywords: flood; estuary; chlorophyll a; dissolved oxygen; nutrients; South Africa; Knysna; Swartvlei

Introduction

Saline coastal lakes are more commonly referred to as coastal lagoons and these can include permanently open or temporarily open systems. Researchers in Australia refer to these estuaries as intermittently closed and open lakes and lagoons (ICOLLs). Similar systems occur in Mexico, New Zealand, Brazil, Uruguay and the U. S. A. (Gobler *et al*. 2006; Haines *et al*. 2006). It is common practice for private and public land to be developed within the floodplains of temporarily open/closed estuaries (TOCE's) and this leads to the frequent artificial breaching of the mouth to prevent the properties from becoming flooded. This practise is not recommended because changing the entrance behaviour may lead to other impacts; drying out of the fringe areas with the subsequent encroachment of terrestrial vegetation, as well as changes in the macrophytes and benthos communities (Haines *et al*. 2006).

Swartvlei is an estuarine lake as defined in Whitfield's (1992) classification of South African estuaries into five types: Permanently open, temporarily open/closed, estuarine lakes, estuarine bays and river mouths. According to Whitfield (1992) estuarine lakes can have either a permanent or temporary connection to the sea. The Swartvlei Estuary is impacted at its head by rail and road bridges and a small resident community. The estuary is frequently closed during the winter months and almost always opened by human intervention in the early spring. The Swartvlei is deeper than Australian ICOLLs that are defined as being < 5 m deep (Gale *et al*. 2006) and this has implications with regards to vertical mixing. Swartvlei is 10 to 12 metres deep, which resists vertical mixing forces and hypoxia and anoxia in bottom water occurs frequently. During the lagoon phase, when the estuary mouth is closed, dissolved oxygen (DO) falls to zero in the bottom water and organic carbon rises to > 40 g m^{-3} as a result of the decomposition of *Zostera* and *Enteromorpha* in the deeper reaches. Nitrate-N and redox potential decrease during this phase, which results in the evolution of H_2S (g) from deeper sediments (Allanson 2001).

There has been mounting scientific evidence over the past century showing that the growing human population is altering the functioning of estuarine ecosystems (Allanson & Baird 1999; Nedwell *et al*. 1999). Flemer and Champ (2006) highlighted two environmental stresses that serve as major concerns for estuarine science, environmental resource management and human health for the near future: namely (1) nutrient over-enrichment (Scheren *et al*. 2004) and (2) freshwater scarcity (e.g. diversion of and/or low freshwater flow to estuaries) (Snow *et al*. 2000a & b; Snow &

Adams 2006). Related to the latter stressor, the construction of dams has changed the quality, quantity and timing of water flowing into estuaries. Chícharo *et al*. (2006) found that nitrates and silicates (referred to as the "new" nutrients) were strongly associated with freshwater and flow, and that ammonia and phosphates (the regenerated nutrients) were mostly dependent on biological activities. By reducing freshwater discharge, the residence time of water in the Guadiana Estuary (south Portugal) increased and this promoted eutrophication. Residence time of an estuary is defined as the time required for a specific flow to replace the storage volume of water in an estuary. A short-term increase in freshwater discharge – freshwater pulse – has been shown to counterbalance this risk by promoting the development of zooplankton (particularly copepods) and thereby exerting a top-down control on the algae.

The South African National Water Act (Act 36 of 1998) requires that a predetermined volume of freshwater be reserved to ensure that each estuary can maintain its ecological function; the ecological reserve. Estuaries are threatened by reductions in river flows and the disruption of natural flooding regimes, through the construction of dams and through excessive water abstraction or transfer (Allanson 2000). The task of a freshwater reserve assessment is to provide quantified information about the frequency, magnitude and duration of particular flows and levels of water quality variables for the Ecological Reserve Category of a target water body (Adams *et al*. 2002). Reserve studies on the Knysna and Swartvlei estuaries are planned for 2007/2008. These studies are based on the response of the abiotic and biotic characteristics of an estuary to a change in flow with an emphasis on high and low flows. Even though floods are considered essential in resetting environmental conditions within estuaries (i.e. flushing out accumulated organic matter and sediments, providing a cue for fish recruitment, introducing nutrients etc.), they can introduce high loads of silt into an estuary, reducing light penetration and suffocating benthic fauna (Morant & Quinn 1999). Researchers rarely have the opportunity to study estuaries following large flood events. A recent flood event (2-3 August 2006) along the southern Cape coast provided scientists with this opportunity.

Variability in annual runoff is expressed as the coefficient of variation (C_v), which is much higher for South African (C_v = 0.8) and Australian rivers (C_v = 0.7) than for European (C_v = 0.29) and North American rivers (C_v = 0.35) (Eyre 1994). This high variability in runoff is also reflected in larger peak and annual floods (relative to the

mean annual runoff) compared with European and North American systems. This is the ratio between the average volume of the 1:100 year flood (Q_{100}) and mean annual flood discharges (Q) which is much higher for Australian rivers ($Q_{100}/Q = 5.08$) than for European ($Q_{100}/Q = 2.2$) and North American systems ($Q_{100}/Q = 3.6$) (Eyre 1994). An understanding of the response of estuaries to floods is therefore important in South Africa.

In other estuaries floods have had the following effects; under flood conditions in the Richmond Estuary, Australia, estuarine processes were bypassed and freshwater, sediment and nutrients were discharged directly onto the continental shelf; i.e. processes brought about by the mixing of fresh water and seawater out to sea. In addition to input from the river, sediment and nutrients may also have been supplied by erosion and scouring of the estuary floor under flood conditions (Eyre & Twigg 1997). The authors did emphasise that estuaries being flushed completely of saltwater is a process usually reserved for tropical systems during high flows, and is not typically observed in temperate estuaries even during extreme events.

Managing nutrient concentrations in estuaries is a major focus of water quality management strategies. However, this can be problematic because estuaries generally act as sinks for nutrients and sediments entering from their catchments (Boesch *et al*. 2001; Hamilton *et al*. 2001; Jiang *et al*. 2004; Flemer & Champ 2006). Studies have routinely shown that over-enrichment of estuaries can lead to phytoplankton blooms, increased organic content in sediments, hypoxia (DO < 2 mg l^{-1}) and even anoxia (no detectable oxygen) (Hamilton *et al*. 2001). Changes in oxygen status alter benthic nitrogen cycling and the adsorption and desorption of phosphorus (Boesch *et al*. 2001; Hamilton *et al*. 2001). Dissolved oxygen (DO) within an aquatic system represents a balance between transport and mixing processes, and biochemical constituents responsible for its utilization (i.e. biological and chemical oxygen demand) (Gale *et al*. 2006). In shallow basins, the dynamics of DO are likely to be dominated by the physical process of vertical mixing from the surface and sediment oxygen demand at depth. During periods of stratification, when vertical mixing is inhibited, the depletion of oxygen is expected through the consumption of oxygen by the sediments. Under persistent stratification, the depletion of oxygen may lead to anoxia, leading to associated changes in sediment redox potential and nutrient release (Gale *et al*. 2006). These conditions are typical of the ectogenic meromictic Swartvlei (Western Cape Province) and Kosi Lakes (KwaZulu-Natal); the

deep basins become filled with dense seawater over which less dense surface water flows (Allanson & Winter 1999).

Objectives

The main aim of this study was to measure changes in water chemistry in response to a pulse in fresh water and to determine how these changes influence phytoplankton biomass. The results from this study may assist researchers in determining the freshwater reserves for the two estuaries, and may contribute to scientific knowledge of the importance of floods in marine dominated estuarine bays and temporarily open/closed estuarine lakes.

Key questions included;

1) Did the pulse of fresh water 'renew' water in deeper reaches of the two estuaries?
2) If strong vertical and horizontal salinity gradients developed following the flood, was there a significant increase in phytoplankton biomass in the river-estuary interface zone (REI)? The REI is regarded as the region in an estuary where vertically averaged salinity is less than 10 ppt and where phytoplankton chlorophyll a (chl a) is highest (Snow *et al*. 2000b).
3) If turbidity in the water-column decreased as the system stabilises, was there an increase in ammonium concentration and a decrease in dissolved oxygen in deeper reaches of the estuaries? This was expected to occur as a result of the mineralization of accumulated organic matter that has settled out of the water-column.

Study sites

The Knysna Estuary is a permanently open estuarine bay (drowned river valley) with a spring-tide prism volume of 19 x 10^6 m^3 and a tidal range of 1.8 m (Allanson *et al*. 2000). The estuary can be subdivided into three distinct sections: (1) The upper estuary, which is influenced by the Knysna River, (2) lagoon sector (between the N2 Bridge and railway bridge) and (3) marine-dominated embayment (below the railway bridge) (Fig. 5.1A). The estuary extends to the Charlesford Weir, approximately 19 km from The Heads (Largier *et al*. 2000). The Knysna River drains a small, well-

vegetated sandstone catchment (~400 km^2) and is characterised by very small sediment and nutrient loads. The mean annual runoff is in the order of 10^8 m^3, which relates to an average flow of 3 m^3 s^{-1}, but the median flow is closer to 1 m^3 s^{-1} (Largier *et al*. 2000).

The Swartvlei system is also situated on the southern South African coast and is subdivided into two distinct components (Fig. 5.1B); the humic-stained large open water area (Swartvlei Lake) above the railway bridge and the shallow, sinuous channel connecting this to the sea (Swartvlei Estuary) (Whitfield 1988). There are three rivers that flow into the estuarine lake; namely the Wolwe, Hoëkraal and Karatara Rivers. They drain a 340 km^2 catchment, supplying approximately 66 x 10^6 m^3 of freshwater per annum to the lake. The catchment land use consists of uncultivated mountain slopes, forestry and agriculture. The water of all three rivers is slightly acidic (pH range of 4 to 7), and heavily stained with humic materials (Silberbauer 1982). The Swartvlei system generally has two distinct phases; the tidal phase and the closed lake phase. As a result, the system is classified as a temporarily open/closed estuarine lake according to the Whitfield (1992) classification scheme.

The sandbar at the mouth of the Swartvlei Estuary is artificially opened before the lake fills up to two metres above the geodetic mean sea level, which usually occurs during autumn and winter. During the tidal phase, seawater can penetrate up to 4 km from the mouth during spring high tides introducing dense, saline water into the lake. This dense water sinks and a halocline develops, which is resistant to vertical mixing. Following mouth closure the water-column becomes wind-mixed and dissolved oxygen in the bottom water drops to zero (Allanson & Howard-Williams 1984). The Swartvlei Lake has a wide littoral shelf which is densely vegetated with *Potamogeton pectinatus*. The shelf is gently sloping to a depth of approximately 2 m depth, and then there is a steep drop-off to a flat lake floor at approximately 12 m (Allanson & Howard-Williams 1984).

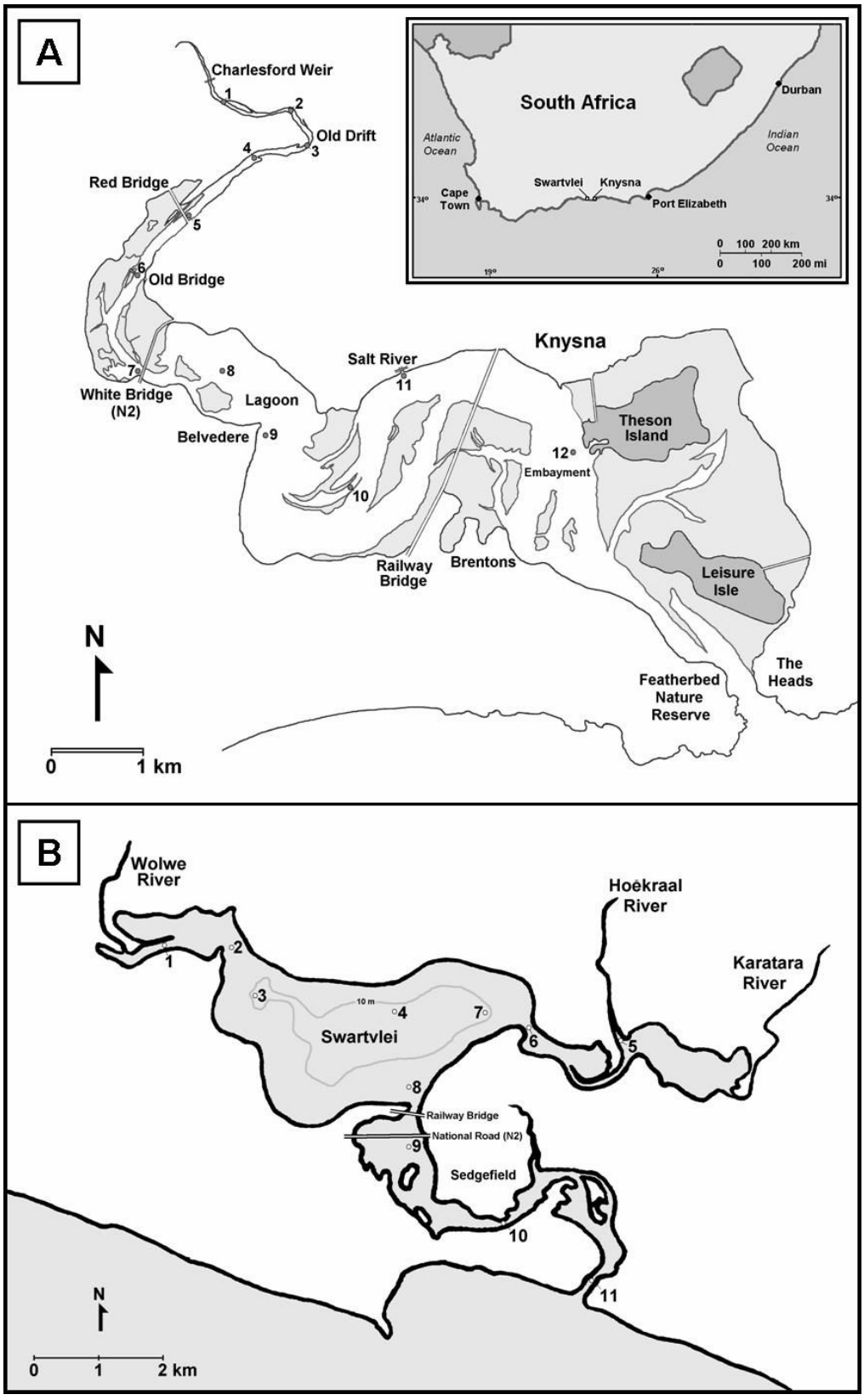

Figure 5.1. Outline of the (A) Knysna and (B) Swartvlei estuaries. Positions of sampling stations are indicated on the plots and the inset shows the location of Knysna and Swartvlei on the south coast of South Africa.

Materials and methods

Sampling took place in the Knysna Estuary 8, 19 and 53 days after a flood event, which started on 2 August 2006. There were 12 sites in total (Fig. 5.1A; Table 5.1) starting at a shallow site just downstream of the Charlesford Weir (16.9 km from the mouth). Physical water parameters (temperature, pH, salinity, dissolved oxygen, redox potential and secchi depth) were sampled at all sites. Samples were collected for nutrient and chl a analyses at all sites excluding sites 8 and 9. Sampling started in the morning, during spring low tide and ended in the afternoon as the tide had turned and the flood tide was starting during all sampling sessions. Locations of the sampling sites were selected to emphasise gradients in water chemistry in relation to salinity gradients.

Sampling in the Swartvlei Estuary took place 7, 20 and 52 days after the start of the flood and there were 11 sites in total (Fig. 5.1B; Table 5.1). Only physical water parameters were measured at sites 2, 4 and 7 on 10 August (day 7) but laboratory water quality analyses of all sites were included from subsequent trips (Fig. 5.1B). Physical parameters were measured using a 650 MDS YSI multiprobe and measurements were taken every second metre from the surface at each site. A Secchi disc was used to determine light attenuation. The vertical attenuation coefficient was determined as described by Cole & Cloern (1987): K (m^{-1}) = 0.4 + (1.09/secchi depth).

Table 5.1.

Coordinates of sampling stations with distances (km) from the mouths of the Knysna and Swartvlei estuaries. Station numbers are shown in Fig. 5.1.

Knysna			Swartvlei		
Station number	**Coordinates**	**Distance (km)**	**Station number**	**Coordinates**	**Distance (km)**
1	34º00'19.32"S; 23º00'34.47"E	16.9	1	33º58'53.70"S; 22º44'08.04"E	11.6
2	34º00'25.29"S; 23º00'00.71"E	16.3	2	33º58'59.83"S; 22º44'25.33"E	10.8
3	34º00'40.74"S; 23º01'02.31"E	15.8	3	33º59'28.10"S; 22º44'35.67"E	10.1
4	34º00'40.25"S; 23º00'35.97"E	15.0	4	33º59'35.99"S; 22º46'00.43"E	8.4
5	34º00'58.10"S; 23º00'03.11"E	14.0	5	33º59'54.51"S; 22º48'11.26"E	11.9
6	34º01'15.21"S; 22º59'34.64"E	13.1	6	33º59'43.04"S; 22º47'17.60"E	9.7
7	34º01'52.92"S; 22º59'26.99"E	11.7	7	33º59'28.98"S; 22º46'46.45"E	9.2
8	34º01'57.59"S; 23º00'12.56"E	10.5	8	34º00'09.03"S; 22º46'05.68"E	7.3
9	34º02'31.52"S; 23º00'22.28"E	9.2	9	34º00'40.86"S; 22º45'58.12"E	6.2
10	34º02'54.20"S; 23º00'49.63"E	7.8	10	34º01'18.96"S; 22º46'59.33"E	3.8
11	34º02'15.06"S; 23º01'27.33"E	6.2	11	34º01'45.41"S; 22º47'53.19"E	0.6
12	34º03'00.30"S; 23º02'38.13"E	3.8			

Phytoplankton biomass was determined using water-column chlorophyll a (chl a) as an index of biomass. Water-column samples were gravity-filtered through plastic Millipore towers using Whatman (GF/C) glass-fibre filters (Snow *et al*. 2000). The samples were collected using a 500 ml weighted pop-bottle and the filters were folded then frozen in tin foil until extraction in the laboratory. Chl a was extracted by placing the filters into glass vials containing 10 ml of 95% ethanol (Merck 4111). The samples were then stored overnight at 1 to 2 ºC. The contents of the vials were filtered and the light absorbance at 665 nm of the supernatant was determined using a spectrophotometer within 24 h, before and after the addition of 0.1 N HCl. The equation used was that of Hilmer (1990), derived from Nusch (1980):

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

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)

Replicate filtered water samples (Whatman GF/C) were collected in acid-stripped containers and 20 µl of 5% $HgCl_2$ (preservative) was added to the 30 ml samples to prevent bacterial transformation of nutrients, i.e. the nitrification of ammonium in particular. The samples were then stored at -20 ºC until analysis within 3 weeks of collection. The defrosted water samples were analysed for total oxidised nitrogen (TOxN; nitrate + nitrite) using the reduced copper cadmium method as described by Bate & Heelas (1975). Ammonium, soluble reactive phosphorus (SRP) and silicate (DSi) were analysed using standard methods (Parsons *et al*. 1984).

Salinity data were plotted as contour plots and dissolved oxygen was plotted as box-whisker plots using Grapher 6.1.21 (Golden Software, Inc.). Before testing for significant differences, the Kolmogorov-Smirnov Test was used to check if the data were parametric. If the data were parametric, then a Students t-test was used to compare two sets of data for significant differences and the Tukey's test was used if there were more than two sets. However, if the data were non-parametric, then a

Mann-Whitney Rank Sum test was used to compare two sets of data and the Kruskal-Wallis Anova on Ranks test was used if more than two sets were compared. Pearsons Product Moment Correlation was used to test the strength of association between variables. All tests were performed using the statistical software package Statistica (Version 7).

Results

Estimated river flows

Rainfall data from the nearby town of George clearly showed a distinct peak in rainfall (230 mm) that fell on 1 August 2006 (Fig. 5.2a). The weather station at George is approximately 30 km and 60 km west of Swartvlei and Knysna estuaries respectively. The average daily rainfall for the year preceding the peak was 1.6 ± 0.2 mm reaching a maximum of 39.5 mm. A second peak, 71.5 mm, occurred within hours of the second sampling session (22-23 August 2006). This is likely to have caused a delay in the time it took for the water-columns in the estuaries to stabilise but the final sampling session was four weeks later, giving the two systems extra time to recover.

Based on river flows measured in the Millwood Forest Reserve (gauge K5H002) (Fig. 5.2b), which represents a third of the ~400 km^2 Knysna River catchment, flows were estimated to be 10.4 $m^3 s^{-1}$, 3.8 $m^3 s^{-1}$, and 1.9 $m^3 s^{-1}$ on 10 and 21 August, and 24 September 2006 respectively; i.e. river flows measured at the gauge were multiplied by three. At the height of the flood, the water level was 4.4 m over the flow gauge. However, accurate flows were limited to 81.1 $m^3 s^{-1}$ when the height was 2.1 m. As a result, flow was roughly estimated to have been > 450 $m^3 s^{-1}$.

The total flow into the Swartvlei Lake, catchment size of 340 km^2, was roughly estimated to be > 500 $m^3 s^{-1}$ based on flow recorded at the Karatara River flow gauge (K4H002) (Fig. 5.2C). This flow gauge only monitors flow from 22 km^2, 6.5% of the total catchment. River flows on the days of sampling were estimated to be 4.6 $m^3 s^{-1}$, 4.2 $m^3 s^{-1}$ and 1.8 $m^3 s^{-1}$ on 9 and 22 August, and 23 September respectively.

Average daily water height in the Swartvlei Lake, using a water level recorder in the lake (K4R002) decreased from 1.7 m (19 May 2006) to 0.6 m above geodetic mean sea level (MSL) following an artificial breaching event (Fig. 5.2D). Despite the mouth being open, a bottleneck in flow under the rail and road bridges caused the

water level in the lake to reach a maximum height of 3.3 m during the flood and the highest daily average of the study period (2.7 m above MSL) was recorded the following day.

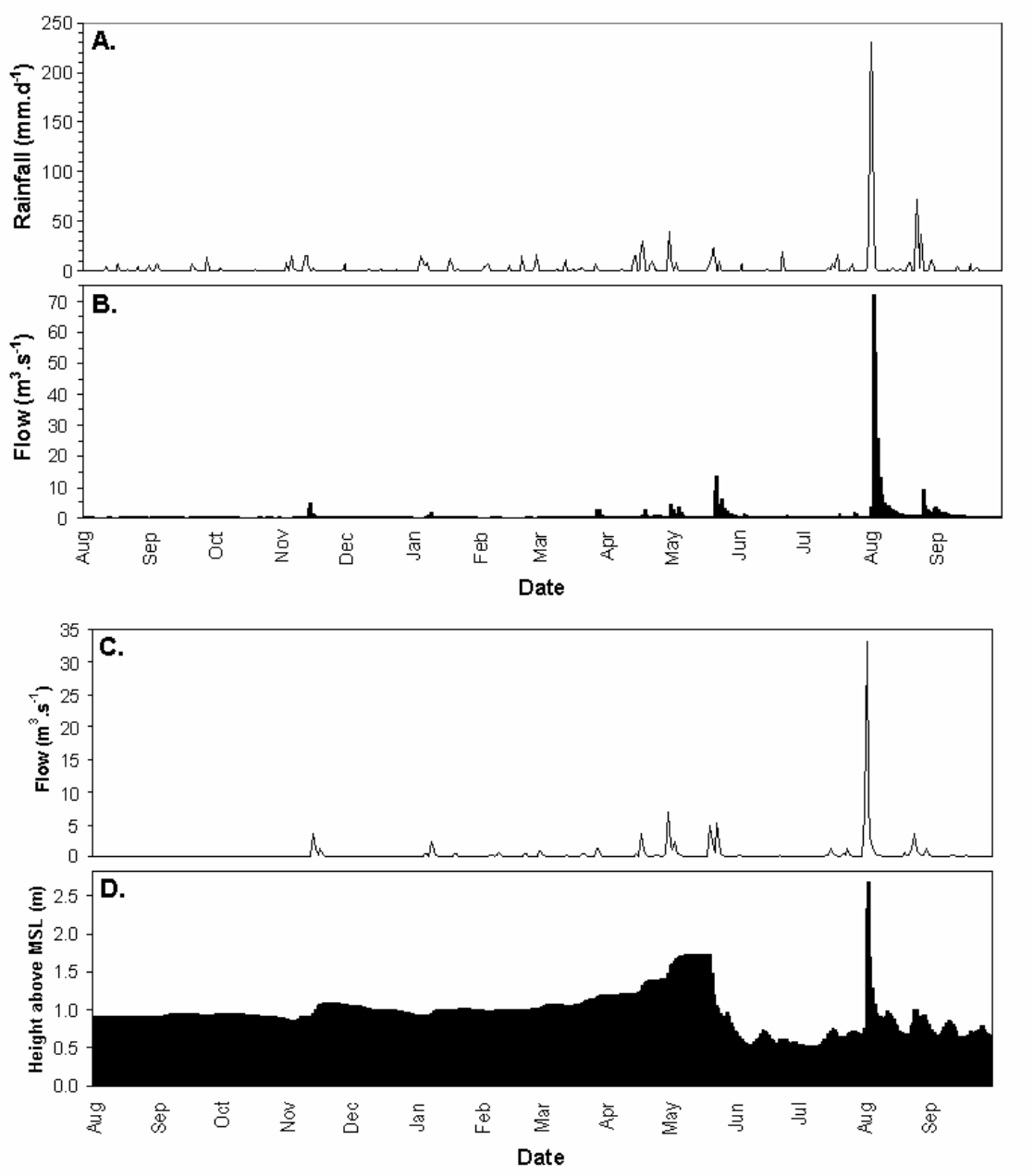

Figure 5.2. Rainfall, flow and height above geodetic mean sea level (MSL) for the Knysna and Swartvlei estuaries during the period August 2005 to October 2006: (A) Daily rainfall (mm) measured at George, a town ~30 and 60 km west of Swartvlei and Knysna estuaries respectively, (B) Knysna River flow measured in the Millwood Forest Reserve (~33% of total flow), (C) Karatara River flow (tributary into Swartvlei; ~6.5% of total flow), and (D) mean height above sea level (m) measured in the Swartvlei Lake.

Knysna Estuary

Sampling started at the head of the estuary during spring low tides and ended during the flood tide on all three sampling sessions, which could potentially mask any stratification in the lower reaches. However, salinity profiles in a previous study (Largier *et al.* 2000) indicated mixed conditions in the lower half of the estuary during both low and high tides except during high flow periods (e.g. 50 m^3 s^{-1} during the 1996-1998 study). Salinity profiles (Fig. 5.3) showed an even distribution of salinity throughout the estuary, ranging from marine at Thesen Island to fresh at the head of the estuary, 8 days after the flood. Over the following 45 days the well mixed saline water in the embayment and lagoon developed into a prominent salt wedge structure, which penetrated upstream creating strong vertical stratification in the upper reaches of the estuary. A further reduction in river flow would be likely to lead to the salt wedge penetrating all the way to the head of the estuary, the halocline then breaking down and the water-column eventually becoming well mixed throughout the system.

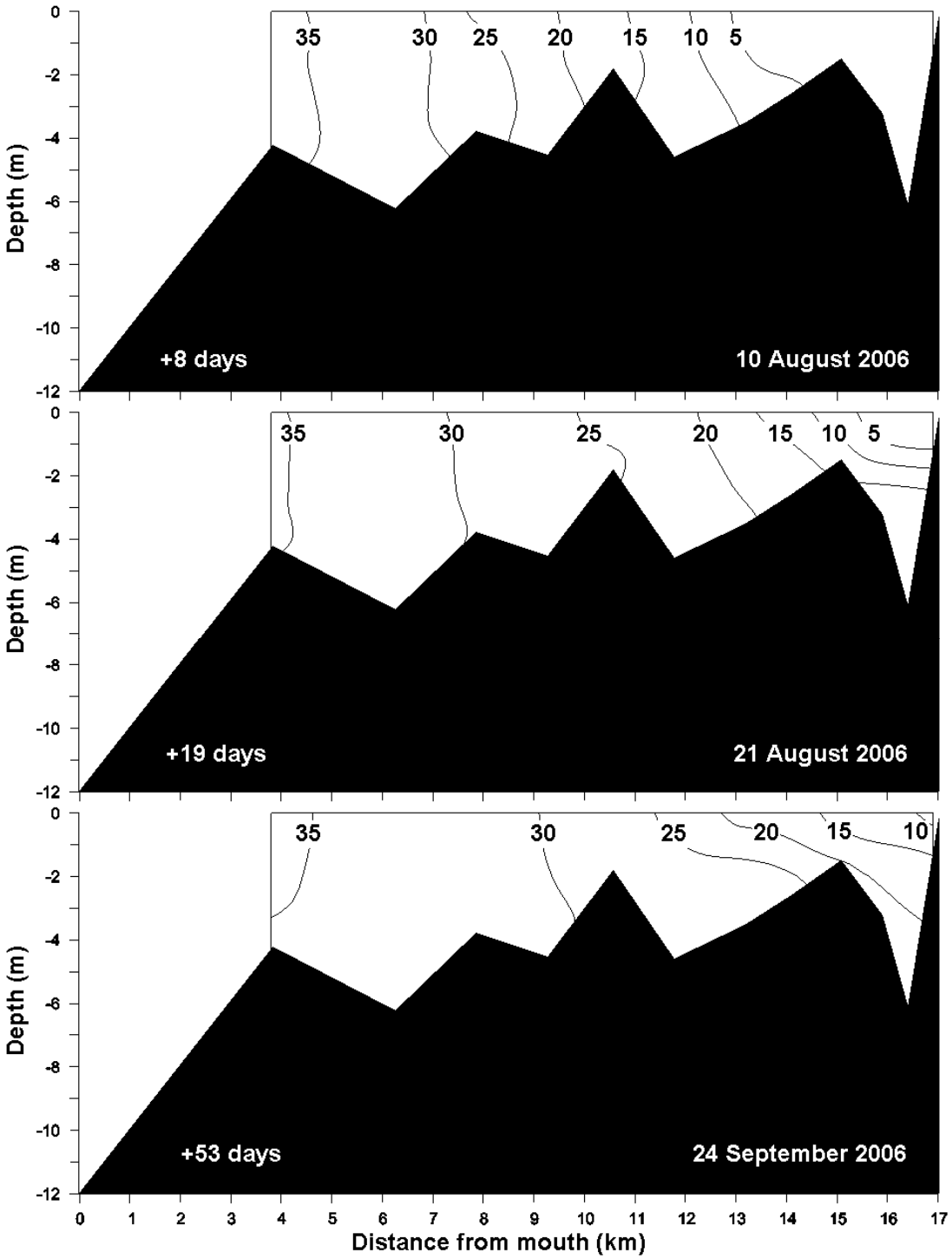

Figure 5.3. Longitudinal salinity distribution in the Knysna Estuary 8, 19 and 53 days after the 2 August 2006 flood. Measurements were taken from 3.8 km to 16.9 km from the mouth of the estuary.

Average temperature in the Knysna Estuary increased with time after the flood (Table 5.2); 13.69 ± 0.06 ºC (+8 days), 14.47 ± 0.06 ºC (+19 days) and 18.00 ± 0.05 ºC (+53 days). Eight days after the flood the water temperature was significantly lower downstream of the N2 Bridge, in the lagoon and bay, than in the estuary above the bridge (T = 25651; $n_{(above\ N2)}$ = 226, $n_{(below\ N2)}$ = 285; $P < 0.001$). Nineteen days after the flood the temperature above the bridge (12.0 ºC to 15.5 ºC) was still significantly lower than below (13.4 ºC to 15.4 ºC) (T = 32428; $n_{(above\ N2)}$= 150, $n_{(below\ N2)}$= 201; $P < 0.001$) and 53 days after the flood the average temperature was higher than the previous two sampling sessions as a result of warmer ambient conditions. The middle reaches of the estuary were warmest as river flow and tidal exchange cooled the extreme upper and lower reaches of the estuary respectively.

Table 5.2.

Average (± SEM) temperatures, pH's and attenuation coefficients (K_d) in the Knysna and Swartvlei estuaries following the 2 August 2006 flood.

Estuary	Date	Temperature (°C)	pH	K_d (m^{-1})
Knysna	10 August	13.69 ± 0.06	7.06 ± 0.04	0.69 ± 0.10
	21 August	14.47 ± 0.06	7.11 ± 0.04	0.39 ± 0.10
	24 September	18.00 ± 0.05	7.69 ± 0.02	0.38 ± 0.03
Swartvlei	9 August	13.65 ± 0.05	7.59 ± 0.02	2.10 ± 0.24
	22 August	14.25 ± 0.06	7.42 ± 0.02	0.88 ± 0.06
	23 September	16.72 ± 0.09	7.84 ± 0.02	0.64 ± 0.06

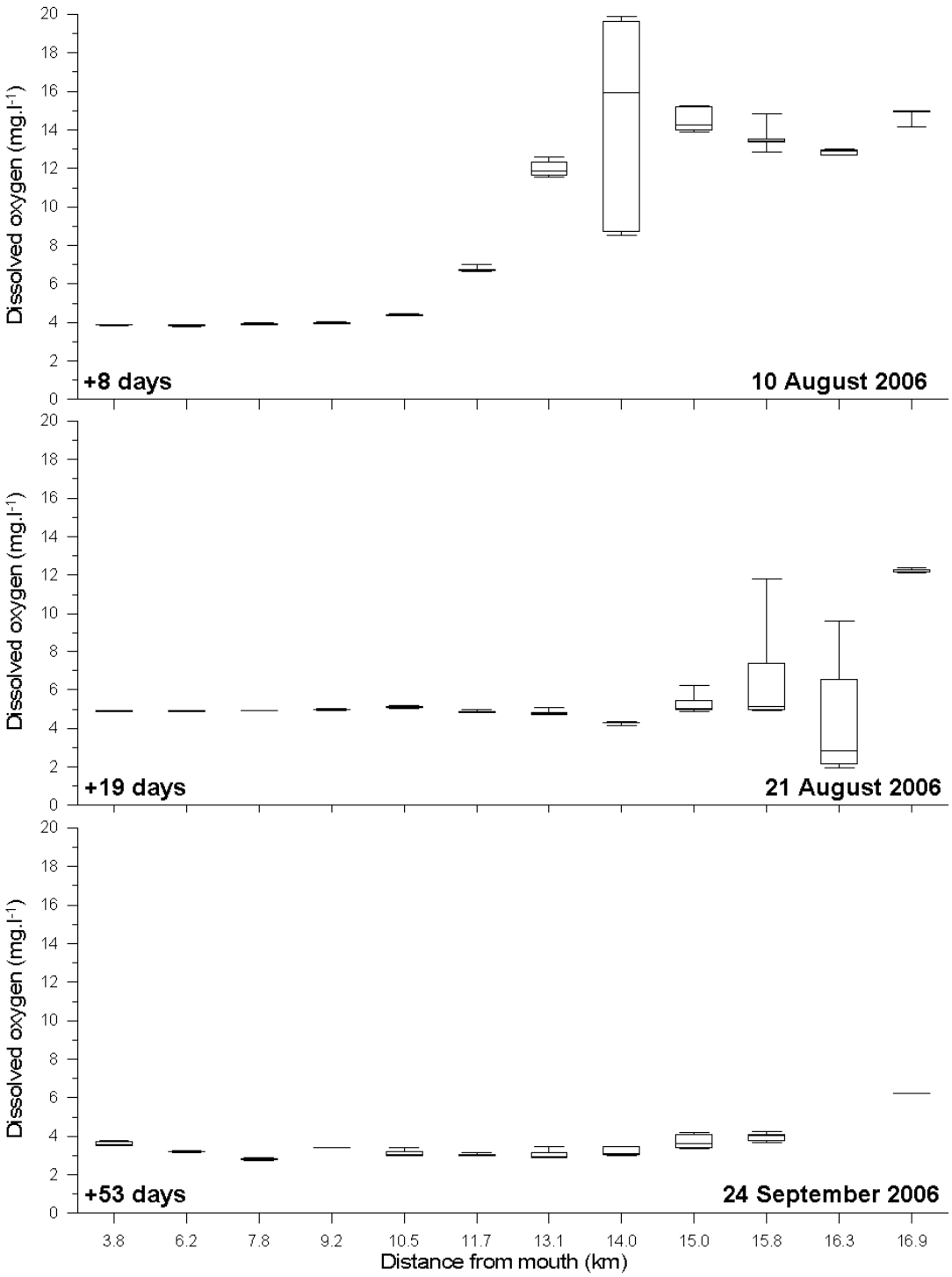

Figure 5.4. Longitudinal DO distribution in the Knysna Estuary following the 2 August 2006 flood. The caps at the end of each box indicate the extreme values (minimum and maximum), the box defines the lower and upper quartiles and the line in the centre of the box is the median.

The Knysna River is high in humic acids and this was reflected in the low average pH measured in the estuary shortly after the flood (Table 5.2). As average salinity in the estuary increased with time after the flood, the average pH increased too but was generally less than 7 in low salinity water (< 5 ppt).

Average light attenuation (K_d) was highest (0.69 ± 0.10 m^{-1}), particularly in the middle reaches of the estuary, 8 days after the flood (Table 5.2). The water was clearer and averages of the attenuation coefficients were similar 19 days (0.39 ± 0.10 m^{-1}) and 53 days after the flood (0.38 ± 0.10 m^{-1}).

Dissolved oxygen (DO) concentrations were high (> 6 mg l^{-1}) in the upper reaches of the estuary (13.1 km and upwards of the mouth) 8 days after the flood (Fig. 5.4). After 19 days, high DO concentrations were only found 15 km and further upstream of the mouth, and after 53 days this was limited to the head of the estuary. DO concentrations of < 2 mg l^{-1} occurred in deep water at the 6 m deep site 2, 16 km from the mouth, 19 days after the flood.

The distribution of nutrients in the Knysna Estuary indicates that the Knysna River is a major source of TOxN and DSi (Fig. 5.5). Vertically averaged TOxN, measured 8 days after the flood, was 21.1 ± 0.5 µM at the head of the estuary and decreased to 0.5 ± 0.2 µM at Thesen Island. Similarly, DSi decreased from 23.7 ± 3.8 µM at the head of the estuary to 2.6 ± 0.7 µM at Thesen Island. Site 2, located 16.3 km from the mouth, was particularly deep (6.2 m) with a small ephemeral stream flowing into it. The stream was flowing strongly 8 and 19 days after the flood but had dried up by the 53rd day, and could have been a source of nutrients at this site. TOxN was 22.0 ± 0.6 µM 8 days after the flood at site 2, slightly higher than measured at site 1 further upstream.

Soluble reactive phosphorous (SRP) was low (< 1 µM) throughout the estuary during the period of the study and was the nutrient most likely to limit primary production (Fig. 5.5). SRP was particularly low in the Knysna River water and only increased near to the mouth of the estuary.

Ammonium concentration peaked 7.8 km from the mouth 8 days after the flood (6.9 ± 0.5 µM), 16.3 km from the mouth 19 days after the flood (7.3 ± 1.4 µM) and then was low (< 1 µM) throughout the estuary 53 days after the flood (Fig. 5.5). After 19 days ammonium was highest in the upper estuary, 13.1-16.3 km from the mouth, particularly at deeper sites where a strong halocline had developed (e.g. the 6.2 m deep site 2). Ammonium in the upper estuary increased significantly with depth (*r* =

0.80; $P < 0.001$; n = 30), salinity ($r = 0.80$; $P < 0.001$; n = 30) and decreasing DO ($r = -0.69$; $P < 0.001$; n = 30). The introduction of organic debris from the flood and the intruding salt wedge as river flow decreased could have contributed to the low DO (a minimum of 1.9 mg l^{-1} was measured at a depth of 6.0 m at site 2).

SRP (or dissolved inorganic phosphorus; DIP) concentrations were consistently low throughout the estuary during the study (ranging from less than 0.1 to 0.9 µM), particularly in the upper reaches of the estuary. Ammonium was highest in the middle reaches of the estuary or in bottom water in deep upper reach sites, so TOxN had the greatest influence on the DIN: DIP trends (Fig. 5.6). The ratio was highest at the head of the estuary soon after the flood, and then decreased over the seven weeks and towards the mouth of the estuary.

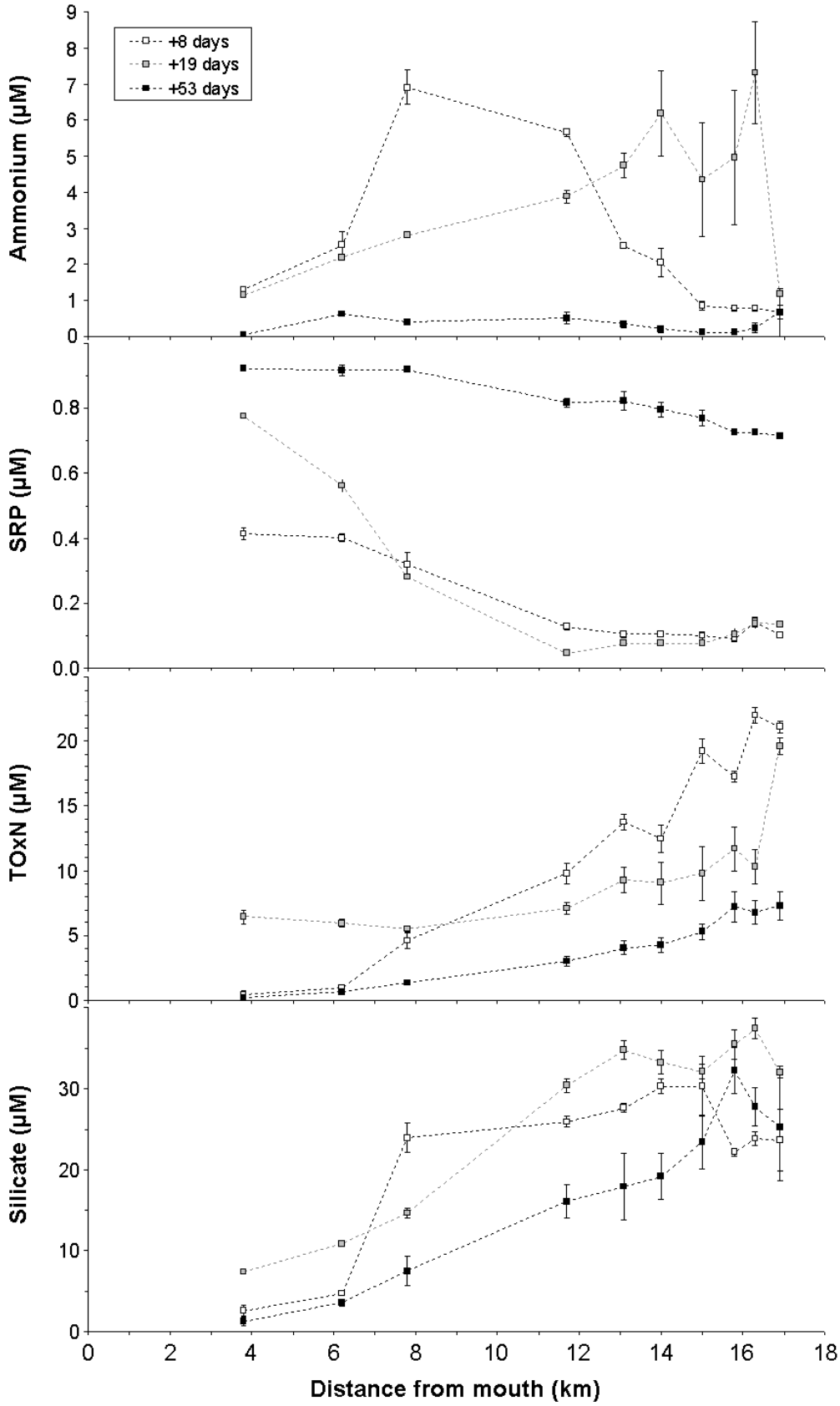

Figure 5.5. Longitudinal nutrient concentrations (µM) in the Knysna Estuary following the 2 August 2006 flood. Standard errors are represented by vertical bars.

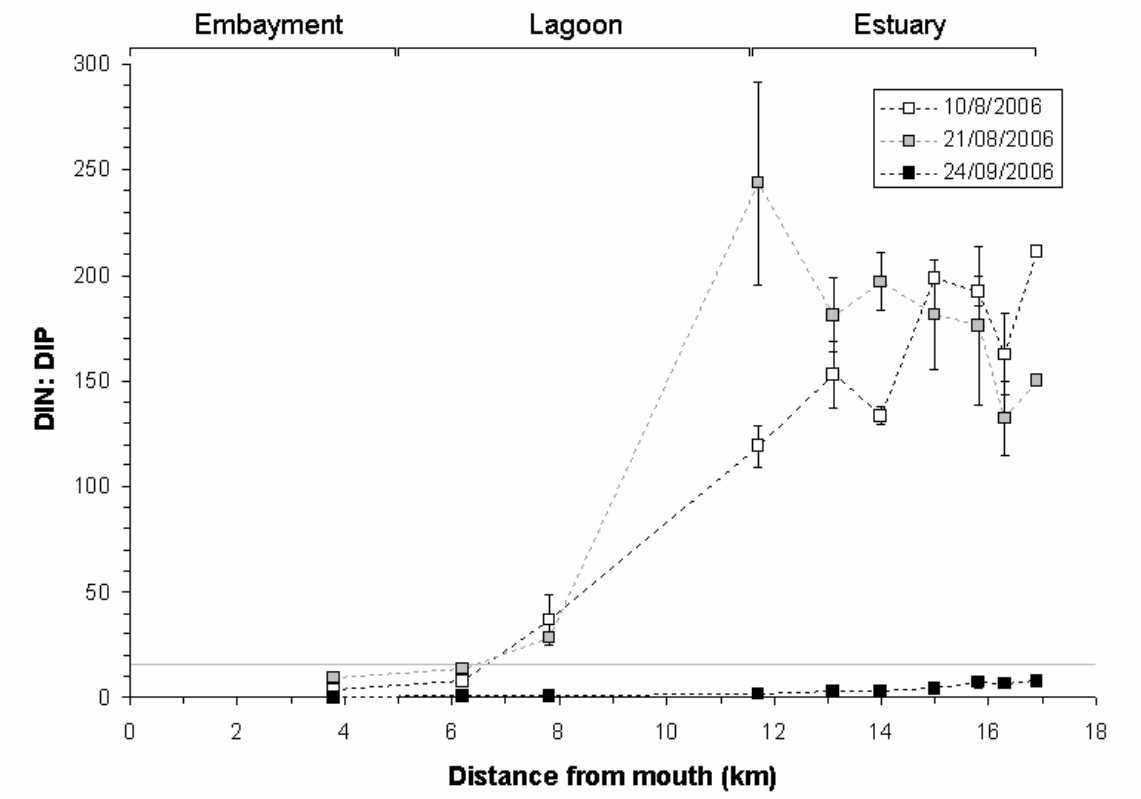

Figure 5.6. The ratios between dissolved inorganic nitrogen (DIN) and dissolved inorganic phosphorus (DIP); DIN: DIP, following the 2 August 2006 flood in the Knysna estuary. The grey line represents the Redfield ratio line.

Average phytoplankton chl a was low (0.30 ± 0.08 µg l^{-1}) throughout the Knysna Estuary 8 days after the flood. Chl a increased slightly towards the mouth of the estuary, 1.63 ± 0.44 µg l^{-1} near Thesen Island (Fig. 5.7). Average phytoplankton chl a (3.33 ± 0.86 µg l^{-1}) in the estuary increased 19 days after the flood and was highest in the middle reaches of the estuary. A maximum vertically averaged chl a of 15.1 ± 5.2 µg l^{-1} was measured 11.7 km from the mouth. A similar peak in vertically averaged chl a was not present 53 days after the flood and average phytoplankton chl a was 1.68 ± 0.10 µg l^{-1}. Average chl a was significantly higher in the estuary 19 and 53 days compared to 8 days after the flood ($H_{0.05,\ 45,\ 53,\ 49}$ = 56.43; P < 0.001).

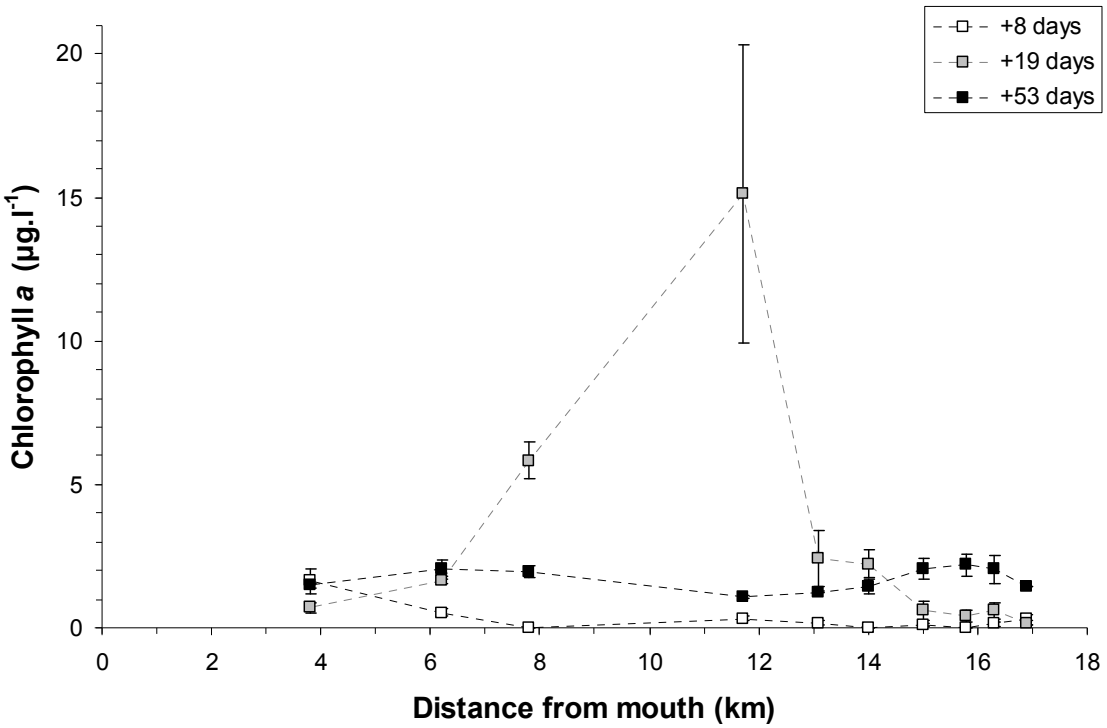

Figure 5.7. Longitudinal phytoplankton chl a in the Knysna Estuary following the 2 August 2006 flood. Standard errors are represented by vertical bars.

Swartvlei Lake and Estuary

The Swartvlei Estuary had been artificially breached and was tidal before the flood. Some vertical stratification was present in the estuary (within 7 km of the mouth) as brackish water flowed out of the lake and a wedge of dense marine water entered through the estuary mouth (Fig. 5.8). Marine water had penetrated ~2.5 km into the estuary after 52 days. Water-column measurements of physical parameters were limited to a depth of 8 m during the first two sampling sessions and to the bottom (> 11 m in the middle of the lake) 52 days after the flood. A salinity of 10 ppt was measured 7-8 m deep throughout the study and surface salinity was consistently < 5 ppt in the lake and < 1 ppt within the tributaries 7 and 20 days after the flood. The slightly weaker river flow after 52 days contributed to the weakening of the halocline within the lake and estuary.

Average temperature in the Swartvlei Lake and Estuary increased from 13.65 ± 0.05 ºC to 14.25 ± 0.06 ºC, 7 to 20 days after the flood respectively (Table 5.2). There was a temperature gradient between the head and mouth of the estuary 7 and 20 days after the flood, i.e. riverwater was cooler than marine water, but this did not

persist for longer. Average water temperature increased to 16.72 ± 0.09 ºC after 52 days. The highest temperatures were recorded at sites with a depth of less than 1.5 m. Temperatures measured in the estuary (0.6 to 6.2 km from the mouth) fell within the range reported by Whitfield (1988); i.e. temperature in the estuary ranged between 10 ºC (winter) and 29 ºC (summer) in shallow areas, and from 15 to 27 ºC in channel surface areas.

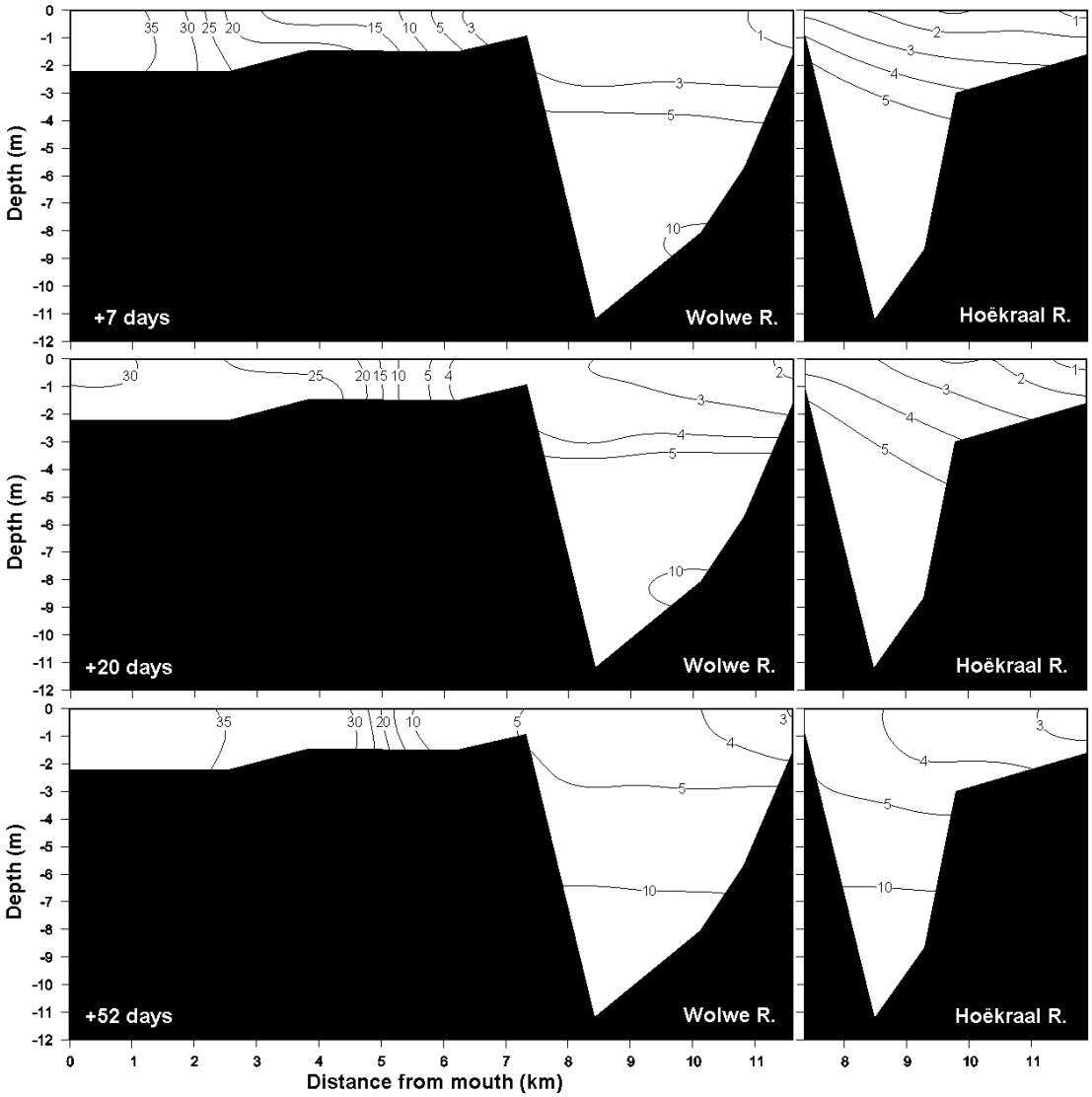

Figure 5.8. Longitudinal salinity distributions in the Swartvlei Lake and Estuary following the 2 August 2006 flood. The x-axes are split to represent salinity contours along the two mouth-tributary axes (Wolwe and Hoëkraal Rivers).

Average DO was similar 7 and 20 days after the flood (8.17 ± 0.16 mg l^{-1} and 8.37 ± 0.25 mg l^{-1} respectively) in the Swartvlei system but there was a significant decrease after 52 days (4.70 ± 0.16 mg l^{-1}) ($H_{0.05,\ 360,\ 217,\ 214}$ = 146.9; $p < 0.001$) (Fig. 5.9). Evidence of elevated BOD was found in deep water (> 11 m), 52 days after the flood, in the lake where ammonium concentration was relatively high (18 µM) and redox potential was negative (-82.0 mV). Ammonium did not exceed 10 µM during the two previous sampling trips, even at depths exceeding 8 m. In addition, DO concentration decreased uniformly +7 and +20 days after the flood but was stratified after 52 days. This indicates that the lake was well oxygenated within the first three weeks after the flood and that anoxic conditions developed 3 to 7 weeks after the flood. Vertically averaged DO was consistently lower in the estuary than in the lake.

High DO concentrations (> 10 mg l^{-1}) were commonly measured in the tributaries and in the shallows between the estuary and lake, particularly 7 and 20 days after the flood. Interestingly, similar results of water super-saturated with DO (180%) were reported 3 weeks after a flood in the Brunswick Estuary, east Australia (Eyre & Ferguson 2006). The super-saturated concentrations were attributed to a phytoplankton bloom but there were no such correlations between phytoplankton chl a and DO during this study.

There was a slight decrease in pH from 7 days (7.59 ± 0.02) to 20 days (7.42 ± 0.02) and then an increase, to more alkaline conditions, 52 days after the flood (7.84 ± 0.02) (Table 5.2). The gradual increase in pH over the 52 days was a result of river flow decreasing and marine water intruding further inland.

The attenuation of light was much higher in the lake (2.9 m^{-1} from 7.2 to 10.1 km) than at the mouth (0.6 m^{-1}) or riverine sites (Wolwe River = 1.1 m^{-1}, Hoekraal River = 2.2 m^{-1}) one week after the floods. Average light attenuation was highest 7 days after the flood compared to +20 and +52 days (Table 5.2). Water enetering the lake from the Hoëkraal / Karatara Tributary was more turbid than the Wolwe Tributary.

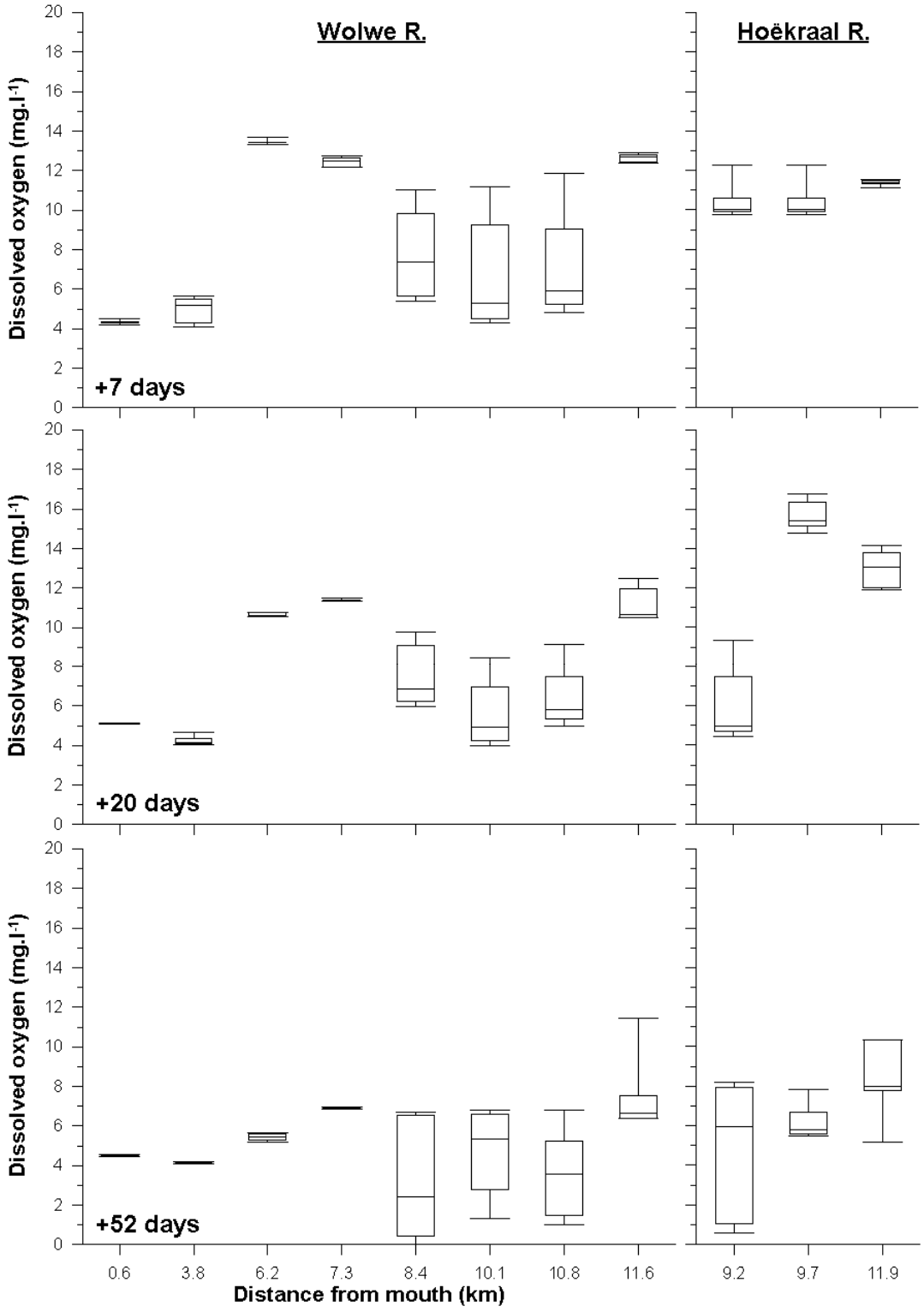

Figure 5.9. DO along the longitudinal axis of the Swartvlei Estuary and Lake following the 2 August 2006 flood. The caps at the end of each box indicate ± standard deviation, the box represents 25% and 75% quartiles and the line in the box the median.

Vertically averaged NH_4^+ was generally low throughout the estuary (ranging from 0.22 ± 0.05 µM near to the mouth to 4.1 ± 0.3 µM measured in the Wolwe Tributary) (Fig. 5.10). However, the gradient from the Wolwe Tributary to the mouth of the estuary was consistent throughout the study. The highest NH_4^+ concentration (17.6 µM) was measured in bottom water from the centre of the lake 53 days after the flood. SRP was generally low throughout the estuary during the entire study, ranging from 0.26 ± 0.03 µM in the Hoëkraal Tributary (+20 days) to 1.1 ± 0.3 µM also measured in the Hoëkraal (+7 days).

For the first 3 weeks after the flood there was a strong gradient in TOxN from the head of the estuary, Wolwe Tributary in particular, to the mouth of the estuary (Fig. 5.10). Concentrations ranged from 12.9 ± 1.6 µM in the Wolwe Tributary (+7 days) to 0.3 ± 0.2 µM measured at the mouth (+52 days). However, a peak in TOxN was found in the lake water; i.e. 7.3 km (8.5 ± 0.6 µM after 20 days) and 8.4 km (10.2 ± 1.6 µM after 52 days). DSi followed a similar gradient to TOxN, decreasing from the Wolwe Tributary to the mouth of the estuary. DSi ranged from 0.8 ± 1.7 µM at the mouth (+52 days) to 66.0 ± 2.5 µM measured in the Wolwe Tributary 20 days after the flood.

The DIN: DIP ratios were not as high in Swartvlei as in Knysna because TOxN was almost half and SRP higher than in Knysna. Fisher *et al*. (1988) only regarded SRP and TOxN of < 0.2 µM and 2.0 µM respectively as being potentially limiting to phytoplankton growth. For this reason it is unlikely that growth was P-limited during this study (SRP ranged from 0.2 to 1.5 µM). However, distinct peaks were evident in the deepest sites in the lake as a result of ammonification (Fig. 5.11). Lowest ratios were consistently measured in the estuary, within 7 km of the mouth, and were < 16 throughout most of the system 7 weeks after the flood.

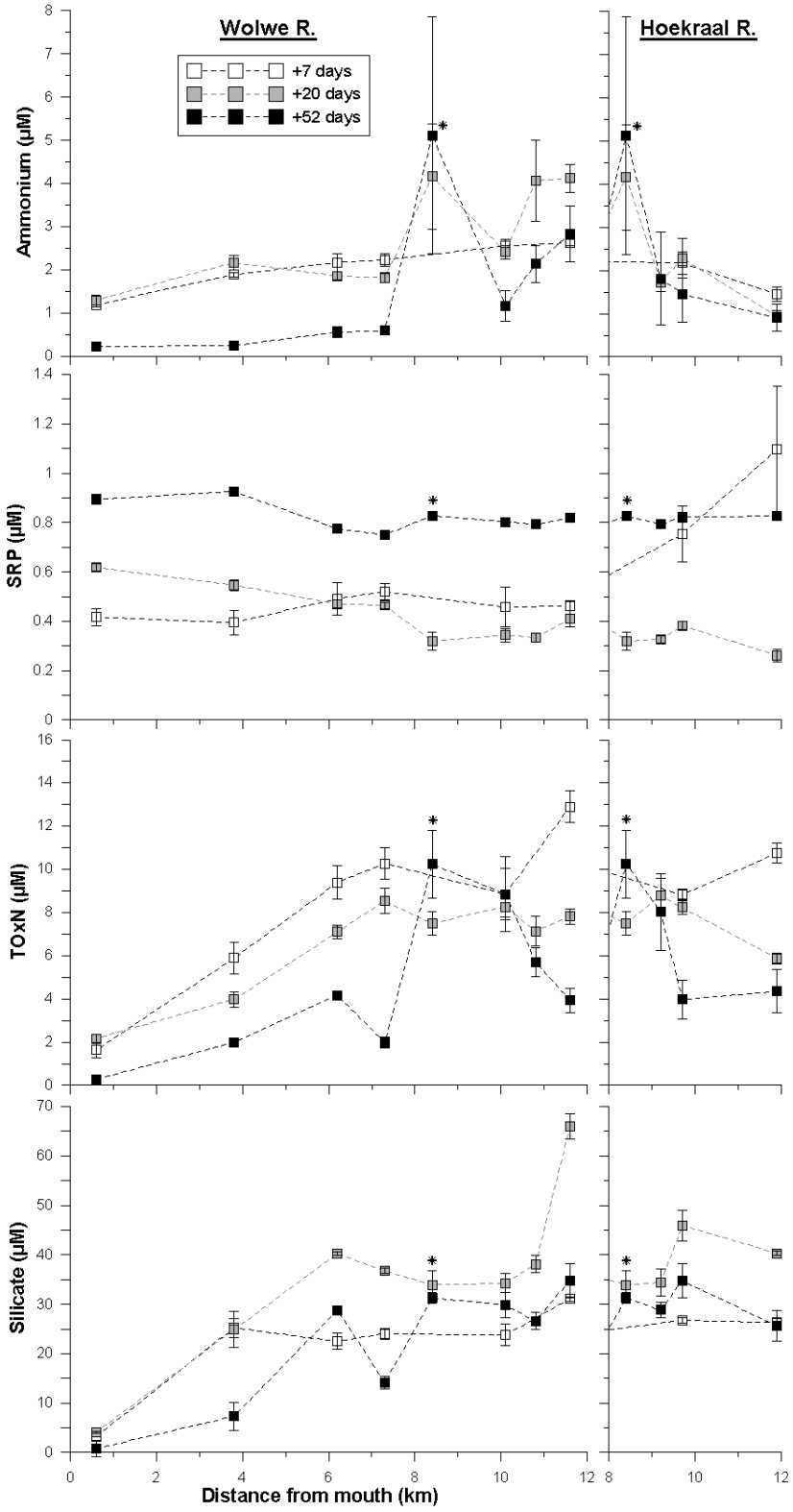

Figure 5.10. Longitudinal nutrient concentrations (µM) in the Swartvlei Lake (7.3 to 11.9 km from mouth) and Estuary (0.6 to 6.2 km from mouth) following the 2 August 2006 flood. Standard error represented by vertical bars. The points where sites in the Wolwe and Hoëkraal River plots overlap is highlighted (✷).

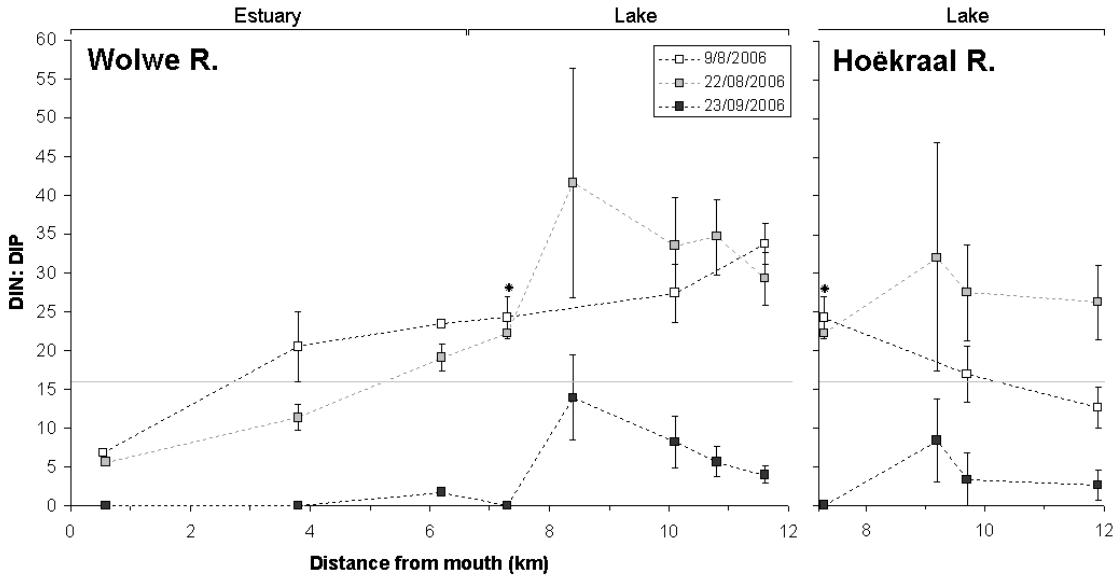

Figure 5.11. The ratios between dissolved inorganic nitrogen (DIN) and dissolved inorganic phosphorus (DIP), DIN: DIP, following the 2 August 2006 flood in the Swartvlei system. The points where sites in the Wolwe and Hoëkraal River plots overlap is highlighted (✷). The grey line represents the Redfield ratio.

There was a poor relationship between phytoplankton chl a and water-column nutrients throughout the study period. One week after the flood, chl a was low (< 1.0 µg l^{-1}) throughout the estuary and lake (Fig. 5.12). After 20 days, distinct maxima were measured at the sites where the Hoëkraal and Wolwe tributaries flowed into the lake; 7.8 ± 4.3 µg l^{-1} (9.7 km from mouth) and 4.6 ± 2.5 µg l^{-1} (10.8 km from the mouth) respectively. These maxima persisted to the following sampling session and increased in concentration; 21.5 ± 10.1 µg l^{-1} and 10.4 ± 2.7 µg l^{-1} respectively. Vertically averaged salinity measured at the interfaces ranged from 2.4 to 5.7 ppt, which are consistent with previous studies of river-estuary interfaces (the sector where maximum water-column chl a occurs and where integrated vertical salinity values are generally less than 10 ppt (Snow *et al*. 2000). The phytoplankton biomass was not significantly correlated to any nutrient 20 and 53 days after the flood.

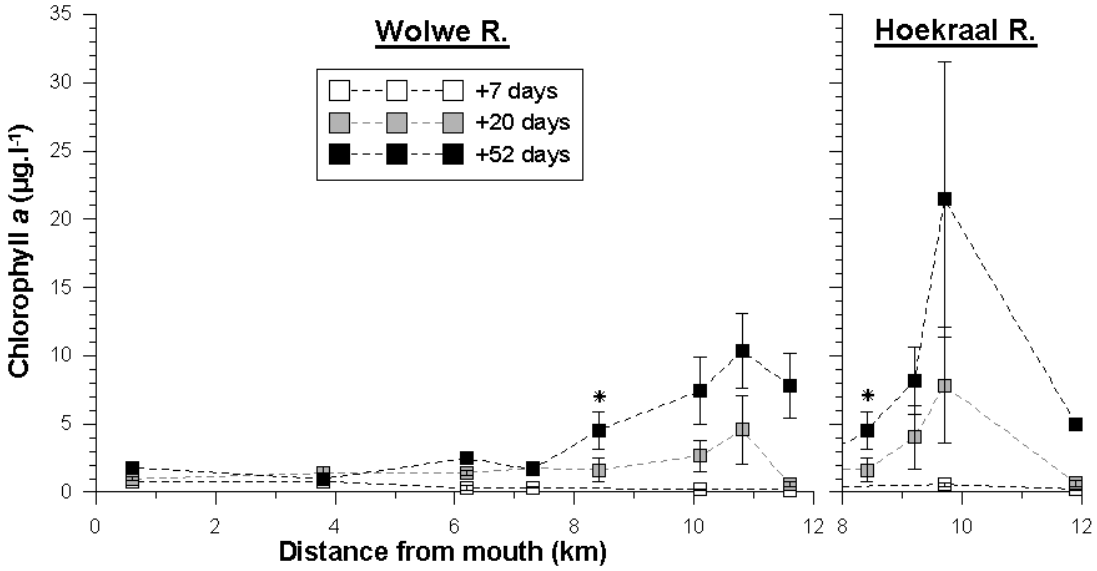

Figure 5.12. Longitudinal water-column chlorophyll a (µg l^{-1}) in the Swartvlei Lake (7.3 to 11.9 km from mouth) and Estuary (0.6 to 6.2 km from mouth) following the 2 August 2006 flood. Standard error represented by vertical bars. The points where sites in the Wolwe and Hoëkraal River plots overlap is highlighted (✷).

Discussion

Knysna Estuary

The major difference affecting the ecological function of both estuaries is their morphology. The Knysna Estuary, a marine dominated estuary, can be subdivided into three distinct sections; i.e. the marine-dominated embayment in the lower reaches, the lagoon in the middle reaches and the estuary in the upper reaches (Largier *et al*. 2000). Switzer (2004) described the estuary as having the largest tidal prism of any South African estuary. The system as a whole is funnel-shaped, being narrow and of low volume in the upper reaches and gradually becoming broader, increasing in volume towards the permanently open and deeply incised mouth. As a result, the entire estuary is well flushed by tidal exchange and the effects of floods are most intense in the upper reaches. The large volume of the lower reaches dissipates the effects of floods and marine water penetrated approximately 5 km upstream from the mouth throughout the study. However, a further decrease in flow of the Knysna River, through abstraction or damming, could lead to saline water intruding further inland for longer periods (Brockway *et al*. 2006).

The pulse of riverwater introduced low temperature water, which was super-saturated with oxygen, was slightly acidic owing to the high concentration of humic acids, and had elevated concentrations of TOxN and DSi. Similar results of reduced temperature and elevated nitrate and silicate concentrations in freshwater were reported by Chícharo *et al*. (2006). Results indicate that once this water mixed with the saline estuary water, the humic acids flocculated and created an area of high turbidity in the middle reaches of the estuary. Ammonium peaked in the middle reaches of the estuary about a week after the flood, possibly as a result of nutrients remaining from the flood, the resuspension of fine sediment or the remineralisation of deposited organic material. This peak moved further upstream as river flow decreased over the following two weeks and there was a gradual decrease in other nutrient concentrations and DO. The reduced river flow led to a gradual increase in pH and an increase in water-column clarity. As the denser lagoon water moved further upstream, the water-column became stratified in the upper reaches of the estuary. Similar results were reported by Largier *et al*. (2000) and Allanson *et al*. (2000) from a 1996-1998 study of the Knysna Estuary. However, Allanson *et al*. (2000) reported a decrease in DO with increasing distance from the mouth. This was attributed to the high biological oxygen demand in the upper reaches and an upwelling event, which introduced cold, oxygen-rich water into the embayment and lagoon. Cold water intrusions, as a result of coastal upwelling, can lead to density currents within the lower reaches of an estuary (Harcourt-Baldwin & Diedericks 2006). The density difference between the plunging water (dense, cold water) and the estuarine water landward of this region sets up a longitudinal gradient along the bed that drives a density current in a landward direction towards the head of an estuary. There was no evidence of an upwelling event throughout the duration of this study and the longitudinal temperature gradient was the reverse of that measured in the 1996-1998 study.

The ammonium concentration in the estuary following a storm in November 2000 (Switzer 2004) was higher than what loads imported in Knysna riverwater could have introduced. It was suggested that the mineralization of urea, which showed a 4-fold increase in concentration following the storm, could have contributed to the ammonium pool. The highest concentration of TOxN during Switzer's study (10.4 µM) was measured following the November 2000 storm but the concentration in the estuary showed a gradual increase over three days after the storm event. This was attributed to the slow release of TOxN, through seepage, from forested areas within

the river's catchment. Trends in SRP following the August 2006 flood closely mirrored those measured following the November 2000 storm event; SRP was 0.2 µM in the estuary a week after the flood and gradually increased to 0.8 µM after 53 days. Switzer (2004) reported a 0.8 µM baseline concentration for the estuary, which decreased to 0.3 µM following the November 2000 storm event. DIP showed near-conservative concentrations in both studies with respect to salinity.

Swartvlei Estuary and Lake

Water from three rivers flow into the tannin-stained Swartvlei Lake: namely the Wolwe, Hoëkraal and Karatara. The water from the lake then flows over a shallow sill, beneath rail and road bridges, through a 7 km long estuary with an intermittently-open estuary mouth. The water in all three rivers is slightly acidic (pH 4-7) and deeply stained with humic materials (Silberbauer 1982). It is likely that the August 2006 flood scoured sediment from the estuary mouth, which had been artificially breached prior to the flood, creating stronger tidal exchange between the sea, estuary and lake.

The pulse of fresh water and the subsequent river flow reduced salinity within the surface 3 m of the lake to less than 3 ppt, and established strong salinity stratification in the estuary. In addition to the riverwater, it is likely that a wedge of marine water had penetrated into the lake and sunk to the bottom of the lake as a dense layer creating a strong halocline with a marked resistance to vertical mixing (Silberbauer 1982). The riverwater flowing into the lake was colder than the rest of the estuary, was super-saturated with DO and was high in nutrients a week after the flood. As flow decreased, the salinity in the lake and estuary gradually increased and most suspended material (most likely flocculated humic material) in the lake's water-column settled out within the first three weeks after the flood. Results suggest that humic material that had accumulated on the sediment surface was mineralised, resulting in hypoxic (< 3 mg l^{-1}) and anoxic conditions in bottom water. The low oxygen environment favoured the release of ammonium into the overlying water and some of this was nitrified once in contact with more oxygenated water. Evidence of this could be seen in the centre of the lake where peaks in ammonium and TOxN concentrations were measured in bottom water and slightly higher in the water-column respectively.

SRP was low in the rivers, lake and estuary water throughout the study, ranging from 0.2 to 1.5 µM and was the nutrient most likely to have limited phytoplankton

growth. However, strong interface regions (REI's) did develop at the confluences between the rivers and the lake where salinity was less than 10 ppt. There were no significant correlations between phytoplankton chl a and nutrients so it is unclear how these chl a maxima developed. Liptrot (1978) found that pH and salinity in the lake favoured the flocculation of humic materials imported in river water, and Silberbauer (1982) described how some of these complexes bind with phosphate ions. It is possible that microalgal cells attach themselves to the floccules where phosphate is more available for uptake but further research is necessary to confirm this.

As flow decreases the mouth will eventually close, wind will mix the water-column of the estuary and lake, salinity is likely to gradually increase to ~18 and a 4 m thick layer of anoxic water is likely to develop, in deeper areas of the lake, above the lake sediment (Silberbauer 1982).

Trends in relation to international research

In small, shallow estuaries with limited tidal exchange, such as Lake Illawara (Australia), the photosysnthesis of microalgae (benthic and pelagic), seagrass and macroalgae can significantly increase DO concentration during daylight hours (Webster *et al*. 2002). Research of the small Wamberal Lagoon and the larger, deeper Smiths Lake have found distinct trends with regards to mixing and the potential for DO becoming depleted at depth (Gale *et al*. 2006). Factors such as wind and tidal mixing are important in mixing the water-column of small, shallow estuaries. As estuary size and depth increases these factors become less effective at mixing the water-column and processes such as heating and baroclinic circulation contribute to the development of vertical stratification. The larger and deeper an estuary is, the increased likelihood of DO depletion at depth. In the Swartvlei, a large and deep coastal lake, DO concentration has frequently been reported as low or completely depleted (Allanson 2001). Results from this study suggest that there was some vertical mixing following the flood but the DO became completely depleted within weeks of the flood at depth, probably as a result of the floods importing and depositing high loads of organic matter into the lake combined with the high residence time of water within the lake basin. Factors such as reduced river flow, decreased amplitude of floods, artificial breaching of the mouth and the two bridges dissecting the system reduce the effectiveness of floods on mixing the water-column. In contrast, the deeply incised mouth of the Knysna Estuary is permanently open and

the funnel-shaped morphology ensures strong tidal exchange occurs, mixing and flushing the entire system on a regular basis. As a result, the estuary is marine dominated and processes are almost entirely dependent on the physico-chemical properties of the seawater.

Conclusions

The key questions were addressed as follows;

The flood and subsequent river flow reversed the longitudinal temperature gradient in the estuary; i.e. temperature increased from the head to the mouth of the estuary, which is contrast to the results of Largier *et al.* (2000) and Allanson *et al*. (2000). The distribution of DO in the Knysna Estuary indicated that the pulse of water did replenish water in the upper half of the estuary, even in a 5.5 m deep site, and that there was no intrusion of upwelled, oxygen-rich water into the estuary. Ammonium concentrations were low throughout the estuary during this period when the water-column was relatively clear. Phytoplankton chl a reached a maximum in the lagoon almost three weeks after the flood but this was in high salinity water (> 30 ppt) and did not persist a further four weeks, so there was no evidence of a river-estuary interface developing in the Knysna Estuary following the flood. It is possible that the residence time of water in the Knysna Estuary was insufficient for a distinct peak in phytoplankton biomass to develop. Residence (or flushing) time refers to the time that it takes to replace water within a particular area. For estuaries, this is mainly dependent on the volume of river inflow and the size and shape of the estuary. Tidal exchange and the state of the mouth (e.g. restricted, unrestricted or closed) can also significantly influence flushing in the lower reaches of an estuary near the mouth. Flushing time is one of the dominant factors that control the degree to which nutrients passing from the catchment to the sea are modified within, for example, an estuary. The extent to which biochemical and biological processes influence nutrient cycling in estuaries is especially controlled by flushing times (Eyre & Balls 1999).

A vertical profile of DO in the Swartvlei Lake showed that stratification only developed 3 to 7 weeks after the flood. DO below the halocline decreased rapidly and negative redox values were recorded above the lake sediment 7 weeks after the flood. This suggests that some flushing of deep, anoxic water in the lake had occurred and that the subsequent mineralisation of humic matter caused anoxic conditions to redevelop only after three weeks. The pulse of fresh water and the

subsequent river flow resulted in phytoplankton biomass maxima developing 20 days after the flood at the river-lake water interface. These maxima persisted, and increased, after a further four weeks. This indicates that a productive interface zone did develop in the less than 10 ppt water. Turbidity in the lake decreased between weeks 1 and 3, and there was an associated increase in ammonium concentration. DO concentration did decrease in the lake, particularly after 7 weeks.

Acknowledgements

This work was funded by the Water Research Commission, South Africa. The authors would like to thank Anusha Rajkaran and Sabine Hoppe-Speer for their assistance in the field, South African National Parks for access to the estuaries, Weather SA for the George rainfall data and the Department of Water Affairs and Forestry (DWAF) for river flow data.

CHAPTER 6

Water quality in South African temporarily open/closed estuaries: A conceptual model

Snow, G.C. and Taljaard, S. 2007. Water quality in South African temporarily open/closed estuaries: A conceptual model. *African Journal of Aquatic Science* **32** (2), 99-111.

Abstract

Of the five types of estuaries found in South Africa, temporarily open/closed estuaries comprise approximately 70% of the total. This paper provides an overview and a simple conceptual model of the water quality features and characteristics of temporarily open/closed estuaries in South Africa. Available literature on estuaries in the warm- and cool-temperate biogeographic regions of South Africa was reviewed and assessed against this model. The structure and water quality characteristics of the estuaries considered supported the conceptual model under the three dominant mouth states: open, semi-open and closed. However, the extent to which biogeochemical processes influence water quality within estuaries is poorly understood, so further research is required if a better understanding of water quality changes in temporarily open/closed estuaries is to be attained.

Keywords: estuary, mouth condition, temporarily open/closed estuaries, water quality

Introduction

There are five main types of estuarine systems in South Africa: permanently open (POE), temporarily open/closed (TOCE), river mouths, estuarine lakes and estuarine bays (Whitfield 1992). An estimated 70% of South African estuaries are TOCEs, which generally have relatively small catchments (Breen & McKenzie 2001). Allanson (2001) discussed the diversity of estuarine types and the individual responses of these systems to physical and chemical determinants in this highly dynamic environment. In addition, he highlighted the need for techniques with which to integrate these features into conceptual and numerical models. The purpose of this paper is to provide an overview of the water quality features and characteristics of TOCEs, focusing on the warm-temperate and cool-temperate biogeographic regions of the Eastern and Western Cape Provinces of South Africa. This paper focuses on the following water quality parameters: salinity, temperature, pH, dissolved oxygen, turbidity and inorganic nutrients.

In estuaries, hydrodynamics or water circulation patterns and factors such as the quality of the inflowing river water play an integral role in determining water quality characteristics. Water circulation patterns, in turn, are largely influenced by run-off patterns. In Australia, for example, most rivers are short and have relatively small catchments. As a result, run-off to estuaries is delivered as short-lived, high energy events (on a scale of days) separated by long dry periods (on a scale of months or years) (Eyre 1998). This has further ripple effects in terms of flushing mechanisms and flushing times within estuaries which, in turn, are important factors influencing water quality processes. For example, in rapidly-flushed systems, nutrients tend to behave conservatively, while internal processes have a much larger effect on nutrient cycling in systems with longer flushing times (Balls 1994).

There are vast differences between the hydrological regimes of estuarine systems in the Northern and Southern Hemispheres. With few exceptions, Southern Hemisphere systems (e.g. in Australia and South Africa) show much higher variability and less consistency in annual run-off patterns, compared to Northern Hemisphere systems (e.g. in Europe and North America). Taking these differences into account, together with differences in tidal exchange and bathymetrical characteristics (e.g. European systems are often deeper than those in Australia and South Africa), conceptual models describing water quality processes in Northern Hemisphere estuaries (Fischer *et al*. 1988) are therefore not necessarily relevant to systems in

Southern Hemisphere continents. Conceptual models describing water quality processes (nutrient cycling in particular) in Southern Hemisphere systems have been developed mainly for Australian subtropical systems, since most of their systems are subtropical (Eyre & Twigg 1997; Eyre 1998; Eyre & Ferguson 2006).

To provide focus to this study, the following approach was followed. A simple conceptual or hypothetical model of the water quality structure and characteristics of TOCEs was developed for South African systems, based on current understanding. The available literature on estuaries in the warm- and cool-temperate biogeographic regions of South Africa (Cape systems) was reviewed and assessed against this model. Preliminary conclusions on whether the water quality structure and characteristics of TOCEs can be explained by such a generic conceptual model are given.

Conceptual model for water quality characteristics in TOCEs

To provide a conceptual model for the water quality characteristics of TOCEs, it is important to provide background information on typical abiotic states that can occur within such estuaries. From this perspective, the three dominant hydrodynamic states in which TOCEs can exist are: (1) mouth open; (2) mouth semi-closed; (3) mouth closed.

In (1), river inflow to the estuary is sufficiently high to keep the mouth open to the sea, allowing seawater intrusion during high tides, and with river inflow introducing fresh water into the upper reaches (Fig. 6.1). The location of the freshwater front within the estuary is largely a function of the volume of river inflow.

In (2), low river input results in an increase in berm height, restricting seawater intrusion during high tides and spring high tides in particular. However, the berm is not high enough to prevent water draining from the estuary into the sea (Fig. 6.1).

In (3), extremely low or no river input results in an increase in the height of the berm, preventing seawater from entering the estuary or water draining from the estuary into the sea. Sporadic overwash into the estuary can occur, depending on berm height and conditions at sea (Fig. 6.1).

Depending on factors such as estuary size, beach profiles and the degree of mouth protection, all three of these states can occur at a given estuary at various times (three-phased systems), or only in the open and closed states (two-phased systems).

These relationships are discussed in further detail below, as part of the hydrodynamic overview. In the following sections, possible water quality characteristics associated with each of the above states are provided.

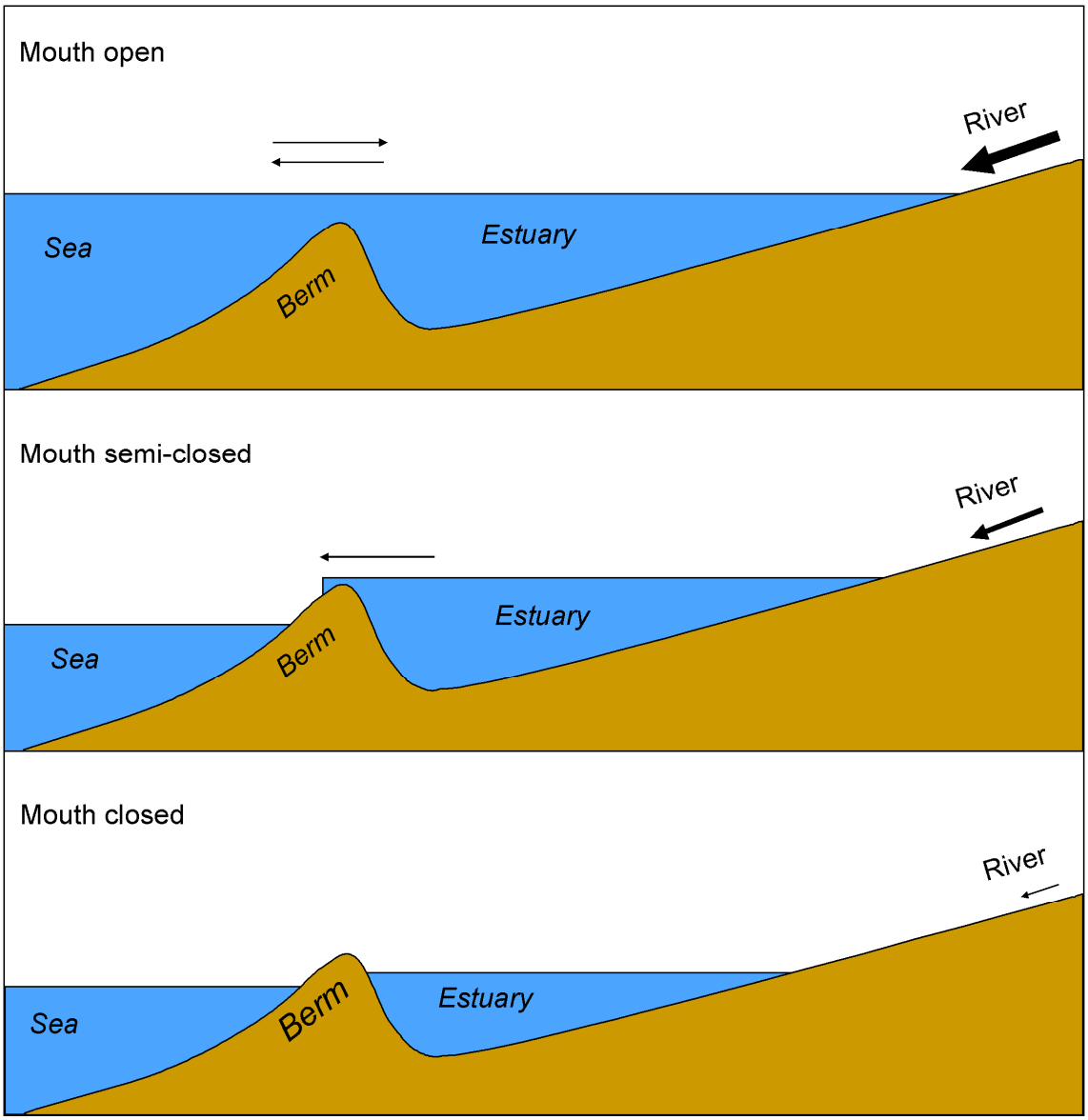

Figure 6.1. Diagrammatic representation of the three dominant states in which TOCE mouths can exist: (1) open, (2) semi-closed and (3) closed.

Salinity

The characteristic salinity distribution patterns expected under each of the three states described above are as follows. When the mouth is open, a longitudinal salinity gradient exists in the estuary (Fig. 6.2). The position of the halocline depends on the rate of river inflow. In some systems, vertical stratification can also develop in this state, usually in systems in which the middle and upper reaches are characterised by deeper water, e.g. the Palmiet Estuary, 75 km south-east of Cape Town.

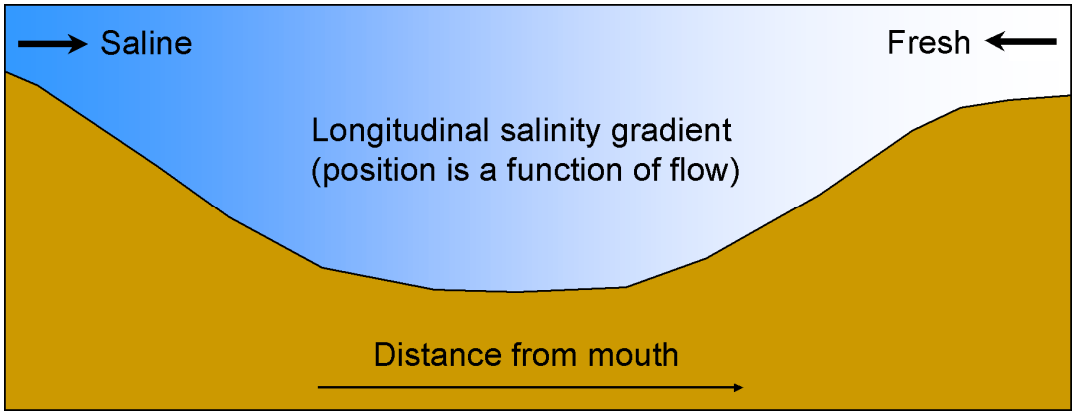

Figure 6.2. Normal salinity conditions in a TOCE when the mouth is open to both seawater and riverwater exchange.

State 2 (mouth semi-closed) is typical during periods of low freshwater input, when a strong longitudinal salinity gradient is created. Inflowing freshwater is restricted to the surface of the estuary, thus trapping more saline water in the deeper areas. At the onset of this state, vertical stratification usually develops as a result of low-density freshwater flowing across higher-density saline waters (Fig. 6.3). Salinity is generally the dominant factor in determining water density variations in estuaries. A change in temperature from 5 ºC to 25 ºC will change the density of water with a salinity of 35 ppt by about 0.006 kg m^{-3}, while a change in salinity from 0 to 30 ppt will change the density by around 0.027 kg m^{-3} (Schumann *et al*. 1999). As a result of entrainment of fresh water into the more saline bottom layer, and the effects of mixing by wind, the estuary gradually changes into a homogeneous, brackish water body. The duration of the stratified conditions depends on river inflow, the strength of wind-mixing forces, and the depth of the estuary. For example, a shallow system

subject to strong windmixing forces will become a homogeneous water body much faster than a deep wind-protected system. Periodic intrusion of seawater (e.g. during spring high tides) may sustain more saline conditions in the deeper waters near the mouth. If the semi-closed state persists for a few months at a time and there is little or no overwash, then salinity throughout the estuary may decrease due to wind induced mixing or entrainment, as the fresh water 'erodes' into the more saline bottom water.

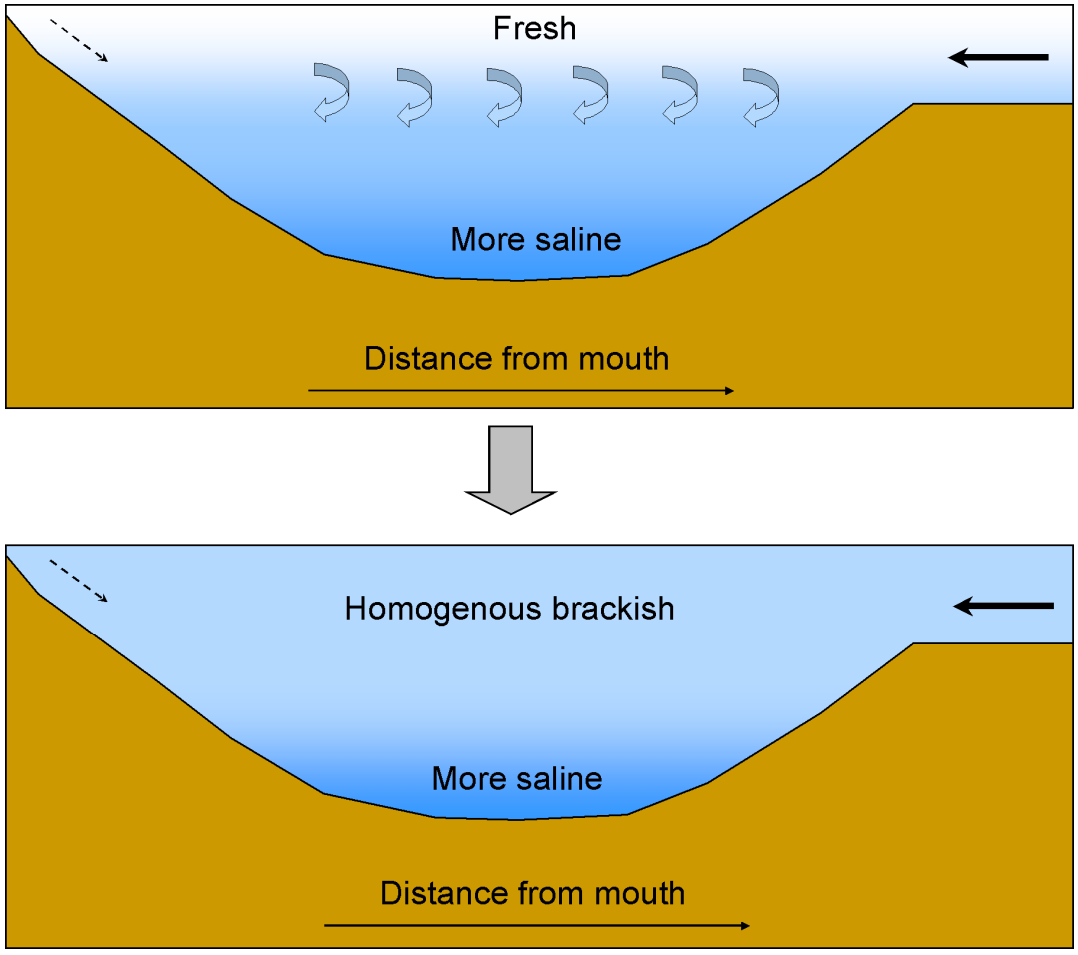

Figure 6.3. Vertically-stratified salinity conditions becoming mixed by wind to become homogeneous brackish in the semi-closed mouth state.

In the closed-mouth state, salinity throughout the water-column is approximately homogeneous (Fig. 6.4), although some vertical and longitudinal stratification may be evident immediately after closure. Depending on the height of the berm and conditions at sea, sporadic overtopping by seawater (or seawater overwash) can occur during this closed state. This results in the intrusion of seawater into the bottom water, the extent of which depends on the volume of seawater entering the estuary and the estuary's bathymetry. Continued river input may result in a system gradually becoming fresher, while the absence of river inflow may result in it gradually becoming more saline; hypersaline in some instances.

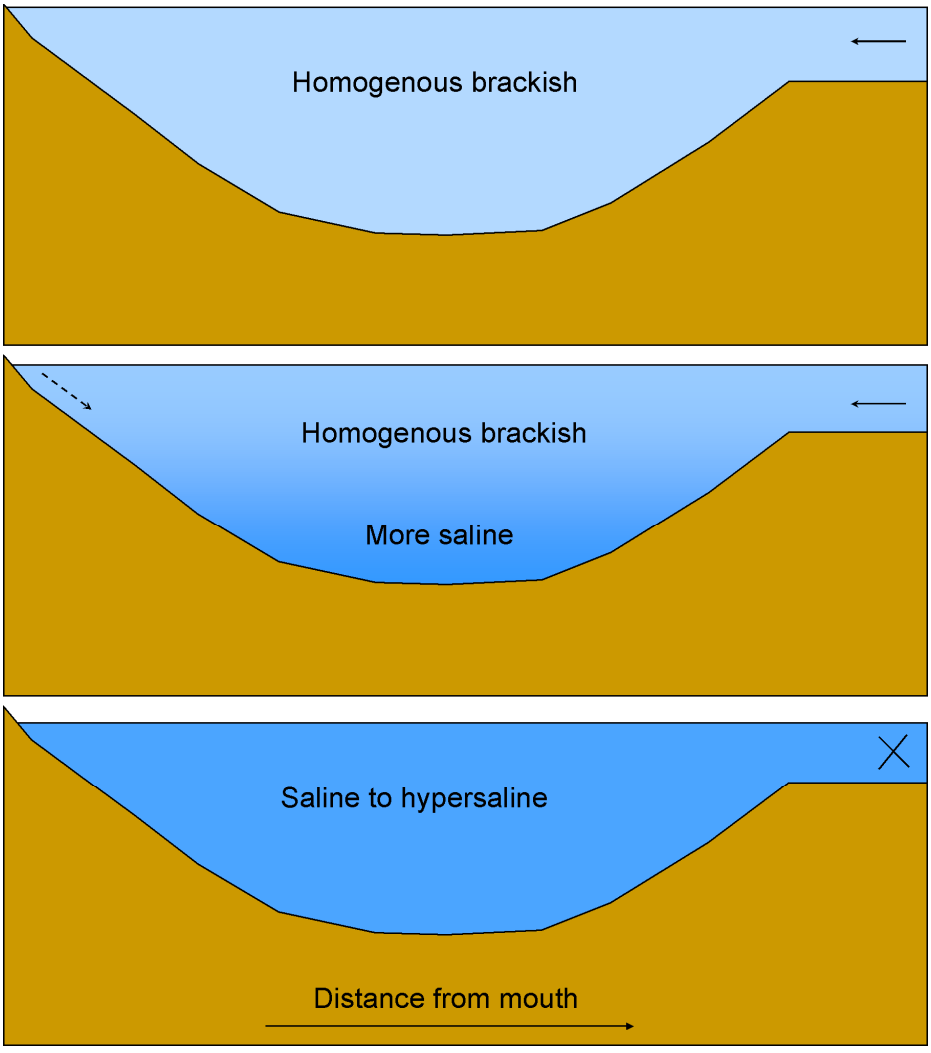

Figure 6.4. The closed-mouth condition when salinity is nearly homogeneous throughout the water-column. Some vertical and longitudinal stratification may be evident immediately after closure. Overwash events may introduce more saline water to deeper reaches of the estuary.

Temperature

In intermittently open estuaries, water temperature is usually a function of seasonal trends in atmospheric temperature. Although the achieved temperature will obviously depend on local atmospheric temperature, strong seasonal signals are expected in temperate regions where winter temperatures typically range between 15–20 °C and summer temperatures between 20–25 °C.

Water temperature in estuaries is also subject to prevailing sea conditions, particularly during the open mouth state. For example, coastal areas along the cool–temperate zone of South Africa, particularly along the West Coast, are regularly subject to upwelling when cold bottom waters are brought to the surface. In these circumstances the temperature of newly-upwelled water can range between 9 and 14 °C, depending on the strength of the upwelling event (DWAF 1995). Along the West Coast, these events are usually most prevalent during spring and summer (Monteiro & Largier 1999), under offshore wind conditions. For the foregoing reasons, when estuaries are in the open-mouth state, sea conditions (e.g. upwelling) can also influence water temperature, particularly in the lower and middle reaches of the estuary. If this happens in summer a strong longitudinal temperature gradient can develop, with the colder water occurring near the mouth and water temperatures becoming significantly higher upstream.

pH

Typically, the pH of estuarine waters is influenced by the inflowing water sources, namely the river and the sea. Seawater pH is known to range between pH 7.9 and 8.2 (DWAF 1995), while that of riverwater is usually a function of catchment characteristics. For example, rivers draining Table Mountain quartzite are usually rich in humic acids, originating from typical vegetation found in these soils, and are characterised by low (~4) pH levels. However, as a result of the strong buffering capacity of seawater, pH levels in estuarine waters are usually within the range 7.0–8.5.

Dissolved oxygen

The typical oxygen distribution patterns found during each of the three mouth states are as follows. During State 1, TOCEs are expected to be well-oxygenated (DO

levels above 6 mg l^{-1}) because there is good water exchange through tidal flushing and river inflow.

In state 2 (mouth semi-closed), strong vertical stratification may prevent proper aeration of bottom waters. Depending on the duration of these stratified conditions and the organic load, bottom waters may become low in oxygen (< 3 mg l^{-1}). If vertical stratification is broken down and through wind-mixing the water-column becomes well mixed again, oxygenated water gets reintroduced into the bottom layers (Fig. 6.5). However, oxygen levels of < 3 mg l^{-1} may still occur in the deeper more saline pools, which are effectively cut off from surface waters as a result of strong vertical stratification persisting at depth.

In State 3, the water-column is expected to be relatively homogeneous, with no marked vertical stratification. As a result, wind-mixing is likely to maintain aerated conditions throughout the water-column. However, lower oxygen levels may still occur in deeper, more stagnant, pools, depending on factors such as organic loading. During an overwash event, low-oxygen bottom water can become replaced with well-oxygenated seawater, depending on the volume of water that enters the system.

In estuaries, a decrease in DO is mainly caused by the degradation of organic material through bacterial activity, which consumes available oxygen. These periods of low or no oxygen can be a result of natural processes, but can also be triggered by nutrients and organic material such as fertilisers, deposition of nitrogen from the atmosphere, erosion of soil containing nutrients, and sewage treatment plant discharges (see United States Geological Survey at http://toxics.usgs.gov). Dissolved oxygen concentrations > 3 mg l^{-1} are unlikely to result in the stress or death of organisms, but concentrations < 2–3 mg l^{-1} can be regarded as hypoxic and often arise due to eutrophication within an estuary. Anoxia is the complete lack of oxygen (0 mg l^{-1}).

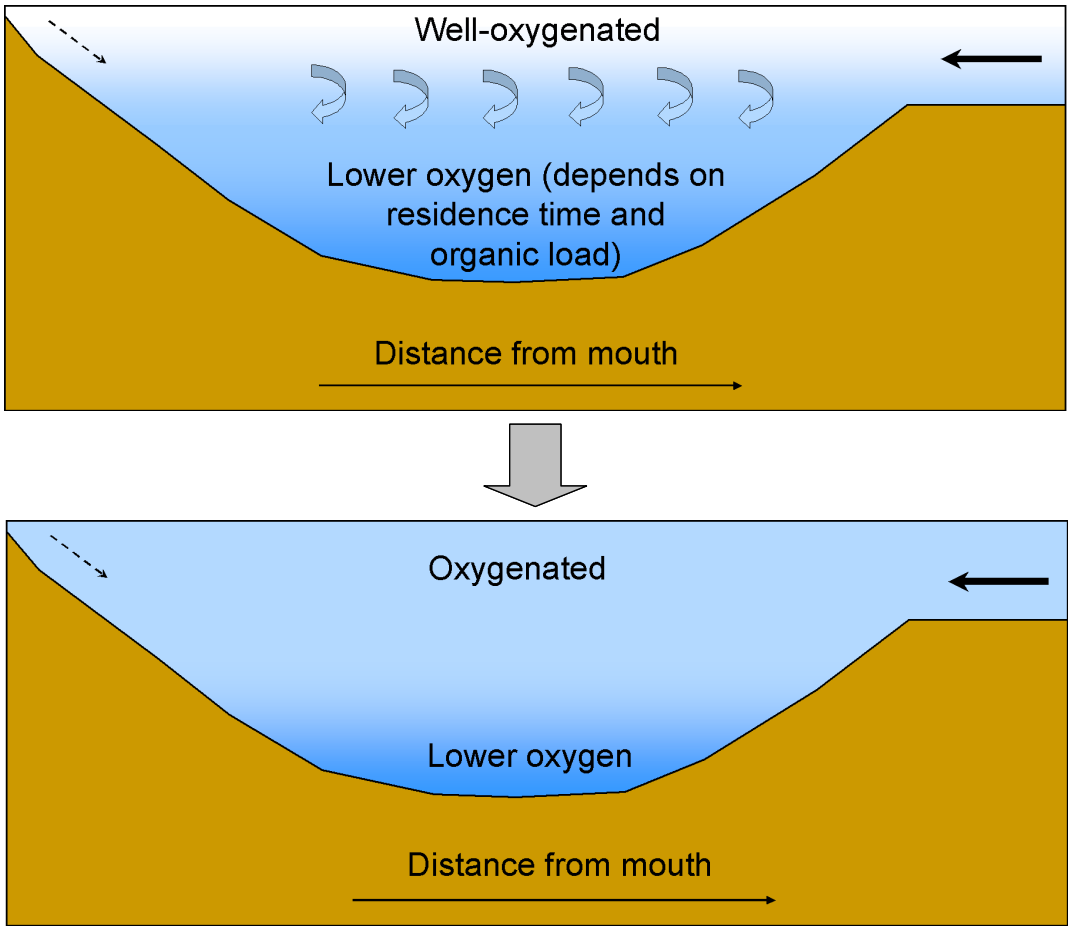

Figure 6.5. Schematic illustration of the progressive change expected in dissolved oxygen characteristics in a TOCE under the semi-closed state.

Turbidity

Turbidity, usually reported in Nephelometric Turbidity Units (NTU), is largely a function of suspended particulate material concentrations in the water sources (river and sea), bottom sediment composition (i.e. mud *versus* sand), water depth and wind-mixing. The turbidity of seawater entering estuaries along the cool and warm temperate regions of South Africa is usually relatively low (< 10 NTU). The turbidity of riverwater flowing into an estuary can vary greatly, depending on catchment geology as well as agricultural practices within the catchment. Suspended silts, clays and colloidal humic acids, which are transported into an estuary via river flow, either flocculate or adsorb to each other. Both processes appear to be a function of salinity and flocculation has been shown to occur at between 0 and 5 ppt salinity (Kemp 1989). The process generally causes a localised turbidity maximum at the river–estuary interface and, unless held in suspension by wind-mixing or other turbulent

forces, the floccules settle out of the water-column without contributing to the planktonic food web.

Sediment characteristics and physical features within the estuary can also affect turbidity. For example, in shallow systems wind-mixing forces can create turbid conditions, characterised by fine bottom sediments, through resuspension. Turbidity characteristics of estuaries are therefore site-specific and can be predicted, taking into account the turbidity characteristics of river inflow (based on knowledge of the catchment geology), bathymetry, bottom sediment composition and prevailing wind forces.

Inorganic nutrients

This overview focuses on dissolved inorganic nitrogen (DIN) and dissolved inorganic phosphate (DIP), considered to be important forms of inorganic nutrients in estuaries.

Although external sources of inorganic nutrients to estuaries are mainly derived from catchments (i.e. river inflow), nutrients can also be introduced from the sea (e.g. tidal intrusion of upwelled waters), groundwater and anthropogenic sources (e.g. sewage) discharging directly into estuaries. Nutrient dynamics within estuaries can also be influenced by a large number of physical and biochemical processes, such as evaporation, sorbtion-desorption, nitrification/denitrification, mineralisation (i.e. degradation of organic matter through bacterial activity) and biological uptake by primary producers (Roy *et al*. 2001).

A simplified schematic illustration of sources and processes that may influence inorganic nutrient concentrations in estuaries is provided in Figure 6.6.

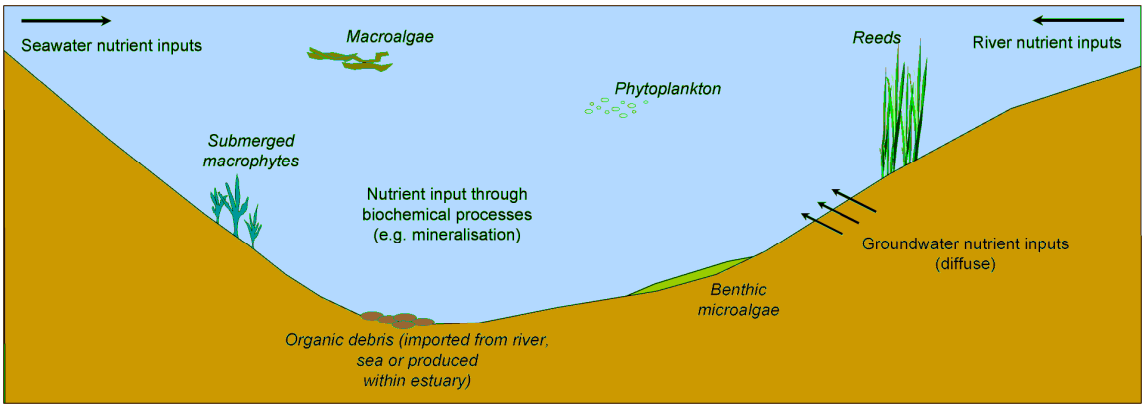

Figure 6.6. A simplified representation of the main potential sources of inorganic nutrients to estuaries.

The inorganic nutrient concentration in an estuary is therefore largely a function of the concentration in the source waters, i.e. the river, sea and/or groundwater, as well as any physical processes (e.g. evaporation) and biochemical processes (e.g. biological uptake and mineralisation) that occur within the estuary.

Inorganic nutrients in estuaries can become biologically available through transformations, so there may not be a direct relationship between the ambient concentration of these variables and the biological response. Instead, there is often a relationship between flux and biological response. The concentration of DIN and DIP measured in the water-column reflects the net effect of the rate at which these nutrients are taken up by primary producers and the rate at which they are regenerated or replaced at that specific position. A very low nutrient concentration could indicate that a particular nutrient has essentially been depleted from the water-column and is therefore limiting primary production, but equally it could simply be the net result of a very rapid uptake by primary producers, loss to the atmosphere (e.g. through denitrification) and slow remineralisation of the nutrient (e.g. due to limited organic loading) (ANZECC 2000).

A generalised characterisation of inorganic nutrient sources and processes, under each of the three dominant states, is as follows.

For State 1, the DIN and DIP concentrations are largely a function of the concentrations in the inflowing river and seawater. Rapid water exchange usually does not allow sufficient residence time within the estuary for marked primary production in the water-column, particularly in the smaller systems characteristic of many of the South African TOCEs. However, in larger TOCEs (e.g. the Great Brak Estuary), residence times in the middle to upper reaches may become sufficient to stimulate water-column productivity during the open state, provided that the limiting nutrients are available. Although physical and biochemical processes and groundwater input may be important for benthic primary production and for macrophytes taking up nutrients through their roots, these are not considered to be major sources in terms of the overall water-column inorganic DIN/DIP pool, although this still needs to be confirmed.

During the open state, one would expect a linear relationship between DIN, DIP and salinity, with the concentrations of DIN and DIP typically being higher at lower salinity values, as illustrated in the property-salinity plot (Fig. 6.7), except in larger TOCEs such as the Great Brak, where longer residence times could allow biological

uptake and/or biochemical processes to alter this relationship. The concept of assessing inorganic nutrient results by comparison to a theoretical line representing conservative mixing of river and seawater has been used and discussed previously by Head (1985) and Balls (1994).

DIN and DIP introduced through river inflow are usually high compared to that introduced via the sea. However, along the cool-temperate West Coast, inorganic nutrient concentration (particularly DIN) is also influenced by the upwelling of colder nutrient-rich bottom water. This is less common along the warm-temperate zone although upwelling is known to also occur along some areas of the south and south-east coasts (Taylor 1992).

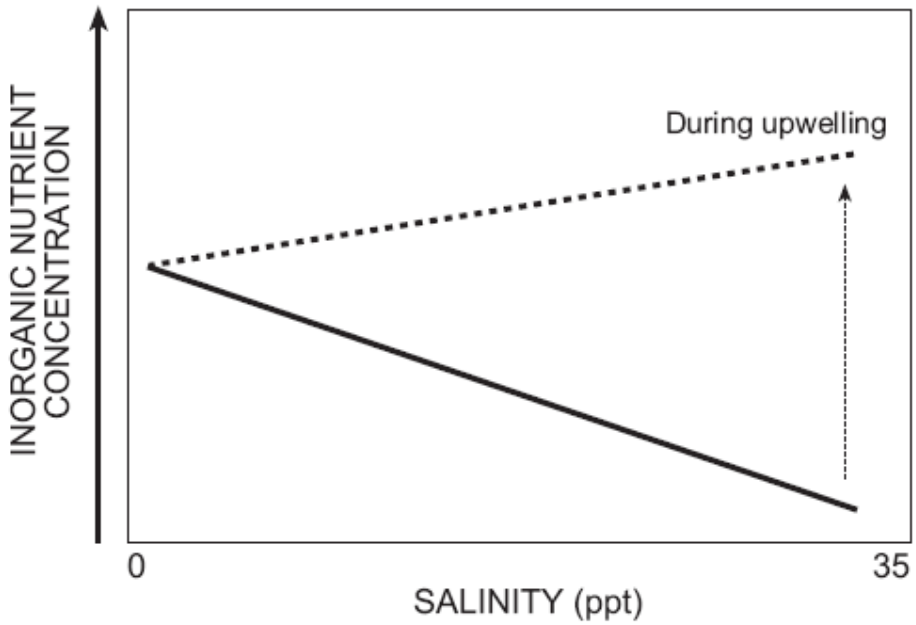

Figure 6.7. Hypothetical relationship between salinity and inorganic nutrient concentrations during the open-mouth state.

At the onset of State 2, DIN and DIP concentrations are largely a function of their concentrations in the inflowing river and seawater. However, higher phytoplankton production, in association with an increase in phytoplankton biomass, is likely to occur as a result of the longer residence time of water, leading to a decrease in DIN and DIP concentrations. River inflow is likely to maintain DIN and DIP levels, to sustain a certain level of water-column primary productivity. A hypothetical relationship between water-column chlorophyll a (chl a) and inorganic nutrient concentrations under the semi-closed state is illustrated in Fig. 6.8.

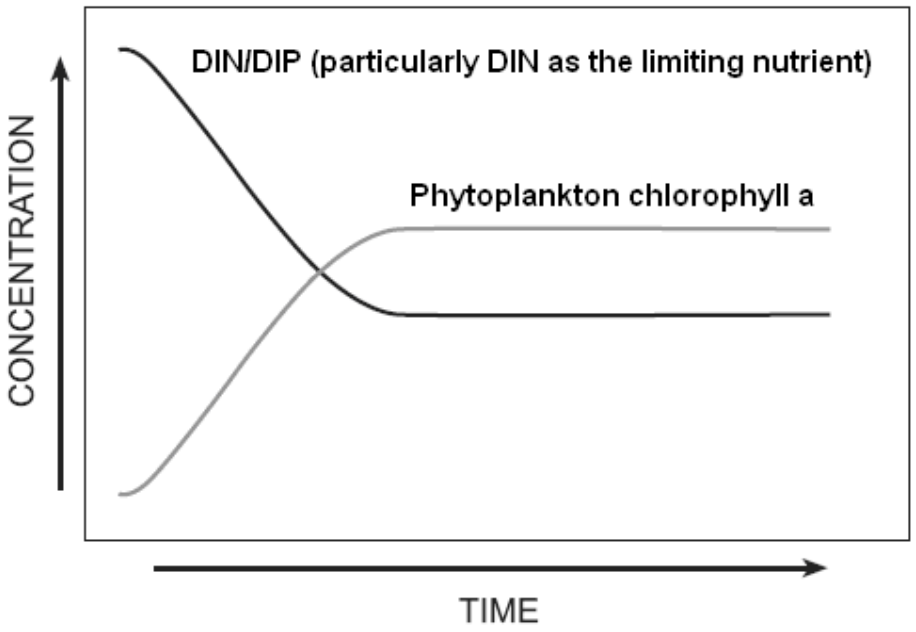

Figure 6.8. A hypothetical relationship between water-column chl a and inorganic nutrient concentrations as a function of time during the semi-closed mouth state.

There are two possible scenarios related to the onset of the closed state (State 3): (a) the mouth is initially open and (b) the mouth is initially in the semi-closed state. In both scenarios, DIN and DIP concentrations are largely a function of the concentrations in the inflowing river and seawater at the time of closure. If the mouth was initially open, then DIN and DIP concentrations would have been high and chl a concentrations low. Following mouth closure, the longer residence time of water would result in an increase in water-column chl a and an associated decrease in DIP and DIN concentrations, until such time as the nutrients (DIN in particular) are depleted (Fig. 6.9). However, if the mouth was semi-closed, then water-column chl a would have been high in relation to nutrient concentrations (Fig. 6.8). Following mouth closure, residence time would increase further, resulting in a short-lived increase in water-column chl a before nutrients (DIN in particular) become depleted. This would result in a decrease in water-column chl a.

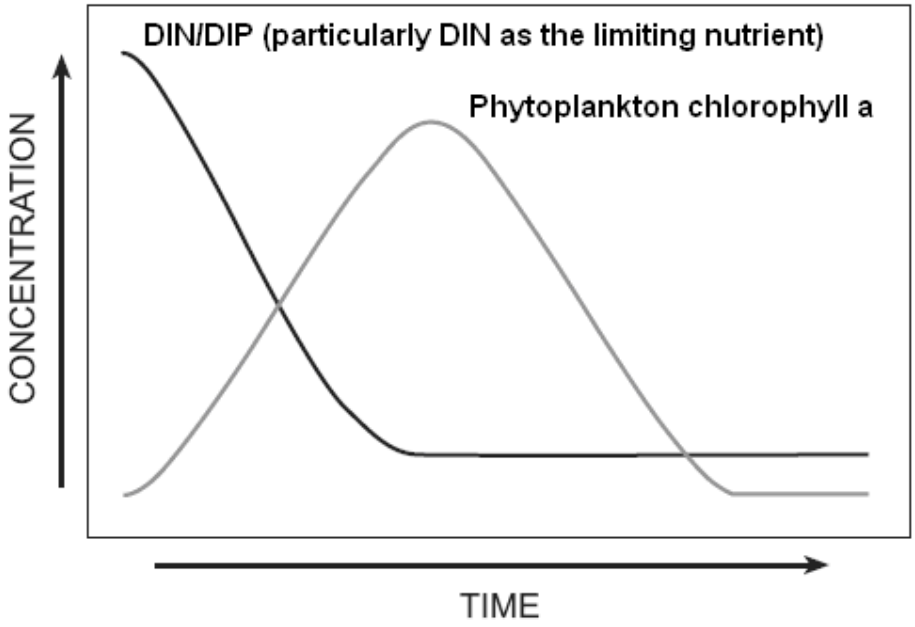

Figure 6.9. Hypothetical relationship between water-column chl a and inorganic nutrient concentrations as a function of time in the closed-mouth state.

It is expected that biochemical processes (e.g. mineralisation) and groundwater input will also become more important sources of DIN and DIP for primary production in TOCEs, during this state. Depending on the rate of these biochemical processes

and groundwater input, as well as sediment disturbance by wind or by the infauna, these sources may also become important sources of DIN and DIP to the water-column. However, very little is known about these biochemical processes in South African TOCEs and this requires further research. This aspect is currently being investigated as part of a Water Research Commission project.

During overwash of seawater under the closed state, DIN and DIP concentrations in the estuary water-column may take on some of the quality characteristics of the seawater, the extent depending on the volume of seawater that enters the system.

Detailed assessment of two case studies

The water quality characteristics in two systems within the cool-temperate zone, the Diep and the Palmiet Estuaries (Fig. 6.10), illustrate the conceptual water quality model.

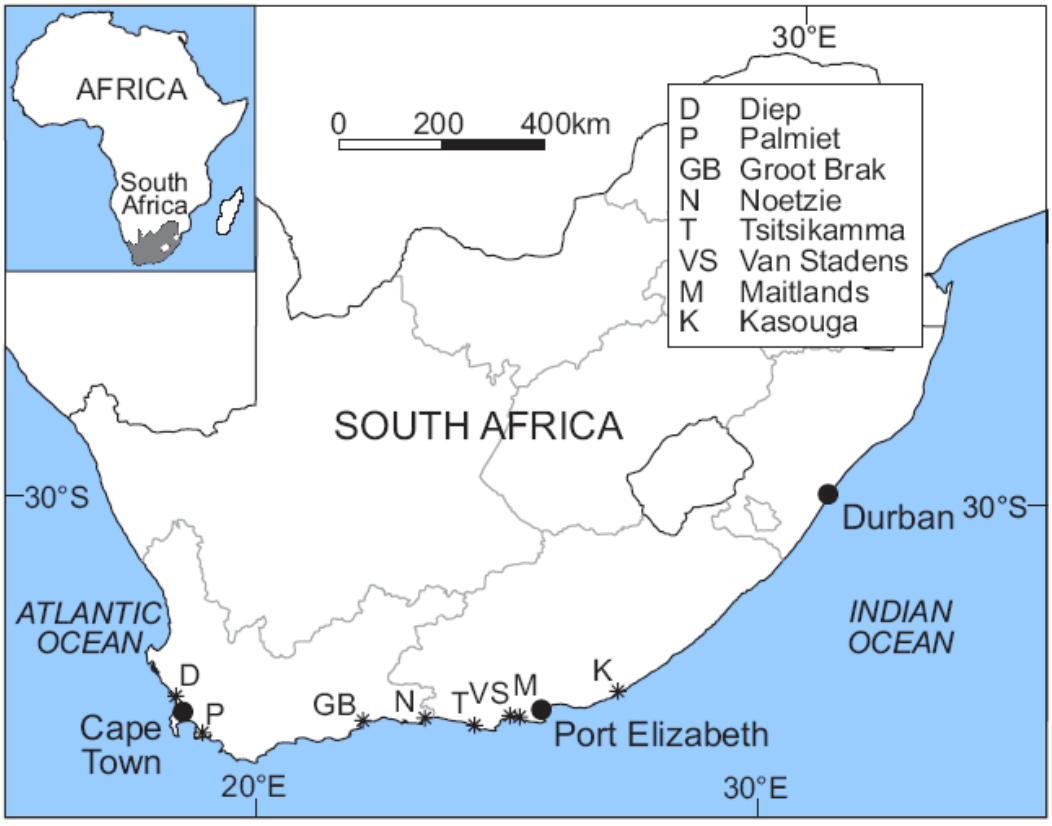

Figure 6.10. Map of South Africa showing locations of estuaries that were compared to the conceptual TOCE model.

Diep Estuary

The Diep Estuary, about 5km north of Cape Town, is divided into two distinct regions: Milnerton Lagoon (below the Otto du Plessis Bridge - 33°54'5"S; 18°28'E) and Rietvlei (between the Blaauwberg Bridge and the Otto du Plessis Bridge - 33°51'S; 18°29'E). During the rainy winter months, the mouth is usually open to the sea. During drier periods (usually in summer), the mouth is closed off by a sandbar (Taljaard *et al*. 1992). Tidal influence is restricted to the lagoon area, which is the focus of this assessment.

Water quality data were collected from the estuary on two occasions: September 1988 (winter) and February 1989 (summer) (Taljaard *et al*. 1992). Sewage effluent is discharged into the system near the Blaauwberg Bridge. In 1988, reed beds (*Phragmites*) in the immediate vicinity of the discharge provided a very efficient wetland, purifying the effluent before it reached the estuary (Taljaard *et al*. 1992). However, in recent years, extensive urban development has resulted in an overloading of the wastewater treatment works, causing major pollution effects within the estuary.

The Diep Estuary is a two-phased system, meaning that it can only be in the open or closed mouth state. This is mainly because the system is slightly larger than most other TOCEs.

Salinity

Open mouth (State 1)

During the winter study (September 1988) the estuary mouth was open. Strong river inflow resulted in the estuary being fresh (< 1.5 ppt) throughout (Taljaard *et al*. 1992). However, a plug of saline water (10–25 ppt) was still evident in a deep (> 2 m) section near the mouth. Saline bottom water remained trapped in the estuary during neap tides but on subsequent spring tides it was usually replaced by seawater, due to strong tidal flow. This suggests that during the open-mouth state, the volume of river inflow largely determines the salinity distribution within the estuary. During higher flows the system will be freshwater-dominated but with strong vertical stratification remaining in the deeper sections near the mouth. Periods of low flow will probably be characterised by strong longitudinal and vertical stratification.

Closed mouth (State 3)

In February 1989 the estuary mouth was closed. Hypersaline and well mixed conditions existed in the estuary with the salinity ranging from 37 ppt near the mouth to 43 ppt near the upper reaches (Taljaard *et al*. 1992), with no vertical stratification evident. In recent years the volume of wastewater from the sewage treatment plant being discharged into the estuary has increased substantially, so it is unlikely that hypersaline conditions will occur in the future and mouth closure is less likely to occur. During the closed-mouth state, salinity in the Diep Estuary is most likely to be homogeneous.

Temperature

During winter (September 1988) temperatures ranged from 13 °C near the mouth to 21 °C in the upper reaches. During summer (February 1989) temperature was relatively uniform and significantly higher, ranging from 24 °C near the mouth to 25 °C in the upper reaches (Taljaard *et al*. 1992). Being situated on the west coast, the upwelling of cold oceanic water may influence local seawater temperatures during summer. Therefore, at times when the open-mouth state occurs during the summer period, temperature near the mouth can decline to around 13 °C.

Temperature distribution patterns in the Diep Estuary show stronger seasonal correlations, rather than correlations with open/closed-mouth states. The temperature regime therefore follows seasonal trends in atmospheric temperatures, as might be predicted in a conceptual model.

pH

pH in the Diep Estuary during periods of high river inflow (September 1988) were similar to those of the inflowing river water and ranged between 7.4 and 7.6. During February 1989, when the system was hypersaline, they ranged from 8.0 to 8.2 throughout the estuary (similar to that of seawater) (Taljaard *et al*. 1992). Therefore, pH in this estuary appears to be linked to the source water entering the system at the time of measurement, e.g. lower pH during high river flow and higher pH under more saline conditions. However, the overall pH range for the system (i.e. 7 and 8.5) was within the range proposed by the conceptual model.

Dissolved oxygen

Open mouth

No dissolved oxygen (DO) measurements were taken in the estuary during the winter open state (September 1989) but one would expect that the system would have been well oxygenated, except in the deeper bottom waters (> 2 m) near the mouth. High total ammonia-N concentrations in this deeper section were indicative of hypoxic or even anoxic conditions (Taljaard *et al*. 1992). This estuary is characterised by strong vertical stratification, particularly in the deeper section near the mouth, during its open phase. As a result, deeper bottom waters (> 2 m) have limited exchange with surface waters and the atmosphere.

Mouth closed

DO concentrations measured in the estuary during the closed state (hypersaline conditions) showed that the system was well oxygenated throughout, with DO saturation levels of about 80% (Taljaard *et al*. 1992). This suggests that in the absence of strong vertical stratification, wind-mixing was sufficient to maintain oxygenated conditions throughout the system.

Turbidity

Turbidity levels in the Diep Estuary appear to be strongly influenced by the characteristics of the source waters, with strong river inflow creating more turbid conditions than under marine-dominated or hypersaline conditions. Average suspended solid concentrations under strong river influence and during the closed state (hypersaline) were 32.4 mg l^{-1} and 11.8 mg l^{-1}, respectively (Taljaard *et al*. 1992). This suggests that river inflow to the system probably introduces turbidity, resulting in the estuary being more turbid when the system is dominated by freshwater.

Inorganic nutrients

Open mouth

During the open state, DIN concentrations appeared to be strongly linked to two sources. In September 1988, strong river inflow resulted in the estuary being

relatively fresh, with nutrient concentrations resembling that of the river, except in the deeper sections near the mouth (which showed a stronger influence from the lower-nutrient seawater). Nitrite-N levels were < 100 µg l^{-1}, while nitrate-N concentrations in the fresher shallower sections ranged between 700 and 980 µg l^{-1}, but were lower (~210 µg l^{-1}) in the deeper pools near the mouth. However, total ammonia-N levels were higher in the deeper pools (560 µg l^{-1}), compared with concentrations measured elsewhere in the system (280 µg l^{-1}). This was probably linked to the longer residence time of water in this area. DIP concentrations followed a similar trend to nitrate, where the concentration in the fresher shallower sections ranged between 620 and 899 µg l^{-1}, but decreased to around 310 µg l^{-1} in the deeper pools.

Closed mouth

DIN and DIP concentrations during January 1989, when the mouth was closed and the estuary hypersaline, did not show any marked trends throughout the system. DIN concentrations were near depletion (nitrite-N: 2.8 µg l^{-1}, nitrate-N: 12.6 µg l^{-1}, total ammonia-N: 70 µg l^{-1}), while DIP concentrations averaged 577 µg l^{-1}.

Palmiet Estuary

The Palmiet Estuary (34°20'S; 18°59'E), 75km south-east of Cape Town, is a small TOCE 1.7 km in length and 300 m at its widest point. The head of the estuary is marked by a series of rocky sills. The channel meanders between rocky banks in the upper reaches of the estuary; scour-holes (4–5 m) are located in these reaches. From about 700 m upstream of the mouth, the channel is located close to the west bank with a broad shallow tidal flat on the east bank. The mouth is close to a rocky bank on the western side, with an extensive and mobile sand spit to the east. The Palmiet River is a 'black water' system draining mainly Table Mountain quartzite, and is characterised by low inorganic nutrient concentrations.

The Palmiet Estuary is a three-phased system and can occur in the mouth-open, semi-closed and closed states. Data on the water quality characteristics of the estuary are available for February 1985 (open), August 1986 (open), February 1988 (closed — only salinity and temperature), January to April 1998 (semi-closed) (Largier 1986; Taljaard *et al*. 1986; Taljaard 1987; Taljaard & Largier 1989; Slinger &

Largier 1990; Largier & Taljaard 1991; Largier *et al*. 1992). Water quality data of the system were obtained mainly from the CSIR (2000).

Salinity

Open mouth

During the dry season this system is marine-dominated, owing to extensive seawater intrusion and limited river inflow. This is probably the dominant state in the system during summer (CSIR 2000). Strong vertical stratification occurs, with the bottom waters being replaced or partially replaced by fresh seawater on the flood tides (particularly spring flood tides). During the wet season (winter), the system is river-dominated and can become completely fresh during ebb tides, with some saline intrusion during flood tides.

Semi-closed mouth

This state occurs quite often in the late summer and was monitored in detail during the summer of 1998 as part of an estuarine flow requirements study (CSIR 2000). At the onset of the semi-closed state, the estuary was strongly stratified. Over the following three months, the depth of the halocline increased, mainly as a result of the turbulence caused by inflowing riverwater. At the end of the sampling period, the estuary was almost completely fresh, except in the deeper scour-holes where a very strong halocline at about 4 m still trapped old saline water (32 ppt). The rate at which this process occurs is a function of the river inflow rate and, if persisting for about five months, the estuary in this state is likely to become completely fresh.

Closed mouth

During a closed state investigated in February 1988, strong vertical stratification persisted, ranging from 5 ppt in surface waters to 34 ppt in bottom waters (Slinger & Largier 1990; CSIR 2000). Although no data were available, it was anticipated that as long as the mouth remained closed, strong stratification would persist for an extended time, mainly because the estuary is protected from wind and turbulence associated with river inflow would also be low (CSIR 2000).

Temperature

Open mouth

During summer, when the mouth is open, the system is usually marine-dominated as a result of saline water intrusion and low river input. Temperatures were largely influenced by the prevailing temperature of the seawater, which varied between 13 °C and 17 °C at that time of year. Upwelling events along this section of coast result in temperatures dropping to 13 °C (CSIR 2000). Riverwater temperatures typically range between 20 °C and 25 °C. The intrusion of cold upwelled oceanic water can result in surface water being distinctly warmer than that at the bottom or surface waters near the mouth. During the wet season (winter), temperatures are largely influenced by that of the riverwater, averaging about 13 °C during winter (CSIR 2000).

Semi-closed and closed mouth

Temperature distribution in the Palmiet Estuary, as in most other TOCEs, showed a strong seasonal signal. As the closed and semi-closed states usually occur during summer, temperatures throughout the estuary will be relatively high. Temperatures measured in the system under these states ranged from 10 °C in winter to 26 °C in summer (CSIR 2000).

pH

Open mouth

During the open marine-dominated state (summer), pH levels in the estuary generally ranged between 7 and 8. Lower pH values have been measured near the head of the estuary associated with the slightly acidic river inflow, but owing to the weak buffering capacity of the riverwater, pH levels rapidly rise once it comes into contact with saline estuarine waters (CSIR 2000). During strong river inflow (winter), when the estuary becomes river-dominated, lower pH levels of between 4 and 6.5 can occur throughout the system (CSIR 2000).

Semi-closed and closed mouth

Although no measurements were taken, it is expected that pH values at the onset of the semi-closed and closed states would range between 7 and 8. As the influence of river water increases, it is expected that pH in the estuary will gradually decrease and may drop to between 4 and 6.5 (CSIR 2000).

Dissolved oxygen

Open mouth

During both the marine- and river-dominated open states the Palmiet Estuary is generally well oxygenated (DO > 6 mg l^{-1}). During the marine-dominated state, strong tidal flushing regularly replaces bottom waters in this small system, while during the river-dominated state, the entire estuary is regularly flushed due to strong river inflow (CSIR 2000).

Semi-closed mouth

The changes in DO concentration in the Palmiet Estuary were studied in detail in the summer of 1998 (CSIR 2000). At the onset of the semi-closed state, when the main flushing mechanism of bottom water during the dry season was cut off, oxygen levels in the bottom waters decreased. This was attributed to the high oxygen demand of decaying organic matter, mainly originating from kelp debris and *Cladophora* that were present at the time. This trend rapidly increased so that, within a week after the onset of the semi-closed state, the water-column below the halocline became hypoxic and, in places, anoxic. However, through entrainment of freshwater into the more saline bottom layer, as well as through the action of wind-mixing forces, low-oxygen water was gradually replaced by well-oxygenated river water. Although most of the estuary was largely re-oxygenated after about two months, leaving only the deeper scour-holes anoxic, it is expected that if this state persisted the entire estuary would be re-oxygenated as the entire water-column would be replaced by well-oxygenated river water through entrainment (CSIR 2000).

Closed mouth

Based on field measurements taken during the semi-closed state, the expectation is that during these conditions, a large portion of the bottom water below the halocline will remain hypoxic and even anoxic. This is mainly a result of the high organic loading in the system from the summer, when kelp debris entered the open mouth and dense *Cladophora* beds developed within the estuary. This situation is likely to persist, or even worsen, whilst the halocline remains intact (CSIR 2000).

Turbidity

Turbidity is not considered to be a major issue in the Palmiet Estuary, since 'black water' systems carry limited suspensoids. However, the dark colour of the humate-stained water does affect light penetration. Measurements were taken only during the semi-closed period (summer 1998) when Secchi disc depths remained at 2 m during the study period (CSIR 2000).

Inorganic nutrients

Open mouth

Under the open marine- and river-dominated states, DIN and DIP concentrations in the estuary were closely associated with salinity, indicating that the nutrient concentrations are mainly influenced by the two water sources. During this state, contribution to the nutrient pool in the water-column from other sources (e.g. remineralisation) appears to be relatively unimportant.

During the marine-dominated open state, marine upwelling may result in elevated DIN and DIP concentrations being introduced to the estuary. Typical of well-oxygenated systems, nitrite-N and total ammonia-N were low. Nitrate-N concentration ranged between 40 and 190 µg l^{-1}, while DIP concentrations ranged between 12 and 30 µg l^{-1} (CSIR 2000).

During the freshwater-dominated open-mouth conditions, concentrations of nitrite-N, total ammonia and DIP were generally similar to those of the marine-dominated state. Nitrate-N concentrations during open-mouth conditions appeared, however, to be much higher (190 to 1 300 µg l^{-1}) indicating nutrient inputs from the

catchment. These inputs are probably associated with agricultural activities, since black water systems are typically low in nutrients (CSIR 2000).

Semi-closed mouth

DIN and DIP levels during the semi-closed state displayed interesting patterns. At the onset of this state in the summer of 1998, DIN and DIP concentration were relatively low, similar to that of the marine-dominated open state (CSIR 2000). DIN and DIP concentrations in the surface layer remained low throughout the three-month study period and even became depleted. Bottom waters below the halocline remained low in nitrite and eventually became depleted of nitrate. However, after about one month, total ammonia and DIP concentrations showed a marked increase in the saline bottom water that was still trapped below the halocline. Total ammonia-N concentrations increased from 30 µg l^{-1} to 2000 µg l^{-1}. Similarly, DIP concentrations increased from 30 µg l^{-1} to 300 µg l^{-1} (CSIR 2000). The increase in these nutrient concentrations was attributed to remineralisation.

Closed Mouth

No data are available for this state. However, it is expected to display similar characteristics to the semi-closed state, where surface water becomes nutrient-depleted and remineralisation becomes an important source inorganic nutrients in bottom waters.

Brief assessments of six other Cape case studies

Water quality related studies for a number of other southern and Eastern Cape estuaries were reviewed, to assess similarities or differences in salinity structure and water quality characteristics, as compared to the conceptual model (Table 6.1).

Salinity

Strong longitudinal and sometimes strong vertical salinity stratification developed during the open phase in all six estuaries. The strengths of the gradients were dependent on estuarine length, bathymetry and the volume of river inflow. The Great Brak Estuary was deepest in its middle and upper reaches; vertical stratification was

therefore strongest in these reaches. A well-developed sandbar at the mouth of the Noetzie Estuary led to this estuary becoming perched, limiting overtopping events to extreme high sea conditions. Breaching of the mouth was followed by the formation of well-developed longitudinal and vertical stratification in the Van Stadens and Maitland Estuaries.

The semi-closed state was not well described in the case studies and is a state not typically found in medium-sized estuaries (e.g. Great Brak Estuary). In the Tsitsikamma Estuary, saline water trapped from a previous open-mouth phase, or overtopping events, caused a strong halocline to develop, particularly in deeper sites.

It was considered likely that extended periods of low or no flow and few overtopping events would result in the estuary becoming more homogeneous. However, this is unlikely to be as significant as recorded in the Palmiet Estuary. Saline water in the Van Stadens and Maitland estuaries appeared to become dissipated through a combination of river flow, groundwater seepage and seepage of estuarine water through the sandbar at the mouth of the estuaries. It was considered unlikely for the classic semi-closed state to occur in the Kasouga Estuary because of the well-developed sandbar at the mouth.

Table 6.1.

Brief descriptions of six estuaries that were compared to the conceptual model.

Estuary	References
Great Brak Estuary (34°03′23′′S; 22°14′25′′E) A medium-sized estuary (7km) with a causeway at the head of the estuary. River flow reduced by Wolwedans Dam, which has reduced the frequency with which the mouth opens. Overwash occurs periodically.	CSIR 1990, CSIR 1992, Taljaard and Slinger 1993, CSIR 1993, CSIR 1994, CSIR 1998
Tsitsikamma Estuary (34°08′05′′S; 24°26′25′′E) A short estuary (~3km) with a small catchment (189km²) and a coastal dunefield adjacent to the lower reaches. These factors keep the mouth semi-closed for most of the year.	Bate *et al.* 1994, Harrison *et al.* 1996,Taljaard *et al.* 2002
Noetzie Estuary (34°04′43′′S; 23°07′46′′E) A near-pristine estuary with a very small catchment (39km²). Water is oligotrophic and humic-stained. Recent development in the catchment has increased the silt load into the estuary.	Harrison *et al.* 1995, Bornman and Adams 2005
Van Stadens Estuary (33°58′01′′S; 25°13′20′′E) An oligotrophic estuary with a small catchment (90km²), which supports largely pristine fynbos, plus some livestock agriculture. Two small dams reduce river flow into the estuary.	Gama *et al.* 2005
Maitland Estuary (33°59′2′′S; 25°17′4′′E) A small estuary with a small catchment (60km²) that consists primarily of dairy farms and a large shrub-thicket nature reserve near the coastline.	Gama *et al.* 2005
Kasouga Estuary (33°39′17′′S; 26°44′08′′E) A short estuary (2.5km long) with a small catchment (39km²). The catchment consists mainly of near-pristine valley thicket, plus some livestock agriculture.	Froneman 2002a and 2002b

During the closed phase, the water-column throughout the Great Brak Estuary was generally homogeneous but the low volume of inflowing riverwater did permit some salinity stratification in the upper reaches of the estuary. The extent of stratification was a function of the volume of river input and the bathymetry of the estuary. Following a large overtopping event, a density-driven circulation pattern developed when higher-salinity water, with a higher density, intruded further upstream. This resulted in a system that was uniformly stratified throughout. Overtopping was regarded as an effective mechanism through which bottom water in an estuary could be renewed during periods of mouth closure (Taljaard & Slinger 1993). However, the extent of the renewal is dependent on the volume of water introduced by the overtopping event and on the bathymetry of the estuary. A similar overtopping event was recorded in the Van Stadens Estuary but the resultant stratification was short-lived, as wind-mixing rapidly eroded it. River flow into the Kasouga Estuary is often so low that water in the estuary is homogeneous throughout and becomes hypersaline. A maximum of 37 ppt was recorded in September 2000 due to the rate of evaporation exceeding the rate of freshwater input. Under these conditions, overtopping events were effective in reducing the salinity in the estuary to a concentration close to that of seawater.

Temperature

Water temperature in the above estuaries was largely affected by atmospheric temperature, the temperature of marine and riverwater entering these systems, and the state of the estuary mouths. During the open state, the temperature of seawater and riverwater strongly influenced the temperature of their lower and upper reaches, respectively, frequently resulting in strong longitudinal and vertical temperature gradients. Marine upwelling also results in a further decrease of water temperature, particularly in the west coast estuaries. During the semi-closed state, water temperature in the estuaries was largely affected by atmospheric temperature and the temperature of riverwater entering these systems. Overwash during the semi-closed state generally introduced cooler marine water into the deeper areas of the lower reaches of the estuaries. During the closed-mouth state, temperature in the estuaries was closely associated with seasonal trends in atmospheric temperatures.

pH

In most of the estuaries studied, pH generally fell within the range 7.0–8.5, as predicted by the TOCE conceptual model. The leaching of tannins in a catchment dominated by fynbos vegetation resulted in the Noetzie River having a relatively low pH of 6.3. However, the pH generally increased to within the expected range from the head of the estuary towards the mouth due to the strong buffering capacity of seawater.

Dissolved oxygen

Strong vertical stratification in the Great Brak Estuary during the open-mouth phase, particularly in its deeper middle and upper reaches, limits the exchange of surface and bottom water. As tidal exchange was insufficient to replenish bottom waters in the middle and upper reaches of the Groot Brak Estuary, hypoxic and even anoxic conditions frequently occurred here. This differs from the conditions predicted by the conceptual model because the Great Brak Estuary is larger than the typical TOCE, thus having reduced tidal exchange efficiency. DO in the Van Stadens and Maitland Estuaries, during the open phase, was as predicted by the model. The estuaries were well oxygenated and even became supersaturated (> 11 mg l^{-1}) following flood events when mats of filamentous green algae developed along the bottom of the Maitland Estuary.

During the closed phase, DO became depleted in the deeper middle and upper reaches of the Great Brak Estuary. However, a large volume of water entered that estuary during an overtopping event and effectively replenished the bottom waters with water of higher DO concentration. Wind-mixing and periods of overwash were sufficient to keep the water-columns in the small Tsitsikamma, Noetzie and Kasouga estuaries well aerated. However, decaying organic matter and poorly-flushed water in scour-holes up to 8 m deep caused DO to become depleted (< 3 mg l^{-1}) in the Maitland and Van Stadens estuaries respectively.

Turbidity

Most of the estuaries considered here have rivers that drain from Table Mountain quartzite vegetated with fynbos, resulting in clear water with varying concentrations of tannins. Secchi depths were generally > 2 m, with low turbidity (< 10 NTU), thus

reflecting the source waters (namely the sea and rivers), which were generally clear. Turbulent river flow re-suspended fine particles at the head of the Noetzie Estuary, but these quickly settled, leaving the rest of the estuary relatively clear.

The Kasouga River drains the Karoo Supergroup, a geological formation high in mudstone. Once eroded, high concentrations of fine sediments enter this estuary and these are easily resuspended by wind-generated turbulence in the shallow water-column. As a result, turbidity in excess of 10 NTU is likely to be measured frequently in this estuary.

In accordance with the TOCE conceptual model, turbidity in these systems is largely influenced by the levels in source waters and/or the extent of resuspension of fines by wind-generated turbulence.

Inorganic nutrients

DIN distribution patterns in the Great Brak Estuary showed similar trends for most surveys, with no marked difference between the open and closed-mouth states. DIN and DIP concentrations were generally low throughout but, on occasion, limited input of DIN from the sea and river was evident through elevated levels measured at stations near the mouth and head of the estuary respectively. However, high total ammonia and DIP concentrations were frequently measured in the deeper (> 2 m) pools of the upper middle reaches, usually coinciding with anoxic conditions, the result of organic degradation and long residence times. These higher concentrations were most likely associated with remineralisation processes. This was even evident during the open-mouth state, when strong vertical stratification resulted in the 'trapping' of these bottom waters.

The Tsitsikamma and Noetzie Estuaries are 'black water' systems, so their nutrient levels were generally low. It is likely that they should remain low during the open and closed states but further studies are needed to test this.

Nutrient concentrations in the Van Stadens Estuary were generally low (DIP < 0.5 mg l^{-1} and DIN < 1.0 mg l^{-1}). As the system moved from open (flows > 1.0 m^3 s^{-1}) to semi-closed (0.5 m^3 s^{-1}) and then to closed (0 m^3 s^{-1}) states, nutrient concentrations, except ammonium, increased as residence time became more optimal then decreased to very low concentrations when there was negligible water exchange. During extended periods of mouth closure there was still a fairly strong

ammonium signal, relative to phosphate and nitrate, possibly as a result of the low DO concentrations, which led to the ammonification of the available oxidised nitrogen (nitrate and nitrite).

Average ammonium concentration in the Maitland Estuary peaked at 11.0 µg l^{-1} during a period when DO concentrations were lowest, coinciding with an extensive growth of cyanophyte mats. These were observed covering the bottom sediments in the middle to upper reaches of the estuary, while some mats floated to the water's surface when dislodged from the bottom. Other nutrient concentrations in the Maitland Estuary (nitrate < 1.0 mg l^{-1}, total phosphate < 1.2 mg l^{-1}) were low, increasing slightly on occasions in response to increased river flow.

Nutrient concentrations in the Kasouga Estuary were extremely low (phosphate and total oxidised nitrogen < 1 mg l^{-1}) and generally homogeneous throughout the estuary during the closed state (Froneman 2002a). Here, Harrison (unpubl. data) recorded phosphate-P, nitrate-N and ammonium-N concentrations of 10–30 µg l^{-1}, 130–240 µg l^{-1} and 0 µg l^{-1}, respectively, in August 1995.

In general, available data on the inorganic nutrient characteristics of selected southern and Eastern Cape TOCE systems were too limited to properly assess similarities and differences with the proposed TOCE conceptual model. However, they did suggest that during the open state the characteristics of the source water are probably a determining factor (e.g. Great Brak Estuary), while processes such as remineralisation could become important during the closed state (e.g. Great Brak and Maitland estuaries).

Conclusions and recommendations

In general, the estuaries considered in this overview fit within the TOCE conceptual model in terms of the predicted values of their salinity, temperature, pH, dissolved oxygen, turbidity and inorganic nutrients under the three dominant states. Most of the estuaries existed as three-phased systems, the only exceptions being the Diep and Great Brak estuaries, which were two-phased systems in which the semi-closed state did not occur. This was mainly because these two estuaries were medium-sized TOCEs, whereas the semi-closed state usually occurs in smaller systems.

The model appears to be rigid because it describes three independent states, but should in fact be regarded as a progression from high flows (open state) to low flows (generally semi-open state) and extremely low or no flow (closed state). If perceived as a progression, it is easier to understand many of the trends in the water quality variables.

The following anomalies were identified, in which the measured results deviated from the predictions in the TOCE conceptual model.

The conceptual model predicts that during the closed state, TOCEs will become well-mixed brackish systems, possibly becoming more saline and even hypersaline. In this regard, the model appears to be somewhat biased to the southern and Western Cape, where rainfall tends to be lower and evaporation higher, as compared to KwaZulu-Natal TOCEs (Perissinotto *et al*. 2004). A key difference between the TOCEs in higher-rainfall areas compared to those in low-rainfall areas with high evaporation is that the former systems tend to become increasingly fresh during the closed state (e.g. Mdloti Estuary) while the latter systems typically become more saline, even hypersaline (e.g. Kasouga Estuary).

Salinity distribution in medium-sized TOCEs (e.g. Great Brak Estuary) also did not follow the predicted salinity distribution patterns under the closed state. Although salinity distribution in these types of estuaries does become more homogeneous compared to the open state, some vertical stratification usually remains in the upper sections, the extent of this depending on its depth and the amount of freshwater still entering the system.

Based on the TOCE conceptual model, low oxygen conditions are generally not expected under the open state. However, in the Great Brak Estuary, low oxygen concentrations were common in bottom waters in the middle and upper reaches

during this state. This was attributed to the system being longer and larger than most typical TOCE systems thereby reducing the efficiency of tidal flushing of bottom waters.

Estuaries subject to anthropogenic interferences, for example nutrient enrichment through wastewater discharges, will not necessarily match the prediction in the TOCE conceptual model. For example, the high inorganic nutrient inputs from the wastewater discharge into the Mhlanga Estuary (Perissinotto *et al*. 2004) are probably masking the variability in nutrient distribution patterns predicted for the closed states in the TOCE model.

In conclusion, this overview has emphasised that the greatest uncertainties in terms of the biogeochemical or water quality characteristics and processes of TOCEs, particularly those in the cool- and warm-temperate zones, are related to the levels of inorganic and organic nutrients in these systems. Further research is therefore urgently required if we are to understand water quality changes in TOCEs.

CHAPTER 7

Relating microalgal spatial patterns to flow, mouth and nutrient status in the temporarily open/closed Mngazi Estuary, South Africa.

Snow, G.C. and Adams, J.B. 2007. Relating microalgal spatial patterns to flow, mouth and nutrient status in the temporarily open/closed Mngazi estuary, South Africa. *Marine and Freshwater Research* **58**, 1032-1043.

Abstract

The Mngazi Estuary, a near pristine and wave-dominated estuary located on the subtropical east coast of South Africa, requires careful management to ensure that land use does not alter its ecological function. This study investigated the quality and quantity of water in the estuary and related these to the microalgae. There was no evidence of a persistent elevated phytoplankton biomass in the region of the estuary where riverwater mixed with brackish estuarine water, even during periods when the estuary mouth was open. Nutrients that determined the microalgal distribution were likely to have come from the mineralisation of organic material, which had been deposited in the estuary following pulses in riverwater. Results indicate that microalgae were P-limited when the mouth was semi-closed and N-limited during the closed mouth phase. Average benthic chlorophyll a (chl a), which ranged from 0.3 to 56.8 μg g^{-1}, was highest in the intertidal middle reaches of the estuary. Organic matter and the proportion of fine sediments (< 125 μm) were highest in these sediments. A conceptual model was developed to predict the response of the estuary if further changes in the quality and quantity of riverwater entering the estuary were to occur.

Key words: Temporarily open/closed estuary; chlorophyll a; carbohydrates; mineralisation; flocculation.

Introduction

There are three main threats to the ecological integrity of estuaries: changes to river flow (Russell *et al*. 2006), eutrophication (Painting *et al*. 2007) and accelerated rates of sedimentation (Manning *et al*. 2007). Rapid population growth, particularly along the coast, has resulted in a decrease in the quality and quantity of freshwater entering estuaries creating a multitude of ecological stresses (Flemer & Champ 2006; Russell *et al.* 2006; Snow & Adams 2006). In semi-arid regions of the world, where the average annual rainfall is below the world average of 860 mm/annum, the demand for freshwater is often higher than is available. As a result, large reservoirs and diversion schemes are constructed to meet the demands of industry, agriculture and domestic use (Otieno & Ochieng 2004). By depriving water to rivers with small catchments, estuaries are at risk of mouth closure, becoming more resistant to scouring during flood events or becoming marine-dominated. In regions where the ratio between mean annual precipitation and mean annual runoff conversion is low the careful management of exploitable water resources is necessary (Allanson 2001).

The quality of water flowing into estuaries is also an area of concern. An increase in the concentrations of nitrogen (N) and phosphorus (P) has been shown to stimulate plant growth and disrupt the balance between the production and metabolism of organic matter (Cloern 2001; Flemer & Champ 2006). Eutrophication occurs when there is an increase in the rate of supply of organic matter into an estuary (Nixon 1995), which is strongly influenced by land use characteristics and the geohydrology of a river's catchment (Cloern 2001; Flemer & Champ 2006). In addition, alterations in the ratios of nutrients, N:P and N:Silicon, can impact on phytoplankton biomass and community composition (Humborg *et al*. 1997; Gilpin *et al*. 2004).

The mixing of riverwater with more saline estuarine water causes the dissolved and suspended particulate matter to flocculate, creating a turbidity maximum in the estuary and a zone of accelerated particle settling (Pierson *et al.* 2002). Floccules are a complex matrix of microbial communities, organic particles (e.g. detritus, extracellular polymers and cellular debris), inorganic particles (e.g. clays and silts) and substantial intrafloc pore spaces (Day 1981; Droppo 2001). The deposition of floccules provides a major food source for the benthos (Yap *et al.* 1987). The rate of flocculation is likely to accelerate exponentially at temperatures exceeding 25 ºC (Jiang *et al.* 2004), increasing the load of deposited material, microbial

decomposition and biological oxygen demand. This process is exacerbated in estuaries with limited tidal exchange, low river flow and vertical salinity stratification. Low oxygen and elevated salinity (Morlock *et al*. 1997) increases the amount of dissolved inorganic nitrogen (DIN; nitrate + nitrite + ammonium) released from the sediment and overlying water (Cloern 2001; Pinckney *et al.* 2001; Gale *et al.* 2006). The transport of solutes across the sediment-water interface in cohesive sediments is enhanced by bioturbation, through the pumping activity of fauna (bioirrigation) or the random movements of fauna within the sediment (enhanced diffusion) (Cook *et al.* 2004).

Temporarily open/closed estuaries (TOCE) have been the focus of a number of studies in recent years: USA (Gobler *et al*. 2005), Australia (Twomey & Thompson 2001; Dye & Barros 2005; Gale *et al*. 2006), South America (Suzuki *et al*. 1998) and South Africa (Perissinotto *et al*. 2003; Skinner *et al*. 2006). Approximately 70% of estuaries in South Africa are TOCE's and are characterised by having small river catchments (Whitfield 1992). The sand barrier located at the mouth of many estuaries typically restricts the input of marine sediment into an estuary, creating a low-energy central basin that functions as a sediment trap (Radke *et al.* 2004) and promotes the deposition of floccules. Deterioration of river catchments or reduced river flows can lead to a gradual accumulation of the settled floccules, decreasing the ratio between basin volume and sediment surface area, making sediment-water exchange processes more important; i.e. the autochthonous supply of nutrients becomes more important in relation to the allochthonous supply (Droppo 2001).

In permanently open estuaries such as the Gamtoos Estuary (Snow *et al.* 2000b; Bate *et al*. 2002) a continuous base flow of freshwater supplied nutrients maintaining a productive phytoplankton community at the river-estuary interface (REI). In contrast, TOCE's have negligible base flows. It is only during large rainfall events that organic matter and nutrients (bound to sediments) enter these estuaries. It was hypothesised that microalgae in the Mngazi Estuary, a TOCE on the east coast of South Africa, would be more dependent on the allochthonous supply of organic matter and nutrients during flood events. The estuary would subsequently depend on the autochthonous supply during extended periods of negligible river flow, particularly during closed and semi-closed mouth conditions. Lake Illawarra, in south-east Australia, is a semi-closed coastal lagoon with low river input and restricted tidal exchange. DIN appeared to limit primary production in the lagoon and as

regenerated nitrogen was released from the sediment the microphytobenthos (MPB), the dominant primary producer, rapidly sequestered it and the relatively small amount that reached the water-column supported the growth of phytoplankton, seagrasses and macroalgae (Webster *et al.* 2002).

The objectives of this study were to determine the spatial patterns of microalgal biomass in the estuary during the open, semi-closed and closed mouth phases then relate these to physico-chemical variables and determine whether a REI exists in the estuary. The research will contribute to our understanding of the functioning of TOCE's, which will enable managers to predict changes in the estuary in response to reduced river flow and increased sediment loads.

Materials and methods

Study area

The Mngazi Estuary is situated to the south of Port St Johns in the north-eastern Eastern Cape Province, South Africa (Fig. 7.1). The estuary is regarded as a three-phase TOCE, having distinct open, closed and semi-closed mouth phases (Snow & Taljaard 2007). The estuary is generally strongly stratified (CSIR 2005) due to limited marine exchange and the bathymetry. Even when the mouth is open the tidal variation is normally 0.2-0.3 m, indicating a constricted mouth that contributes to the stratification. A sandy barrier regularly develops across the mouth of the estuary, particularly at river flows less than 0.2 $m^3 s^{-1}$. The Mngazi River catchment is largely rural and impacts include: high levels of deforestation for fuel and housing material, soil erosion largely as a result of free ranging livestock and water abstraction. During this study there was extensive bait digging in the intertidal zone of the middle and lower reaches of the estuary using spades, destabilising the sediment and potentially releasing nutrients, such as ammonium and phosphate, into the estuary.

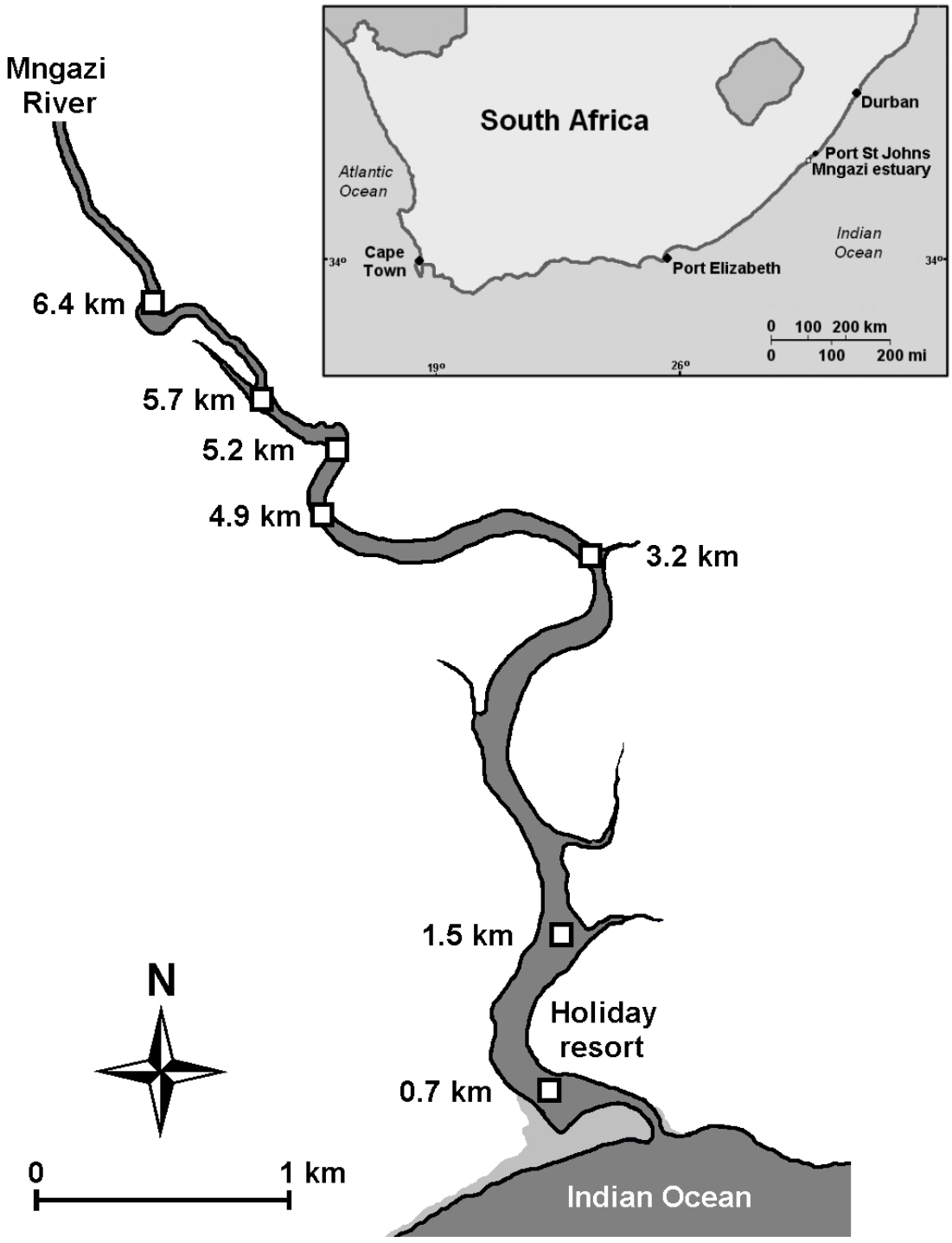

Figure 7.1. Outline of the Mngazi Estuary with the distances of the sampling stations indicated on the plot. The inset shows the location of the estuary on the east coast of South Africa.

River flows

The catchment of the Mngazi River is steep so pulses of riverwater and floods into the estuary are common. During the period January 2003 to August 2005 there were at least 8 freshwater pulses or floods, with flows exceeding 10 m^3 s^{-1} (CSIR 2005). Flow measured at gauge T7H001 represents 41.2% of the mean annual runoff in the catchment and is limited to a maximum flow of 88 m^3 s^{-1} (Fig. 7.2). The highest daily averaged flows recorded were in excess of 70 m^3 s^{-1} (September 2002) and 60 m^3 s^{-1} (September 2004). Large floods are regarded as important mechanisms required to scour the main channel, breach the estuary mouth, replenish nutrient- and oxygen-depleted water within the estuary and introduce fine particulate material into the estuary. The geology of the river catchment is made up of some mudstone and sandstone but predominantly shale, which erodes into fine clay with high alumina content (Blyth & de Freitas 1984).

The estuary was sampled on six occasions: twice during each of the closed (June and November 2003), semi-closed (June 2002 and January 2003) and open phases (March and June 2005). There were five sampling sites during the first 4 sampling sessions that were located 0.7, 1.5, 3.2, 5.2 and 6.4 km from the mouth (Fig. 7.1). There were six sites during the final 2 sessions that were 0.7, 1.5, 3.2, 4.9, 5.7 and 6.4 km from the mouth. The first 4 sampling surveys focused on benthic microalgae whereas the last 2 surveys investigated phytoplankton size fractionation.

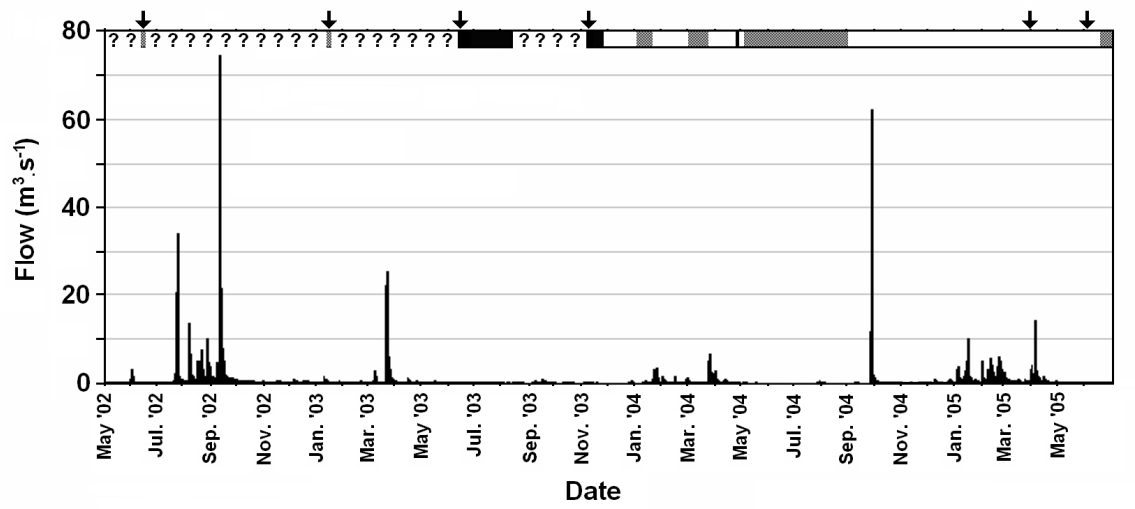

Figure 7.2. Average daily flow data for the Mngazi River (T7H001), 01 May 2002 to 30 June 2005 (courtesy of the South African Department of Water Affairs and Forestry). The gauge monitors runoff from 315 km^2 of the 591 km^2 catchment. Horizontal bar at top of graph indicates mouth state (? = unknown, black = closed, hatched = semi-closed and white = open) and arrows indicate sampling dates.

Water quality

Water quality variables were recorded at each site using a multiprobe; YSI 650MDS handheld unit and sonde. Secchi depth (m) was used as a measure of water clarity. Filtered water samples (Whatman GF/C) were analysed for total oxidised nitrogen (TOxN; nitrate + nitrite) using the reduced copper cadmium method as described by Bate and Heelas (1975). Ammonium, soluble reactive phosphorus (SRP) and silicate (DSi) were analysed using standard spectrophotometric methods (Parsons *et al.* 1984).

Sediment particle size

Sediment to a depth of approximately 1 cm was collected from directly below the area where each benthic chlorophyll a (chl a) replicate was collected. In the laboratory each sediment sample was dried at 105 ºC to constant weight. Samples were then disaggregated using a mortar and pestle then shaken through a series of steel mesh sieves (mesh apertures of 500, 250, 125, and 63 µm). The dry sieve method could underestimate the proportion of cohesive sediments (< 63 µm) but it was decided that the error would be small because South African estuary sediment

has a relatively high sand content; results from six estuaries (refer to Chapter 2) showed that the >125 µm fraction contributed approximately 80% of the sediment. Each fractionated sediment size class was weighed and the dry weight of each fraction was expressed as a percentage (w/w) of the total weight.

Phytoplankton chlorophyll a

Water samples (500 ml) were gravity filtered through Whatman GF/C filters, which were then stored in the dark of a cooler box until they could be frozen. The chl a was extracted by placing the frozen filters into 10 ml of 95% ethanol (Merck 4111). After extraction for 24 hours, spectrophotometric determinations of chl a were performed according to Nusch (1980). Absorbance was measured before and after acidification of extracts with 0.1 N HCl. Phytoplankton cells in samples collected in March and June 2005 were size fractionated by filtering 500 ml through nitex, Whatman GF/D then Whatman GF/C filters, separating the cells into > 20.0 µm (micro-phytoplankton), 2.7-20.0 µm (nano-phytoplankton) and 1.2-2.7 µm (pico-phytoplankton) size classes respectively.

Benthic chlorophyll a

Four replicate intertidal benthic samples were collected at low tide from each site by scraping surface sediment (< 2 mm depth) from within premarked circles (20 mm internal diameter) just above the estuarine water level. Four subtidal samples were collected from each site using a 20 mm internal diameter corer attached to an extension pole and the surface sediment was scraped from the core. Both intertidal and subtidal samples were stored in the dark of a cooler box until they could be frozen. The samples were freeze-dried, approximately 0.1 g was added to 4 ml of 95% ethanol (Merck 4111) and then stored for 24 hours at 0 °C. Once the chl a had been extracted the samples were whirlimixed, filtered through Whatman GF/C filters and the extract was analysed on a Waters M-45 high performance liquid chromatograph (HPLC).

All of the scatter plots and bar graphs were produced using Microsoft Office Excel 2003, and the contour plots of phytoplankton size fractions were produced using Grapher 6.1.21 (Golden Software, Inc.). Before testing for significant differences, the Kolmogorov-Smirnov Test was used to check if the data were

parametric. If the data were parametric, then a Students t-test was used to compare two sets of data for significant differences and the Tukey's test was used if there were more than two sets. However, if the data were non-parametric, then a Mann-Whitney Rank Sum test was used to compare two sets of data and the Kruskal-Wallis Anova on Ranks test was used if more than two sets were compared. Pearsons Product Moment Correlation was used to test the strength of association between variables. All tests were performed using the statistical software package Statistica (Version 7).

Results

Salinity

Based on average monthly flows of the Mngazi River (CSIR 2005), the flow rates into the Mngazi Estuary were 0.34 $m^3 s^{-1}$ (June 2002), 0.33 $m^3 s^{-1}$ (January 2003), 0.20 $m^3 s^{-1}$ (June 2003), 0.12 $m^3 s^{-1}$ (November 2003), 0.75 $m^3 s^{-1}$ (March 2005) and 0.19 $m^3 s^{-1}$ (June 2005). A large flood with flow in excess of 88.7 $m^3 s^{-1}$ occurred in September 2004, which scoured the mouth of the estuary (Fig. 7.2). Freshwater pulses following the flood kept the estuary in an open state. The estuary was semi-closed in June 2002 and January 2003, closed in June and November 2003 and open in March and June 2005 (Fig. 7.3). The longitudinal salinity gradient at the surface was relatively weak during the closed phase, particularly in June 2003 (surface salinity ranged from 13.3 ppt 6.4 km from the mouth to 21.8 ppt near the estuary mouth). There were strong vertical gradients in the mid to upper reaches of the estuary (the difference between surface and bottom salinity exceeded 13.0 ppt). The two sites nearest to the mouth were well mixed, probably due to exposure to coastal winds. These results suggest that during the closed phase when river input was low, the estuary became well stratified in the middle to upper reaches of the estuary, the sites nearest the mouth were generally well mixed due to coastal winds and the water in the estuary was brackish (10 to 20 ppt).

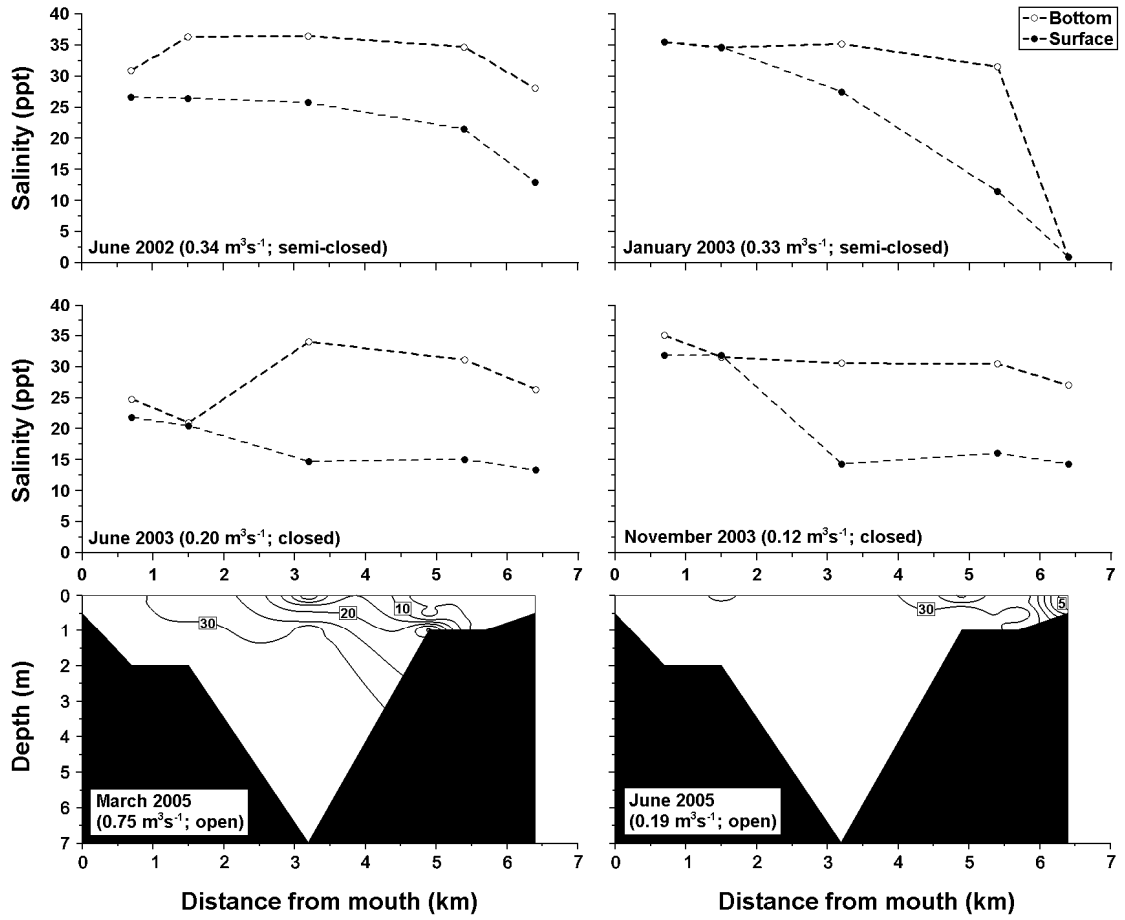

Figure 7.3. Salinity profiles in the Mngazi Estuary during the period June 2002 to June 2005. State of the mouth and estimated river flow are included in each plot.

Salinity at the site nearest to the head of the estuary ranged from fresh to 14.3 ppt during the period of study and changes can be attributed to flow and mouth condition. During periods of low flow and closed mouth conditions, water in the estuary increased above mean sea level through marine overtopping events and freshwater flow. This results in the upper reaches of the estuary becoming deeper and brackish water penetrating further upstream. When the estuary mouth was open and river flow was 0.75 m^3 s^{-1} a full salinity gradient was measured in the estuary. When the mouth was partially open there was a relatively weak freshwater signal (13 ppt at the surface) at the head of the estuary in June 2002 and the estuary was vertically stratified throughout. However, a salinity of 1.0 ppt was measured near to the head of the estuary in June 2003, the mid to upper reaches of the estuary were strongly stratified and the two sites nearest to the mouth were well mixed.

During open-mouth phases, a strong longitudinal salinity gradient was restricted to the upper half of the estuary. In March 2005 the vertically averaged salinity (± standard error of the mean) ranged from 0.06 ± 0.05 ppt (6.4 km) to 31.3 ± 2.7 ppt (3.2 km), and in June 2005 it ranged from 4.8 ppt (6.4 km) to 31.3 ± 2.5 ppt (5.7 km). These salinity data indicate that river input was lower in June 2005 and high salinity water penetrated further upstream than in March. There was strong salinity stratification in the middle and lower reaches of the estuary in March 2005, with a distinct thin layer of freshwater at the surface and increasing to 30 ppt within a metre of the surface. Salinity at the two sites nearest to the head of the estuary were near-fresh (< 0.5 ppt) and well mixed. It is unlikely that such strong stratification occurs in the middle and lower reaches of the estuary frequently and would require an extended period of stable weather conditions for the halocline to develop.

Temperature, dissolved oxygen and light attenuation

In intermittently open estuaries, water temperature is usually a function of seasonal trends in atmospheric temperature. Water temperature in the Mngazi Estuary ranged from 21.1 ºC to 28.1 ºC during summer and 15.8 ºC to 20.7 ºC during winter, averaging 24.9 ± 0.4 ºC and 18.8 ± 0.4 ºC respectively. There was a significant difference in temperature between summer and winter (t = -10.66; p < 0.001; n = 32). During winter (June 2002, 2003 and 2005) the surface water was generally cooler than that measured at the bottom and the pattern was reversed during the summer months (January 2003, November 2003 and March 2005) (Table 7.1). Water temperature during the study was generally highest near the head of the estuary and decreased towards the mouth.

Average DO concentration in the Mngazi Estuary was higher in June 2003 compared to June 2005 (Table 7.1). Hypoxic conditions (< 24%) were present in the bottom water of the middle and lower reaches of the estuary in March 2005. Factors such as water residence time (the time it takes for a certain volume of water to be replaced), bacterial decay of organic matter, temperature and vertical stratification can influence DO concentration.

The average secchi depth ranged from 0.21 ± 0.08 m in March 2005 (open) to 1.14 ± 0.05 m in June 2003 (Table 7.1). In June 2003, water clarity was relatively even throughout the estuary, with secchi depth ranging from 1.00 to 1.26 m. In contrast, secchi depth in March 2005 was low at the head of the estuary, 0.08 m, and

Table 7.1.

Average temperature, dissolved oxygen (DO), secchi depth and average phytoplankton chl a in the Mngazi Estuary from June 2002 to June 2005 (± standard error).

Date (mouth state; flow (m^3 s^{-1}))	Temperature (ºC)	DO (%)	Secchi depth (m)	Phytoplankton chl a (µg l^{-1})
06/2002 (semi-closed; 0.34)	18.7 ± 1.0	No data	0.52 ± 0.20	8.02 ± 1.93
01/2003 (semi-closed; 0.33)	26.5 ± 0.5	No data	0.70 ± 0.25	1.68 ± 0.11
06/2003 (closed; 0.20)	18.6 ± 0.7	71.8 ± 9.80	1.14 ± 0.05	5.00 ± 1.49
11/2003 (closed; 0.12)	26.0 ± 0.6	No data	0.52 ± 0.27	3.24 ± 0.93
03/2005 (open; 0.75)	22.6 ± 0.1	30.1 ± 7.77	0.21 ± 0.08	13.37 ± 0.92
06/2005 (open; 0.19)	19.5 ± 0.3	44.5 ± 2.26	0.25 ± 0.37	10.78 ± 0.86

increased to the mouth of the estuary (0.59 m) as water clarity improved. A number of factors could have contributed to the high turbidity: phytoplankton cells, resuspension of fine sediment through tidal currents and the river input of suspended particulate matter. However, there was a concave relationship between secchi depth and distance from the mouth, which indicates that the estuary behaved as a sink for suspended particulate matter. During the closed and semi-closed mouth phases, the water in the middle reaches of the estuary was frequently the most turbid. Average phytoplankton chl a was low (Table 7.1), < 10 µg l^{-1}, and evenly spread throughout the surface water of the estuary (Fig. 7.6) suggesting that the water-column was mostly affected by the resuspension of fine sediment or the flocculation of particulate material in this part of the estuary.

Inorganic nutrients

Sampling during the closed, semi-closed and open mouth states took place once in summer and once in winter for each state respectively, which indicates that the mouth state was not seasonal during the period of study. Samples were collected for nutrient analyses during the closed and semi-closed mouth phases.

Ammonium ranged from 0.1 to 9.8 µM during the period of study and peaked in the bottom water of the middle reaches in June of 2002 and 2003 (Fig. 7.4). This area of the estuary was the most turbid and vertically stratified at the time of sampling. SRP was low throughout the estuary during the semi-closed state but increased to concentrations ranging from 5.4 to 19.8 µM during the closed state. During the period of study TOxN ranged from below detectable limits to 3.7 µM. TOxN, together with DSi, is regarded as a 'new' nutrient and is usually imported in riverwater. During this study, there was only evidence of this in June 2002 where concentrations decreased from 2.3 µM at the head to < 0.5 µM in the mid-lower reaches of the estuary. DSi measured in the surface water in January 2003, decreased exponentially from 372 µM at the head of the estuary to 20 µM at the mouth along the longitudinal axis, which indicated that the estuary was acting as a sink for DSi.

Based on the results of the study, ammonium concentrations could be interpreted as being consistently available to microalgae, SRP as being limiting during the semi-closed mouth phase and TOxN as being generally low, particularly

during winter when the estuary mouth is closed (Figure 7.4). TOxN is generally imported into estuaries in the riverwater and it would be expected that the release of ammonium from the sediment was the most important source of nitrogen during periods of low flow.

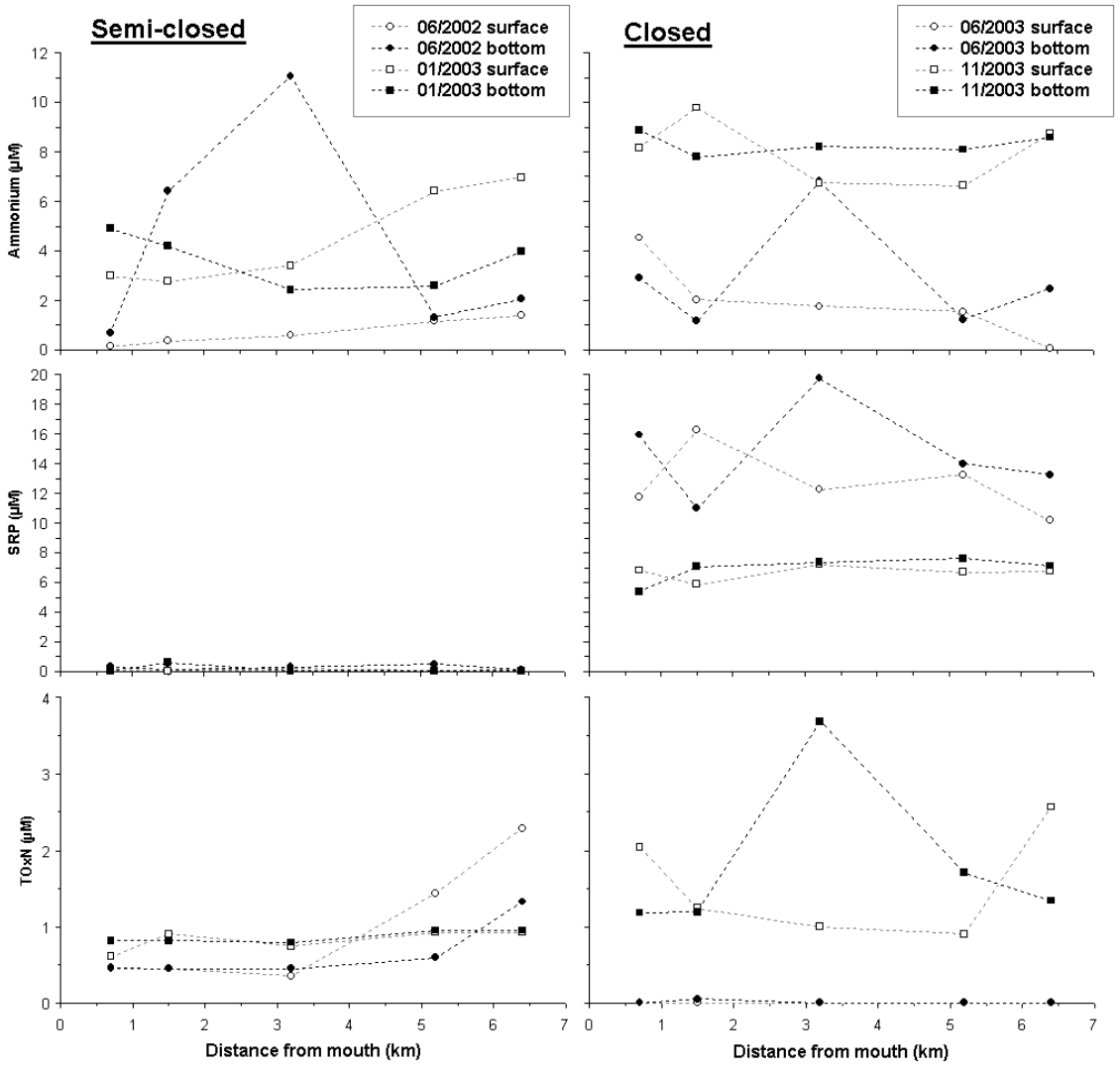

Figure 7.4. Ammonium, SRP and TOxN relative to distance from the mouth of the Mngazi Estuary mouth during semi-closed (June 2002 and January 2003) and closed (June 2003 and November 2003) mouth conditions.

When the dissolved inorganic nitrogen and phosphorus ratios (DIN: DIP) were considered, phosphorus was the most likely nutrient limiting microalgal growth at most sites (i.e. the ratio was > 16) when the mouth was semi-closed and this switched to nitrogen during the closed mouth phase (Fig. 7.5). DIN and DIP concentrations of less than 2.0 µM and 0.2 µM respectively were regarded as being potentially limiting to phytoplankton growth (Fisher *et al*. 1988).

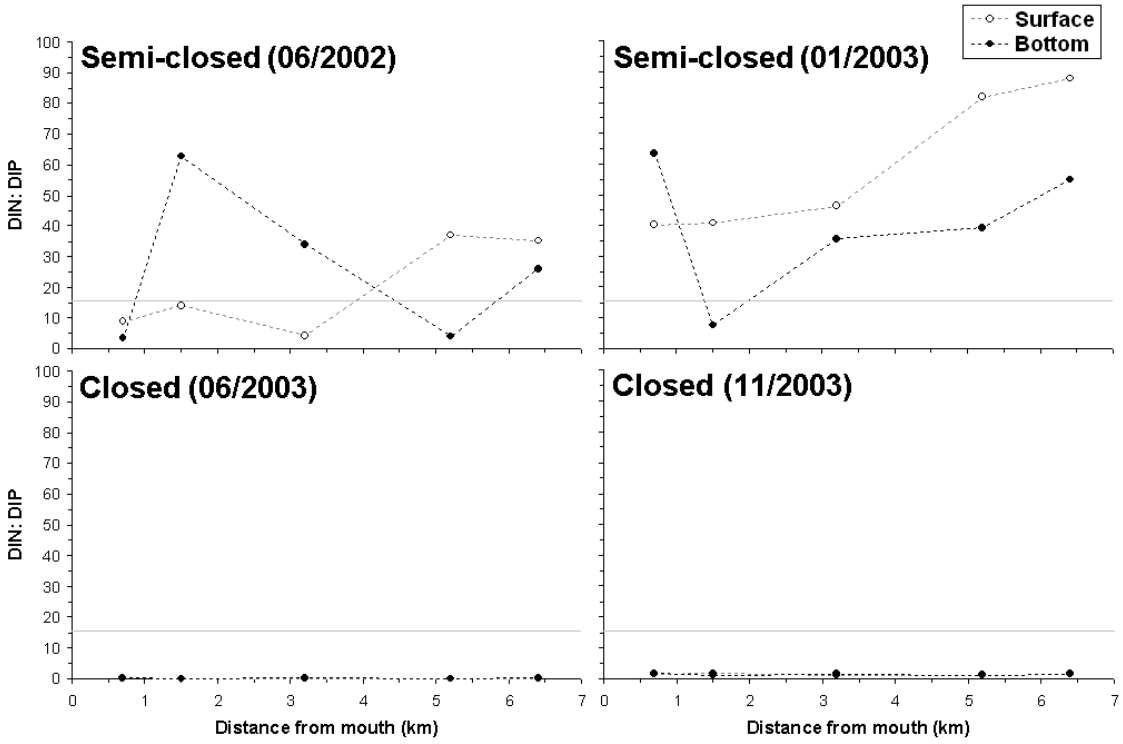

Figure 7.5. Dissolved inorganic nitrogen (DIN; TOxN + NH_4^+) and phosphorus (DIP; SRP) ratios in relation to distance from the mouth, June 2002 to November 2003. The DIN: DIP of 16: 1 is represented by grey lines.

Phytoplankton biomass (chl a)

Phytoplankton chl a concentration ranged from 0.5 to 18.1 µg l^{-1}, 1.0 to 20.7 µg l^{-1} and 5.0 to 25.3 µg l^{-1} during the closed, semi-closed and open mouth conditions respectively (Fig's. 7.6 and 7.7). The average concentration during the open phase was significantly higher than when the mouth was closed or semi-closed ($H = 43.64$; $P < 0.001$). Chl a reached higher maxima during summer months (25.3 µg l^{-1} and 20.7 µg l^{-1} in summer and winter respectively) but the difference was not significant.

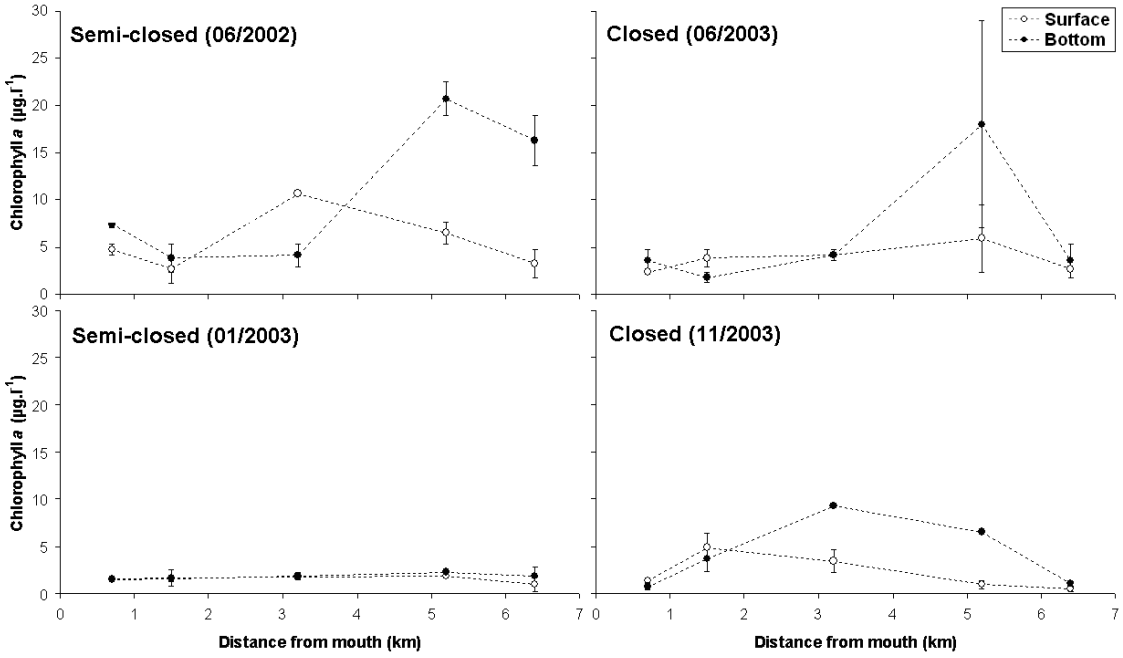

Figure 7.6. Phytoplankton chl a in relation to distance from the mouth of the estuary during the semi-closed (June 2002 and January 2003) and closed (June 2003 and November 2003) mouth conditions. Standard error represented by vertical bars.

Average phytoplankton chl a was highest when the mouth of the estuary was open (10.8 ± 0.9 µg l^{-1} in June 2005 and 13.9 ± 0.9 µg l^{-1} in March 2005). This could be the result of benthic microalgal cells becoming resuspended into the water-column or by nutrients becoming available in marine water or released from resuspended sediment. There were no discernable trends in phytoplankton chl a distribution in the estuary in relation to mouth condition. Chl a was either low (<5 µg l^{-1}) and evenly distributed in January 2003 or reached a maximum in the middle reaches of the estuary in November 2003. Samples were collected at the surface and bottom of the

estuary on these dates. In March 2005, detailed distribution plots (Fig. 7.7) show a distinct maximum in the middle reaches of the estuary (3.2 km from the mouth) and this occurred at depths ranging from 3 to 4 m. If only the surface and bottom phytoplankton chl a were considered, then a similar trend occurred to the two previous summer sampling sessions. Nano-phytoplankton (phytoplankton collected 2.7 to 20.0 µm pore size filter) dominated the phytoplankton, reaching a maximum of 17.9 µg l^{-1} at a depth of 3.5 m.

In winter, phytoplankton peaked at the surface, 3.2 km from the mouth in June 2002 (Fig. 7.6). However, a more distinct peak occurred at the bottom, 5.2 km from the mouth, in June 2002, 2003 and 2005 (Fig's. 7.6 and 7.7). This peak, dominated by micro- and nano-phytoplankton (Fig. 7.7), occurred at a depth of 3 to 4 m. The chl a peak that occurred near the surface, 3.2 km from the mouth was dominated by smaller pico-phytoplankton.

Peaks in phytoplankton chl a occurred at vertically averaged salinities ranging from 18.9 ppt to 33.6 ppt. This range is much higher than the salinity of <10 ppt as defined for a river-estuary interface zone (Bate *et al*. 2002), which suggests that river input was not the principal factor that determined the distribution of phytoplankton biomass. It is more likely that nutrients were evenly distributed throughout the estuary and that a combination of nutrients released from sediment through remineralisation and from riverwater supported the phytoplankton biomass.

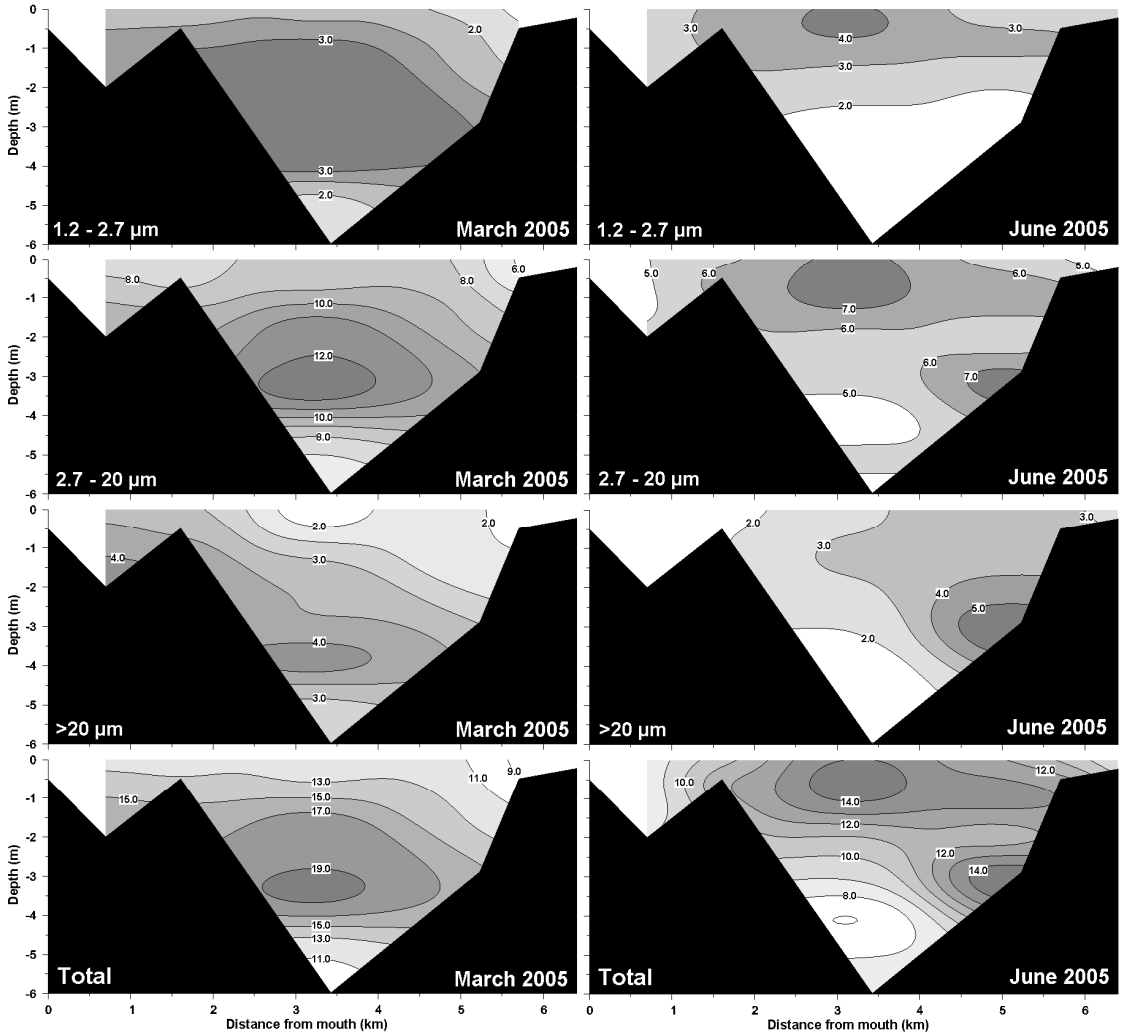

Figure 7.7. Micro- (nitex; >20.0 µm), nano- (GF/D; 2.7 - 20 µm), pico- (GF/C; 1.2 - 2.7 µm) and total phytoplankton chl a (µg l^{-1}) relative to distance from the mouth of the Mngazi Estuary in March and June 2005 (open mouth phase).

Sediment particle and organic spatial patterns

The intertidal zone in the Mngazi Estuary, January 2003, was dominated by coarse sediment particles (> 125 µm) (Fig. 7.8). The highest proportion of muddy sediments (< 125 µm) and organic matter was measured in the middle reaches of the estuary, 3.2 km from the mouth; mud 31.7 ± 6.6% and organic matter 5.65 ± 0.52%. Sediment at the mouth and head of the estuary contained very little muddy sediment (< 1.3%) and organic matter (< 2%) and was dominated by coarse sand of marine and fluvial origins respectively. The organic content was significantly correlated to mud (n = 25; r = 0.69; P < 0.001) and moisture contents (n = 25; r = 0.93; P < 0.001).

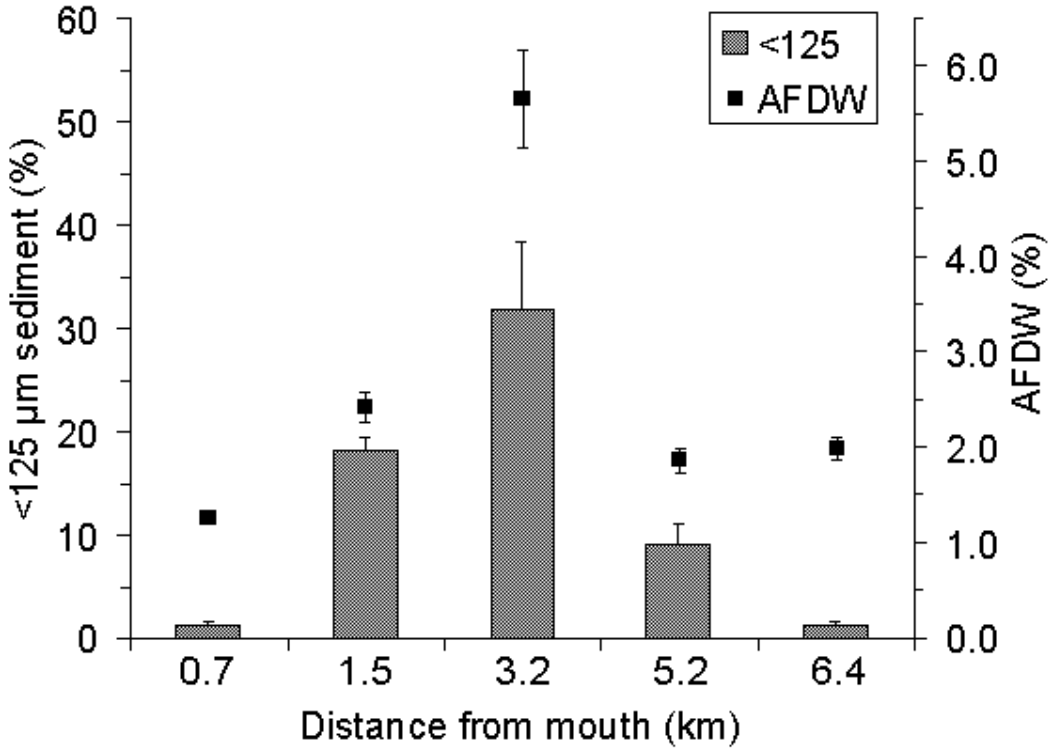

Figure 7.8. Intertidal mud (<125 µm) and organic matter (AFDW) contents in relation to distance from the Mngazi Estuary mouth (January 2003). Standard error bars displayed.

Benthic chlorophyll a

Benthic chl a ranged in concentration from 2.1 to 56.8 µg g^{-1} and 0.3 to 15.7 µg g^{-1} in the intertidal and subtidal zones respectively (Fig. 7.9). The average chl a in the intertidal zone, 22.5 ± 3.5 µg g^{-1}, was significantly higher than in the subtidal zone,

4.2 ± 0.7 µg g^{-1} ($n_{inter.}$ = 22, $n_{sub.}$ = 28; T = 797.0; P < 0.001). Benthic biofilms tend to be patchy but the highest chl a concentrations were closely associated with the fine, cohesive sediments found in the middle reaches of the estuary. Intertidal chl a, measured in January 2003, was significantly correlated to ash-free dry weight and water content in the surface sediment (r = 0.92; P < 0.001 and r = 0.89; P < 0.001 respectively), and with the 63-125 µm sediment particle size fraction (r = 0.61; P < 0.001). There were no correlations with overlying water quality suggesting that benthic microalgal biomass was dependent on the substrate, particularly that high in organic matter, as a source of nutrients.

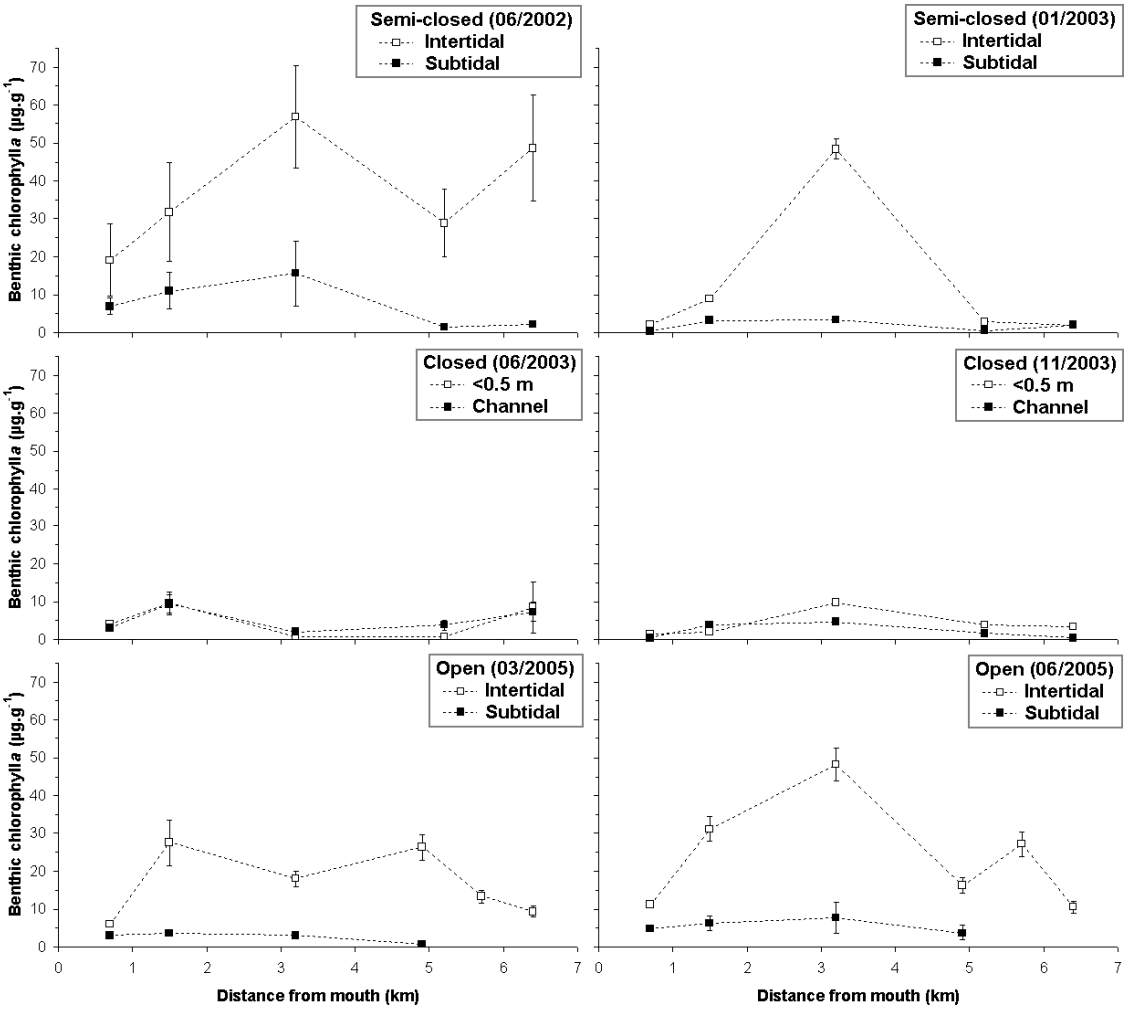

Figure 7.9. Benthic chl a (µg g^{-1}) in relation to distance from the mouth (km). Intertidal and subtidal concentrations given for semi-closed and open mouth conditions, and shallow (close to estuary bank) and channel concentrations given for closed mouth conditions. Standard error represented by vertical bars.

Discussion

The Mngazi Estuary was predominantly in an open or semi-closed mouth state during the period of study and was only closed for short periods of time. The estuary mouth was partially protected from direct wave action and limited sediment was available for mouth closure, so very little flow (0.05-0.2 m^3 s^{-1}) was required to maintain a semi-closed mouth state. After the flood in September 2004, the mouth stayed open for an extended period (~8 months) and during this period the tidal amplitude was close to a metre in the estuary, indicating significant scouring of sediment from the mouth region during the flood (CSIR 2005). The increase in tidal flow maintained open mouth conditions for longer. The neighbouring Mngazana Estuary was sampled shortly after the start of a heavy rainfall event and turbidity was high at the head of the estuary (Secchi depth = 0.05 m and turbidity was > 1700 NTU), which indicates a high sediment load from riverwater following heavy rainfall events. Water clarity increased towards the mouth of the Mngazi Estuary in March 2005; Secchi depth increased from 0.08 m to 0.59 m. In addition, sediment in the middle reaches of the estuary had a high organic matter and fine sediment (< 125 µm) content, which indicates that the estuary acts as a sink for suspended sediment. It is expected that during periods of strong river flow when the mouth is open, high concentrations of suspended and dissolved organic matter, nutrients (DSi in particular) and fine sediment are imported into the estuary.

River flow into the Mngazi Estuary was negligible for long periods and conditions calm, with little or no tidal exchange as a result of a well-developed sandbar in the mouth region. These stable conditions frequently resulted in a weak longitudinal salinity gradient, relative to the open state, and a strong vertical salinity gradient throughout most of the estuary. The water in the lower reaches of the estuary was prone to vertical mixing, presumably due to coastal winds. This type of mixing is typical of exposed and shallow estuaries, generally < 2 m, such as the Kasouga Estuary (Eastern Cape, South Africa) (Froneman 2000 & 2002) and Wamberal Lagoon (New South Wales, Australia) (Gale *et al*. 2006). According to the conceptual model of TOCE's developed by Snow & Taljaard (2007), the water-column is likely to be well mixed through wind-mixing but factors such as low river input, overtopping events and extended periods of calm weather conditions can lead to some vertical and longitudinal stratification. This was the case in slightly deeper estuaries, generally > 2 m, such as the Mpenjati Estuary (KwaZulu-Natal, South

Africa) (Perissinotto *et al*. 2002) and Smiths Lake (New South Wales, Australia) (Gale *et al*. 2006) where strong longitudinal and vertical stratification were observed during the closed state.

There has been a lack of published research on North American TOCE's but a recent study of Mecox Bay in New York State has found similar trends to South African TOCE's (Gobler *et al*. 2005). Freshwater flowing into the Mecox Bay Estuary was high in DIN (140 ± 20 µM) so the productivity appeared to be P-limited during the wet winter months (DIN: DIP was >100), and this decreased to less than one in summer. There was a trend to smaller cells, pico- and nanophytoplankton, during low nutrient conditions. Small cells are known to be more efficient at proliferating under oligotrophic conditions (Parsons & Takahashi 1973).

There was no evidence of a distinct phytoplankton chl a maximum in the Mngazi Estuary at the river-estuary interface (REI zone) where salinity was less than 10 ppt. Instead, chl a was generally highest at low flows at depths of up to four metres, in the middle reaches of the estuary. The nanophytoplankton, sizes ranging from 2.7 to 20.0 µm, dominated the phytoplankton. A second maximum was located near the bottom in the mid to upper reaches of the estuary in June 2005 and was possibly the result of flocculated material, with an elevated microalgal content, that had settled out of the water-column and was pushed upstream by the saline wedge during flood tides. This process of flocculated material being forced upstream by tidal currents was found in the macrotidal Humber, Trent and Ouse estuaries, United Kingdom (Uncles *et al*. 2006).

Water temperature often exceeded 25 ºC in summer and the river flow was regularly < 0.3 m^3 s^{-1} for long periods between freshwater pulses. These conditions were regarded as ideal for the deposition of fine mud particles through the process of flocculation (Jiang *et al*. 2004; Ruan 1991). There was a distinct change in sediment content, based on January 2003 findings, from coarse sand (> 125 µm) of marine origin in the lower reaches, to sand with high mud and organic contents in the middle reaches and to coarse fluvial sand at the head of the estuary. The most probable source of the fine sediment with a high organic content (> 7%) in the middle reaches is through the process of flocculation, a process well described by Day (1981). Research in the Tamar Estuary, England, found that the majority of floccules settled out of the water-column during the ebb tide (86%) of which only 8% was outside the turbidity maximum zone (Manning & Bass 2006). If the level of sedimentation

increases significantly over time, then there is a reduced contact time between organic matter and dissolved oxygen in the water-column. This increases the amount of sulphate reduction in the sediment and reduces the sediment quality for benthic invertebrates (Radke *et al*. 2004). It is likely that the mineralisation of the organic matter from these sediments together with the increased nutrient flux through processes such as bioturbation supports the high benthic microalgal biomass in this region (chl a exceeded 50 µg g^{-1} in June 2002). Benthic microalgae in the upper sediment of the Mdloti Estuary (KwaZulu-Natal, South Africa) were also significantly correlated to the more silty sediment in the middle reaches of the estuary (Mundree *et al*. 2003).

Conclusions

Based on results from this study and published findings in other estuaries, a simple conceptual model describing the functioning of the Mngazi was produced. Pulses in river flow were considered to be an important driving factor controlling the biogeochemistry of the Mngazi Estuary, so the model was based on changes occurring within the estuary relative to flow and mouth phase (Fig. 7.10).

High amplitude floods introduce high concentrations of dissolved and suspended organic matter, sediments and nutrients into the estuary. The currents from the flood and the resulting tidal exchange replenish oxygen-depleted water in deeper reaches of the estuary. As riverwater flowing into the estuary decreases, the estuary enters a deposition stage in which dissolved and particulate organic matter, fine sediments, microalgal cells and nutrients aggregate into floccules, settle out of suspension and are deposited in the middle reaches of the estuary. During this stage, there is likely to be strong longitudinal and vertical stratification, a sandbar begins to redevelop in the mouth region and the mineralisation of nutrients from the deposited floccules begins. Phytoplankton chl a is highest during this period in the middle to upper reaches of the estuary.

With a further reduction in river flow, the estuary gradually becomes semi-closed and the longitudinal stratification weakens. Strong vertical stratification should develop throughout most of the estuary, particularly in water deeper than 2 m, and the deposition of flocculated material continues, albeit at a much lower rate. The mineralisation of nutrients begins to deplete oxygen concentrations within deeper reaches of the estuary and a microphytobenthic community develops into the

dominant primary producers, particularly in the fine intertidal sediments in the middle reaches of the estuary. This increases nutrient recycling within the sediment, limiting the release of nutrients to the phytoplankton. The water-column gradually shifts from being nitrogen to phosphorus limited for microalgal growth.

During short periods when river flow is negligible, the estuary mouth closes and the water behind the sand berm becomes perched above mean sea level resulting in the intertidal zone becoming flooded. The water-column is likely to become homogenous through wind-mixing and DO is uniform, 3-4 mg l^{-1}, throughout the estuary and possibly lower in deeper regions of the estuary where pockets of saline water are trapped. The water-column will become clear throughout most of the estuary and a well developed subtidal MPB community will develop. There will be limited release of nutrients to the water-column through bioturbation sustaining a weak phytoplankton community of small celled species (nano- and picoplankton). Occasional overtopping during storm events is likely to reintroduce oxygenated water into the bottom water.

This model can be used to predict changes in the estuary in response to catchment activities. Two typical scenarios for estuary catchments relevant to estuary managers are reduced flow and increased catchment utilization. For example a reduction in the frequency and intensity of floods would result in the estuary shifting from a river-dominated to a more wave-dominated system, causing the estuary to be in the closed phase more often and for longer periods. There would be little allochthonous supply of organic matter and nutrients. Remineralisation would be reduced thus influencing the availability of nutrients for microalgae, particularly phytoplankton. The estuary could become N limited during the closed, low river flow phase. Reduced flocculation and particle settling could change the substrate type influencing the distribution and biomass of benthic microalgae that are usually associated with high organic matter and fine sediments. Thus the lack of longitudinal sediment and salinity gradients would reduce habitat and species diversity.

An increase in agricultural activities in the catchment in the absence of large impoundments would increase sediment and organic matter inputs from the catchment. The increased mineralisation of organic matter is likely to support a higher biomass of benthic microalgae, particularly in the intertidal zone, and increase biological oxygen demand (BOD) in deeper reaches of the estuary. This may increase the risk of hypoxic or anoxic conditions occurring in deeper areas of the

estuary (> 2 m). Responses are expected to be similar in estuaries described as intermittently open estuaries, barred-estuaries, barrier-built estuaries, and intermittent open and closed lakes and lagoons (ICOLLs).

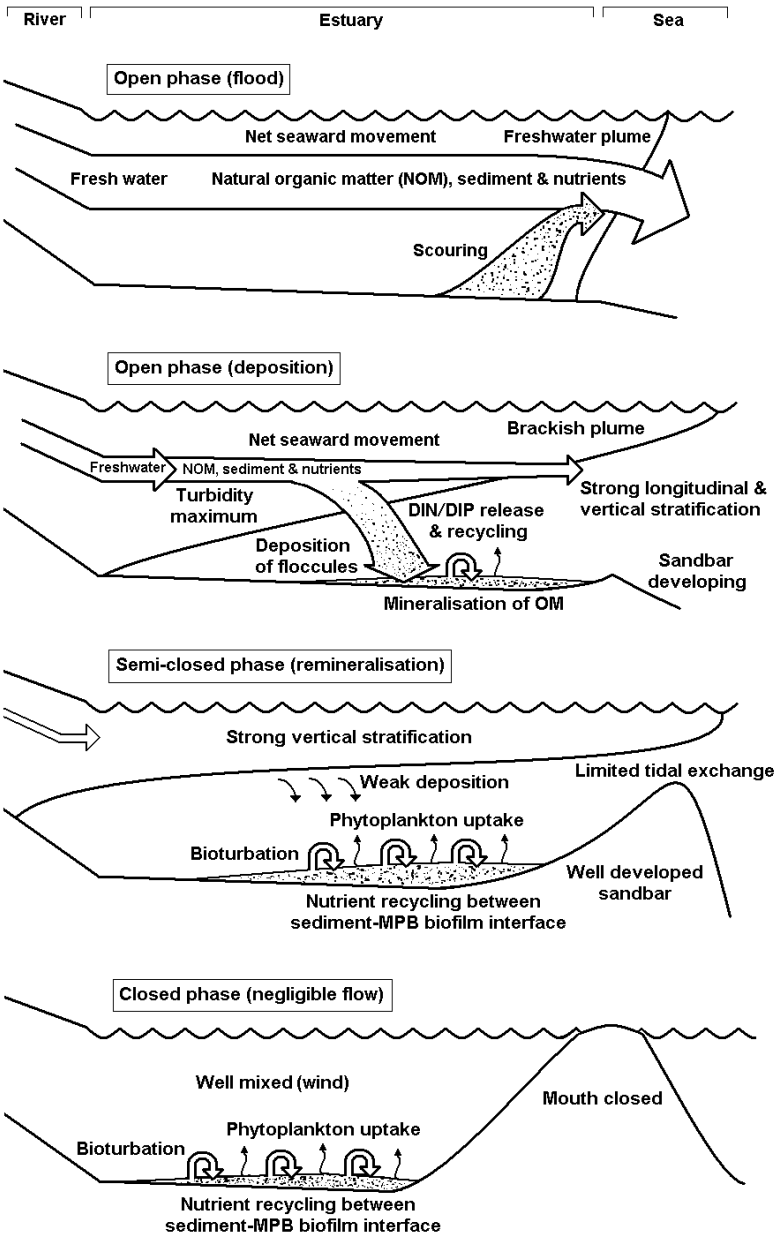

Figure 7.10. A conceptual model of the transition from open (flooded) to closed mouth conditions in the Mngazi Estuary. River input influences the mouth phase, stratification and the import of dissolved and particulate organic matter, sediments and nutrients (TOxN and DSi in particular). As river flow decreased, there was a trend towards the water-column becoming well-mixed, nutrients being mineralised from organic matter in the sediment and the microphytobenthos (MPB) dominating primary production (modified from Eyre & Twigg, 1997).

CHAPTER 8

The value of microalgae in the determination of the freshwater requirements of South African estuaries

The results presented in the preceding chapters are discussed in an attempt to answer the main questions of this thesis:

1) What factors determine the spatial patterns of microalgal biomass, phytoplankton and MPB in South African estuaries?

2) How is MPB biomass related to sediment carbohydrates and sediment stability?

3) How can microalgae be used to determine the freshwater inflow requirements of estuaries?

Phytoplankton

Previous research on permanently open estuaries showed that estuaries with large catchments have adequate freshwater to support numerous phytoplankton communities and a rich pelagic food chain (Adams *et al*. 1999). This was the case in the Sundays (Hilmer & Bate 1990 & 1991) and Gamtoos (Snow *et al*. 2000b) estuaries but in others that have a strongly seasonal rainfall (Berg Estuary) or are starved of riverwater through abstraction and impoundments (Kromme Estuary) there is much lower phytoplankton biomass (Fig. 8.1). Based on results from the Kromme Estuary, an almost complete reduction of freshwater prevented salinity stratification from occurring. In addition, large human-made impoundments have been found to act as DSi sinks and any increases in the rate of phytoplankton production within these reservoirs, particularly as a result of eutrophication, increases the rate of DSi depletion (Hessen 1999). As a result, phytoplankton biomass was low (< 10 µg l^{-1}) in comparison to the nearby Gamtoos and Sundays estuaries that frequently exceed 30 µg l^{-1}. In the Berg Estuary, river flow was extremely seasonal, with almost no flow during summer but with strong flow during winter. The mouth of the estuary has been artificially deepened and stabilised behind concrete walls resulting in strong tidal exchange and a well mixed water-column in the lower reaches.

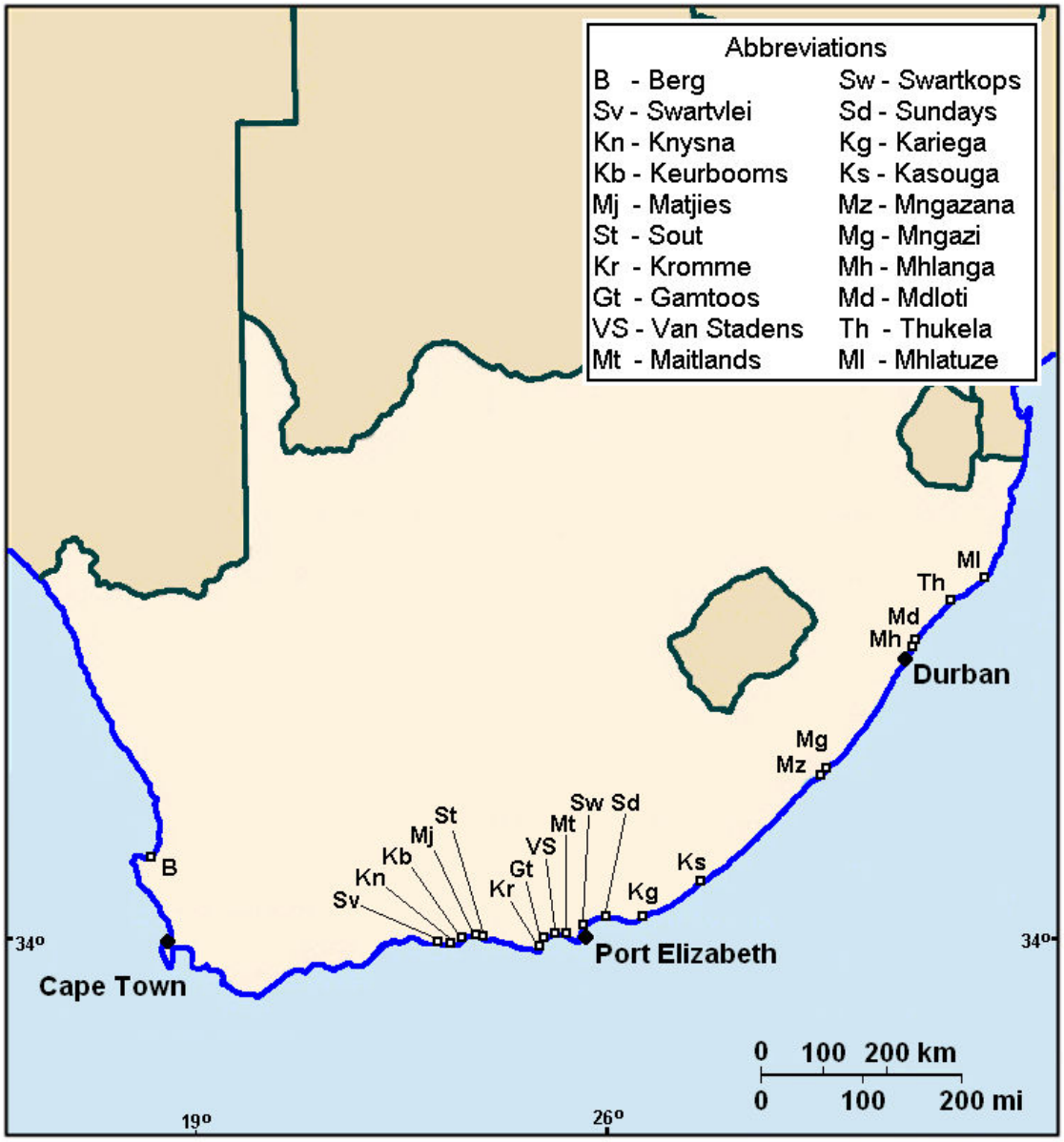

Figure 8.1. Locations of South African estuaries mentioned in the text (Chapter 8).

The tidal prism is large and water level fluctuations occur ~70 km from the mouth. Saline intrusion is restricted to within a few kilometres of the mouth in winter but penetrates 43 km in summer (Taljaard 2004). Even when there was longitudinal salinity stratification, the water-column was well mixed as a result of the strong tidal exchange. Phytoplankton biomass was low in this estuary (< 10 µg l^{-1}) suggesting that the strong turbulence of the water-column did not support a high phytoplankton biomass and that residence time was too short in winter for a high biomass to develop. Flagellates and, in much lower abundance, diatoms dominated the phytoplankton in the Kromme, Berg and Mngazi estuaries but it is not known what

proportion of the flagellates were heterotrophic; i.e. potentially imposing a top-down pressure on microalgal biomass through herbivory, so further research is urgently necessary to quantify this.

Following the August 2006 floods along the southern Cape coast a persistent phytoplankton bloom (chl a > 20 µg l^{-1}) developed in the Swartvlei Lake at the confluences of the tributaries and the brackish water in the lake. This indicates that the continuous river input into the lake created a productive interface zone in the lake itself. However, a similar peak of chl a occurred in the Knysna Estuary four weeks after the flood and this did not persist for long because of the strong tidal exchange through the deep bay and mouth that rapidly flushed nutrients from the system.

Phytoplankton in the Mngazi Estuary, a 3-phase TOCE, occasionally exceeded 20 µg l^{-1} during all mouth phases but there was no evidence of a river-estuary interface zone (REI), probably as a result of low river flow and nutrient concentrations. During the semi-closed mouth state the nutrient concentration in the riverwater was relatively low (TOxN was < 2.5 µM and SRP below detectable levels); potentially limiting microalgal growth, hence the highest biomass was generally measured in the middle reaches of the estuary at depths of up to 4 m where NH_4^+ was highest. Phytoplankton in the Ems Estuary, Netherlands, was largely dependent on the recycling of organic matter for N and P; the accumulation, remineralisation and burial of particulate organic matter was instrumental in maintaining high primary production (van Beusekom & de Jonge 1998). It is likely that microalgae in the Mngazi Estuary were also dependent on the remineralisation of organic matter in the sediments as a consistent nutrient supply. This would have been highest in the middle reaches of the estuary where organic content in the sediment was highest (AFDW > 5%) but further research is required to confirm this. The TOCE conceptual model (Snow & Taljaard 2007) provided insight into the nutrient dynamics in response to mouth state in the Mngazi Estuary and the spatial patterns of microalgae that followed. The conceptual model will hopefully provide a useful tool for estuarine researchers and managers charged with setting the freshwater inflow requirements of small estuaries.

Benthic microalgae

The spatial patterns of the MPB were shown to be affected by the quantity and quality of riverwater as well as the biogeochemical parameters of the sediment. Intertidal chl a was significantly correlated to SRP in Swartkops Estuary (r = 0.47) but based on the Redfield ratio of 16:1 (DIN: DIP) microalgal growth appeared to be stiochiometrically nutrient limited at a number of sites. In addition, based on the results of studies in six permanently open estuaries (Chapter 2), benthic chl a content was significantly correlated to TOxN. However, although statistically significant, the coefficient was low (0.15) indicating that the association may not be ecologically significant on its own and that the association is coincidental. By contrast, microalgal spatial patterns were strongly related to biogeochemical parameters of the sediment. Benthic chl a was significantly correlated to the proportion of < 125 µm sediment (r = 0.31), organic matter (r = 0.53) and moisture contents (r = 0.62) of the sediment in at least five of the six estuaries, Sundays Estuary excluded, during the period of study. This could suggest that the remineralisation of nutrients from the degradation of organic matter is a valuable source of nutrients. In addition, sediment with a high organic content is likely to have a higher moisture holding capacity than coarse sediments with a low organic content providing a more stable environment for MPB, particularly in the intertidal zone that is exposed every tidal cycle and prone to desiccation.

The results of this study have highlighted, using correlation analyses, the close association between microalgal biomass and organic matter. The association with water-column nutrients has been much weaker but these can be made available through transformations, which may mask a direct relationship between the ambient concentration of these variables and the biological response (van Beusekom & de Jonge 1998; Snow & Taljaard 2007). In addition, organic matter can be derived from the MPB itself, creating a continuous cycle of nutrients and organic matter. A number of studies have focussed on nutrient budgets within South African estuaries (Scharler *et al*. 1997; Dupra *et al*. 2001; Switzer 2004); Knysna, Kromme, Gamtoos, Swartkops, Sundays, Thukela and Mhlatuze, but few have tried to integrate microalgae within these type of studies. Recent research in the Colne Estuary, U. K., has focussed on the flux of nutrients across the sediment-water interface, scaled the flux measurements up to calculate nutrient budgets for the entire estuary and estimated the amount of DIN assimilated by the MPB (Dong *et al*. 2000; Thornton *et*

al. 2002; Thornton *et al*. 2007). The loading of nutrients from rivers to the ocean is not straight forward. A large fraction of nitrogen is transformed by bacterial mediated processes within the estuarine sediment, which is available to benthic microalgae, before passing through estuaries into the ocean (Thornton *et al*. 2007). Similar research is necessary in South African estuaries to determine the relationship between benthic microalgae and nutrient loads entering the estuaries, which then can be used to relate microalgae (biomass and community structure) to the quality and quantity of freshwater.

Biostabilisation

Since Holland *et al*. (1974) quantified the importance of diatom biofilms on marine sedimentation processes, a number of studies have focussed on the role that the extracellular polymeric substance (EPS) exuded, primarily from diatoms, has on sediment stabilisation. The phytoplankton, diatoms in particular, are capable of releasing transparent extracellular particles (Passow 2002), which play an important role in the sedimentation and biogeochemical cycling of matter. However, the production of carbohydrates in the water-column is far less than that produced by the MPB (Goto *et al*. 2001). Colloidal carbohydrate, of which EPS is a fraction, was highest in the muddier sediment generally found in the middle reaches of the estuaries in this study. There was strong multicollinearity between chl a, carbohydrate (all fractions), organic matter, moisture and mud contents. This is partly the result of the larger surface area to volume ratio and cohesive nature of fine particle sediments. However, the secretion of EPS into the sediment matrix is an important food source for bacteria (Yallop *et al*. 2000) that influences the flux of oxygen and nutrients across the sediment-water interface (Woodruff *et al*. 1999) and is significantly related to sediment stability (Yallop *et al*. 2000). As a result, the sediment in areas of high microalgal biomass generally resists erosion, which could affect channel-morphology.

Recent research has shown that the use of herbicides reduces the growth rates and photosynthetic activity of diatoms, reducing surface sediment stability by the absence of a cohesive biofilm (Mason *et al*. 2003). The subsequent accelerated erosion leads to increased turbidity in the water-column, which has potential knock-on effects such as reduced photosynthesis of submerged macrophytes. The data presented in this thesis indicated that the conditions that favour the accumulation of

fine sediment and organic material in estuarine sediments supported high biomass of benthic microalgae, which is likely to increase the stability of the sediment through the production of colloidal carbohydrates. Further research into the critical erosion threshold, using a portable annular flume, is necessary to confirm this. Conditions that favour the sedimentation of particulate material include reduced river flow through increased abstraction and the impoundment of rivers, which decrease the frequency and intensity of floods in estuaries.

Contributions to Resource Directed Measures (Determination of the freshwater requirements of estuaries)

There are two components in the determination of RDM for estuarine ecosystems: Abiotic (or driving) and biotic (or response). The data requirements for microalgae in estuaries include the measurement of driving and response components together, i.e. the process recognises that these components are inter-related (Table 8.1). Due to budget constraints only limited field work can take place and the objective is to obtain some data on the microalgae rather than nothing. Recommendations to improve the data requirements based on the results, and understanding gained from the research presented in this thesis, are discussed below.

Table 8.1.

Data requirements on microalgae for the Intermediate Determination of RDM in estuaries (DWAF 1999).

DATA REQUIRED	PURPOSE
(1) Chlorophyll-a measurements taken at 5 stations (at least) at the surface, 0.5 m, and 1 m depth intervals thereafter. Cell counts of dominant phytoplankton groups i.e. flagellates, dinoflagellates, diatoms and blue-green algae. **(3)** Measurements must be taken coinciding with typically high and low flow conditions.	To determine phytoplankton biomass and dominant phytoplankton types. Phytoplankton biomass is an index of eutrophication while changes in the dominant phytoplankton groups indicate changes in response to water quality and quantity. **(2)** A study of this nature is probably only necessary in large permanently open estuaries where phytoplankton are important primary producers. Measurements for different flow conditions are required to establish natural variability.
Intertidal and subtidal benthic chlorophyll-a measurements taken at 5 stations (at least). Epipelic diatoms need to be collected for identification. These measurements must be taken coinciding with a typically high and low flow condition (in temporarily closed estuaries measurements must include open as well as closed mouth conditions).	**(4)** To determine benthic microalgal biomass and dominant epipelic diatom species. Benthic microalgae are important primary producers in shallow estuaries or those with large intertidal areas. **(5)** Epipelic diatom composition can indicate changes in water quality. Measurements for different flow and mouth conditions are required to establish natural variability.
Simultaneous measurements of flow, light, salinity, temperature, nutrients and substrate type (for benthic microalgae) need to be taken at the sampling stations during both the phytoplankton and benthic microalgae surveys.	**(6)** Measurements of different abiotic parameters are required to determine their effect on phytoplankton and benthic microalgae. In turn, this information is used to estimate reference conditions and predict the implication of future runoff scenarios. Change in microalgal biomass and composition indicates changes in water quality that is strongly related to freshwater input. These data are used to set the Resource Quality Objectives for both water quality and quantity. Microalgal field excursions should coincide with the hydrodynamic and water quality field exercise.

A detailed discussion of six points, inserted in Table 8.1, is included:

1) The main aim of a freshwater requirement study is to collect a representative range of abiotic and biotic variables that allows the microalgal specialist to discern any longitudinal and vertical trends that might be present. A minimum of five sampling sites have been used in the past and to date have proven to be both cost and analytically effective. The accumulated data (abiotic variables, phytoplankton biomass and community structure) are needed to develop an index of eutrophication based on phytoplankton biomass and community structure.

   Dominant phytoplankton groups can also provide information about eutrophication and imbalances in macronutrients. According to Stoermer & Smol (1999), eutrophication frequently leads to changes in microalgal communities at the class or division level, typically favouring a decrease in diatoms and an increase in non-silicified flagellates. The eutrophication of lentic inland waters (impoundments in particular) favours the growth of diatoms and the subsequent uptake of silicate. As a result, water flowing from these systems into estuaries has a nutrient imbalance (low Si:P and N:P) and can potentially limit diatom growth and microalgal biomass, particularly if silicate concentration is less than 2 µM (Egge & Aksnes 1992).

   The flagellates are a poorly defined group and are made up of taxa from a number of phyla. For the purposes of this study the flagellates refer to flagellated cells (one or more flagella) that did not fall in the other four groups of phytoplankton (Table 8.2). Cells can be autotrophic (contain chloroplasts) or heterotrophic (chloroplasts are absent). In future RDM studies, attempts will need to be made to distinguish autotrophic and heterotrophic groups. Two methods that can be used are the use of an epifluorescence microscope (Haas 1982) or the use of the Autotrophic Index (AI), a ratio of ash-free dry weight to chl a (Collins & Weber 1978). The AI is used to distinguish the effects of inorganic nutrient and organic enrichment. A high index score indicates a high organic load relative to chl a, which is likely to support a high biomass of heterotrophs, a high biological oxygen demand and a relatively low biomass of autotrophs.

Table 8.2.

Classification, based on Shipunov (2007), and brief description of phytoplankton groups used in the RDM method for determining the freshwater inflow requirements of estuaries.

Group	Classification	Description
Diatoms	Kingdom Protista Superphylum Chromista Phylum Chromophyta Class Bacillariophyceae	Mostly unicellular. Encased in a species-specific silica cell wall (frustule).
Chlorophytes	Kingdom Protista Superphylum Chloronta Phylum Chlorophyta Class Chlorophyceae	A division of green algae. Predominantly aquatic photosynthetic eukaryotes containing chlorophylls *a* and *b*, and store food as starch.
Dinoflagellates	Kingdom: Protista Superphylum Alveolate Phylum Dinozoa Class Dinoflagellata	Mostly marine plankton. Possess two flagella: One extends to the posterior and the other forms a lateral circle around the cell. Cell wall is made up of flattened vesicles often supporting cellulose plates (theca). Can be mixotrophic.
Cyanophytes	Domain: Prokaryota Kingdom: Bacteria	Bacteria capable of photosynthesis. Many species are capable of fixing atmospheric nitrogen. Many cyanobacteria form filaments and individual cells have a thick gelatinous cell wall.
Flagellates	Kingdom Protista Examples come from a range of Phyla.	Organisms in these studies are considered to be flagellates if they possess one or more whip-like organelles (flagella), do not fall within the other 4 groups and can be mixotrophic.

2) Permanently open estuaries (POE's) with a continuous base flow support distinct phytoplankton communities at the interface between the riverwater and the estuary (Snow *et al*. 2000). Smaller estuaries are more likely to close periodically (temporarily open-closed estuaries; TOCE) and phytoplankton community structure changes over the closed-open-closed cycle. Recent studies of the Van Stadens (Fig. A.4), Maitlands, Kasouga, Kariega, Mdloti and Mhlanga estuaries (Froneman 2002 a & b; Perissinotto *et al*. 2004; Gama *et al*. 2005; Skinner *et al*. 2006) have contributed to our knowledge but additional data are still needed to enable us to generate conceptual models of phytoplankton dynamics within these estuaries. In addition, mouth closure in small TOCE's makes these systems more prone to the effects of eutrophication in the form of macroalgal growth and anoxia.

A number of studies have investigated temporal and spatial changes in the contribution to the total chl a from different phytoplankton size classes; previous research has found a trend from larger cells occurring during eutrophic conditions to smaller cells of different species becoming more dominant during oligotrophic conditions (Parsons & Takahashi 1973). However, the phytoplankton in the oligotrophic Van Stadens Estuary (Gama *et al*. 2005) was consistently dominated by the larger microphytoplankton (cells larger than 20 µm) and nanophytoplankton (cells between 2.7 and 20 µm in size) dominated the eutrophic Mdloti and Mhlanga estuaries (Perissinotto *et al*. 2004) and mesotrophic Mngazi Estuary (Snow & Adams 2007). As a result, the relationship between phytoplankton cell size and the trophic status of estuaries in South Africa is not always clear and this trend should be used with caution until further investigations can clarify the situation. Although the RDM method indicates that phytoplankton studies should only take place in POE's, measurements are continuing in TOCE's until we understand the role of these primary producers in this type of estuary. Microalgae are at the base of the foodchain in all estuaries and should not be left out of any environmental assessment.

3) Some estuaries have a seasonal flow pattern (e.g. the Berg Estuary has a high flow in winter and a low flow in summer) and can therefore be sampled during typical high and low flow conditions. However, a number of estuaries, particularly along the south Cape coast, do not have strong seasonal flow patterns. In a recent freshwater requirement study of the Sout Estuary, southern Cape coast, which falls within a national reserve giving it high ecological importance, there were no

flow gauges in the river catchment area and no biological information. In cases such as these, specialists need to make a judgement on what the average flow, microalgal community structure and biomass should be based on a growing database of information from other RDM studies. Where no flow data exist, attempts can be made to sample during high and low flow conditions by using rainfall data as a proxy of flow. The Berg Estuary study (Chapter 4) showed that two sampling sessions, coinciding with low and high flow conditions as outlined in the RDM procedure, was insufficient to accurately determine changes in microalgal biomass and community structure. Instead, predictions had to be made based on results available from other South African estuaries. In large estuaries such as the Berg, more than two sampling sessions will likely be necessary to determine the relationship between flow and phytoplankton response. As an example, eight sampling sessions were required to determine the optimal flow rate needed to maintain a maximum phytoplankton biomass in the river-estuary interface zone (REI) of the Gamtoos Estuary (Snow *et al.* 2000b).

1 & 4) No comprehensive biological guidelines have been developed using benthic and water-column chl a as indicators of an estuary's trophic status, specifically for use in the RDM process. Based on data collected during this study, together with other available data, phytoplankton and benthic chl a classification schemes were developed (Table 8.3 and 8.4 respectively). As the amount of available data increases, including eutrophic and hyper-eutrophic examples of estuaries, trophic indices based on microalgal biomass can be developed.

The average phytoplankton chl a (± standard deviation) of 933 samples from the Berg, Kromme, Sundays, Knysna, Swartvlei, Sout, Matjies, Gamtoos, Mngazana and Mngazi estuaries (Snow *unpublished data*), and Mhlatuze Estuary (Gama *unpublished data*) was 8.64 ± 19.26 $\mu g.l^{-1}$. Concentrations ranged from 0 to 263.3 $\mu g.l^{-1}$ with a median concentration of 3.43 $\mu g.l^{-1}$. Based on the median and quartile values, a phytoplankton chl a classification scheme was developed (Table 8.3).

Table 8.3.

Classification scheme of median phytoplankton chl a for a whole estuary obtained using microalgal biomass data from freshwater requirement studies.

Biomass class	Median chl a
Very low	Less than 1.0 µg l^{-1}
Low	1 to 3.5 µg l^{-1}
Medium	3.5 to 8.0 µg l^{-1}
High	>8.0 µg l^{-1}

It is important to note that the phytoplankton chl a concentrations used to develop the foregoing classification scheme were collected using the methods described in the RDM process and reported as the median concentration in the estuary. Water filtered for chl a analyses should ideally be used for nutrient analyses to reduce costs and obtain more accurate correlation results between phytoplankton biomass and water chemistry.

This classification scheme can be used in future RDM studies to place the microalgal biomass results in context and provide comparisons on a whole estuary basis. For example data collected during a well-developed but very localised phytoplankton bloom in the Sundays Estuary were analysed and the median concentration was 4.8 µg l^{-1}. As a result, phytoplankton biomass in the estuary on that sampling date can be classified as medium in comparison to other estuaries. Additional sampling sessions are likely to make the classification more accurate. In contrast, the Sout Estuary is a small permanently open estuary with very few impacts within the catchment area. The median phytoplankton chl a concentration was 0.3 µg l^{-1}. As a result, this estuary can be classified as having a very low phytoplankton biomass, probably typical of an oligotrophic estuary. Spatial data are also presented in an RDM study to indicate where the highest phytoplankton biomass can be found along the longitudinal gradient and whether a REI, a region of high microalgal biomass, exists in the estuary.

A similar classification scheme was developed using intertidal benthic chl a (Table 8.4). The average benthic chl a (± SD) of 527 samples from the Sout, Berg, Gamtoos, Keurbooms, Mngazi, Sundays and Swartkops estuaries (Snow,

unpublished data) was 11.4 ± 13.3 µg g^{-1}. Based on median and quartile values a benthic chl a classification scheme was developed (Table 8.4).

Occasionally, a very localised microalgal bloom occurs in the water-column or in the vicinity of a groundwater seepage site but the chl a is low in the rest of the estuary. By basing the classification scheme on the median chl a of the estuary, the localised peak does not carry as much weight as if average chl a was used. Instead, the estuary is only likely to go up a class if chl a is elevated, through eutrophication, throughout the estuary.

Table 8.4.

Intertidal benthic microalgal biomass classification scheme based on median chl a contents and concentrations obtained using chl a data from freshwater requirement studies.

Biomass class	Median chl a content (median chl a concentration)
Low	< 3.5 µg g^{-1} (< 11 mg m^{-2})
Medium	3.5 to 7.2 µg g^{-1} (11 to 23 mg m^{-2})
High	7.2 to 13.4 µg g^{-1} (23 to 42 mg m^{-2})
Very high	>13.4 µg g^{-1} (> 42 mg m^{-2})

Benthic chl a data used to develop the intertidal benthic chl a classification scheme (Table 8.4) were collected using the intermediate RDM process. The classification scheme is only provided for intertidal benthic chl a, the main focus of this study. In addition, the organic content of the sediment, measured as ash-free dry weight (AFDW), should be measured together with benthic chl a; an AFDW in excess of 3% should be regarded as high, likely to support a higher microalgal biomass and typical of more eutrophic systems.

Chl a data reported in the scheme are likely to be regarded as low, which is attributed to the median of at least five sites from the intertidal zone along the estuary length being used; biomass is usually low in the sandy sediments near to the mouth and head of estuaries. In addition, there were no available data from eutrophic or hyper-eutrophic permanently open estuaries along the South African coast.

Water within the Sout and Keurbooms estuaries is typically clear and low in nutrients and hence can be regarded as oligotrophic. In contrast, DIN in the Sundays and Gamtoos estuaries (up to 70 µM) and DIP in the Swartkops (up to 30 µM) were much higher. However, phytoplankton blooms and associated anoxia events are uncommon and it is likely that these estuaries are mesotrophic. This is particularly evident when compared to known eutrophic sites such as the temporarily open-closed Mhlanga and Mdloti estuaries on the KwaZulu-Natal coast (Perissinotto *et al*. 2004) and the Colne Estuary (Snow, unpublished data) on the east coast of England (Table 8.5). Wastewater treatment works empty high nutrient and organic loads into these estuaries supporting much higher microalgal biomass. Median benthic microalgal content in the Colne Estuary was 156 µg g^{-1}, a magnitude higher than the 'very high' class in Table 8.4.

Table 8.5.

Intertidal benthic chl a, phytoplankton chl a and water-column nutrient ranges in the Mhlanga and Mdloti estuaries (Perissinotto *et al*. 2004) and averages (± SD) in the Colne Estuary (Snow, unpublished data).

Estuary	DIN (µM)	DIP (µM)	Phytoplankton chl a (µg l^{-1})	Benthic chl a
Mhlanga	17.1 – 418	7.8 – 81.7	0.7 – 303	1.7 – 313 mg m^{-2}
Mdloti	29.6 – 1236	0.1 – 13.5	0.9 – 111	1.3 – 391 mg m^{-2}
Colne	220 ± 196 SD	137 ± 167 SD	No data	111 ± 72.6 SD µg g^{-1}

5) Epilithic diatom indices have been developed and are successfully implemented for South African rivers (Harding *et al*. 2005; Taylor *et al*. 2005). Complicating factors in estuaries such as muddy substrates and tidal exchange introduce a broad suite of environmental variables not found in rivers, preventing the development of similar indices in estuaries. The RDM method requires that epipelic diatoms be collected and the dominant species identified. Diatoms are generally regarded as the most dominant group of microalgae in estuarine sediment. Based on results from six permanently open estuaries along the south-eastern coast of South Africa (Chapter 2), benthic microalgal biomass was closely associated with fine sediment (< 125 µm), organic matter and water content of the

intertidal sediment. The relationship between benthic microalgae and water-column chemistry was much weaker. It is unlikely that lists of dominant benthic diatoms living on soft sediments, generally based on two sampling sessions, will provide sufficient information about the quality and quantity of estuarine water to identify broad trends. It is hoped that the growing database of diatom relative abundance data and environmental variables will contribute to the development of an index, similar to that used in rivers.

6) Simultaneous measurements of abiotic variables need to be taken when sampling for phytoplankton and benthic microalgae. This is essential to identify close associations between the abiotic and biotic variables. The conceptual models presented in Chapter 6 describe the typical dynamics of abiotic variables in TOCE's, which could help microalgal specialists identify microalgal response. We also need to know about nutrient pulses in inflowing water and whether phytoplankton and the MPB are capable of luxury consumption, i.e. retain nutrients within the microalgal biomass.

   The measurement of abiotic variables includes the measurement of sediment type. This is usually achieved using the time-consuming hydrometer method or a series of sieves. The type of sediment that supports the highest MPB biomass, which is usually closely associated to eutrophication, contains high organic and silt contents. The silt content can be easily measured by wet-sieving a known dry mass of sediment through a 63 µm sieve and measuring the dry mass of sediment retained by the sieve. In addition, the organic content using the ash-free dry weight method (550 ºC combustion) can be measured to provide information about the level of eutrophication. High organic content in sediment is likely to support a high biomass of bacteria, utilising available oxygen and remineralising nutrients. This typically occurs in areas of deposition and decay below phytoplankton blooms or thick macrophytes beds. In future RDM studies it is recommended that silt and organic content only be measured instead of the full particle size range. Microalgal biomass and community composition can then be compared between sites and estuaries relative to mud and organic contents.

Synthesis of results and suggestions for future research

Phytoplankton biomass has generally been highest in estuaries with adequate freshwater to provide a continuous base-flow. The river input that is most likely to support the highest phytoplankton biomass, at the river-estuary interface, should replace water in the estuary every three tidal cycles or approximately 42 days. In cases when river input was higher than this, such as in the Berg Estuary during periods of high rainfall, or lower, such as in the freshwater starved Kromme Estuary, then phytoplankton biomass is likely to be low. This is likely to be exacerbated by strong tidal exchange through broad and deep estuary mouths (e.g. Knysna and Berg estuaries).

Results suggest that riverwater from pristine catchments is likely to be dominated by freshwater diatom taxa but reduced river flow, particularly through the construction of impoundments, is likely to increase the ratio of flagellates to diatoms. In addition, the uptake of dissolved silicate by diatoms in impoundments is likely to deplete silicate in riverwater, limiting diatom growth in estuaries. Further research is needed to investigate the flagellate: diatom ratio and to investigate changes in phytoplankton community composition in relation to changing stoichiometric N:Si and P:Si ratios.

Large floods introduce high loads of suspended particulate matter into estuaries, increasing turbidity and generally resulting in high biological and chemical oxygen demands once strong salinity stratification develops, particularly in deep systems (e.g. Swartvlei Lake). The remineralisation of organic matter in these deposits can be an important source of nutrients in nutrient-poor estuaries (e.g. Mngazi and Mngazana estuaries). In addition, nutrients entering these estuaries or those that are transformed within the estuary may be taken up very quickly, masking the close association between water-column nutrients and phytoplankton biomass.

The MPB and associated colloidal carbohydrates were closely associated with the mud, organic and moisture contents of the sediment they inhabit and are likely to be closely associated with sediment stability as a result of the binding properties of the exuded exopolymers. All of these variables were generally highest in intertidal sediment bordering the deeper middle reaches of the permanently open estuaries in this study. Factors that influence the loads and the sedimentation of fine particulate matter in estuaries (e.g. the construction of large impoundments, water abstraction and poor land-use practices) are likely to affect microalgal biomass and influence

sediment stability. As an example, the Churchill and Mpofu dams in the catchment of the Kromme River effectively trap fine particulate matter and reduce the frequency and intensity of floods. This has resulted in the coarse sediment of marine origin penetrating far upstream into the Kromme Estuary. The effects of the reduced sedimentation of organic matter (of allochthonous and autochthonous supply) and the remineralisation of nutrients that are available to microalgae at the sediment-water interface remain largely unknown in South African estuaries.

Future research should aim to link river and estuarine water quality processes. To achieve this, loads of N and P entering estuaries as well as the flux of these inorganic nutrients across the sediment-water interface should be determined, then related to microalgal biomass and community composition. These results could assist managers determine the freshwater requirement to maintain an estuary's microalgal status. In addition, further research is needed to determine the effects of point-source discharges, particularly from wastewater treatment works and septic tanks, on microalgal biomass and community composition. This is necessary in small TOCE's where pulses of discharges from these point sources are likely to occur over busy holiday periods and have a significant affect on the ecology.

References

Adams, J. B., Bate, G. C. and O'Callaghan, M. 1999. Primary producers. In: Allanson, B.R., Baird, D. (Eds.), *Estuaries of South Africa*. Cambridge University Press, Cambridge, pp. 91–117.

Adams, J.B., Bate, G.C., Harrison, T.D., Huizinga, P., Taljaard, S., Van Niekerk, L., Plumstead, E.E., Whitfield, A.K. and Wooldridge, T.H. 2002. A method to assess the freshwater inflow requirements of estuaries and application to the Mtata estuary, South Africa. *Estuaries* **6B**, 1382-1393.

Allanson, B. R. 2000. Sensitivity of estuaries. *African Journal of Aquatic Science* **25**: 9-12.

Allanson, B. R. 2001. Some factors governing the water quality of microtidal estuaries in South Africa. *Water SA* **27**(3): 373-386.

Allanson, B. R. and Howard-Williams, C. 1984. A contribution to the physico-chemical limnology of Swartvlei. *Archiv fur Hidrobiologie* **99**: 133-159.

Allanson, B. R. and Read, G. H. L. 1995. Further comment on the response of south east coast estuaries to variable freshwater flows. *Southern African Journal of Aquatic Sciences* **21**: 56-71.

Allanson, B.R. and Baird, D. (eds.). 1999. *Estuaries of South Africa*. Cambridge University Press, 340 pp.

Allanson, B.R., Winter, D., 1999. Chemistry. In: Allanson, B.R., Baird, B. (eds.), *Estuaries of South Africa*, Cambridge University Press, pp. 53-90.

Allanson, B. R., Maree, B. and Grange, N. 2000. An introduction to the chemistry of the water-column of the Knysna Estuary with particular reference to nutrients and suspended solids. *Transactions of the Royal Society of South Africa* **55**:141-162.

Archibald, R. E. M. 1983. The diatoms of the Sundays and Great Fish Rivers in the Eastern Cape Province of South Africa. *Bibliotheca Diatomologica*. J Cramer. 431 pp.

Austen, I., Andersen, T. J. and Edelvang, K. 1999. The influence of benthic diatoms and invertebrates on the erodibility of an inter-tidal mudflat, the Danish Wadden Sea. *Estuarine, Coastal and Shelf Science* **49**(1): 99-111.

Australia and New Zealand Environment and Conservation Council (ANZECC). 2000. *Australian and New Zealand guidelines for fresh and marine water quality*. National Water Quality Management Strategy No 4. Canberra, Australia.

Baird, D. and Heymans, J. J. 1996. Assessment of the ecosystem changes in response to freshwater inflow of the Kromme River Estuary, St. Francis Bay, South Africa: a network analysis approach. *Water SA* **22**: 307-318.

Balls, P. W. 1994. Nutrient inputs to estuaries from nine Scottish east coast rivers; influence of estuarine processes on inputs to the north sea. *Estuarine, Coastal and Shelf Science* **39**: 329-352.

Bally, R., McQuaid, C. M. and Pierce, S. M. 1985. Primary productivity of the Bot River estuary, South Africa. *Transactions of the Royal Society of South Africa* **45**: 333-345.

Bate, G. C. 2006. *Olifants/Doring catchment ecological water requirements study: microalgal specialist report*. DWAF RDM report on estuarine component.

Bate, G. C. and Adams, J. B. 2000. The effects of a single freshwater release into the Kromme estuary: Overview and interpretation for the future. *Water SA* **26**: 329-332.

Bate, G. C. and Heelas, B. V. 1975. Studies on the nitrate nutrition of two indigenous Rhodesian grasses. *Journal of Applied Ecology* **12**: 941-952.

Bate, G. C., Adams, J. B. and Wooldridge, T. H. 1994. *Tsitsikamma River: a biological survey of the Tsitsikamma River Estuary*. Report on the Algoa Water Resources System Analysis. Report B 33/1/1200, Department of Water Affairs and Forestry, Pretoria. 56pp.

Bate, G. C., Whitfield, A. K., Adams, J. B., Huizinga, P. and Wooldridge, T. H. 2002a. The importance of the river-estuary interface (REI) zone in estuaries. *Water SA* **28**, 271-279.

Bate, G. C., Adams, J. B. and van der Molen, J. S. 2002b. *Diatoms as indicators of water quality in South African river systems*. WRC Report 814/1/02.

Bate, G. C., Smailes, P. A. and Adams, J. B. 2004. *Benthic diatoms in the rivers and estuaries of South Africa*. WRC Project No. K5/ 1107.

Bellinger, B. J., Abdullahi, A. S., Gretz, M. R. and Underwood, G. J. C. 2005. Biofilm polymers: relationship between carbohydrate biopolymers from estuarine mudflats and unialgal cultures of benthic diatoms. *Aquatic Microbial Ecology* **38**: 169-180.

Bickerton, I. B. and Pierce, S. M. 1988. *Estuaries of the Cape part II: Synopses of Available Information on Individual Systems*, Report No. 33 Krom (CMS 46) and Kabeljous (CMC 47), CSIR Research Report 432.

Blanchard, G. F., Paterson, D. M., Stal, L. J., Richard, P., Galois, R., Huet, V., Kelly, J., Honeywill, C., de Brouwer, J. F. C., Dyer, K. R., Christie, M. and Seguignes, M. 2000. The effect of geomorphological structures on potential biostabilization by microphytobenthos on intertidal mudflats. *Continental Shelf Research* **20**: 1243–1256.

Blyth, F. G. H. and de Freitas, M. H. 1984. *A Geology for Engineers*. 7th edition. The Bath Press, Bath, 325 pp.

Boesch, D. F., Brinsfield, R. B. and Magnien, R. E. 2001. Chesapeake Bay eutrophication: Scientific understanding, ecosystem restoration, and challenges for agriculture. *Journal of Environmental Quality* **30**: 303-320.

Boney, A. D. 1989. *Phytoplankton*. London, Edward Arnold.

Bornman, T. G. and Adams, J. B. 2005. *Present state of the Noetzie Estuary*. Institute for Environmental and Coastal Management, Nelson Mandela Metropolitan University. IECM Research Report No. 45. 43pp.

Branch, G. M. and Day, J. A. 1984. The ecology of South African estuaries. Part XIII. The Palmiet River estuary in the south-western cape. *South African Journal of Zoology* **19**: 63-77.

Breen, C. M. and McKenzie, M. (eds.). 2001. *Managing Estuaries in South Africa: An Introduction*. Institute of Natural Resources, Pietermaritzburg.

Brockway, R., Bowers, D., Hoguane, A., Dove, V., Vassele, V., 2006. A note on salt intrusion in funnel-shaped estuaries: Application to the Incomati estuary, Mozambique. *Estuarine, Coastal and Shelf Science* **66**: 1-5.

Cahoon, L. B., Nearhoof, J. E., Tilton, C. L. 1999. Sediment grain size effect on benthic microalgal biomass in shallow aquatic ecosystems. *Estuaries* **22**: 735-741.

Campbell, E. E., Knoop, W. T. and Bate, G. C. 1991. A comparison of phytoplankton biomass and primary production in three east Cape estuaries, South Africa. *South African Journal of Science* **87**: 259-264.

Cartaxana, P., Mendes, C. R., van Leeuwe, M. A. and Brotas, V. 2006. Comparative study on microphytobenthic pigments of muddy and sandy intertidal sediments of the Tagus estuary. *Estuarine, Coastal and Shelf Science* **66**: 225-230.

Chan, T. U., Hamilton, D. P., Robson, B. J., Hodges, B. R. and Dallimore, C. 2002. Impacts of hydrological changes on phytoplankton succession in the Swan River, Western Australia. *Estuaries* **25**(6B): 1406-1415.

Chícharo, L., Chícharo, M.A. and Ben-Hamadou, R. 2006. Use of a hydrotechnical infrastructure (Alqueva Dam) to regulate planktonic assemblages in the Guadiana estuary: Basis for sustainable water and ecosystem services management. *Estuarine, Coastal and Shelf Science* **70**(1-2): 3-18.

Cloern, J. E. 2001. Our evolving conceptual model of the coastal eutrophication problem. *Marine Ecology Progress Series* **210**: 223-253.

Cole, B. and Cloern, J. E. 1987. An empirical-model for estimating phytoplankton productivity in estuaries. *Marine Ecology Progress Series* **36**(3): 299-305.

Collins, G. B, and Weber, C. T. 1978. Phycoperiphyton (algae) as indicators of water quality. *Transactions of the American Microscopical Society* **97**: 36-43.

Cook, P. L. M., Revill, A. T., Butler, E. C. V. and Eyre B. D. 2004. Carbon and nitrogen cycling on intertidal mudflats of a temperate Australian estuary. II. Nitrogen cycling. *Marine Ecology - Progress Series* **280**: 39–54.

Cooper, J.A.G., Wright, C.I. and Mason, T.R. 1999. Geomorphology and sedimentology. In: Allanson, B.R., Baird, D. (Eds.), *Estuaries of South Africa*. Cambridge University Press, Cambridge, pp. 5–25.

CSIR. 1990. Great Brak. *Environmental study with reference to a management plan for the Wolwedans Dam and the Great Brak River mouth*. CSIR Report EMA-C 9036. Stellenbosch.

CSIR. 1992. *Great Brak Management Programme*. Interim Report. Report on the monitoring results for the period April 1990 to March 1992. CSIR Report EMAS-C 92083. Stellenbosch.

CSIR. 1993. *Great Brak Management Programme*. Interim Report. Report on the monitoring results for the period April 1992 to March 1993. CSIR Report EMAS-C 93051. Stellenbosch.

CSIR. 1994. *Great Brak Management Programme*. Interim Report. Report on the monitoring results for the period April 1993 to March 1994. CSIR Report EMAS-C 940013. Stellenbosch.

CSIR. 1998. *Great Brak Management Programme*. Interim Report. Report on the monitoring results for the period April 1997 to March 1998. CSIR Report ENV/S-C 98122. Stellenbosch.

CSIR. 2000. *Palmiet River Water Resource Management Plan*. Summary Report: Requirements of the estuary. CSIR Report ENV-S-C 2000-48. Stellenbosch.

CSIR. 2005. *Kromme Estuary Ecological Reserve Study*. Specialist report: Hydrodynamics and Water Quality. Prepared by CSIR for CES. Report No. KSCR-IR-0001.

CSIR. 2005. *Preliminary Assessment of the Hydrodynamics of the Mngazi and Mngazana Estuaries*. CSIR Environmentek interim report, Stellenbosch, South Africa, 20 pp.

Davis, M. W. and Lee, H. II. 1983. Recolonization of sediment-associated microalgae and effects of estuarine infauna on microalgal production. *Marine Ecology Progress Series* **11**: 227-232.

Day, J. H. (ed.). 1981. *Estuarine Ecology with Particular Reference to Southern Africa*. A.A. Balkema, Cape Town. 411 pp.

de Brouwer, J. F. C., de Deckere, E. M. G. T. and Stal, L. J. 2003. Distribution of extracellular carbohydrates in three intertidal mudflats in Western Europe. *Estuarine, Coastal and Shelf Science* **56**: 313–324.

de la Rey, P. A., Taylor, J. C., Laas, A., van Rensburg, L. and Vosloo, A. 2004. Determining the possible application value of diatoms as indicators of general water quality: A comparison with SASS 5. *Water SA* 30(3): 325-332.

Department of Water Affairs and Forestry. 1995. *South African Water Quality Guidelines for Coastal Marine Waters*. Volume 1: Natural Environment. Pretoria.

Department of Water Affairs and Forestry. 1999. *Resource Directed Measures for Protection of Water Resources*. Volume 5: Estuarine Ecosystems Version 1.0, Pretoria.

Department of Water Affairs and Forestry. 2005. *Kromme/Seekoei Catchments Reserve Determination Study* – Technical Component. Kromme Estuary. Prepared by CSIR for CES. Report No. KSCR-IR-0001.

Dong, L. F., Thornton, D. C. O., Nedwell, D. B., Underwood, G. J. C. 2000. Denitrification in the sediments of the River Colne Estuary, England. *Marine Ecology Progress Series* **203**, 109–122

Droppo, I. G. 2001. Rethinking what constitutes suspended sediment. *Hydrological Processes* **15**: 1551-1564.

Dubois, M. K. A., Gilles, J. K., Hamilton, P. A., Reber, P. A. and Smith, F. 1956. Colorimetric method for determination of sugars and related substances. *Analytical Chemistry* **28**: 350-356.

Dupra, V., Smith, S.V., Marshall Crossland, J.I. and Crossland, C.J. 2001. *Estuarine systems of sub-Saharan Africa: carbon, nitrogen and phosphorus fluxes*. LOICZ Reports & Studies No. 18, LOICZ, Texel, The Netherlands. 83 pp.

Du Preez, D. R. 1996. *The Structure and Ecophysiology of the Surf Diatom, Anaulus australis Drebes et Schultz*. Unpublished Ph.D., University of Port Elizabeth (now NMMU), South Africa.

Dye, A. and Barros, F. 2005. Spatial patterns of macrofaunal assemblages in intermittently closed/open coastal lakes in New South Wales. *Estuarine, Coastal and Shelf Science* **64**, 357-371.

Dyer, K. R. 1979. *Estuarine Hydrology and Sedimentation*. Cambridge University Press. 230pp.

Egge, J. K. and Aksnes, D. L. 1992. Silicate as regulating nutrient in phytoplankton competition. *Marine Ecology Progress Series* **83**, 281-289.

Emmerson, W. D., Watling, H. R. and Watling, R. J. 1982. *Community Analyses in the Kromme and Swartkops Estuaries and in the Algoa Bay Region*. University of Port Elizabeth. Zoology Department. Report No. 16. 128 pp.

Eyre, B. 1994. Nutrient biogeochemistry in the tropical Moresby River estuary system, North Queensland, Australia. *Estuarine, Coastal and Shelf Science* **39**:15-31.

Eyre, B. 1998. Transport, retention and transformation of material in Australian estuaries. *Estuaries* **21**(4A): 540-551.

Eyre, B. D. and Balls, P.W. 1999. A comparative study of nutrient processes along the salinity gradient of tropical and temperate estuaries. *Estuaries* **22**, 313-326.

Eyre, B. and Ferguson, A. J. P. 2006. Impact of a flood event on benthic and pelagic coupling in a sub-tropical east Australian estuary (Brunswick). *Estuarine, Coastal and Shelf Science* **66**: 111-122.

Eyre, B.D. and Twigg, C. 1997. Nutrient behaviour during post-flood recovery of the Richmond River estuary Northern NSW, Australia. *Estuarine, Coastal and Shelf Science* **44**: 311-326.

Fielding, P. J., Damstra, K. St. J. and Branch, G. M. 1991. Benthic diatom biomass, production and sediment chlorophyll in Langebaan Lagoon, South Africa. *Estuarine Coastal Shelf Science* **27**: 413-426.

Figge, K., Köster, R., Thiel, H. R. and Wieland, P. 1980. Schlickuntersuchungen im Wattenmeer der deutschen Bucht (Zwischenbericht über ein Forschungsprokekt des KFKI). *Die Kuste* **35**:187-204.

Fisher, T. R., Harding, L. W., Stanley, D. W. and Ward, L. G. 1988. Phytoplankton, nutrients, and turbidity in the Chesapeake, Delaware, and Hudson estuaries. *Estuarine, Coastal and Shelf Science* **27**: 61-93.

Flemer, D.A. and Champ, M.A. 2006. What is the future fate of estuaries given nutrient over-enrichment, freshwater diversion and low flows? *Marine Pollution Bulletin* **52**: 247-258.

Friend, P. L. Ciavola, P., Cappucci, S. and Santos, S. 2003. Bio-dependent bed parameters as a proxy tool for sediment stability in mixed habitat intertidal areas. *Continental Shelf Research* **23**: 1899–1917.

Froneman, P.W. 2000. Preliminary study of the food web structure of two contrasting estuaries along the eastern Cape coast. *Southern African Journal of Aquatic Sciences* **25**: 13-22.

Froneman, P. W. 2002a. Response of plankton to three different hydrological phases of the temporarily open/closed Kasouga, South Africa. *Estuarine, Coastal and Shelf Science* **55**: 535-546.

Froneman, P. W. 2002b. Seasonal changes in selected physico-chemical and biological variables in the temporarily open/closed Kasouga, Eastern Cape, South Africa. *African Journal of Aquatic Science* **27**: 117-123.

Froneman, P.W. 2002. Response of the biology to three different hydrological phases in the temporarily open/closed Kariega estuary. *Estuarine, Coastal and Shelf Science* **55**: 535-546.

Gale, E., Pattiaratchi, C. and Ranasinghe, R. 2006. Vertical mixing in intermittently closed and open lakes and lagoons, and the dissolved oxygen response. *Estuarine, Coastal and Shelf Science* **69**: 205-216.

Gama, P. T., Adams, J. B., Schael, D. M. and Skinner, T. 2005. *Phytoplankton Chlorophyll a Concentration and Community Structure of Two Temporarily Open/Closed Estuaries*. Prepared for Water Research Commission, WRC No. 1255/1/05, Pretoria.

Garcia, I. 2005. *Removal of Natural Organic Matter by Enhanced Coagulation in Nicaragua*. Licentiate Thesis, Department of Chemical Engineering and Technology, Royal Institute of Technology, Stockholm, Sweden.

Gilpin, L. C., Davidson, K. and Roberts, E. 2004. The influence of changes in nitrogen: silicon ratios on diatom growth dynamics. *Journal of Sea Research* **51**: 21-35.

Gobler, C. J., Cullison, L. A., Koch, F., Harder, T. M. and Krause, J. W. 2005. Influence of freshwater flow, ocean exchange, and seasonal cycles on phytoplankton : nutrient dynamics in a temporarily open estuary. *Estuarine, Coastal and Shelf Science* **65**(1-2): 275-288.

Goto, N., Mitamura, O. and Terai, H. 2001. Biodegradation of photosynthetically produced extracellular organic carbon from intertidal benthic algae. *Journal of Experimental Marine Biology and Ecology* **257**: 73-86.

Grange, N. and Allanson, B. R. 1995. The influence of freshwater inflow on the nature, amount and distribution of seston in estuaries of the Eastern Cape, South Africa. *Estuarine, Coastal and Shelf Science* **40**: 402-420.

Haas, L. W. 1982. Improved epifluorescence microscopy for observing planktonic microorganisms. *Annales I'Institut Oceanographique,* Paris **58** (S): 261-266.

Haines, P.E., Tomlinson, R.B. and Thom, B.G. 2006. Morphometric assessment of intermittently open/closed coastal lagoons in New South Wales, Australia. *Estuarine , Coastal and Shelf Science* **67**: 321-332.

Hamilton, D.P., Chan, T., Robb, M.S., Pattiaratchi, C.B. and Herzfeld, M. 2001. The hydrology of the upper Swan River estuary with focus on an artificial destratification trial. *Hydrological Processes* **15**: 2465-2480.

Hanlon, A. R. M., Bellinger, B., Haynes, K., Xiao, G., Hofmann, T. A., Gretz, M. R., Ball, A. S., Osborn, A. M. and Underwood, G. J. C. 2006. Dynamics of extracellular polymeric substance (EPS) production and loss in an estuarine, diatom-dominated, microalgal biofilm over a tidal emersion – immersion period. *Limnology and Oceanography* **51**: 79-93.

Harcourt-Baldwin, J. -L. and Diedericks, G.P.J. 2006. Numerical modelling and analysis of temperature controlled density currents in Tomales Bay, California. *Estuarine, Coastal and Shelf Science* **66**: 417-428.

Hardin, G. 1968. The tragedy of the commons. *Science* **162**: 1243-1248.

Harding, W. R., Archibald, C. G. M. and Taylor, J. C. 2005. The relevance of diatoms for water quality assessment in South Africa: A position paper. *Water SA* **31**(1): 41-46.

Harrison, T. D., Cooper, J. A. G., Ramm, A. E. L. and Singh, R. A. 1995. *Health of South African Estuaries, Palmiet - Sout*. Catchment and Coastal Environmental Programme, Executive Report. Durban: CSIR.

Harrison, T. D., Cooper, J. A. G., Ramm, A. E. L. and Singh, R. A. 1996. *Health of South African Estuaries, Groot (Wes) – Great Fish*. Catchment and Coastal Environmental Programme, Executive Report. Durban: CSIR.

Haynes, K., Hofmann, T. A., Smith, C. J., Ball, A. S., Underwood, G. J. C. and Osborn, A. M. 2007. Diatom-derived carbohydrates as factors affecting bacterial community composition in estuarine sediments. *Applied and Environmental Microbiology* **73**(1): 6112–6124.

Head, P. C. (Ed) 1985. *Practical Estuarine Chemistry*. Estuarine and brackish-water sciences association handbook. Cambridge University Press.

Hessen D.O. 1999. Catchment properties and the transport of major elements to estuaries. In: Nedwell D.B. & D. Raffaelli (eds) *Advances in Ecological Research*, Volume 29. Academic Press, London, pp 1-41.

Hillebrand, H. and Kahlert, M. 2002. Effect of grazing and water-column nutrient supply on biomass and nutient content of sediment microalgae. *Aquatic Botany* **72**:143-159.

Hilmer, T. 1990. *Factors Influencing the Estimation of Primary Productivity in Small Estuaries*. Ph.D. thesis, University of Port Elizabeth, South Africa.

Hilmer, T. and Bate, G. C. 1990. Covariance analysis of chlorophyll distribution in the Sundays River Estuary, Eastern Cape. *South African Journal of Aquatic Science* 16 37-59.

Hilmer, T. and Bate, G.C. 1991. Vertical migration of a flagellate-dominated bloom in a shallow South African estuary. *Botanica Marina* **34**: 113-121.

Hirji, R., Johnson, P., Maro, P. and Chiuta, T. M. (eds.). 2002. *Defining and Mainstreaming Environmental Sustainability in Water Resources Management in Southern Africa*. SADC, IUCN, SARDC, World Bank: Maseru, Harare, Washington DC. 318 pp.

Horner-Rosser, S. M. J. and Thompson, P. A. 2001. Phytoplankton of the Swan-Canning Estuary: a comparison of nitrogen uptake by different bloom assemblages. *Hydrological Processes*. **15**: 2579-2594.

Holland, A. F., Zingmark, R. G. and Dean, J. M. 1974. Quantitative evidence concerning the stabilization of sediments by marine benthic diatoms. *Marine Biology* **27**: 191-196.

Humborg, C., Ittekkot, V., Cociasu, A. and Bodungen, B. 1997. Effect of Danube River dam on Black Sea biogeochemistry and ecosystem structure. *Nature* **386**, 385-388.

Hustedt, F. 1976. *Die Süsswasser-flora Mitteleuropas*. Otto Koeltz Science Publishers. Koenigstein, Germany. 466 pp.

Jerling, H. J. and Wooldridge, T. H. 1994. The mesozooplankton of a freshwater-starved estuary. In: Dyer, K.R.. & Orth, R.J. (eds). *Changes in fluxes in estuaries*. Olsen and Olsen Publishers, Denmark.

Jesus, B., Mendes, C. R., Brotas, V. and Paterson, D. M. 2006. Effect of sediment type on microphytobenthos vertical distribution: Modelling the productive biomass and improving ground truth measurements. *Journal of Experimental Marine Biology and Ecology* **332**: 60– 74.

Jezewski, W. A. and Roberts, C. P. R. 1986. *Estuarine and Lake Freshwater Requirements*. Technical Report No. TR 129, DWA Pretoria.

Jiang, G., Zhou, H., Ruan, W., Yao, S., Zhang, Z. and Zhao, L. 2004. Influence of water temperature on mud particle deposition - Laboratory tests. *Journal of Coastal Research* **43**: 59-66.

Kemp, W. M. 1989. Estuarine chemistry. In *Estuarine Ecology*, ed. J.H. Day, C.A.S. Hall and W.M. Kemp, pp. 79-143. John Wiley & Sons, Inc.

Köster, M., Dahlke, S. and Meyer-Reil, L. –A. 2005. Microbial colonization and nutrient dynamics of sandy and muddy sediments in hypertrophic coastal waters of the Southern Baltic Sea (Nordrügensche Bodden). *Aquatic Microbial Ecology* **39**: 69-83.

Krammer, K. 2000. The Genus *Pinnularia*. In: Lange-Bertalot (Ed), *Diatoms of Europe* Vol. 1. Koeltz Scientific Books, Königstein, 703 pp.

Krammer, K. and Lange-Bertalot, H. 1991. *Süsswasser von Mitteleuropa. Bacillariophyceae. Teil 3: Centrales Fragillariaceae and Eunotiaceae*. Gustav Fischer Verlag. Stuttgart. 230 pp.

Lange-Bertalot, H. and Krammer, K. 1989. *Achnanthes* eine monographie der Gattung. *Bibliotheca Diatomologica*. Band 18. J Cramer. Berlin. 393 pp.

Largier, J. L. 1986. Structure and mixing in the Palmiet, South Africa. *South African Journal of Marine Science* **4**: 139-152.

Largier, J. L. and Taljaard, S. 1991. The dynamics of tidal intrusion, retention and removal of seawater in a bar-built estuary. *Estuarine, Coastal and Shelf Science* **33**: 325-338.

Largier, J. L., Slinger, J. H. and Taljaard, S. 1992. The stratified hydrodynamics of the Palmiet – a prototypical bar-built estuary. In: *Dynamics and Exchanges in Estuaries and the Coastal Zone*. Coastal and Estuaries Studies 40. Prandle, D (ed.), pp 135 – 153. Springer and Verlag, New York.

Largier, J. L., Attwood, C., Harcourt-Baldwin, J. -L. 2000. The hydrographic character of the Knysna Estuary. *Transactions of the Royal Society of South Africa* **55**(2): 107-122.

Liptrot, M. R. M. 1978. *Community Metabolism and Phosphorus Dynamics in a Seasonally Closed South African Estuary*. M.Sc. thesis, Rhodes University, South Africa, 130 pp.

Lubke, R. and de Moor, I. (eds). 1998. *Field Guide to the Eastern and Southern Cape Coasts*. University of Cape Town Press, Cape Town. 559 pp.

Lucas, A. B. 1986. *The distribution of chlorophyll pigments in relation to cyclic, sporadic and episodic events in the Great Fish River estuary*. MSc thesis, Rhodes University, Grahamstown, South Africa.

Lucas, C. H., Widdows, J. and Wall, L. 2003. Relating spatial and temporal variability in sediment chlorophyll *a* and carbohydrate distribution with erodability of a tidal flat. *Estuaries* **26**(4A): 885-893.

Lund, J. W. G., Kipling, C. and Lecren, E. D. 1958. The inverted microscope method of determining algal numbers and the statistical basis of estimations by counting. *Hydrobiologia* **11**: 143-170.

MacIntyre, H. L., Geider, R. J. and Miller, D. C. 1996. Microphytobenthos: the ecological role of the “secret garden” of unvegetated, shallow-water marine habitats. I. Distribution, abundance and primary production. *Estuaries* **19**:186-201.

Manning, A. J. and Bass, S. J. 2006. Variability in cohesive sediment settling fluxes: Observations under different estuarine tidal conditions, *Marine Geology* **235** (1-4): 177-192.

Manning, A. J., Bass, S. J. and Dyer, K. R. 2006. Floc properties in the turbidity maximum of a mesotidal estuary during neap and spring tidal conditions. *Marine Geology* **235**(1-4): 193-211.

Manning, A. J., Friend, P. L., Prowsec, N. and Amos, C. L. 2007. Estuarine mud flocculation properties determined using an annular mini-flume and the LabSFLOC system. *Continental Shelf Research* **27**, 1080–1095.

Mason, C. F., Underwood, G. J. C., Baker, N. R., Davey, P. A., Davidson, I., Hanlon, A., Long, S. P., Oxborough, K., Paterson, D. M. and Watson, A. 2003. The role

of herbicides in the erosion of salt marshes in eastern England. *Environmental Pollution* **122**: 41-49.

Miller, D. C., Geider, R.J. and MacIntyre, H. L. 1996. Microphytobenthos: The ecological role of the 'Secret Garden' of unvegetated, shallow-water marine habitats. 2. Role in sediment stability and shallow-water food webs. *Estuaries* **19**: 202-212.

Monteiro, P. M. S. and Largier, J. Ll. 1999. Thermal stratification in Saldanha Bay (South Africa) and subtidal, density-driven exchange with the coastal waters of the Benguela upwelling system. *Estuarine, Coastal and Shelf Science* **49**: 877-890.

Morant, P. and Quinn, N. 1999. Influence of man and management of South African estuaries. In: Allanson BR and Baird D (eds.) *Estuaries of South Africa*. Cambridge University Press, Cambridge. 289-321.

Morlock, S., Taylor, D., Giblin, A., Hopkinson, C. and Tucker, J. 1997. Effects of salinity on the fate of inorganic nitrogen in sediments of the Parker River estuary, Massachusetts. *Biological Bulletin* **193**: 290-292.

Mukheibir, P. and Sparks, D. 2006. *Climate Variability, Climate Change and Water Resource Strategies for Small Municipalities*. Prepared for Water Research Commission, WRC Report No. 1500/1/06, Pretoria.

Mundree, S., Perissinotto, R. and Nozais, C. 2003. Seasonal variations in the vertical distribution of benthic microalgae in the upper sediment of the Mdloti estuary, South Africa. *Botanica Marina* **46**: 323-331.

Nedwell, D. B., Jickells, T. D., Trimmer, M., Sanders, R., 1999. Nutrients in estuaries. *Advances in Ecological Research – Estuaries.* Academic Press, San Diego. **29**: 43-92.

Nedwell, D. B., Raffaelli, D. G. and Fitter, A. H. (eds.). 1999. *Advances in Ecological Research - Estuaries* **29**. Academic Press, San Diego.

Nikulina, V. N. 2003. Seasonal dynamics of phytoplankton in the inner Neva Estuary in the 1980s and 1990s. *Oceanologia* **45**(1): 25-39.

Nixon, S. W. 1995. Coastal marine eutrophication: a definition, social causes, and future concerns. *Ophelia* **41**: 199-219.

Nusch, E. A. 1980. Comparison of different methods for chlorophyll and phaeopigment determination. *Archiv für Hydrobiologie, Beiheft Ergebnise der Limnologie* **14**: 14-36.

Otieno, F. A. O. and Ochieng, G. M. M. 2004. Water management tools as a means of averting a possible water scarcity in South Africa by the year 2025. *Water SA* **30**(5): 120-124.

Painting, S. J., Devlin, M. J., Malcolm, S. J., Parker, E. R. Mills, D. K., Mills, C., Tett, P., Wither, A., Burt, J., Jones, R. and Winpenny, K. 2007. Assessing the impact of nutrient enrichment in estuaries: Susceptibility to eutrophication. *Marine Pollution Bulletin* **55**: 74–90.

Parsons, T. R. and Takahashi, M. 1973. Environmental control of phytoplankton cell size. *Limnology and Oceanography* **18**: 511-515.

Parsons, T.R., Maita, Y., Lalli, C.M., 1984. *A Manual of Chemical and Biological Methods for Seawater Analysis*, Pergamon Press, N.Y. 173 pp.

Passow, U. 2002. Production of transparent exopolymer particles (TEP) by phyto- and bacterioplankton. *Marine Ecology Progress Series* **236**: 1-12.

Paterson, D. M. and Black, K. S. 1999. Water flow, sediment dynamics and benthic biology. *Advances in Ecological Research* **29**, 155-193.

Perissinotto, R., Nozais, C. and Kibirige, I. 2002. Spatio-temporal dynamics of phytoplankton and microphytobenthos in a South African temporarily-open estuary. *Estuarine, Coastal and Shelf Science* **55**: 47-58.

Perissinotto, R., Nozais, C., Kibirige, I. and A. Anandraj. 2003. Planktonic food webs and benthic pelagic coupling in three South African temporarily open estuaries. *Acta Ecologica* **24**: S307–S316.

Perissinotto, R., Blair, A., Connell, A., Demetriades, N. T., Forbes, A. T., Harrison, T. D., Iyer, K., Joubert, M., Kibirige, I., Mundree, S., Simpson, H., Stretch, D., Thomas, C., Thwala, X. and Zietsman, I. 2004. *Contributions to Information Requirements for the Implementation of Resource Directed Measures for Estuaries. Volume 2: Responses of the Biological Communities to Flow Variation and Mouth State in Two Kwazulu-Natal Temporarily Open / Closed Estuaries*. Report prepared for Water Research Commission by the Consortium for Estuarine Research and Management, WRC Report No. 1247/2/04.

Perkins, R. G. Honeywill, C. Consalvey, M., Austin, H. A., Tolhurst, T. J. and Paterson, D. M. 2003. Changes in microphytobenthic chlorophyll a and EPS resulting from sediment compaction due to dewatering: opposing patterns in concentration and content. *Continental Shelf Research* **23**:575-586.

Pierson, W. L., Bishop, K., Van Senden, D., Horton, P. R. and Adamantidis, C. A. 2002. *Environmental Water Requirements to Maintain Estuarine Processes*. Environmental Flows Initiative Technical Report Number 3, Commonwealth of Australia, Canberra.

Pinckney, J. and Zingmark, R. G. 1993. Biomass and production of benthic microalgal communities in estuarine habitats. *Estuaries* **16**: 887-897.

Pinckney, J. L., Paerl, H. W., Tester, P. and Richardson, T. L. 2001. The role of nutrient loading and eutrophication in estuarine ecology. *Environmental Health Perspectives* **109**: 699-706.

Radke, L. C., Prosser, I. P., Robb, M., Brooke, B., Fredericks, D., Douglas, G. B. and Skemstad, J. 2004. The relationship between sediment and water quality, and riverine sediment loads in the wave-dominated estuaries of south-west Western Australia. *Marine and Freshwater Research* **55**: 581-596.

Reddering, J. S. V. 1988. Prediction of the effects of reduced river discharge on the estuaries of the south-eastern Cape Province, South Africa. *South African Journal of Science* **84**: 726-730.

Reddering, J. S. V. and Esterhuysen, K. 1983. *Sedimentation in the Kromme Estuary*. ROSIE Report No.6, University of Port Elizabeth, South Africa.

Riethmüller, R. Heineke, M. Kühl, H. and Keuker-Rüdiger, R. 2000. Chlorophyll a concentration as an index of sediment surface stabilisation by microphytobenthos? *Continental Shelf Research* **20**:1352-1372.

Rodriguez, F. D. G. 1993. *The Determination and Distribution of Microbenthic Chlorophyll a in Selected South Cape estuaries*. MSc thesis, University of Port Elizabeth, South Africa.

Round, F. E. 1981. *The Ecology of Algae*. Cambridge University Press, London.

Round, F. E., Crawford, R. M. and Mann, D. G. 1990. *The Diatoms*. Biology and Morphology of the Genera. Cambridge University Press, London. 747 pp.

Roy, P. S., Williams, R. J., Jones, A. R., Yassini, I., Gibbs, P. J., Coates, B., West, R. J., Scanes, P. R., Hudson, J. P. and Nichol, S. 2001. Structure and function of south-east Australian estuaries. *Estuarine, Coastal and Shelf Science* **53**: 351-384.

Ruan, W. J. 1991. The mechanism analysis of fine sediment in the flowing water. *Marine Science* **5**: 46-49.

Russell, M. J., Montagna, P. A. and Kalke, R. D. 2006. The effect of freshwater inflow on net ecosystem metabolism in Lavaca Bay, Texas. *Estuarine, Coastal and Shelf Science* **68**: 231-244.

Rysgaard, S., Christensen, P. B. and Nielsen, L. P. 1995. Seasonal variation in nitrification and denitrification in estuarine sediment colonized by benthic microalgae and bioturbating infauna. *Marine Ecology Progress Series* **126**:111-121.

Scharler, U. M., Baird, D. and Winter, P. E. D. 1997. *Diversity and Productivity of Biotic Communities in Relation to Freshwater Inputs in Three Eastern Cape Estuaries*. Report to the Water Research Commission, WRC Report No. 463/1/98.

Scheren, P. A. G. M., Kroeze, C., Janssen, F. J. J. G., Hordijk, L. and Ptasinski, K. J. 2004. Integrated water pollution assessment of the Ebrie Lagoon, Ivory Coast, West Africa. *Journal of Marine Systems* **44**: 1-17.

Schoeman, F. R. and Archibald, R. E. M. 1976. The Diatom Flora of Southern Africa. *Special Report WAT 50.* CSIR. South Africa.

Schumann, E. 2004. Estuarine hydrodynamics (temperature and salinity). In *Berg River Baseline Monitoring Programme, first annual report*. Anchor Environmental Consultants, University of Cape Town.

Schumann, E. H., Largier, J. L. and Slinger, J. H. 1999. Estuarine hydrodynamics. In: *Estuaries of South Africa*, ed. B.R. Allanson and D. Baird, pp. 27-52. Cambridge University Press.

Shipunov, A. B. 2007. *Systema Naturae*. The classification of living organisms (Version 5.6). Nature Precedings. (http://precedings.nature.com/documents/241/version/1).

Silberbauer, M., 1982. Phosphorus dynamics in the monimolimnion of Swartvlei. *Journal of the Limnological Society of southern Africa* **8**(2): 54-60.

Simonsen, R. 1987. *Atlas and Catalogue of the Diatom Types of Friedrich Hustedt*. Volumes 1 to 3. J. Cramer, Stuttgart.

Skinner, T., Adams, J. B. and Gama, P. T. 2006. The effect of mouth opening on the biomass and community structure of microphytobenthos in a small oligotrophic estuary. *Estuarine, Coastal and Shelf Science* **70**: 161-168.

Slinger, J. H. and Largier, J. L. 1990. The evolution of thermohaline structure in a closed estuary. *South Africa Journal of Aquatic Science* **16**(1/2): 60-77.

Slinger, J. H. and Taljaard, S. 1994. Preliminary investigation of seasonality in the Great Berg estuary. *Water SA* **20**: 279-288.

Smith, D. J. and Underwood, G. J. C. 2000. The production of extracellular carbohydrates by estuarine benthic diatoms: The effects of growth phase and light and dark treatment. *Journal of Phycology* **36**: 321-333

Snow, G. C. 2000. *Structure and Dynamics of Microalgae in the Gamtoos Estuary*. MSc Thesis. Botany Department, University of Port Elizabeth, South Africa. 104 pp.

Snow, G. C. and Adams, J. B. 2006. Response of micro-algae in the Kromme Estuary to managed freshwater inputs. *Water SA* **32**: 71-80.

Snow, G. C. and Adams, J. B. 2007. Relating microalgal spatial patterns to flow, mouth and nutrient status in the temporarily open/closed Mngazi estuary, South Africa. *Marine and Freshwater Research*, In press.

Snow, G. C., Adams, J. B. and Bate, G. C. 2000a. The effects of a single freshwater release into the Kromme Estuary: Microalgal response. *Water SA* **26**: 301-310.

Snow, G. C., Adams, J. B. and Bate, G. C. 2000b. Effect of river flow on estuarine microalgal biomass and distribution. *Estuarine, Coastal and Shelf Science* **51**: 255-266.

Snow, G. C. and Taljaard, S. 2007. Water quality in South African temporarily open/closed estuaries: A conceptual model. *African Journal of Aquatic Sciences*, 32:99-111.

Stal, L. J. 2003, Microphytobenthos, their extracellular polymeric substances, and the morphogenesis of intertidal sediments. *Geomicrobiology Journal* **20**: 463-478.

Stoermer, E. F. and Smol, J. P. (eds.) 1999. *The Diatoms: Applications for the Environmental and Earth Sciences*. Cambridge University Press, United Kingdom.

Sullivan, M. J. 1999. Applied diatom studies in estuarine and shallow coastal environments. In: *The Diatoms: Applications for the Environmental and Earth Sciences* (Ed. By E.F. Stoermer and J.P. Smol), pp. 334-351. Cambridge University Press, Cambridge.

Sündback, K., Carlson, L., Nillson, C., Jönsson, B., Wulff, A. and Odmark, S. 1996. Response of benthic microbial mats to drifting green algal mats. *Aquatic Microbial Ecology* **10**: 195-208.

Suzuki, M. S., Ovalle, A. R. C. and Pereira, E. A. 1998. Effects of sand bar openings on some limnological variables in a hypertrophic tropical coastal lagoon of Brazil. *Hydrobiologia* **368**: 111-122.

Switzer, T. B. 2004. *The Role of Water-column and Benthic Communities in the Spatial and Temporal Production and Uptake of Nutrients in Controlling the Trophic Status of the Knysna River Estuary, South Africa*. Ph.D. thesis, University of Cape Town, Cape Town, South Africa.

Taljaard, S. 1987. *Nutrient circulation in the Palmiet River Estuary: A summer study*. CSIR Research Report 633. Stellenbosch.

Taljaard, S. 2004. *Berg River Baseline Monitoring – Initialisation Report: Estuarine water quality part II – nutrients*. Anchor Environmental Consultants, University of Cape Town, Cape Town.

Taljaard, S. and Largier, J. L. 1989. *Water circulation and nutrient distribution in the Palmiet River Estuary: A winter study*. CSIR Research Report 680. Stellenbosch.

Taljaard, S. and Slinger, J. H. 1993. *Investigation into the flushing efficiency of 1. A freshwater release and 2. Seawater overwash in the Great Brak Estuary*. CSIR Research Report 713. Stellenbosch.

Taljaard, S., De Villiers, S., Fricke, A. H. and Kloppers, W. S. 1992. *Water quality status of the Rietvlei/Milnerton Lagoon system (Diep River Estuary) – 1988/89*. CSIR Data Report EMAS-D 92007. Stellenbosch.

Taljaard, S., Eagle, G. A. and Hennig, H. F. –K. O. 1986. The Palmiet Estuary: A model for water circulation using salinity and temperature measurements over a tidal cycle. *Water SA* 12(3): 119-126.

Taljaard, S., Adams, J. B., Huizinga, P., van Niekerk, L., Whitfield, A. K. and Wooldridge, T. H. 2002. *Determination of the preliminary Ecological Reserve on a rapid level for the Tsitsikamma Estuary, including a desktop assessment on other estuaries in the K80 tertiary catchment.* Department of Water Affairs and Forestry, Pretoria, South Africa. 52pp.

Taylor, D. I. 1992. The influence of upwelling and the short-term changes in concentrations of nutrients in the water-column on fluxes across the surface of a salt marsh. *Estuaries* 15 (1): 68-74.

Taylor, J. C., Harding, W. R., Archibald, C. G. M. and van Rensburg, L. 2005. Diatoms as indicators of water quality in the Jukskei-Crocodile river system in 1956 and 1957, a re-analysis of diatom count data generated by BJ Cholnoky. *Water SA* **31**, 237-246.

Thornton, D. C. O., Dong, L. F., Underwood, G. J. C. and Nedwell, D. B. 2002. Factors affecting microphytobenthic biomass, species composition and production in the Colne estuary. *Aquatic Microbial Ecology* 27: 285-300.

Thornton, D. C. O., Dong, L. F., Underwood, G. J. C. and Nedwell, D. B. 2007. Sediment–water inorganic nutrient exchange and nitrogen budgets in the Colne Estuary, UK. *Marine Ecology Progress Series* **337**, 63-77.

Tolhurst, T. J., Underwood, T. J., Perkins, R. G. and Chapman, M. G. 2005. Content versus concentration: Effects of units on measuring the biogeochemical properties of soft sediments. *Estuarine, Coastal and Shelf Science* 63: 665-673.

Tolhurst, T.J., Jesus, B., Brotas, V. and Paterson, D.M., 2003. Diatom migration and sediment armouring—an example from the Tagus estuary, Portugal. *Hydrobiologia* 503: 183–193.

Turpie, J. K. and Clark, B. M. 2005. The influence of freshwater flow on the Berg River Estuary: A conceptual model. Anchor Environmental Consultants – specialist report. 34 pp.

Twomey, L. and Thompson, P. 2001. Nutrient limitation of phytoplankton in a seasonally open Bar-Built Estuary: Wilson Inlet, Western Australia. *Journal of Phycology* **37**: 16-29.

Uncles, R. J., Stephens, J. A. and Law, D. J. 2006. Turbidity maximum in the macrotidal, highly turbid Humber Estuary, UK: Flocs, fluid mud, stationary suspensions and tidal bores. *Estuarine, Coastal and Shelf Science* 67: 30-52.

Underwood, G. J. C. and Kromkamp, J. 1999. Primary production by phytoplankton and microphytobenthos in estuaries. *Advances in Ecological Research* **29**: 93-153.

Underwood, G. J. C. 2000. Changes in microalgal species composition, biostabilisation potential and succession during saltmarsh restoration. In: *British Saltmarshes* (Ed. By B.R. Sherwood, B.G. Gardiner and T. Harris). Linnean Society of London, Forrest Text, Cardigan Press, 418 pp.

Underwood, G. J. C. 2002. Adaptations of tropical marine microphytobenthic assemblages along a gradient of light and nutrient availability in Suva Lagoon, Fiji. *European Journal of Phycology* **37**: 449-462.

Underwood, G. J. C. and Kromkamp, J. 1999. Primary production by phytoplankton and microphytobenthos in estuaries. *Advances in Ecological Research* **29**: 93-153.

Underwood, G. J. C. and Paterson, D. M. 2003. The importance of extracellular carbohydrate production by marine epipelic diatoms. *Advances in Botanical Research* **40**: 183-240.

Underwood, G. J. C. and Smith, D. J. 1998b. Predicting epipelic diatom exopolymer concentrations in intertidal sediments from chl. a. *Microbial Ecology* **35**: 116-125.

Underwood, G. J. C., Paterson, D. M. and Parkes, R. J. 1995. The measurement of microbial carbohydrate exopolymers from intertidal sediments. *Limnology and Oceanography* **40**: 1243-1253.

Underwood, G. J. C., Philips, J. and Saunders, K. 1998. Distribution of estuarine benthic diatom species along salinity and nutrient gradients. *European Journal of Phycology* **33**: 173-183.

van Beusekom, J. E. E. and de Jonge, V. N. 1998. Retention of phosphorus and nitrogen in the Ems Estuary. *Estuaries* **21**(4A), 527-539.

van der Lee, W. T. B., 2000. Temporal variation of floc size and settling velocity in the Dollard estuary. *Continental Shelf Research* **20**: 1495-1511.

van Duyl, F. C., de Winder, B., Kop, A. J. and Wollenzien, U. 1999. Tidal coupling between carbohydrate contents and bacterial activities in diatom-inhabited intertidal mudflats. *Marine Ecology Progress Series* **191**: 19-32.

Webster, I. T., Ford, P. W. and Hodgson, B. 2002. Microphytobenthos contribution to nutrient-phytoplankton dynamics in a shallow coastal lagoon. *Estuaries* **25**(4a), 540-551.

Welker, C. Sdrigotti, E., Covelli, S. and Faganeli, J. 2002. Microphytobenthos in the Gulf of Trieste (Northern Adriatic Sea): Relationship with Labile Sedimentary Organic Matter and Nutrients. *Estuarine, Coastal and Shelf Science* **55**: 259–273.

Whitfield, A.K. 1988. The role of tides in redistributing macrodetrital aggregates within the Swartvlei estuary. *Estuaries* **11**(3): 152-159.

Whitfield, A. K. 1992. A characterisation of southern African estuarine systems. *South African Journal of Aquatic Science* **18**: 89-103.

Whitfield, A. K. 1999. Ichthyoplankton diversity, recruitment and dynamics. In Allanson, BR & Baird D (Eds). *Estuaries of South Africa*. Cambridge University Press, pp 209-218.

Winterwerp, J. C. 2002. On the flocculation and settling velocity of estuarine mud. *Continental Shelf Research* **22**: 1339–1360.

Woodruff, S. L., House, W. A., Callow, M. E. and Leadbetter, B. S. C. 1999. The effects of biofilms on chemical processes in surficial sediments. *Freshwater Biology* **41**: 73-89.

Yallop, M. L., Paterson, D. M. and Wellsbury, P. 2000. Interrelationships between rates of microbial production, exopolymer production, microbial biomass, and sediment stability in biofilms of intertidal sediments. *Microbial Ecology* **39**: 116-127.

Yap, H. T., von Oertzen and J.-A., Schiewer, U. 1987. Effects of turbidity caused by nutrient loading on benthic dynamics. *Continental Shelf Research* **7**(11-12): 1439-1444.

Appendix

Figure A.1. The Mpofu Dam, approximately 45% full, located approximately 4 km from the tidal head of the Kromme Estuary, Eastern Cape Province.

Figure A.2. The mouth region (open) of the temporarily open/closed Mngazi Estuary, Eastern Cape Province. Mngazi River Bungalows Resort is slightly to the right of centre.

Figure A.3. The flood-tide delta of the permanently open Mngazana Estuary, Eastern Cape Province. The mouth and entrance to creek 1 are to the right of the image.

Figure A.4. A well developed sand berm at the mouth region of the temporarily open/closed Van Stadens Estuary, Eastern Cape Province.

Figure A.5. Tannin-stained upper reaches of the Knysna Estuary, Western Cape Province, approximately 2 km below the Chelmsford Weir (10 August 2006).

Figure A.6. Flood debris against a house located in the upper reaches of the Knysna Estuary, Western Cape Province. (10 August 2006).

Figure A.7. Knysna Embayment; picture taken from South African National Parks offices, Thesen Island (10 August 2006).

Figure A.8. Dense beds of *Potamogeton pectinatus* L., colonising the shallow littoral zone of the Swartvlei Lake, Western Cape Province (9 August 2006).

Figure A.9. Maximum water level recorded during August 2006 floods at Pine Lake Marina, western shores of Swartvlei Lake (9 August 2006).