Ecosystem Functioning of Selected Estuaries on the East Coast of South Africa

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ABSTRACT

River inflow is one of the most important factors influencing the density and biomass of estuarine biotic communities. The aim of this study was to obtain an understanding of the ecosystem functioning of selected estuaries on the east coast of South Africa and to represent their dynamics through ecosystem models. The responses of water column nutrients, plankton density and biomass to inlet phase changes for 16 temporarily open/closed estuaries (TOCEs) in South Africa were first determined. This analysis demonstrated that inlet phase and the duration of mouth closure were the most important factors determining plankton density and biomass of the analysed TOCEs. Estimates of planktonic standing stocks for four of these estuaries revealed that stocks can be from 26 to 10 000 times higher during the closed compared to the open phase. Also, slightly higher variability of planktonic density and biomass was recorded during the closed phase of TOCEs. The second major thrust of this study was to analyse the variability and temporal stability of planktonic and macrobenthic invertebrate density and biomass in two KwaZulu-Natal estuaries over a dry/wet cycle. The results suggest that “stable” variability and species synchronization could be the mechanisms whereby the estuarine biota of these systems compensate for environmental changes and attain a degree of environmental homeostasis. The third major thrust involved an assessment of the spatio-temporal variations in the elemental composition and stoichiometry of suspended and sediment detritus, zooplankton and macrobenthic taxa from two estuaries over a dry/wet cycle. Significant seasonal variations in the elemental composition of detritus, zooplankton and macrobenthic species were found, with the variations in the elemental content of sediment and suspended detritus being related to the seasonal changes in river inflow, while the among-taxa variability was mainly explained by feeding mode. Finally, static seasonal carbon and nitrogen ecosystem network models were developed for the East Kleinemonde, Mlalazi and Mpenjati estuaries to investigate their nutrient dynamics and ecosystem functioning. The results indicated that the East Kleinemonde and Mpenjati estuaries were mainly dependent on primary producers during the dry season, especially the high standing stocks of phytoplankton and microphytobenthos. Similarly, the dependency on detritus was higher during the wet season due to the high riverine imports during this season. Consequently, higher detritivory was recorded in all three study systems during the wet season. Cycling of nitrogen was higher than of carbon on a seasonal basis, with higher recycling of nitrogen during the dry season implying a lower availability of this element due to reduced freshwater inflow and nutrient input during the low rainfall period. System indices indicated that the organization of these systems was higher during the dry season, while the overheads on imports and exports peaked during the wet season. The ecosystem models analysed here provide an initial insight into the overall carbon and nitrogen dynamics of estuaries on the east coast of South Africa.

PREFACE

The work described in this PhD thesis was carried out in the School of Life Sciences, University of KwaZulu-Natal, Westville, from April 2010 to November 2012, under the supervision of Dr Ursula Scharler and Prof. Alan Whitfield.

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

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DECLARATION 1 – PLAGIARISM

I, **Kelly Ortega-Cisneros** declare that

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

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

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

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DECLARATION 2 - PUBLICATIONS

DETAILS OF CONTRIBUTION TO PUBLICATIONS that form part and/or include research presented in this thesis are as follows:

Publication 1

Ortega-Cisneros, K.; Scharler, U.M.; Whitfield, A.K. 2014. Inlet mouth phase influences density, variability and standing stocks of planktonic assemblages in temporarily open/closed estuaries. Estuarine, Coastal and Shelf Science 136: 139-148.

Publication 2

Ortega-Cisneros, K.; Scharler, U.M. In press. Variability and temporal stability of communities in estuaries (Mlalazi and Mpenjati, South Africa). Marine Ecology Progress Series.

Publication 3

Ortega-Cisneros, K.; Scharler, U.M. In prep. Temporal patterns in planktonic and macrobenthic elemental composition and stoichiometry in two sub-tropical estuaries, KwaZulu-Natal, South Africa.

Publication 4

Ortega-Cisneros, K.; Scharler, U.M.; Whitfield, A.K. In prep. Comparative analysis of carbon and nitrogen dynamics of three estuaries on the east coast of South Africa using network analysis.

Contribution from researchers other than my supervisor Dr U.M. Scharler and co-supervisor Prof. A.K. Whitfield:

This thesis aimed to build ecosystem models for three estuaries on the east coast of South Africa, and fieldwork was conducted in two out of the three systems. Data for various communities of the East Kleinemonde Estuary were obtained from:

Dr Nicola James (Southern African Institute for Aquatic Biodiversity) provided fish seine net catch and length data for the construction of the ecological models of the East Kleinemonde Estuary (Chapter 4).

Prof. William Froneman (Rhodes University) provided zooplankton biomass data for the construction of the ecological models of the East Kleinemonde Estuary (Chapter 4).

Prof. Tris Wooldridge (Nelson Mandela Metropolitan University) provided macrobenthos species composition and density for the construction of the ecological models of the East Kleinemonde Estuary (Chapter 4).

Signed: …………………………………………………………

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GENERAL INTRODUCTION

Estuaries are among the most productive aquatic ecosystems and they provide several ecosystem services to society, e.g. nutrient cycling, nursery areas for fish and invertebrates, food production, recreation and cultural services (Constanza et al., 1997). In South Africa, approximately 280 estuaries have been recorded and 182 of them have been classified as temporarily open/closed estuaries or TOCEs (Whitfield, 1992). This is the most predominant type of estuary in South Africa, accounting for more than 65% of the estuaries (Whitfield, 1992). These environments are highly dynamic and are usually closed off from the sea by a sandbar when freshwater inflow is low (Whitfield, 1992). During river flooding, these estuaries increase in volume and the water level eventually surpasses the height of the sandbar, thus opening the mouth to the sea (Whitfield, 1992). TOCEs are mainly concentrated on the east coast of South Africa, with 84 and 93 TOCEs recorded on the warm temperate and subtropical biogeographical region respectively (Perissinotto et al., 2010).

Permanently open estuaries are very scarce in South Africa, accounting for ~ 13 % of the estuaries in this country (Whitfield, 1998). These environments constitute exceptionally important nursery areas for a wide variety of fish taxa (Whitfield, 1998) and invertebrates (Emmerson, 1994; Papadopoulos et al., 2002). They are characterized by a moderate tidal prism ($1 - 10 \cdot 10^6$ m^3), mean salinities ranging from 10-40 and have both tidal and riverine mixing processes (Whitfield, 1992). These systems usually rely on their relatively large tidal prism to maintain their permanent connection to the sea; however they can close to the sea during extreme droughts (Whitfield, 1998). Only 37 estuaries were recorded as permanently open prior to 1990 (Reddering and Rust, 1990) and the number of POEs has probably decreased in the recent decades due to increasing freshwater abstraction from their catchments (Whitfield, 1998).

One of the main threats to estuaries is habitat degradation, mainly through freshwater abstraction from catchments. The consequences of freshwater starvation are primarily dependent on human population densities, estuary type and the effectiveness of management policies. For instance, freshwater abstractions in the catchment of temporarily open/closed estuaries may result in extended mouth closure and shorter open phases, thus having significant negative effects on estuarine biota (e.g. Perissinotto et al., 2010; Wooldridge, 1991). Similarly, a reduction of freshwater inflow into permanently open estuaries usually results in hypersaline conditions (Whitfield and Bruton, 1989), smaller estuary channel dimensions (Reddering, 1988), as well as changes in estuarine biota (e.g. Allanson and Read, 1995; Baird and Heymans, 1996; Grange and Allanson, 1995; Schlacher and Wooldridge, 1996). Very few studies have holistically analysed the effects of fluctuations in river inflow on the functioning of estuarine systems in South Africa (Heymans et al., 2002; Scharler, 2012; Scharler and Baird, 2005). It is thus important to explore the overall functioning of these types of estuaries in terms of biomass, productivity and connectivity with adjacent environments to understand their ecology, services and predict their responses to different environmental threats.

The overarching aim of this study was to increase our understanding of estuaries on the east coast of South Africa by exploring the functioning of permanently open and temporarily open/closed systems through time, and to represent their dynamics using ecological models. Based on the predominance of TOCEs in South Africa, it is expected that they fulfil important ecosystem services along the coastline despite their usually small size (e.g. nursery areas, export of biomass and nutrients to the sea, recreational areas). The direct input of rainfall and freshwater inflow to TOCEs determines the timing of opening events and their overall functioning, especially the marked changes between the open and closed phases. For instance, minimum phytoplankton and microphytobenthic biomass have been associated with open-mouth conditions of TOCEs in South Africa (e.g. Perissinotto et al., 2000; Skinner et al., 2006), and low zooplankton abundance and biomass have also been recorded during open-mouth conditions (e.g. Kibirige et al., 2006; Whitfield, 1980).

The existence of possible patterns associated with small scale temporal variability in TOCEs has important implications for the functioning and management of these highly vulnerable systems. However, almost no studies have attempted to determine the responses of TOCEs to inlet phase changes in a small temporal scale. Thus Chapter 1 had a primary aim of determining the responses of water column nutrients, phytoplankton biomass, zooplankton density and biomass to inlet phase changes in temporarily open/closed estuaries. In addition, the temporal variability of these nutrients and biotic components were assessed in relation to the duration of closure/opening of a system, as well as estimating the potential differences in planktonic standing stocks between open and closed phases in selected systems.

Estuarine biota are influenced by the considerable range of physico-chemical fluctuations at various spatial and temporal scales (McLusky and Elliott, 2004), especially cyclic changes such as seasonal fluctuations of temperature, salinity and river inflow. The Environmental Homeostasis Hypothesis suggests that estuarine variability may provide estuarine communities with the ability to reach stability by adapting to the changes in the environment (Elliott and Quintino, 2007). Temporal stability (S) is a measure of the stability of a community or population through time (Lehman and Tilman, 2000) and has been widely used to evaluate the stability of terrestrial and aquatic communities, where it has shown its usefulness as a stability index. The temporal stability has however not been used to investigate the stability of complex systems such as estuaries (but see Pinto et al., 2013) and overall the stability of estuarine systems is poorly understood. Chapter 2, therefore, aimed to determine the variability and temporal stability of phytoplankton biomass, zooplankton and macrobenthic density and biomass in two subtropical estuaries (one POE and one TOCE) influenced by marked seasonal fluctuations in rainfall and river inflow.

Seasonal fluctuations in rainfall and river inflow alter the nutrient input to estuaries, which can modify the community structure, primary and secondary production and food web dynamics of these systems. Ecological stoichiometry theory has been used to provide a better understanding of trophic dynamics, nutrient cycling and consumer-resource interactions by studying the balance of elements involved in ecological interactions and processes (Sterner and Elser, 2002). Ecological stoichiometry studies in estuaries have mainly documented the elemental composition and ratios of particulate organic matter in water and sediment, with almost no studies covering the nutrient content and ratios of benthic and pelagic organisms. Since the seasonal variations in rainfall and river inflow have been shown to produce changes in the density and biomass of estuarine communities in South Africa, it is expected that the nutrient content and elemental ratios of estuarine organisms will also be affected by these seasonal changes. Therefore, Chapter 3 aimed to provide an insight into the stoichiometry of sub-tropical estuaries by analysing the spatio-temporal variations in the elemental composition and ratios of suspended and sediment detritus, zooplankton and benthic macroinvertebrate species in two contrasting estuaries on the KwaZulu-Natal coast over a dry/wet cycle.

The influence of the seasonal variations in river inflow and nutrient input to estuaries has been investigated at the population and community-level (e.g. Andersen and Hessen, 1991; Mulder and Bowden, 2007; Schoo et al., 2012); however there is a conspicuous lack of studies examining the role of nutrients at the ecosystem-level in estuaries. A holistic analysis of the role of nutrients such as carbon and nitrogen in estuarine foodwebs has the potential to provide a comprehensive understanding of ecosystem functioning in estuaries. Despite its importance, almost no studies have simultaneously evaluated the dynamics of carbon, nitrogen and phosphorus at the ecosystem-level in estuaries (Baird et al., 2008; 2011). Ecological network analysis (ENA) is one of the few methodologies that provides information on whole-system functioning, and important ecosystem features such as nutrient recycling and trophic dynamics. In this sense, Chapter 4 investigated the carbon and nitrogen dynamics based on quantified flow networks in three estuaries using ecological network analysis, and examines the importance of fluctuations in riverine inflow in the ecosystem functioning of these estuaries.

Overall, this study has developed an environmental and biological baseline for two estuaries with contrasting mouth status (one permanently open and one temporarily open/closed) in KwaZulu-Natal, and using existing information from other estuaries in South Africa to provide an insight into the dynamics, variability and ecosystem functioning of estuaries in relation to freshwater inflow. Outcomes of this work therefore constitute a framework for future comparison and management of estuarine ecological functioning in South Africa.

The main objectives of this study were:

1. To characterise the responses of water column nutrients and plankton (phytoplankton biomass, zooplankton density and biomass) to inlet phase changes in selected temporarily open/closed estuaries on the east coast of South Africa;
2. To determine the variability and temporal stability of phytoplankton biomass, zooplankton and macrobenthic density and biomass in two sub-tropical estuaries over a dry/wet cycle;
3. To determine spatial and temporal variations in the elemental composition of abiotic sources, zooplankton and benthic macroinvertebrate species in two sub-tropical estuaries with contrasting mouth status in KwaZulu-Natal over a dry/wet cycle;
4. To compare the structure and functioning of three estuaries on the east coast of South Africa using ecological network analysis based on ecosystem models of quantified trophic links specific to each estuary over a dry/wet cycle.

Inlet mouth phase influences density, variability and standing stocks of planktonic assemblages in temporarily open/closed estuaries

1.1. Introduction

Estuaries are one of the most productive ecosystems on a per unit area basis and provide several important services to the coastal zone and society, e.g. they act as nursery areas for many fish and invertebrate species, play a major role in sustaining coastal fisheries, and support important recreational and cultural activities (Constanza et al., 1997). It is thus not surprising that estuaries are also among the most impacted of all aquatic systems, primarily because of human reliance on their services but also the concentration of people within the coastal zone (Elliott and Whitfield, 2011). This problem is exacerbated in estuaries with an intermittent connection to the sea because they have a high potential to become eutrophic due to the relatively long water residence time during their closed phase (Haines, 2006).

Estuaries with an intermittent connection to the sea (also known as estuarine or coastal lagoons) potentially occupy 13% of coastal areas around the world or approximately 32 000 km of coastline (Barnes, 1980), and dominate some stretches of coast, e.g. approximately 70% of the total number of estuaries in South Africa and 50% of the estuaries in New South Wales, Australia. Despite the fact that these systems are internationally widespread, comparatively little information is available on their hydrological and biological aspects.

Detailed studies on small temporally open/closed estuaries (TOCEs; Whitfield, 1992) only started in the late 1970s, driven primarily by Begg (1978; 1984) who provided a comprehensive overview of the main features of a large number of TOCEs on the northeastern coast of South Africa. The number of investigations on the biological communities within this type of estuary increased considerably during the late 1990s (Cowley and Whitfield, 2001; Haines, 2006; Nozais et al., 2001; Panosso and Esteves, 2000; Perissinotto et al., 2000; Suzuki et al., 1998; Walker et al., 2001), with relatively more studies focusing on the dynamics of phytoplankton, macrophytes and fishes when compared to zooplankton, macrobenthos and meiobenthos (James et al., 2007; Perissinotto et al., 2000). Literature suggests that the majority of published studies on estuaries with an intermittent connection to the sea arose from research in three countries, namely South Africa, Australia and Brazil.

Most TOCEs in South Africa are located within the subtropical and warm temperate biogeographical regions. Rainfall patterns vary significantly across the country and follow a seasonal pattern (summer rainfall) in the subtropical region, while it is mostly bi-modal rainfall (spring and autumn) in the warm temperate region and winter rainfall in the cool temperate region (Davies and Day, 1998). South African TOCEs are highly dynamic systems but are usually closed off from the sea by a sandbar during low rainfall periods when freshwater inflow is too low to breach the sandbar at the mouth. On the other hand, during higher rainfall periods or when the river floods, these estuaries increase in volume in response to large freshwater inputs which then breach the sand berm (Whitfield, 1992). The outflow phase is usually associated with a rapid decline in estuarine water volume, a characteristic that is more pronounced in 'perched' systems. River flow and tidal exchange from the sea are the principal factors controlling the physico-chemical properties of TOCEs during the open phase (Perissinotto et al., 2003; Whitfield, 1992). The open phase can last from days to weeks and ends due to the regeneration of a sandbar at the estuary mouth. Likewise, the closed phase may fluctuate from days to years, mainly linked to river flood events which usually trigger a renewed open phase (Perissinotto et al., 2010).

Several authors have reported significant biological changes between the open and closed phases in these systems (Whitfield et al., 2008). For instance, higher phytoplankton biomass has been recorded during the closed phase of few estuaries in Australia and South Africa (Maher et al., 2011; Perissinotto et al., 2010 and references therein). Furthermore, higher zooplankton abundances and biomass have also been documented in a small number of South African estuaries during the closed phase (Kibirige et al., 2006 and references therein). These changes have been mainly associated with inlet status, freshwater inflow and nutrient concentrations. Despite these findings, almost no studies have focused on determining possible patterns in the small scale responses of TOCEs to inlet phase changes. This type of information is of crucial importance for the establishment of management strategies for these systems (Lill et al., 2013; Whitfield et al., 2012a), particularly due to their high susceptibility to anthropogenic pressures in terms of both climate (e.g. increased rainfall, prolonged drought events) and other globally common impacts on estuaries (e.g. eutrophication, catchment degradation).

This study gathered available information on dissolved inorganic nutrients (nitrogen and phosphorus), phytoplankton biomass, zooplankton density and biomass from intermittently open estuaries to determine the responses of water column nutrients and planktonic communities to inlet phase changes. The specific objectives were; (1) to determine if dissolved inorganic nutrient concentrations, phytoplankton biomass, zooplankton density and biomass vary between the open and closed phases of these systems; (2) to examine the temporal variability of dissolved nutrient concentrations, phytoplankton biomass, zooplankton density and biomass in relation to the duration of closure/opening of a system; and (3) to estimate the potential differences in planktonic standing stocks between open and closed phases in selected systems.

1.2. Material and Methods

A bibliographic survey was conducted on the Institute for Scientific Information (ISI) website and through Web Search, looking for the terms “barred-estuaries”, “barrier-built estuaries”, “intermittently open estuaries”, “intermittently open and closed lakes and lagoons”, “temporarily open/closed estuaries”, “seasonally closed estuaries” and “shallow coastal lagoons” in order to track literature on locations where estuaries with an intermittent connection to the sea have been reported. However, data availability on these systems which complied with our criteria (see below) for inclusion in the analysis in order to fulfill above objectives was comparatively small, and data on South African estuaries dominated the analysis. Therefore, the subsequent data analysis concentrated on 16 South African estuaries (Objective 1), from which five estuaries were analysed in detail due to data availability (Objective 2). Information that could be ascertained from the literature on intermittently closed estuaries from other parts of the world was included in the discussion section for comparative purposes.

Our literature search was then focused on studies describing changes in dissolved inorganic nutrients and planktonic communities between open and closed phases of intermittently open/closed estuaries from South Africa. Relevant literature was identified through the ISI website, Web Search, university library on-line catalogues and the South African Environmental Observation Network (SAEON) database. Our search was restricted to studies with available information on dissolved inorganic nitrogen (DIN: nitrate + nitrite + ammonia) and phosphorus concentrations (DIP: orthophosphate), phytoplankton chlorophyll-a concentrations (chlorophyll-a), zooplankton density and biomass. These parameters were selected in our analysis because firstly, nutrients and phytoplankton chlorophyll-a (as a proxy of phytoplankton biomass) have been widely used as indicators of water quality and, secondly, planktonic communities show a relatively fast response to physical disturbances such as estuary mouth opening events.

The criteria used to select a publication for our analysis were that data from at least one of the five parameters during both open and closed phases was included in the study. A total of 18 publications were thus selected, of which three studies were local reports, two were postgraduate theses and 13 were peer-reviewed scientific publications (Table 1.1). In addition, one set of unpublished personal data was used in the analysis (Table 1.1). Data for each parameter, together with inlet status, were extracted from tables and/or graphs that were presented in these publications. The units used in this study were DIN and DIP (μM), phytoplankton chlorophyll-a ($mg \cdot m^{-3}$), zooplankton density ($ind \cdot m^{-3}$) and biomass ($mg\ DW \cdot m^{-3}$). If values in the publications were expressed in other units, these were converted to reflect the units stated above.

Table 1.1. List of study locations, analysed parameters and references used in this review.

Region	Estuary	Parameter	Condition[1]	Reference
Warm temperate	East Kleinemonde	Chlorophyll-a Zooplankton	Good	Whitfield et al. (2008); van Niekerk et al. (2008)
Warm temperate	Kasouga	Chlorophyll-a DIP Zooplankton	Good	Froneman (2002c, 2004a, c)
Subtropical	Little Manzimtoti	Chlorophyll-a Nutrients	Fair	Forbes and Demetriades (2008)
Subtropical	Lovu	Chlorophyll-a Nutrients	Fair	McLean (2008)
Warm temperate	Maitland	Chlorophyll-a	Fair	Gama (2008)
Subtropical	Manzimtoti	Chlorophyll-a Nutrients	Poor	McLean (2008)
Subtropical	Mbokodweni	Chlorophyll-a Nutrients	Poor	McLean (2008)
Subtropical	Mdloti	Chlorophyll-a Nutrients Zooplankton	Fair	Anandraj et al. (2007); Anandraj et al. (2008); Deale et al. (2013); Forbes and Demetriades (2008); Nozais et al. (2001); Perissinotto et al. (2004)
Subtropical	Mhlanga	Chlorophyll-a Nutrients Zooplankton	Fair	Forbes and Demetriades (2008); Perissinotto et al. (2004)
Warm temperate	Mngazi	Chlorophyll-a DIN	Good	Snow and Adams (2007)
Subtropical	Mngeni	DIN	Poor	McLean (2008)
Subtropical	Mpenjati	Chlorophyll-a Nutrients Zooplankton	Fair	Anandraj et al. (2007); Kibirige and Perissinotto (2003b); Ortega-Cisneros unpublished data; Perissinotto et al. (2002)
Subtropical	Ngane	Chlorophyll-a Nutrients	Fair	Forbes and Demetriades (2008)
Warm temperate	Nyara	Chlorophyll-a Zooplankton	Excellent	Perissinotto et al. (2000); Walker et al. (2001)
Subtropical	Tongati	Chlorophyll-a Nutrients	Poor	Forbes and Demetriades (2008)
Warm temperate	Van Stadens	Chlorophyll-a DIN	Fair	Gama (2008)

[1] According to Whitfield and Baliwe (2013)

The extent of the data sets differed between studies on a temporal and spatial scale, some studies provided information from each sampling station (from three to seven stations), and others presented data from two sampling dates to time series of up to a year. The differences in spatial and temporal resolution among studies and the unbalanced design precluded us from conducting further statistical tests in order to reduce bias in the results. To reduce the differences in the number of data points available for a particular sampling date and estuary, and because none of the studies included in our analysis reported significant spatial differences in planktonic density and biomass, data points from the different sampling stations along each estuary were pooled and averaged for a particular date. The resulting dataset comprised one data point for each sampling date in a particular estuary and included one to several data points for the open and closed phase of each estuary. The number of available studies differed among the parameters, i.e. 15 and 18 data sets were analysed for DIP and DIN respectively, 23 data sets for phytoplankton chlorophyll-a, 7 and 9 for zooplankton density and biomass respectively. These were analysed to determine differences in the concentrations of these parameters between phases, and to ascertain their variability.

To understand the temporal variability of dissolved nutrients and planktonic communities, data points for each sampling date in a particular estuary were plotted against the number of days that a system remained open or closed. The exact day of a breaching event and specific sampling dates were necessary to calculate the number of days that an estuary was in the open or closed phase and thus establish a relationship between planktonic density and biomass at a certain time. Unfortunately, this type of information was not provided in all the publications and this analysis was therefore conducted only for the East Kleinemonde, Mdloti, Mhlanga, Mpenjati and Nyara estuaries. Four studies were available for the analysis of DIN and DIP, 6 studies for the analysis of phytoplankton chlorophyll-a, with 4 and 5 for zooplankton density and biomass respectively.

In order to quantify changes on the planktonic density and biomass through time, data points per study were grouped according to the number of days that an estuary was open or closed, and concentrations were pooled in intervals of 50 days. A fifty-day interval was chosen to ensure that most analysed systems were represented within a particular interval. Four intervals were established corresponding to 1) from mouth breaching to 50 days after, 2) from 51 to 100 days after mouth breaching, 3) 50 days after mouth closure and 4) from 51 to 100 days after mouth closure. Data points from each interval were averaged and those values were then standardized against the mean and SD obtained from the intervals (mean of zero and one standard deviation, z-score) in order to ascertain the degree of change in concentrations between systems. The standardized values, which show by how many standard deviations the score differs from the mean, were plotted for each interval to represent the temporal dynamics in these concentrations from the closed to open phase.

For four of the above analysed estuaries, specific volume estimates of the lowest and highest water level for the open and closed phases were available. Differences in planktonic standing stocks between the open and closed mouth phases were calculated based on volume estimates for the East Kleinemonde (van Niekerk et al., 2008), Mdloti (Perissinotto et al., 2004), Mhlanga (Perissinotto et al., 2004) and Mpenjati estuaries (Ortega-Cisneros, unpublished data) in South Africa. To estimate standing stocks, a volume estimate for the open and closed phase, together with the mean phytoplankton chlorophyll-a concentrations, zooplankton density and biomass values during each phase, were used.

1.3. Results

Of the 16 systems in South Africa which satisfied the selection criteria, six are located within the warm temperate region and ten in the subtropical region. Estuaries in the subtropical region are mainly perched and therefore have limited tidal exchange when in an open phase. The analysed systems range from a poor to excellent condition (Table 1.1) (Whitfield and Baliwe, 2013). Surface areas of these estuaries range from 0.01-0.48 km^2 in the subtropical region and from 0.11-0.17 km^2 for the estuaries in the warm temperate region.

1.3.1. Nutrients

Dissolved inorganic nitrogen (DIN) and phosphorus (DIP) concentrations exhibited similar responses to the open and closed phase (Fig. 1.1a, b). Mean DIN concentrations varied from 0.02 to 478.7 μM during the open phase in the studied environments and from 0.02 to 677.4 μM during the closed phase. DIN concentrations (Mean ± SD) were higher during the open phase (72.82 ± 109.63) than during the closed phase (53.17 ± 119.48). The median value during the open phase (16.00 μM) was also higher than during the closed phase (7.36 μM). On the other hand, DIP concentrations ranged from 0.23 to 66.3 μM during the open phase and from 0.12 to 252.8 μM during the closed phase. Mean DIP concentrations were lower and less variable during the open phase (14.56 ± 17.99) than during the closed phase (30.03 ± 48.84). The median value during the open phase (5.67 μM) was higher than during the closed phase (5.00 μM). Higher coefficients of variation were recorded for both DIN (CV=2.23) and DIP (CV=1.63) during the closed phase compared to the open phase (CV_{DIN}: 1.50; CV_{DIP}: 1.24).

1.3.2. Chlorophyll-a

Chlorophyll-a concentrations (mg·m^{-3}) varied strongly between the open and closed phases (Fig. 1.1c). Chlorophyll-a concentrations ranged from 0.001 to 53 mg·m^{-3} during the open phase and from 0.07 to 145.3 mg·m^{-3} during the closed phase for all estuaries. Overall, mean chlorophyll-a concentrations during the open phase (7.91 ± 12.76) were lower and less variable than those of the closed phase (12.11 ±

21.80). Similarly, the median value during the open phase (2.76 mg·m^{-3}) was lower than during the closed phase (3.48 mg·m^{-3}). The coefficient of variation was also higher during the closed phase (CV = 1.80) than during the open phase (CV = 1.61).

1.3.3. Zooplankton density and biomass

Zooplankton density (ind.m^{-3}) and biomass reached higher mean and median values during the closed phase (Fig. 1.1d, e). Density values ranged from 78 to 46 264 ind·m^{-3} during the open phase and from 184 to 1 815 106 ind·m^{-3} during the closed phase. Mean zooplankton densities were lower during the open phase (6 994 ± 11 309) than during the closed phase (266 661 ± 396 678). The median value during the open phase (2 006 ind·m^{-3}) was also lower than during the closed phase (46 661 ind·m^{-3}). The coefficient of variation during the closed phase (CV = 1.49) was lower than during the open phase (CV = 1.62).

Zooplankton biomass ranged from 0.21 to 348.10 mg DW·m^{-3} during the open phase and from 0.33 to 2 287.50 mg DW·m^{-3} during the closed phase. Similarly, mean zooplankton biomass was lower during the open phase (50.12 ± 77.10) than during the closed phase (278.73 ± 452.80). The median value during the open phase (18.83 mg DW·m^{-3}) was also lower than during the closed phase (58.62 mg DW·m^{-3}). The coefficient of variation during the open phase (CV = 1.54) was slightly lower than during the closed phase (CV = 1.62).

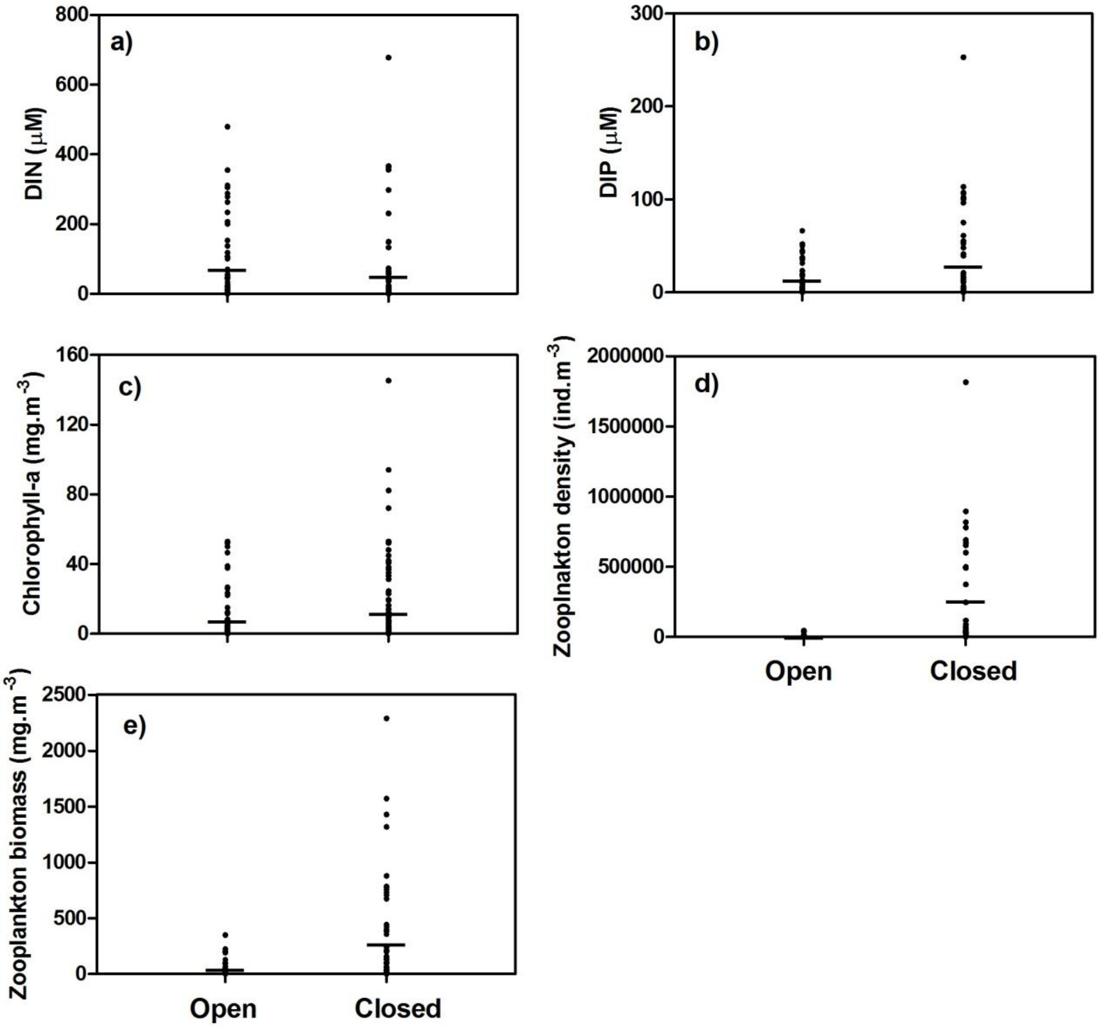

Figure 1.1. Plot of selected abiotic and biotic parameters during the open and closed phases in temporarily open/closed estuaries from South Africa: (a) Dissolved inorganic nitrogen concentrations (µM), (b) Dissolved inorganic phosphorus concentrations (µM), (c) Phytoplankton chlorophyll-a concentrations (mg·m^{-3}), (d) Zooplankton density (ind·m^{-3}) and (e) Zooplankton biomass (mg·m^{-3}). Horizontal lines represent the means.

1.3.4. Temporal dynamics of dissolved inorganic nutrients and planktonic communities

Highest DIN and DIP values overall were observed within the first 25 days after mouth breaching and closure in the analysed estuaries (Fig. 1.2a, b). DIN concentrations were higher within the first 25 days after mouth breaching compared to the period after mouth closure for all analysed systems. Very high DIP concentrations were observed during the closed phase of these estuaries, with DIP concentrations peaking 10 days after mouth closure at the Mhlanga Estuary during March 2002 and February 2003. Similarly, DIP concentrations peaked after 25 days of mouth closure during April 2004 at the Mdloti Estuary. At the Mpenjati Estuary, DIP concentrations were highest after 18 days of mouth breaching during May 2010 (Fig. 1.2b).

In terms of chlorophyll-a, it was clear that higher concentrations were recorded during the closed phase of all studied estuaries (Fig. 1.2c). High variability in chlorophyll-a concentrations was observed among systems but, in general, it was evident that when chlorophyll-a concentrations for the same system were compared between phases, chlorophyll-a concentrations were higher during the closed phase. Similarly, peaks in chlorophyll-a concentration were observed after around 10 days of mouth closure at the Mdloti, Mhlanga and East Kleinemonde estuaries. Moreover, a gradual increase in chlorophyll-a concentrations with time of mouth closure was observed at the East Kleinemonde and Mpenjati estuaries. At the Nyara Estuary, no clear trend on chlorophyll-a concentrations was identified.

Zooplankton density and biomass tended to follow the trends observed for chlorophyll-a concentrations, with higher concentrations during the closed phase of all systems (Fig. 1.2d, e). As with chlorophyll-a concentrations, a marked variability in zooplankton density and biomass was observed among estuaries, with some systems reaching extremely high densities and biomass. Very high zooplankton densities and biomass levels were observed at the Mdloti Estuary after approximately 10 days of mouth closure, which peaked at about 40 days of mouth closure. Similarly, the Mhlanga Estuary exhibited the highest density and biomass after 11 days of mouth closure during February 2003. At the Mpenjati and Nyara estuaries, an increase in zooplankton density and biomass was observed with time of closure, however, estimates of recovery time were not determined in these estuaries due to lack of data.

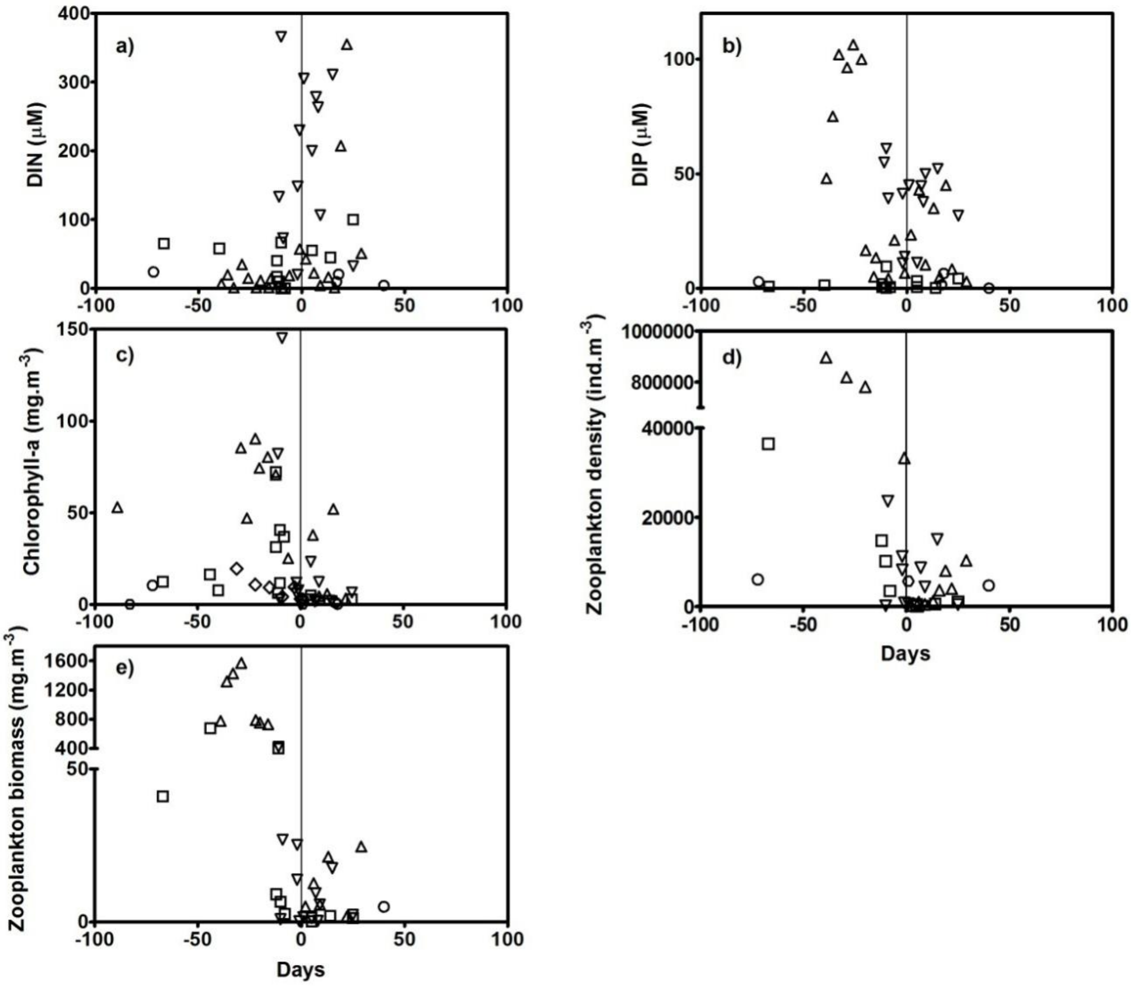

Figure 1.2. Short-term variability of selected abiotic and biotic parameters versus the number of days a TOCE spent in the open and closed phases. (a) Dissolved inorganic nitrogen concentrations (μM), (b) Dissolved inorganic phosphorus concentrations (μM), (c) Phytoplankton chlorophyll-a concentrations (mg·m^{-3}), (d) Zooplankton density (ind·m^{-3}) and (e) Zooplankton biomass (mg·m^{-3}). Negative values indicate days after mouth closure and positive values indicate days after mouth breaching. Symbols indicate particular estuaries (rhombus = East Kleinemonde; squares = Mdloti; triangles = Mdloti; inverted triangles = Mhlanga; circles = Mpenjati and hexagon = Nyara).

Standardized values that had been calculated for each variable and estuary for the 50 day intervals allowed us to quantify the differences between concentrations during the open and closed phases. Chlorophyll-a concentrations, zooplankton density and biomass were significantly higher during the first 50 days of closed phase compared to the first 50 days of the open phase in all analysed systems (Fig. 1.3 a-c). This pattern is less clear in the interval of 50 to 100 days after moth closure, with values both above and below the mean for all three plankton variables.

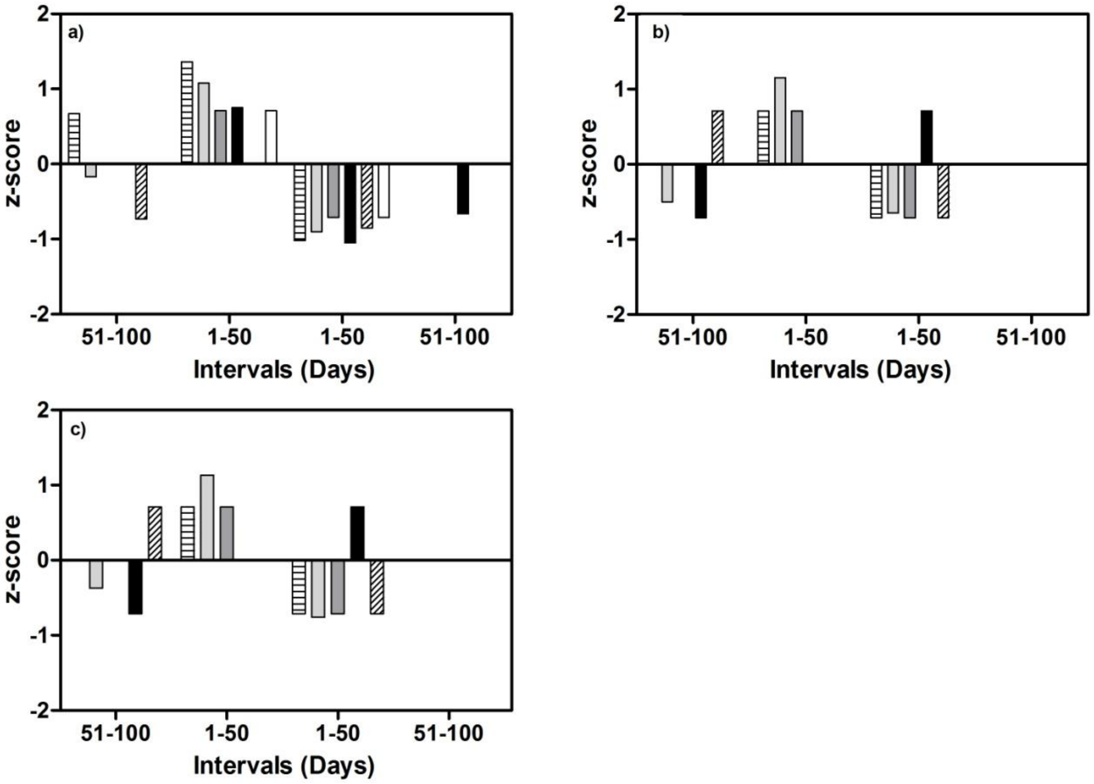

Figure 1.3. Plot of selected biotic parameters aggregated by intervals of 50 days during the open and closed phases of six different studies. Data were standardized to a mean of zero and a SD of 1. (a) Phytoplankton chlorophyll-a concentrations ($\text{mg}\cdot\text{m}^{-3}$), (b) Zooplankton density ($\text{ind}\cdot\text{m}^{-3}$) and (c) Zooplankton biomass ($\text{mg}\cdot\text{m}^{-3}$). Negative values indicate days after mouth closure and positive values indicate days after mouth breaching. Colors indicate estuary (white = East Kleinemonde, horizontal bars = Mdloti, light grey = Mdloti; dark grey = Mhlanga; black = Mpenjati, diagonal bars = Nyara).

1.3.5. Plankton standing stocks per estuary

Phytoplankton standings stocks during the closed phase were approximately 76 times higher than during the open phase in the East Kleinemonde Estuary (Fig. 1.4a). Similarly, zooplankton abundance standing stocks were $41 \cdot 10^6$ and $3\,476 \cdot 10^6$ individuals during the open and closed phase respectively (Fig. 1.4b). In terms of biomass, zooplankton standing stocks fluctuated from 574 to 15 026 g DW during the open and closed phase respectively (Fig. 1.4c). Standing stocks in the East Kleinemonde Estuary were up to 85 times higher during the closed phase compared to the open one.

In the Mdloti Estuary, phytoplankton standing stocks during the closed phase were about 193 times higher than during the open phase (Fig. 1.4a). Mean zooplankton standing stocks in terms of abundance were $17 \cdot 10^6$ and $169\,618 \cdot 10^6$ individuals during the open and closed phase respectively (Fig. 1.4b). Likewise, mean zooplankton biomass standing stocks fluctuated from 42 g to 113 245 g DW during the open and closed phase respectively (Fig. 1.4c). Differences in standing stocks between phases were up to 10 000 times higher during the closed phase of this estuary.

In the Mhlanga Estuary, phytoplankton standing stocks were 242 times higher during the closed phase compared to the open one (Fig. 1.4a). Mean zooplankton abundance varied from 89 $\cdot$ 10^6 to 18 932 $\cdot$ 10^6 individuals during the open and closed phase respectively (Fig. 4b). Similarly, mean standing stocks were 115 g and 68 666 g DW during the open and closed phase respectively (Fig. 1.4c). Differences of up to 600 times were observed between the standing stocks of the open and closed phases in the Mhlanga Estuary.

In the Mpenjati Estuary, phytoplankton standing stocks during the closed phase were approximately 26 times higher than during the open phase (Fig. 1.4a). Mean zooplankton standing stocks in terms of abundance ranged from 510 $\cdot$ 10^6 to 9 080 $\cdot$ 10^6 individuals during the open and closed phase respectively (Fig. 1.4b). Zooplankton standing stocks varied from 15 375 g to 162 407 g DW during the open and closed phase respectively (Fig. 1.4c). Zooplankton standing stocks were up to 18 times higher during the closed phase when compared to the open one.

From the above it is apparent that mean standing stocks of phytoplankton and zooplankton strongly differed between the open and closed phases for the four analysed estuaries. A marked variability was observed among the standing stocks of these systems, with the highest phytoplankton and zooplankton standing stocks being recorded during the closed phase at the Mhlanga and Mpenjati estuaries. The lowest and highest differences between standing stocks were observed at the Mpenjati and Mdloti estuaries respectively.

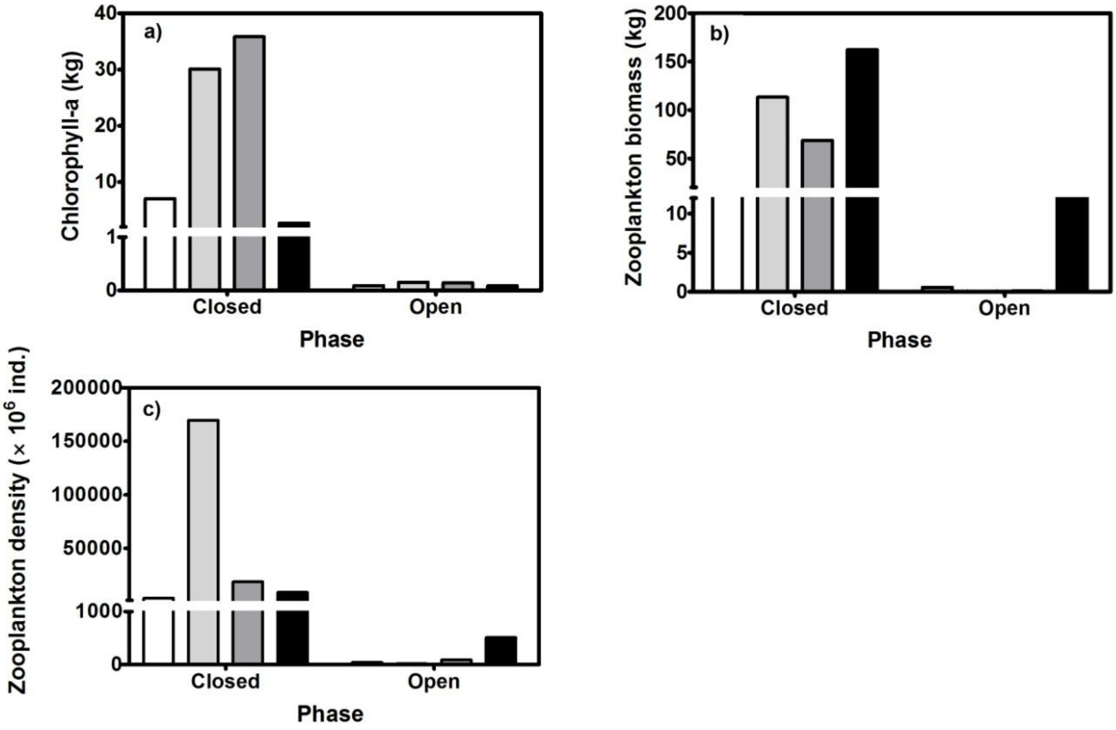

Figure 1.4. Standing stocks during the open and closed phases for the four analysed estuaries to represent differences between these phases: (a) Phytoplankton standing stocks (kg), (b) Zooplankton abundance standing stocks (·10 ^{6} ind.) and (c) Zooplankton biomass standing stocks (kg). Colors indicate estuary: white = East Kleinemonde; light grey = Mdloti; dark grey = Mhlanga; black = Mpenjati).

1.4. Discussion

Our literature review indicated the presence of estuaries with an intermittent connection to the sea in 16 countries: Australia, Borneo, Brazil, Ghana, India, Malaysia, Mexico, New Zealand, Portugal, South Africa, Spain, Sri Lanka, Togo, United States of America, Uruguay and Vietnam (Koh et al., 2012; Kwei, 1977; Lae, 1994; Moreno et al., 2010; Pereira Coutinho et al., 2012; Perissinotto et al., 2010 and references therein; Raz-Guzman and Huidobro, 2002; Schallenberg et al., 2010; Tung et al., 2006). These results indicate that TOCEs are common coastal features worldwide, sometimes accounting for the majority of estuaries in certain countries (Maher et al., 2011; Whitfield, 1992). Our results suggest that even in locations where these systems can be abundant, the number of TOCEs per coastline has not been quantified (e.g. Brazil, Netto et al., 2012). It is highly likely that TOCEs are common along the coastlines of other countries, but this has yet to be recorded in the scientific literature.

Lower concentrations of dissolved inorganic nutrient concentrations were expected after prolonged closed phase due to depletion following uptake by phytoplankton, particularly if they are not replenished by freshwater and/or marine inputs (Froneman, 2006). Our analyses showed higher DIN and DIP concentrations during the open phase of the analysed systems, particularly during approximately 25 days after mouth breaching. The high DIN and DIP concentrations recorded during open phases may be attributed to the increase in nutrient availability related to higher rainfall and river inflow at the onset of the wet season.

The availability of nutrients in intermittently open/closed estuaries is directly influenced by the inlet status and the duration of closure/opening (Taljaard et al., 2009), e.g. large amounts of new nutrients from riverine input are imported into these systems at the onset of the open phase but most are flushed out of the estuary without being assimilated. As residence time increases during the open phase, the nutrient-rich freshwater flow will generate an increase in primary production. After estuarine closure, these nutrient concentrations will decrease over time as river flow declines and they are absorbed by sediments and taken up by primary producers (Taljaard et al., 2009). As shown by our data, very high nutrient concentrations can also be found just after mouth closure, agreeing with the model proposed by Taljaard et al. (2009) which indicates that in TOCEs assimilation processes by phytoplankton usually take place during the closed phase due to higher water residence time. Nutrient availability will decline after extended mouth closure because of uptake by microalgae, as well as the reduced riverine inputs during the closed phase (Taljaard et al., 2009).

Higher phytoplankton chlorophyll-a concentrations during the closed phase are to be expected, primarily because of reduced flushing and stable water column conditions during this phase (Froneman, 2002b; Nozais et al., 2001; Skinner et al., 2006). Our analyses showed that higher chlorophyll-a concentrations were associated with the closed phase of most systems. Similarly, the analysis of the standardized values from the closed to the open phases showed that concentrations during the open phase (1-50 days after mouth breaching) were significantly lower than the concentrations recorded during the closed phase (interval of 50 days after mouth closure) for all estuaries. Our results agree with previous studies that reported significantly higher chlorophyll-a concentrations during the closed phase of TOCEs in Australia (Haines, 2006), Brazil (Suzuki et al., 1998), New Zealand (Lill, 2010) and Portugal (Pereira Coutinho et al., 2012) (Table 1.2).

Table 1.2. Examples of global TOCEs where significantly higher chlorophyll-a concentrations have been recorded during the closed phase.

Estuary	**Location**	**Size (km^2)**	**Chlorophyll-a concentration (mg·m^{-3})**		**Reference**
			Open	**Closed**	
Curl Curl	Australia	0.06	6.7[1]	7.1[1]	Haines (2006)
Dee Why	Australia	0.30	2[1]	5.6[1]	Haines (2006)
Kianga	Australia	0.13	1.8	78.5	Haines (2006)
Middle	Australia	0.40	1.3	44.1	Haines (2006)
Nangudga	Australia	0.62	11.8	31	Haines (2006)
Cabiunas	Brazil	0.34	0.9	6.6	Branco et al. (2008)
Grussai	Brazil	0.80	70	200	Suzuki et al. (1998)
14 estuaries in Otago	New Zealand	< 1	lower (no data available)	higher (no data available)	Lill (2010)
Albufeira	Portugal	1.3	9.8	40.9	Pereira Coutinho et al. (2012)
Óbidos	Portugal	7	63.7	289.3	Pereira Coutinho et al. (2012)
St. André	Portugal	1.7-2.5	15	52.5	Pereira Coutinho et al. (2012)

[1] Median values

An increase in zooplankton abundance was predicted during the estuary closed phase due to anticipated higher food availability, higher residence time of the water body and reduced disturbance associated with flushing events. As with chlorophyll-a concentrations, our analyses confirmed that zooplankton density and biomass decreased markedly from the closed to the open phase in the five analysed estuaries. Higher abundance and biomass of zooplankton during the closed phase have been reported in a range of South African TOCEs, from pristine to eutrophic systems. For example, Kibirige et al. (2006) reported significantly lower total zooplankton abundance and biomass during 16 open phases in the eutrophic Mhlanga Estuary (South Africa) for the period March 2002 – March 2003, which corroborated the earlier findings of Whitfield (1980) from the same system. The latter author attributed this result to the flushing of zooplankton out of the estuary during the river outflow phase and short water residence time during the brief tidal phase.

An increase in zooplankton density and biomass during the closed phase have been recorded in other small intermittently open/closed estuaries in Australia and Brazil, due primarily to the favorable physical and biological conditions pertaining to this phase (Branco et al., 2008; Rissik et al., 2009; Santangelo et al., 2007). However, extended mouth closure can cause differential responses on the functioning of TOCEs. For instance, the freshwater deprived Kasouga Estuary was separated from the sea for almost three years before experiencing mouth breaching during November 2001. In this system, chlorophyll-a concentrations and zooplankton biomass increased after mouth breaching probably because freshwater inflow provided this estuary with a source of nutrients and this input stimulated planktonic growth during the open phase (Froneman, 2002c). Similar patterns in chlorophyll-a concentrations were reported for Smiths Lake in New South Wales, Australia after a prolonged inlet closure (Everett et al., 2007) and the nutrient limited Mecox Bay in New York, USA (Gobler et al., 2005). Therefore, the duration of the closed phase is another crucial factor in determining the responses of planktonic communities to open phases.

An analysis of the temporal variability indicated that most data points were concentrated within the 50 days after mouth breaching and closure. Furthermore, most analysed systems experienced longer closed phases when compared to open ones (e.g. they are predominantly closed systems). From our analysis, it was apparent that planktonic concentrations peaked within 10 days of mouth closure. Our results agree with those from Anandraj et al. (2008), who determined that phytoplankton concentrations reached pre-breaching levels 15 days after mouth closure at the Mdloti Estuary. Similarly, Deale et al. (2013) determined that zooplankton density and biomass exceeded pre-breaching levels nine days after mouth closure in the same estuary. These are the only studies dealing with small scale temporal variability of planktonic communities of TOCEs in South Africa. Overall, planktonic communities showed a similar recovery time after mouth closure in the analysed estuaries independently of their trophic status, degree of tidal exchange (perched versus unperched) or frequency of breaching events. For instance, the eutrophic Mdloti and Mhlanga estuaries breached nine and 16 times respectively during March 2002 - March 2003 (Perissinotto et al., 2004), but their responses to inlet opening were similar to that reported for more pristine estuaries which usually breach once per year. Moreover, perched and unperched estuaries (e.g. East Kleinemonde and Nyara estuaries) also showed similar responses to inlet opening, even when unperched systems are strongly influenced by overwash events which can introduce nutrient rich water to the estuary and enhanced phytoplankton primary production on the lower reaches (Froneman, 2004c; Gama et al., 2005).

In general, it seems that the favourable conditions during the closed phase (e.g. high water residence time and low physical disturbance) contribute to an increase of planktonic biomass during this phase. However, the frequency between successive opening events is another key factor in determining the higher planktonic production during the closed phase, as adequate periods of mouth closure are required for planktonic communities to build-up their biomass (Whitfield, 1980). It is vital to highlight here that the responses observed for the planktonic biomass can be rapidly modified if open and closed phases are affected by anthropogenic and climate changes (e.g. stronger or more frequent rainfall events, drought episodes, increased processed wastewater outfalls into estuaries, artificial breaching).

Variability (as coefficient of variation, CV) was similar among the different parameters analysed in this study, but they were usually higher during the closed phase. As shown by the analysis of standardized values, chlorophyll-a concentrations, zooplankton density and biomass can differ between intervals of 0-50 and 51-100 days during mouth closure, confirming that concentrations during the closed phase can be more variable. Due to data availability, it was not possible to determine the smaller scale temporal variability of these concentrations during the open phase. It is possible that as the closed phase is the dominant one for most of our studied systems, this incorporates the influence of seasonal and other environmental changes (e.g. pollution events) and may thus show higher variability, which are not usually captured during the much shorter open phase.

The importance of accounting for dilution and volume changes in systems with intermittent connections to the sea is an important issue, with Rissik et al. (2009) reporting an increase of up to 100% in the maximum volume of the Dee Why Lagoon (Australia) during a closed phase. They determined that if the dilution effect was not considered in their estimates, population growth rates of phytoplankton and of the copepod *Acartia* sp. would have been underestimated by as much as 25% during the closed phase. Similarly, marked differences in planktonic standing stocks between phases were calculated for the East Kleinemonde, Mdloti, Mhlanga and Mpenjati estuaries, with the Mhlanga and Mdloti systems showing the most striking differences. These results suggest that marked differences in planktonic standing stocks between phases are common in most TOCEs.

There are more than 180 TOCEs along the South African coast and most of them open to the sea frequently during high rainfall periods. If the large differences in water volume reported for the four analysed estuaries are similar for other TOCEs, then the total export of nutrients and detritus to the ocean during the opening phase of these systems will be significant and perhaps comparable in importance with the export from the more limited number of larger permanently open estuaries. The differences in standing stocks between phases not only have important implications for an understanding of the structure and functioning of TOCEs, but also for the ecology of the adjacent nearshore marine environment, since exports from these systems have been shown to significantly contribute to coastal productivity (Lamberth and Turpie, 2003; Schlacher and Connolly, 2009; Vorwerk, 2006).

Despite differences in trophic state, frequency of breaching events or degree of tidal exchange, the five analysed estuaries showed similar responses to inlet opening, which were consistent with the univariate analysis of dissolved nutrients, planktonic density and biomass from 16 estuaries in South Africa and with literature from TOCEs in Australia, Brazil, New Zealand and Portugal. This might indicate that overall TOCEs showed a similar response to inlet openings, however, the reduced number of systems analysed here precludes us from making further conclusions. Future research should focus on evaluating the smaller scale temporal variability of TOCEs to determine if patterns in their responses to inlet openings can be generalized.

Our findings provide insights into the dynamics of TOCEs, which is of upmost importance for the appropriate management of these systems. Our study also highlights the importance of maintaining natural open and closing regimes in TOCES, since alterations in these phases may have far reaching consequences for coasts where intermittently open/closed estuaries are abundant, especially where the nearshore marine environment is oligotrophic and coastal organisms depend on pulses of nutrients, detritus and biomass exports for their productivity (Abrantes and Sheaves, 2010; Deegan, 1993; Schlacher et al., 2008).

Variability and temporal stability of communities in estuaries (Mlalazi and Mpenjati, South Africa)

2.1. Introduction

Diversity has long been considered to be a mechanism promoting stability in a community, with high-diversity communities being more resistant to species invasion and to environmental fluctuations (Cottingham et al., 2001; Stachowicz et al., 2002; Tilman, 1999 and references therein). The diversity-stability relationship has been tested in diverse ecosystems such as grasslands, microbial communities and rocky shore benthic assemblages (e.g. Bulleri et al., 2012; Grman et al., 2010; McGrady-Steed and Morin, 2000; Romanuk and Kolasa, 2002; Steiner et al., 2005; Tilman, 1999), with most authors reporting a positive relationship between diversity and stability at community level (but see Valdivia and Molis, 2009) and a negative relationship at population level. Consequently, diversity (e.g. species richness and evenness) is predicted to promote community stability through two main insurance mechanisms: 1) a buffering effect, where higher species richness reduces the temporal variance of a community-level property (e.g. abundance, biomass) and 2) a performance-enhancing effect, where higher species richness produces an increase in the temporal mean of a community-level property such as abundance or biomass (The Insurance Hypothesis, Yachi and Loreau, 1999). These two mechanisms are influenced by the individual species responses to environmental fluctuations and the degree of asynchrony of these responses (Yachi and Loreau, 1999) as follows.

Environmental variability has been shown to play an important role in influencing the strength of diversity effects on stability (Campbell et al., 2011; Romanuk and Kolasa, 2002). Evidence suggests that under high environmental forcing, additional mechanisms such as species dominance and asynchrony of responses can contribute to the stability of a community (Grman et al., 2010; Langenheder et al., 2012; Loreau and de Mazancourt, 2008). Species dominance can promote stability due to the effect of better performing species, which exhibit lower temporal variance and higher capacity to resist disturbances (Grman et al., 2010; Langenheder et al., 2012; Valdivia et al., 2013). Similarly, asynchronous species fluctuations can foster stability by compensating the decrease of less-tolerant species with the increase of better performing ones (Loreau and de Mazancourt, 2008; Tilman, 1999). Stability can also be enhanced through the effect of statistical averaging of the different species fluctuations within a community by cancelling out the independent responses to environmental fluctuations (Doak et al., 1998). Statistical averaging is highly influenced by species richness and evenness because in communities with low species richness and evenness, total variances will be mainly influenced by the fluctuations of dominant species (Cottingham et al., 2001; Steiner et al., 2005). Moreover, temporal stability can be promoted by a faster increase of the mean of a total community property with increasing species diversity when compared to its variance. This mechanism known as overyielding takes place when a species performs better in a multi- than a mono-specific assemblage (Lehman and Tilman, 2000).

Finally, other mechanisms such as species traits (Flöder and Hillebrand, 2012), food chain length and omnivory (Long et al., 2011) can also determine the stability of community properties. The interaction effect of these different mechanisms in community stability in relation to different environmental forcing is not yet clear. Stability and its mechanisms have been studied in a wide range of ecosystems, however, no studies have evaluated the stability of communities in environments with a high degree of environmental forcing such as estuaries. An exception is the study by Pinto et al. (2013), who evaluated if the temporal stability of specifically the macrobenthos community can be used as a measure of estuarine stability in relation to ecosystem services provision in the Mira and Mondego estuaries, Portugal.

Estuaries are considered to be naturally stressed systems due to the high variability in their physico-chemical characteristics. Estuarine biota are exposed to strong fluctuations in salinity, tidal influence, pH, nutrient levels, river inflow and even mouth status, but they are able to survive and even thrive in this stressful environment (Elliott and Quintino, 2007). It has been hypothesized that this natural variability constitutes a subsidy for estuarine biota to flourish, rather than a stress (Costanza et al., 1992; Elliott and Quintino, 2007); which provides estuarine communities with an opportunity to persist under the fluctuating environmental conditions, rather than perish, exhibiting high resilience after disturbances (e.g. floods, droughts). Consequently, the Environmental Homeostasis Hypothesis (Elliott and Quintino, 2007) suggests that the variable, and perceived stressful, estuarine environment might provide estuarine communities with the capacity to reach stability by compensating to the changes in the environment. However, this natural stress typical of estuaries constitutes an advantage only for those species that are able to tolerate the variable environmental conditions. These are relatively few when compared to e.g. oceanic environments, and estuaries are thus characterized by a comparatively low species diversity (Elliott and Whitfield, 2011; Whitfield et al., 2012b). As stated by Elliott and Quintino (2007), estuarine ecosystems are able to function successfully despite their low diversity, although diversity has been put forward as a necessity for efficient ecosystem function in the biodiversity-ecosystem functioning debate (Loreau et al., 2001).

Estuarine systems are influenced by numerous environmental factors and are characterized by a large range of physico-chemical fluctuations at various spatial and temporal scales (McLusky and Elliott, 2004). Estuaries are also controlled by cyclic changes, e.g. seasonal fluctuations of temperature, salinity and river inflow. River inflow constitutes one of the most important factors influencing estuaries and its communities (Adams et al., 1999; Montagna et al., 2002; Wooldridge, 1999). One example of such marked seasonal changes occurs in the KwaZulu-Natal coast, South Africa, which experiences a subtropical climate with two marked seasons. The rainy season is characterized by strong river inflow to estuaries (October to April) and a dry season (May to September) by lower rainfall and negligible river inflow to estuaries. This marked seasonal variation in environmental conditions produces spatio-temporal changes in the estuarine planktonic and macrobenthic invertebrate communities in this region (Perissinotto et al., 2010 and references therein). It can be expected that this natural variability will influence the stability of these estuarine communities.

In this study, two KwaZulu-Natal (KZN) estuaries were used as examples to determine the variability and temporal stability of community-level properties such as phytoplankton biomass, zooplankton and macrobenthic density and biomass over a dry/wet cycle. Specifically, our objectives were: 1) to determine if the studied community-level properties (i.e. phytoplankton biomass, zooplankton and macrobenthic invertebrate density and biomass) vary significantly through time and space in both estuaries, 2) to determine if the variability (as coefficient of variation, CV) of these community-level properties significantly changes through time, and 3) to estimate the temporal stability of these community-level properties and determine the main drivers of stability in these estuaries.

2.2. Materials and Methods

In order to determine spatio-temporal changes and the temporal stability of the planktonic and benthic communities in the Mlalazi and Mpenjati estuaries, time-series of planktonic and macrobenthic communities were produced. Biological and environmental samples were collected at both estuaries between May 2010 and May 2011. Quarterly samplings (five seasons) were conducted at three stations in the Mpenjati Estuary and four stations in the Mlalazi Estuary (Fig. 2.1). The difference in number of stations was based primarily on the differences in estuary length. The stations were located so that representative samples were collected in the upper, middle and lower reaches of the respective estuaries. The duration of our study (13 months) allowed us to include major seasonal environmental fluctuations and adequately describe the stability of these estuarine communities under a seasonal cycle.

2.2.1. Study sites

The Mlalazi Estuary (28° 56'42" S; 31°48' 58" E) is classified as a permanently open system (POE, Fig. 2.1). The catchment area is approximately 492 km^2 (DEAT, 2001) and the estuary length is length of approximately 10 km. The estimated mean annual runoff is 122 · 10^6 m^3 (Jezewski et al., 1984) and the estuary area is 95.86 ha (van Niekerk and Turpie, 2012). Subsistence farming, sugar cane and commercial forestry accounts for approximately 46% of the catchment usage, with 53% of the catchment considered undegraded and 1% urban (coastal village of Mtunzini and the town of Eshowe).

The Mpenjati Estuary (30°58'21''S, 30°17'02''E) is a temporarily open/closed system (TOCE; Whitfield, 1992) and is also located in the South African subtropical region (Fig. 2.1). The catchment area is approximately 101 km^2, with an axial length of 1.1 km. The estimated mean annual runoff is $20.7 \cdot 10^6$ m^3 (Jezewski et al., 1984) and the estuary area is 11.6 ha (van Niekerk and Turpie, 2012). Most of the catchment is used for banana and sugar cane farming. Both systems are located in the subtropical biogeographical region and part of nature reserves. These systems are considered to have no main negative anthropogenic impacts on their catchments (Whitfield and Baliwe, 2013).

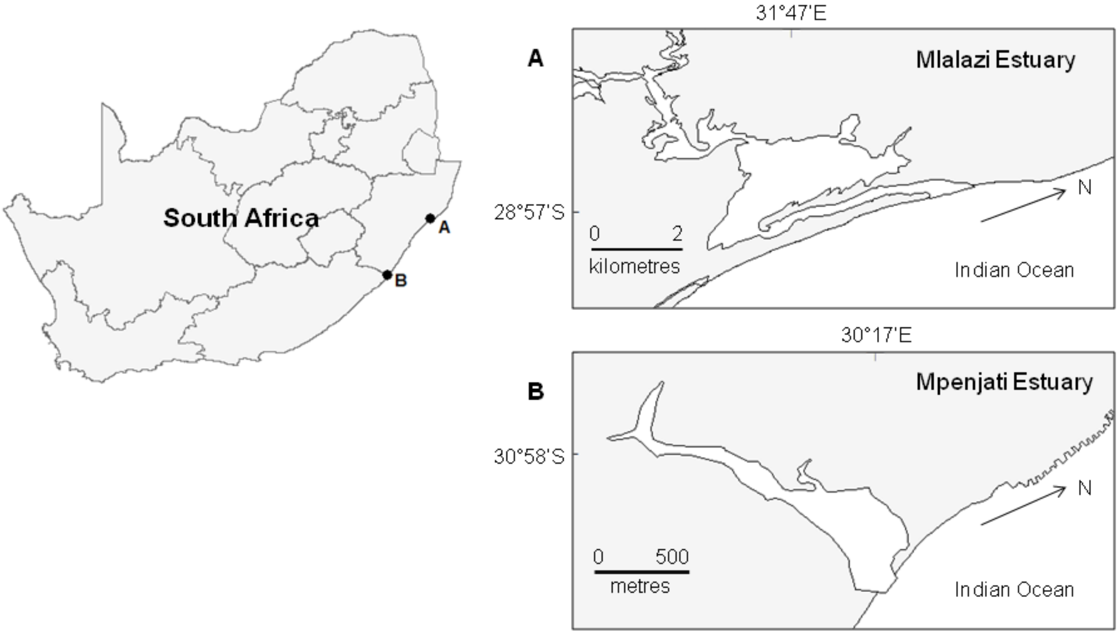

Figure 2.1. Maps of the two study systems (Mlalazi and Mpenjati estuaries) on the east coast of South Africa.

2.2.2. Environmental variability

In order to determine if the seasonal fluctuations in rainfall and river inflow produce spatio-temporal variations in the abiotic environment of these estuaries, physico-chemical measurements (salinity, temperature, pH, and dissolved oxygen) were taken at ca. 50 cm depth and bottom of the water column using a YSI 6920 water quality logger at each station to determine the variability in space and time of these parameters in both systems. Simultaneously, triplicate sub-surface water samples were collected for the determination of Total Suspended Solids (TSS), dissolved inorganic nitrogen and phosphorus and particulate organic carbon and nitrogen (POC and PON). Triplicate sediment samples were also taken for the determination of sediment nutrient concentrations (particulate C and N) and mud content (%). Methodological details are specified in Appendix 2.1.

2.2.3. Biological variability

To determine the variability and temporal stability in the planktonic and macrobenthic communities of the Mlalazi and Mpenjati estuaries, data were collected as follows:

Phytoplankton biomass: Triplicate sub-surface samples were collected from May 2010 to May 2011, and serially filtered through 20 µm Nitex mesh, 2 µm membrane filters, and 0.72 µm GF/F filters. The pigments were extracted using 90 % acetone for 24 hours in the dark at -4°C. Phytoplankton chlorophyll-a concentrations (as a proxy for phytoplankton biomass) were estimated using a Turner Trilogy fluorometer (California, US) (Holm-Hansen and Riemann, 1978). Microplankton, nanoplankton and picoplankton size fractions were used as proxies of species composition for the phytoplankton community.

Zooplankton abundance and biomass: Daytime zooplankton samples were collected using a hyperbenthic sled with a 200 µm mesh plankton net from September 2010 to May 2011. The sled was towed at a speed of 1-2 knots at each station for 27 meters. Samples were preserved in 5% formalin with Rose Bengal. In the laboratory, samples were suspended in 1 to 5 l solutions, depending on the concentration of organisms. From each solution, three 20 ml subsamples were withdrawn at mid-depth, while stirring continuously to ensure homogenous suspension (Jerling and Wooldridge, 1991b; Perissinotto and Wooldridge, 1989b). Organisms were then identified to species level and counted with a dissecting microscope. The coefficient of variation between subsamples was always less than 10%. Biomass (as dry weight) was determined for the most numerically dominant species of the zooplankton in terms of density and biomass after oven-drying for 48 h at 60°C.

Macrobenthic invertebrate abundance and biomass: Three replicate samples were collected with a Zabalocki-type Ekman grab (sampling area 0.024 m^2) during September 2010 and a van Veen grab (sampling area 0.025 m^2) during the other sampling sessions. Both types of grab sample the same area and depth (ca. 10 cm). Three replicate samples were collected at each station, each consisting of three grab samples to collect sufficient material for subsequent analysis. Samples were collected using a 500 µm sieve and preserved in 5% formalin with Rose Bengal. In the laboratory, organisms were identified, counted and weighed after oven-drying for 48 h at 60°C.

2.2.4. Data analysis

To test for significant spatio-temporal changes in the environmental and biological data at both estuaries (Objective 1), PERMANOVA analysis (Anderson et al., 2008) was performed using seasons and stations as factors. All environmental variables (Table 2.1) were log- transformed and normalized to account for the differences in units among variables (Clarke and Gorley, 2006). A similarity matrix based on Euclidean distance was produced to represent the relationship among stations and seasons.

For the analysis of zooplankton and macrobenthic communities, only the most abundant species (collectively accounting for 99 % of mean density or biomass) were considered. All groups accounting for < 1 % of mean density or biomass were not included in our analysis in order to reduce bias towards their contributions. Phytoplankton biomass, zooplankton and macrobenthos density and biomass data were square-root transformed to balance the contributions between more abundant and rare taxa. Separate PERMANOVAs based on Bray-Curtis similarity matrix were conducted and a significance level of 0.05 was used. PERMANOVA analyses were conducted in PRIMER 6 (Clarke and Gorley, 2006).

To determine if the variability of the community-level properties significantly changed through time in both estuaries (Objective 2), the coefficient of variation (CV) defined as the ratio between the standard deviation and its mean ($CV=\delta/\mu$) was used. Higher values of CV represent a high variability. CV of phytoplankton biomass, zooplankton and macrobenthic density and biomass were calculated for each station among seasons in both estuaries to determine the variability in these community-level properties. In order to test for significant temporal changes in the variability (CV) of each community-level property, a one-way ANOVA was performed using seasons as factors. To test if the variability changed among community-level properties and estuaries, a two-way ANOVA was conducted. Possible significant differences between pairs of samples were determined with a Tukey HSD post-hoc test. Normality and homoscedasticity were evaluated for each community-level property prior to the analyses, and all CV values were $\log_{10}$ transformed in order to fulfil the assumptions of parametric tests. ANOVA tests were conducted using the software package IBM SPSS Statistics 21 at a significance level of 0.05.

To determine the temporal stability of the analysed community-level properties and its stabilizing mechanisms (Objective 3), the temporal stability (S) was used as a stability measure. S is an index of the stability of a community or population along time (Lehman and Tilman, 2000). In this study, community stability was estimated as the ratio between the temporal mean of a particular community-level property and its standard deviation. S is the inverse of the coefficient of variation, thus higher values indicate higher temporal stability. S was calculated for each station and community-level property in both systems.

Four possible mechanisms contributing to temporal stability (*S*) were evaluated. The community-wide synchrony (φ_c) (Loreau and de Mazancourt, 2008), considered an important mechanism contributing to community stability was calculated as:

$$\varphi_c = \sigma^2_{cT} \qquad {}^{s}_{i=1}\sigma_{ci}$$

where σ^2_{cT} is the variance of the total community-level property (e.g. phytoplankton biomass, zooplankton density) and σ_{ci} is the standard deviation in the abundance or biomass of species i, in a community with s species. φ_c ranges from 0 (perfect asynchrony) and 1 (perfect synchrony), with higher synchrony values meaning that most species within a community are positively correlated and fluctuate in a similar manner. This statistic is independent of the magnitude and distribution of species abundances/biomasses and variances, which allow for quantitative comparisons of communities with different species richness (Loreau and de Mazancourt, 2008). Other important stabilizing mechanisms such as species richness (Margalef, 1958), species evenness (Pielou, 1975) and species dominance (Simpson, 1949) indices were calculated for each community-level property and estuary using the routine DIVERSE in Primer 6 (Clarke and Warwick, 1994). A Pearson product-moment correlation was run to determine the relationship between the temporal stability and stabilizing mechanisms (i.e. dominance, species richness, synchrony). Correlation tests were conducted in IBM SPSS Statistics 21 using a significance level of 0.05.

2.3. Results

2.3.1. Spatio-temporal changes in abiotic factors

The inlet of the Mlalazi Estuary remained open throughout the study period, whereas the mouth of the Mpenjati Estuary was open and closed for similar periods of time during this study. Mouth breaching occurred twice during the study period due to river flooding at the end of October 2010 and the beginning of May 2011. This estuary was open to the sea from the end of October to the beginning of April 2011.

Temperature measurements were relatively stable along the axis of each estuary, temperature ranged from 20.1°C (May 2010) to 27.7°C (February 2011) at the Mlalazi Estuary. At Mpenjati Estuary, temperature varied from 20.1°C (June 2010) to 24.8°C (February 2011). A marked salinity gradient was observed during November 2010 at the Mlalazi Estuary, salinities ranged from 5 in the upper reaches of the estuary to 30 in the lower reaches. At the Mpenjati Estuary, no salinity gradient was observed during the study period, with the exception of November 2010, when salinities ranged from 5 on the upper reaches to 20 in the lower reaches. Measurements of pH were uniform among seasons and stations in the Mlalazi Estuary. Mean pH ranged from 7.0 (May 2011) to 7.8 (September and November 2010). At the

Mpenjati Estuary, pH measurements were also uniform among stations but were significantly higher during September 2010 (8.6).

At the Mlalazi Estuary, mean DIN concentrations were highest during May 2010 (dry season) and lowest during February 2011 (end of wet season). At the Mpenjati Estuary, mean DIN concentrations were highest during September 2010 (dry season) and lowest during February 2011 (end of wet season). Mean DIP reached highest and lowest concentrations during May and February 2011 respectively at both estuaries. TSS also showed lowest concentrations during February 2011 and reached highest values during September 2010. Conversely, POC and PON were significantly higher during February 2011. In the Mpenjati Estuary, TSS exhibited highest concentrations during September 2010 and lowest during May 2010. POC and PON showed very similar patterns, with concentrations of both being lowest during November 2010 and highest during September 2010.

All analysed environmental variables showed significant seasonal differences at the Mlalazi Estuary, with the exception of the pH and sediment mean grain size. Similarly, a significant interaction effect of season × station was observed by TSS, POC and PON of sediment and water, mud content and sediment organic matter. Salinity showed significant differences among seasons and stations at this estuary, lower salinities were recorded at the upper reaches of the estuary throughout the study period (Table 2.1). At the Mpenjati Estuary, all environmental variables showed significant seasonal differences. Significant interaction effects of season × station were recorded by DIP, TSS, POC and PON of the sediment, mud content and sediment organic matter (Table 2.1). In both systems, salinities were lowest during the wet season.

Table 2.1. PERMANOVA for environmental parameters recorded at the Mlalazi and Mpenjati estuaries.

Parameter	**Significant variations**	
Water column parameters	**Mlalazi Estuary (POE)**	**Mpenjati Estuary (TOCE)**
pH	No significant differences	Seasons
Salinity	Seasons, Stations	Seasons
Temperature (°C)	Seasons	Seasons
DIN (µM)	Seasons	Seasons, Stations × Seasons
DIP (µM)	Seasons	Seasons
TSS ($mg \cdot l^{-1}$)	Seasons, Stations, Stations × Seasons	Seasons, Stations, Stations × Seasons
Particulate Organic Carbon ($\mu g \cdot l^{-1}$)	Seasons, Stations, Stations × Seasons	Seasons
Particulate Organic Nitrogen ($\mu g \cdot l^{-1}$)	Seasons, Stations, Stations × Seasons	Seasons
Sediment parameters		
Particulate Organic Carbon of the sediment (%)	Seasons, Stations, Stations × Seasons	Seasons, Stations, Stations × Seasons
Particulate Organic Nitrogen of the sediment (%)	Seasons, Stations, Stations × Seasons	Seasons, Stations, Stations × Seasons
Mud content (%)	Seasons, Stations × Seasons	Stations, Stations × Seasons
Sediment organic matter (%)	Seasons, Stations, Stations × Seasons	Seasons, Stations, Stations × Seasons

2.3.2. Spatio-temporal changes in biological communities

Phytoplankton biomass

Mean total phytoplankton biomass (as chl-a) in the Mlalazi Estuary ranged from 0.53 ± 0.42 $mg \cdot m^{-3}$ in May 2011 (dry season) to 9.75 ± 2.23 $mg \cdot m^{-3}$ in November 2010 (wet season). In the Mpenjati Estuary chlorophyll-a concentrations ranged from 0.16 ± 0.06 (SD) in May 2011 (abnormal dry season with high rainfall) to 10.40 ± 2.26 $mg \cdot m^{-3}$ to September 2010 (dry season). Nanoplankton size fraction was the largest contributor to total phytoplankton biomass in the Mlalazi estuary, accounting for 49 and 69 % of the total biomass during May 2011 and May 2010 respectively. The picoplankton size fraction was usually the second largest contributor to phytoplankton biomass (but not in November 2010), accounting for 15 and 44 % of the total biomass during November 2010 and May 2011 respectively. In the Mpenjati estuary, the nanoplankton size fraction was also the largest contributor to total phytoplankton biomass, with the exception of May 2010 and May 2011. It comprised between 37 and 89 % of the total biomass during May 2011 and September 2010 respectively. Picoplankton was the second largest contributor to total biomass, fluctuating from 10 to 59 % during August 2010 and May 2011.

PERMANOVA analysis detected significant differences between seasons for the phytoplankton chlorophyll-a concentrations in the Mlalazi and Mpenjati estuaries (Table 2.2, 2.3). These differences are explained by the marked differences in chlorophyll-a concentrations between the wet and dry seasons, with high chlorophyll-a concentrations recorded during the wet season at the Mlalazi Estuary and during the dry season at the Mpenjati Estuary.

Zooplankton density and biomass

Mean zooplankton density varied from 3 920 ± 3 150 ind·m^{-3} in June 2010 (dry season) to 58 671 ± 37 969 ind·m^{-3} in November 2010 (wet season) at the Mlalazi Estuary and from 6 053 ± 1 925 ind·m^{-3} in September 2010 (dry season) to 46 264 ± 23 233 ind·m^{-3} in November 2010 (wet season) at the Mpenjati Estuary. Zooplankton density was dominated by the copepod *Acartia* sp. at the Mlalazi estuary, accounting for 74 and 98% of the total density during May 2011 and September 2010 respectively. The copepod *Pseudodiaptomus hessei* contributed to the total density with 1 to 23 % during September 2010 and May 2011 respectively. The copepod *P. hessei* was the dominant species at the Mpenjati estuary, representing 12 to 82 % of the total density during May 2011 and November 2010. *A. natalensis* was the second largest contributor to the total density, accounting for 14 to 63 % of the total density during September 2010 and November 2011.

A significant interaction effect of season × station was detected on zooplankton density in the Mlalazi Estuary by PERMANOVA analysis (Table 2.2). Contrarily, significant seasonal changes in zooplankton density were only observed at the Mpenjati Estuary (Table 2.3). Significant variations were based in differences between samples of low and high zooplankton density at both estuaries.

Mean zooplankton biomass varied from 0.003 ± 0.002 (SD) g·m^{-3} on June 2010 (dry season) to 0.064 ± 0.060 (SD) g·m^{-3} on November 2010 (wet season) at Mlalazi Estuary. At Mpenjati Estuary, zooplankton biomass ranged from 0.028 ± 0.024 (SD) g·m^{-3} on February 2011 (wet season) to 0.348 ± 0.341 (SD) g·m^{-3} on May 2011 (abnormal high rainfall during the dry season). At the Mlalazi estuary, *Acartia* sp. was the largest contributor to the total biomass accounting for 20 to 86 % during November 2010 and February 2011. The copepod P. *hessei* contributed from 1 to 20 % to the total biomass during September 2010 and May 2011 respectively. The mysid *Mesopodopsis africana* and *Rhopalophthalmus* sp. collectively contributed from 2 to 78 % of the total biomass during February 2011 and November 2010. The copepod *P. hessei* accounted for 1 to 52 % of the total biomass at the Mpenjati estuary during May and February 2011, similarly the mysid *M. africana* contributed from 60 (November 2010) to 98 % (September 2010 and May 2011) to the total biomass at this estuary.

PERMANOVA analysis found a significant season × station interaction effect at the Mlalazi and Mpenjati Estuary (Table 2.2, 2.3). However, no significant pair-wise comparisons were recorded among stations at the Mpenjati Estuary.

Macrobenthic invertebrate density and biomass

Mean macrobenthic density ranged from 2 797 ± 2 329 ind·m^{-2} in February 2011 (wet season) to 29 350 ± 30 248 ind·m^{-2} in September 2010 (dry season) at the Mlalazi Estuary. In the Mpenjati Estuary, macrobenthic density ranged from 4 938 ± 4 619 ind·m^{-2} in November 2010 (wet season) to 20 044 ± 7 034 ind·m^{-2} in May 2011 (abnormal high rainfall during the dry season). At the Mlalazi estuary, the largest contributor to the total density was the tanaid *Apseudes digitalis* accounting for 29 to 38 % during May and February 2011. The contribution of the macrobenthic species to the total density varied seasonally, i.e. the bivalve *Brachidontes virgilae* was the second largest contributor to total density during November 2010 and May 2011, but the amphipod *Corophium triaenonyx* was the second most abundant species during September 2010. At the Mpenjati Estuary, polychaetes from the family Spionidae accounted for 23 to 78 % of the total density during May 2011 and November 2010. The polychaetes *Desdemona ornata* (14-25 %) and *Capitella capitata* (1-25%) also largely contributed to the total density in this estuary.

A significant season × station interaction effect in the macrobenthos density was recorded at the Mlalazi Estuary (Table 2.2) and a significant main effect of season at the Mpenjati Estuary (Table 2.3). PERMANOVA analysis reported significant differences in the macrobenthos density between dry and wet months at both systems.

Mean macrobenthic biomass varied from 7.891 ± 9.221 g·m^{-2} in May 2011 (dry season) to 98.039 ± 165.54 g·m^{-2} in November 2010 (wet season) at the Mlalazi Estuary. In the Mpenjati Estuary, macrobenthic biomass ranged from 0.114 ± 0.082 g·m^{-2} in November 2010 (wet season) to 2.055 ± 2.088 g·m^{-2} in May 2011 (abnormal dry season). At the Mlalazi Estuary, the bivalves *B. virgilae* and *Dosinia hepatica* dominated the macrobenthos biomass together they accounted for 38 to 92 % of the total biomass. At the Mpenjati Estuary, polychaetes from the family Spionidae largely contributed to the macrobenthic biomass (6-85 %), similarly the polychaetes *Ceratonereis keiskama* and *Dendronereis arborifera* together accounted for 8 to 56 % of the total biomass.

A significant season × station interaction effect was detected on the macrobenthos biomass at the Mlalazi and Mpenjati estuaries (Table 2.2 and 2.3). However, no significant pair-wise comparisons were recorded between stations at the Mpenjati Estuary.

Overall, significant seasonal variations were recorded for the planktonic and benthic communities of both estuaries. Marked increases in phytoplankton biomass, zooplankton density and biomass were observed during the wet season in the Mlalazi Estuary. Contrarily, significant decreases in these community properties were recorded during the wet season at the Mpenjati Estuary. At both systems, macrobenthos density and biomass were significantly higher during the dry season (or closed phase for the TOCE Mpenjati). Significant spatial differences were recorded for the zooplankton and benthic communities at the Mlalazi but not at the Mpenjati Estuary.

Table 2.2. PERMANOVA results of biological parameters for the Mlalazi Estuary. Values in bold indicate significant differences.

Source	df	SS	MS	Pseudo-F	P(perm)	Unique perms	P(MC)
Chlorophyll-a concentrations							
Season	4	18622	4655.4	7.3185	**0.0002**	4990	0.0004
Station	3	2482.2	827.4	1.3007	0.2788	4978	0.272
Residuals	12	7633.4	636.11				
Total	19	28737					
Macrobenthos density							
Season	3	18765	6254.9	13.103	**0.0001**	9930	0.0001
Station	3	33212	11071.00	23.191	**0.0001**	9919	0.0001
Sea × St	9	21229	2358.80	4.9413	**0.0001**	9855	0.0001
Residuals	32	15276	477.36				
Total	47	88481					
Macrobenthos biomass							
Season	3	29846	9948.7	6.6358	**0.0001**	9891	0.0001
Station	3	51547	17182	11.461	**0.0001**	9886	0.0001
Sea × St	9	36346	4038.4	2.6937	**0.0001**	9858	0.0001
Residuals	32	47976	1499.2				
Total	47	1.66E+05					
Zooplankton density							
Season	4	7492.3	1873.1	6.9986	**0.0002**	4978	0.0002
Station	3	2970.6	990.21	3.6999	**0.0004**	4981	0.0006
Sea × St	12	8550.3	712.53	2.6623	**0.0004**	4973	0.0004
Residuals	18	4817.4	267.64				
Total	37	23349					
Zooplankton biomass							
Season	3	6478.4	2159.5	2.6386	**0.0135**	9940	0.0159
Station	3	8061.7	2687.2	3.2834	**0.0016**	9935	0.0032
Sea × St	9	19414	2157.1	2.6357	**0.0008**	9899	0.0017
Residuals	14	11458	818.42				
Total	29	43969					

Table 2.3. PERMANOVA results of biological parameters for the Mpenjati Estuary. Values in bold indicate significant differences.

Source	**df**	**SS**	**MS**	**Pseudo-F**	**P(perm)**	**Unique perms**	**P(MC)**
Chlorophyll-a concentrations							
Season	4	21887	5471.9	14.462	**0.0002**	4986	0.0002
Station	2	681.28	340.64	0.9003	0.4846	4993	0.4744
Residuals	8	3026.9	378.36				
Total	14	25596					
Macrobenthos density							
Season	3	19183	6394.3	8.335	**0.0001**	9923	0.0001
Station	2	3908.6	1954.30	2.5475	0.0257	9939	0.0279
Sea × St	6	7735	1289.20	1.6804	0.0633	9934	0.0705
Residuals	21	16110	767.17				
Total	32	47340					
Macrobenthos biomass							
Season	3	26143	8714.3	10.112	**0.0001**	9925	0.0001
Station	2	7740.2	3870.1	4.4909	**0.0001**	9940	0.0004
Sea × St	5	12436	2487.3	2.8863	**0.0004**	9917	0.0007
Residuals	19	16373	861.76				
Total	29	61565					
Zooplankton density							
Season	3	5881.9	1960.6	16.253	**0.0001**	9948	0.0001
Station	2	1252.4	626.22	5.1911	**0.0005**	9951	0.0025
Sea × St	6	1653.4	275.56	2.2843	0.0260	9924	0.0316
Residuals	12	1447.6	120.63				
Total	23	10235					
Zooplankton biomass							
Season	3	20254	6751.3	17.054	**0.0001**	9954	0.0001
Station	2	4009.7	2004.8	5.0643	**0.001**	9959	0.0021
Sea × St	6	13280	2213.3	5.5909	**0.0003**	9933	0.0002
Residuals	12	4750.6	395.88				
Total	23	42294					

2.3.3. Variability of the community-level properties

No significant seasonal differences in the coefficient of variation (CV) of the different community-level properties were detected at the Mlalazi and Mpenjati estuaries ($p > 0.05$). This indicated that the changes in seasons did not influence the variability of the community-level properties, even when significant differences among seasons were reported for phytoplankton biomass, as well as zooplankton and macrobenthic invertebrate density and biomass. Although significant seasonal changes were recorded for particular estuarine assemblages within these systems, the variability in each parameter remained relatively constant through time.

Our results indicate that phytoplankton biomass (as chl-a) showed the lowest variability among the community-level properties in both estuaries, varying from 0.16 ± 0.13 for the Mlalazi Estuary and from 0.24 ± 0.18 for the Mpenjati Estuary (Fig. 2.2). Contrarily, macrobenthic biomass showed highest variability and varied from 0.82 ± 0.37 at the Mlalazi Estuary and from 0.73 ± 0.39 at the Mpenjati Estuary (Fig. 2.2). No significant interaction effect of type of community-level property × estuary in the CV was reported by the two-way ANOVA test ($F_{(4,141)}$=1.199, p= 0.314). However, significant differences were reported in the CV among community-level properties (p = 0.019). The CV of phytoplankton biomass was significantly different ($p < 0.01$) from the CV of all other community properties. Significant differences were also found between the CV of macrobenthos biomass and zooplankton density and biomass (p=0.002 and p=0.045 respectively).

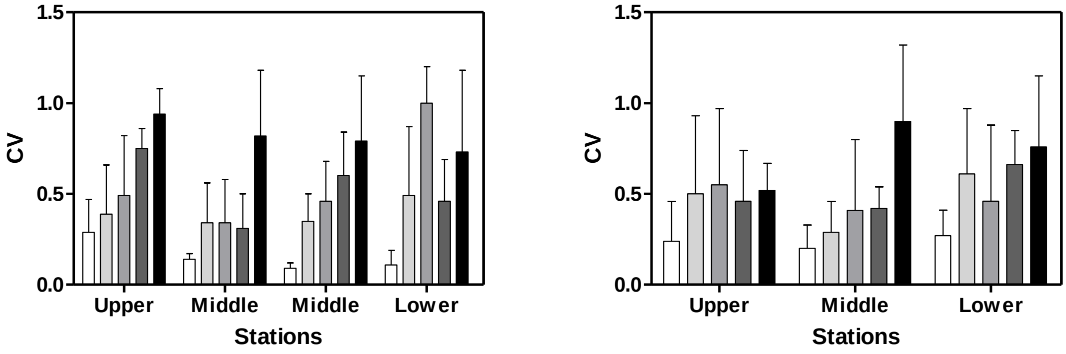

Figure 2.2. Mean variability among stations of the analysed community level-properties at the Mlalazi (left) and Mpenjati (right) estuaries. Colours indicate community-level property (white= phytoplankton biomass, light grey= zooplankton density, grey=zooplankton biomass, dark grey= macrobenthos density and black=macrobenthos biomass).

2.3.4. Stability of community-level properties

Temporal stability of community-level properties showed small variations, but was overall slightly lower in the Mpenjati Estuary. Community stability varied from 0.87 ± 0.61 (mean among stations, zooplankton biomass) to 1.75 ± 0.31 (macrobenthos density) at the Mlalazi Estuary (Fig. 2.3a). Similarly, *S* ranged from 0.72 ± 0.09 (macrobenthos biomass) to 1.15 ± 0.58 (zooplankton density) at the Mpenjati Estuary (Fig. 2.3a).

Regarding the possible stabilizing mechanisms of community stability, the overall lowest and highest species synchrony was recorded for the macrobenthos and phytoplankton biomass at the Mlalazi Estuary respectively (Fig. 2.3b). Zooplankton biomass and macrobenthos density exhibited the lowest and highest species synchrony respectively in the Mpenjati Estuary (Fig. 2.3b), highlighting the differences in synchrony for a given community property between systems. Macrobenthos density showed the lowest species dominance in both systems, zooplankton density and biomass showed the highest species dominance at the Mlalazi and Mpenjati estuaries respectively (Fig. 2.3c). Consequently, the lowest species richness was recorded for the zooplankton density and the highest by the macrobenthos biomass at both systems (Fig. 2.3). It is probably that if the phytoplankton species composition would have been analysed, this would have had the highest species richness among community-level properties. Similarly, the stability of phytoplankton species could exhibit a different trend what there is when using size classes.

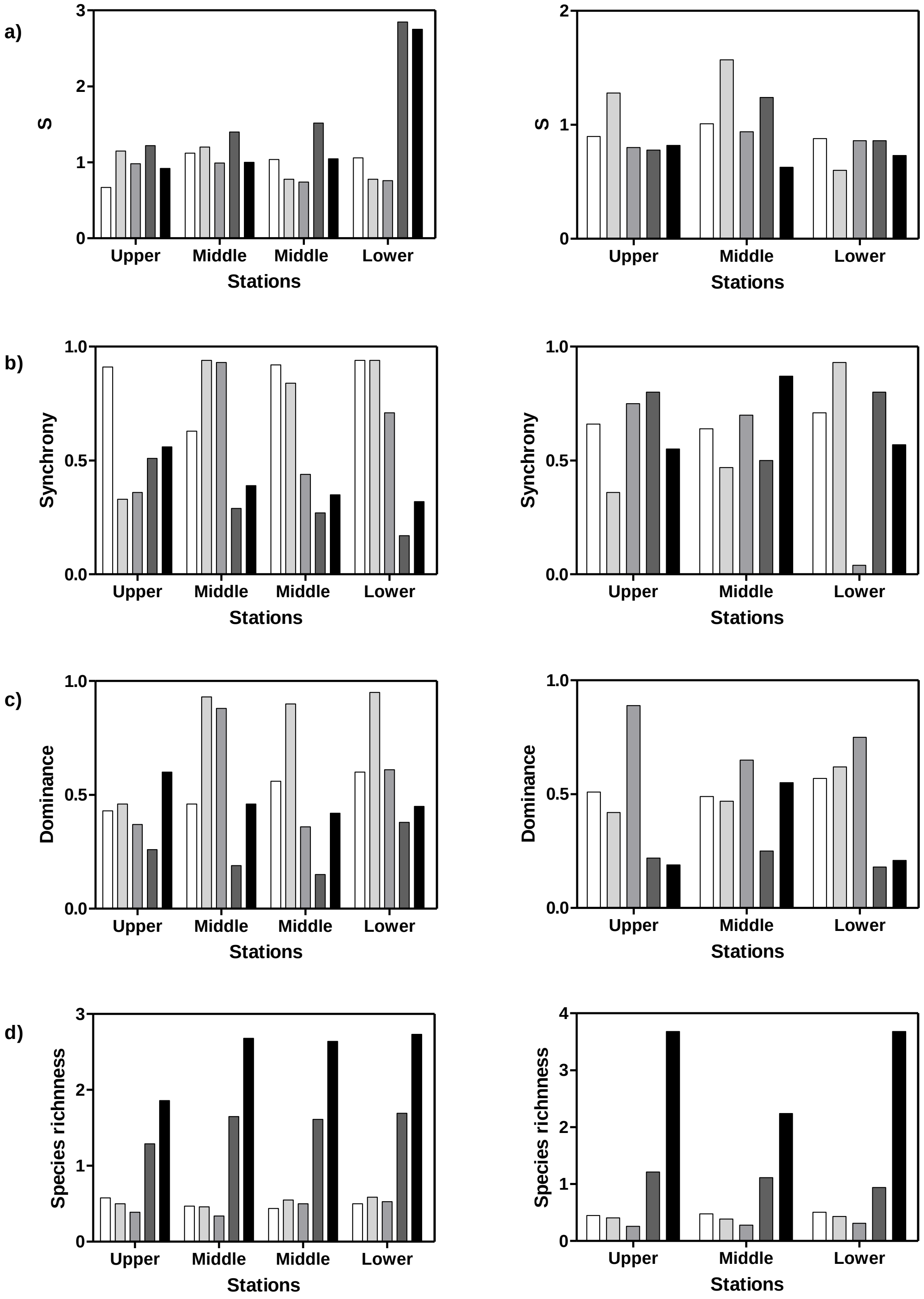

Figure 2.3. Differences in community attributes at the Mlalazi (left) and Mpenjati (right) estuaries, a) Temporal Stability b) Species synchrony, c) Species dominance and d) Species richness. Description of parameters are listed in Figure 2.2.

Pearson correlation analysis showed that the temporal stability (S) significantly increased with species richness at the Mlalazi Estuary (Table 2.4). *S* significantly decreased with an increase in species synchrony at this system (Table 2.4). At the Mpenjati Estuary, temporal stability showed no significant correlations with species diversity, dominance, evenness or synchrony. However, *S* showed a significant positive correlation with mean total community property (density/biomass) (Table 2.4).

When the temporal stability and stabilizing mechanisms data from both systems were pooled, Pearson correlation analysis showed a significant negative correlation between the temporal stability and species synchrony, but with none of the other stabilizing mechanisms. This may indicate that species synchrony was the most important mechanism influencing community stability in these systems (Table 2.4).

Table 2.4. Pearson correlation between the temporal stability and community properties at the Mlalazi and Mpenjati estuaries. Only significant correlations are included ($p < 0.05$).

Parameters	**r^2**	**n**	***p***
Mlalazi Estuary			
Synchrony	-0.550	20	0.012
Species richness	0.512	20	0.021
Mpenjati Estuary			
Mean total community property	0.735	15	0.002
Both systems			
Synchrony	-0.521	35	0.001

2.4. Discussion

In this study, we determined the spatio-temporal variations of environmental variables and biological communities, as well as the variability and temporal stability of five different community-level properties (e.g. phytoplankton biomass, zooplankton and macrobenthos density and biomass) in two estuaries in South Africa. Environmental variables showed significant spatio-temporal variations at both estuaries throughout the study period. Our study also detected significant spatio-temporal changes in the communities of the permanently open Mlalazi Estuary, whereas significant seasonal changes, but not spatial, were observed in the communities of the Mpenjati Estuary. Contrarily, the variability of these community-level properties did not show significant seasonal changes, however significant differences were reported for the variability among properties, with macrobenthos biomass exhibiting the highest variability in both systems and phytoplankton biomass the lowest. The temporal stability (*S*) was similar among community-level properties and between systems, and species synchrony was found to be the main driver explaining the stability of these communities in both systems.

Phytoplankton biomass, zooplankton density and biomass responded differently to the dry/wet cycle in each type of estuary. In the permanently open Mlalazi Estuary, chlorophyll-a concentrations, zooplankton density and biomass were significantly higher during the wet season when compared to the dry season. Similar differences were observed in the temporarily open/closed Mpenjati Estuary except that the highest chlorophyll-a concentrations and zooplankton biomass were recorded during the closed phase (dry season). The highest planktonic density and biomass during the wet season in the Mlalazi Estuary are attributed to the increase in freshwater inflow and nutrient input to this estuary during the wet season (Adams et al., 1999; Wooldridge, 1999). Conversely, the high water residence time and low physical disturbance during the closed phase in TOCEs are key to explaining the increased phytoplankton and zooplankton biomass recorded during this phase, despite river inflow being at a minimum (Froneman, 2006; Skinner et al., 2006; Whitfield, 1980).

Macrobenthic invertebrate density and biomass were significantly lower during the wet season (increased river inflow) in both types of systems. Contrarily, Gladstone et al. (2006) found no significant differences in the macrobenthic community structure at the entrance barriers of four TOCEs in New South Wales, following two mouth breachings. However, Netto et al. (2012) reported a reduction of 50 and 90 % in zoobenthic biomass and density in Camacho lagoon, South Brazil due to the scouring and flushing of sediments after a mouth breaching. In this study, macrobenthic invertebrate communities, independently of estuary type, showed a similar negative response to increased river flow and mouth opening during the wet season. The significant reduction in macrobenthic density and biomass during this season could be attributed to the increased freshwater scour which created instability and habitat loss for the remaining zoobenthos.

Despite significant seasonal differences reported for planktonic and benthic density and biomass in both estuaries, the variability (as $CV = \sigma/\mu$) of phytoplankton biomass (as chl-a), zooplankton and macrobenthic invertebrate density and biomass did not show significant seasonal differences through the study. This suggests that the marked seasonal environmental changes do not influence the variability of these communities in both systems. We propose that the lack of significant seasonal differences in the variability of the analysed community-level properties rather indicate that seasonal reductions in the mean of these properties were compensated by a simultaneous decrease in the standard deviation. This compensatory effect (“stable” variability) has been found to be an insurance mechanism against the effects of disturbances in a rocky shore assemblage and a marine subtidal system (Bulleri et al., 2012; Long et al., 2011).

Our analysis also found that both estuaries showed similar variability (as $CV = \sigma/\mu$) in the analysed community-level properties, with lowest CV for the phytoplankton biomass and highest for the macrobenthos biomass in both systems. These differences can be attributed to either an increase in the standard deviation, a decrease in the mean of a community-level property or both (Bulleri et al., 2012; Lehman and Tilman, 2000) and in our study, the differences in the variability are attributed to an increase in the standard deviation (in relation to its mean) from phytoplankton to macrobenthos communities. Our results are in agreement with Steiner et al. (2005), who found significantly lower variability of primary producers than consumers at the population-level in a multitrophic aquatic system (composed of bacteria, algae, heterothropic protozoa and rotifers), and attributed their results to the influence of stable dominant species at lower trophic levels. In our study, the nanoplankton size fraction was constantly dominant throughout the study period in spite of the marked seasonal changes in biomass (Ortega-Cisneros unpublished data), it is thus probably that the effect of stable dominant taxa could also have explained the lower variability of the phytoplankton biomass reported here.

The temporal stability (*S*) of the total mean density and biomass of planktonic and benthic communities was similar between estuaries. In contrast to the variability results, the temporal stability did not vary among community-level properties. *S* values in our study areas were considerably lower than those reported for other systems exposed to high environmental fluctuations e.g. rocky shore benthic assemblages (Bulleri et al., 2012; Valdivia et al., 2013). Our *S* values were however comparable with *S* of macroinvertebrate communities in the Mira and Mondego estuaries in Portugal (Pinto et al., 2013). Unfortunately, it was not possible to compare the temporal stability (*S*) of the community properties analysed here with those of other estuaries, due to the unavailability of such studies.

Stability is mainly controlled by species synchrony, statistical averaging, species dominance and richness (Doak et al., 1998; Lehman and Tilman, 2000; Loreau and de Mazancourt, 2008; Tilman, 1999). In this study, the temporal stability of the estuarine communities at the Mlalazi Estuary was mainly driven by species synchrony and richness. None of these mechanisms had an effect on the *S* of the community-level properties at the Mpenjati Estuary, but *S* was significantly correlated to the mean community-level property because slightly higher *S* values were associated to a few very high mean zooplankton densities at this estuary. Similarly, Pinto et al. (2013) reported a stronger association of *S* with species abundance than to species richness of macrobenthic communities at the Mira and Mondego estuaries. The lack of a significant species richness-stability relationship in the Mpenjati Estuary could be related to a higher influence of environmental variability in this system (closed and open phases, narrower range of *S* values) when compared to the Mlalazi Estuary (Romanuk and Kolasa, 2002). The lack of a positive relationship between species richness and stability in the Mira, Mondego (Pinto et al., 2013) and Mpenjati estuaries (this study) confirms that a high diversity is not necessary for the successful functioning of complex systems as estuaries (Elliott and Quintino, 2007). In terms of species synchrony, asynchronous fluctuations are enhanced by interspecific competition and the presence of species with differential responses to stress (Tilman, 1999). Synchronous fluctuations have been related to a higher degree of environmental forcing and are expected to increase with an increasing gradient of environmental stress (Bertness and Callaway, 1994; Houlahan et al., 2007; Valone and Barber, 2008), although biological interactions can also generate positive covariances (Bulleri et al., 2012; Steiner et al., 2005). It is thus suggested that the prevalence of environmental forcing (e.g. natural stress) in estuarine systems is key to explaining the role of species synchrony as a main driver of the community stability in these systems. Overall, we suggest that stable variability and species synchronization could be the mechanisms used by the estuarine biota of these two systems to compensate for environmental changes and reach environmental homeostasis (*sensu* Elliott and Quintino, 2007).

Our mesoscale results did not provide support for the diversity-stability relationships in the planktonic and benthic communities of these two estuaries, since species richness was not the main stability driver in both studied systems. Species synchrony was overall the most important mechanism contributing to community stability in these estuaries, similar results have been reported in other systems exposed to a high degree of environmental forcing (Bulleri et al., 2012; Campbell et al., 2011). Despite the statistically significant variations in total community density and biomass throughout the study, the variability of the analysed community-level properties did not vary seasonally in both estuaries. This stable variability constitutes a mechanism to cope with environmental fluctuations in these systems. In conclusion, our results suggest that species synchrony and stable variability are the mechanisms responsible for the environmental homeostasis of these two estuaries. Our study reiterates the need for the establishment of specific management programs for estuaries, which recognize that estuarine environmental variability constitute a subsidy to their biota and not an stress. However, it is first necessary to determine thresholds in the measures of stability which allows us to detect state changes within and across estuarine systems.

Temporal patterns in planktonic and benthic elemental composition and stoichiometry in two sub-tropical estuaries, KwaZulu-Natal, South Africa

3.1. Introduction

Ecological stoichiometry focuses on studying the balance of elements in ecological interactions and processes (Sterner and Elser, 2002). Ecological stoichiometry has helped to provide a better understanding of ecosystem functioning and dynamics, because it allows comparisons among organisms and ecosystems by expressing their composition in biogeochemical terms (Sterner and Elser, 2002). Several principles have been developed in ecological stoichiometry theory, which aim to explain the characteristics of elemental composition and ratios in ecosystems. Stoichiometric homeostasis is one of these principles and refers to the capacity of an organism to maintain a constant chemical composition regardless of variations in the composition of its resource nutrient content (Sterner and Elser, 2002). Homeostasis can be either weak, when an organism changes its elemental composition according to its resource (e.g. autotrophs are assumed to have a weak homeostasis), or strict, when the elemental composition is kept constant despite the variability in the elemental ratios of food sources (e.g. most heterotrophs) (Elser and Urabe, 1999; Hessen and Bjerkeng, 1997; Wang et al., 2012). Latter studies have however indicated that heterotrophs may not be as strictly homeostatic as assumed (Chrzanowski and Grover, 2008; DeMott, 2003; DeMott and Pape, 2005; Mulder and Bowden, 2007). Persson et al. (2010) analysed 132 datasets from published studies to test the generality of the strict homeostasis assumption; they found that autotrophs were generally less homeostatic than heterotrophs but heterotrophic species also differ in their degree of homeostatic regulation. Moreover, these authors suggest that the degree of homeostasis is strongly influenced by environmental and physiological factors.

Stoichiometry imbalances are caused by the differences in elemental composition between primary producers (i.e. nutrient poor, high C:nutrient ratio) and consumers (i.e. nutrient rich, low C:nutrient ratio), which use diverse physiological mechanisms to compensate for the elemental imbalance (Elser et al., 2000; Sterner and Hessen, 1994). Large elemental imbalances between primary producers and consumers can modify the growth, reproduction and nutrient release of an organism, with consequences at the community and ecosystem level (Elser et al., 1988; Elser and Urabe, 1999; Sterner and Hessen, 1994). Consumer-driven nutrient recycling is caused by imbalances in consumer-resource stoichiometry, e.g. when species with a low N:P ratio accumulate P and recycle N in excretion (Elser et al., 1988). The excreted material will eventually release the surplus nutrients back into the environment, where this will be assimilated by the primary producer once again. Consumer-driven nutrient recycling may thus determine the overall nutrient limitation regime experienced in a system (Elser et al., 1988; Elser and Urabe, 1999).

Overall, consumer nutrient content is ultimately determined by diverse environmental and biological factors e.g. calcium concentrations, pH and temperature, or when the nutrient content of its resource is constant (Chrzanowski and Grover, 2008; McGrath and Quinn, 2000; Tan and Wang, 2009).

Ecological stoichiometry theory has been used to determine ecosystem level changes by analysing the quality and quantity of nutrients and food sources (e.g. Glibert et al., 2011; Sterner and Elser, 2002). Nutrient imbalances therefore have further implications for consumers, e.g. extra expenditure of energy in compensatory feeding and processing the surplus C to maintain their nutrient ratio (Elser et al., 1988). For instance, terrestrial and freshwater herbivores facing strong nutritional constraints exhibited reduced gross growth efficiency (DeMott et al., 1998; Elser et al., 2000). Lower abundances of primary producers and herbivorous consumers have also been recorded under nutrient limitations, with these constraints affecting the survival, reproductive output and biochemical composition of the herbivores, which will ultimately impact the food quality and quantity of higher order consumers (Schoo et al., 2012). In this sense, nutrient enrichment (e.g. from pollution) may result in long-term alterations in the community structure, secondary production and food web dynamics of ecosystems, by alleviating the nutrient limitations of certain consumers and facilitating their growth (Cross et al., 2003; Glibert et al., 2011; van de Waal et al., 2009).

The empirical support for ecological stoichiometry theory has been mainly derived from studies on zooplanktonic species, with less research focused on benthic taxa (Frost et al., 2003). Similarly, most stoichiometric studies have been conducted in terrestrial and freshwater environments (Sterner and Elser, 2002). There is, however, a scarcity of stoichiometry studies on detritus-based food webs, despite the fact that they constitute the dominant energy pathway in most aquatic ecosystems (Cross et al., 2003 and references therein). In this sense, almost no studies have been conducted to investigate the stoichiometry of estuarine organisms at the population or community-level.

Estuaries are highly productive ecosystems and provide several important ecosystem services, including nursery areas for invertebrate and fish species and high nutrient recycling (Constanza et al., 1997). Estuaries are nonetheless among the most impacted of all marine-linked ecosystems (Lotze et al., 2006). The growing demand for freshwater, food, recreation and waste disposal in the river catchment impose numerous threats to estuaries, including habitat degradation and pollution (Hale et al., 2004; Ridgway and Shimmield, 2002). These activities alter the concentrations of nutrients such as nitrogen and phosphorus entering estuaries due to increased erosional processes, effluents from fertilized urban and agricultural land and sewage treatment plants (Howarth et al., 2002; Nedwell et al., 2002). The application of the ecological stoichiometry theory to estuaries can provide better insights into the functioning of these systems, e.g. stoichiometry analysis showed that sewage derived particulate organic matter enhanced the growth of P-rich consumers and modified the community structure towards P-rich taxa in a stream in Austria (Singer and Battin, 2007).

The KwaZulu-Natal coast is located on the north-eastern side of South Africa and is influenced by a subtropical climate characterized by two marked seasons, the rainy season (October – April) with strong river inflow to estuaries and the dry season (May - September) with lower average rainfall and negligible river inflow to estuaries. The seasonal variability in rainfall and river inflow have been found to produce significant spatio-temporal changes in the estuarine communities of this region (Chapter 1 and 2, Perissinotto et al., 2010 and references therein). If seasonal variations in rainfall and river inflow cause temporal variations in estuarine communities, it can be expected that these temporal changes may influence the elemental composition and ratios of estuarine planktonic and benthic communities.

This study aims to provide an insight into the understanding of the stoichiometry in selected sub-tropical estuaries by analysing the elemental composition of abiotic sources, as well as that of the zooplankton and benthic macroinvertebrate species in two estuaries with contrasting mouth status in KwaZulu-Natal. The specific objectives were to:

1) Determine carbon and nitrogen biomass of the abiotic and biotic components of the Mlalazi and Mpenjati estuaries from May 2010 to May 2011.
2) Examine if the elemental composition (% C, % N and C:N ratio) of suspended and sediment detritus exhibit spatio-temporal variations at the Mlalazi and Mpenjati estuaries.
3) Establish if the elemental composition and ratios of selected zooplankton and macrobenthic species vary seasonally at the Mlalazi and Mpenjati estuaries.
4) Determine if the elemental composition of invertebrates differed among zooplankton and macroinvertebrate species.
5) Analyse the carbon and nitrogen signatures of abiotic and biotic components to determine their trophic position at the Mlalazi and Mpenjati estuaries.

3.2. Materials and Methods

3.2.1. Study sites

This study was conducted in two estuaries on the KwaZulu-Natal coast within the subtropical biogeographical region (Fig. 3.1). The Mlalazi Estuary (28° 56'42" S; 31°48' 58" E) is a permanently open system with a catchment area of approximately 492 km^2 (DEAT, 2001) and an estuarine area of 95.86 ha (van Niekerk and Turpie, 2012). Subsistence farming, sugar cane and commercial forestry accounts for approximately 46% of the catchment usage, with 53% of the catchment considered undegraded and 1% urban.

The Mpenjati Estuary (30°58'21"S, 30°17'02"E) is a temporarily open/closed system (Whitfield, 1992) which experienced two mouth openings during the study period. This estuary was connected to the sea from the end of October 2010 to mid-April 2011 and from mid-May to July 2011 during an abnormal wet winter. Its catchment area is approximately 101 km^2 and the estuary area is 11.6 ha (van Niekerk and Turpie, 2012). Most of the catchment is used for banana and sugar cane farming. A waste water treatment plant is located alongside the upper reaches of estuary and discharges treated water into the system. Both systems are part of nature reserves and considered to be in a good condition (Whitfield and Baliwe, 2013).

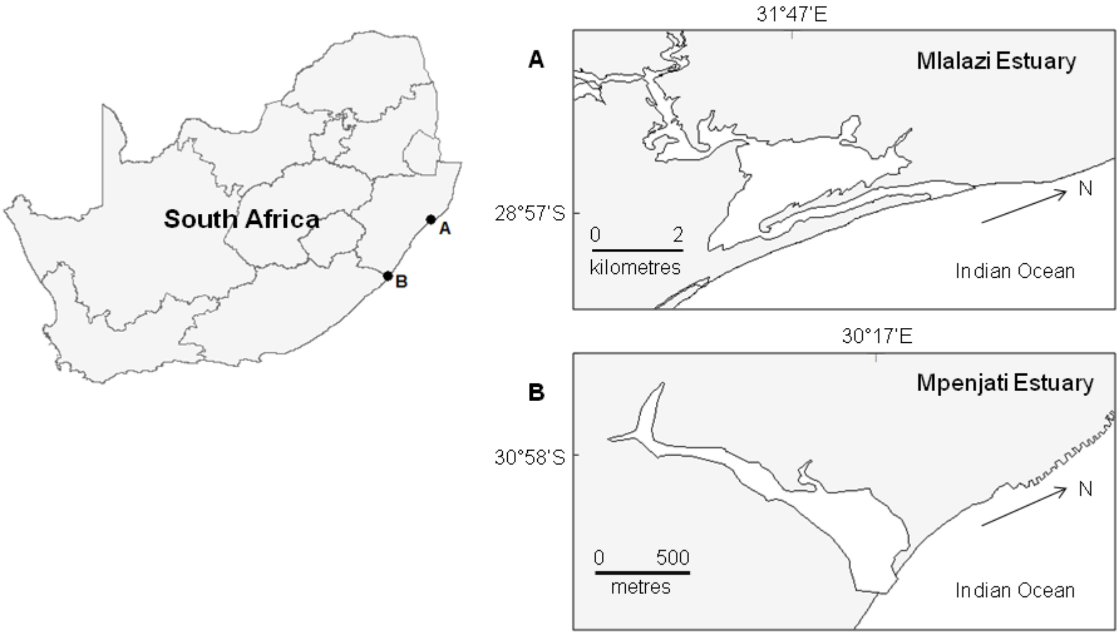

Figure 3.1. Map of the study areas (Mlalazi and Mpenjati estuaries) on the east coast of South Africa.

3.2.2. Sampling procedure

Biological and environmental samples were collected at the Mlalazi and Mpenjati estuaries between May 2010 and May 2011. Quarterly monitoring was conducted at three stations in the Mpenjati Estuary and four stations in the Mlalazi Estuary (Fig. 3.1), with the difference in number of stations being based primarily on the differences in estuary length. The stations were located so that representative spatial samples were collected in the upper, middle and lower reaches of the respective estuaries.

For the determination of seston (particulate organic carbon and nitrogen), triplicate water samples were collected in acid washed plastic bottles from ca. 50 cm depth at each station and subsequently stored in the dark on ice. Three 100 ml samples of water filtrate from seston determinations were collected in acid washed bottles and frozen at -20 °C for the determination of dissolved inorganic nitrogen (DIN: nitrate+nitrite+ammonia). Sediment nutrient concentrations (C, N) were determined by collecting three sediment samples at each station to a depth of 2.5 cm using a twin-corer (internal diameter 2 cm).

For the determination of size-fractionated chlorophyll-a, triplicate sub-surface samples were serially filtered through 20 µm Nitex mesh, 2 µm membrane filters, and 0.72 µm GF/F filters. Similarly, three sediment samples to a depth of 3 cm were taken using a sediment corer (internal diameter 2 cm) at each station and placed in 50 ml polyethylene bottles for the determination of microphytobenthos. Pigments were extracted by placing the sediment core into 10 ml of 90 % acetone for 24 hours in the dark at -4°C.

Zooplankton samples were collected using a hyperbenthic sled with a 200 µm mesh plankton net during daytime. The sled was towed at a speed of 1-2 knots at each station for 27 m. Two replicates were collected at each station and then preserved in 5% formalin with Rose Bengal. Macrobenthos samples were collected using a Zabalocki-type Ekman grab (sampling area 0.024 m^2, 10 cm depth) at each station during September 2010. For the rest of the study, macrobenthos samples were collected with a van Veen grab (sampling area 0.025 m^2, 10 cm depth). Three replicates were collected at each station, each consisting of three grab samples to collect sufficient material for subsequent analysis. Macrobenthos samples were filtered using a 500 µm sieve and the contents preserved in 5% formalin using Rose Bengal to aid the extraction of invertebrates.

3.2.3. Biomass determinations

Phytoplankton and microphytobenthos chlorophyll-a concentrations (as a proxy for biomass) were estimated using a Turner Trilogy fluorometer (Sunnyvale, California, US) based on the method of Holm-Hansen and Riemann (1978). Zooplankton samples were suspended in 1 to 5 L solutions, depending on the concentration of organisms. From each solution, three 20 ml subsamples were withdrawn at mid-depth, while stirring continuously to ensure homogenous suspension (Jerling and Wooldridge, 1991a; Perissinotto and Wooldridge, 1989a). Organisms were then identified and counted using a dissecting microscope. Dry weight was determined for the most representative species of the zooplankton (accounting for 99 % of the abundance) after oven-drying for 48 h at 60°C. Similarly, individuals from macrobenthos samples were identified, counted and weighed after oven-drying for 48 h at 60°C.

3.2.4. Elemental analysis

Seston samples (Particulate organic carbon and nitrogen) were filtered through a 0.72 µm pre-combusted and pre-weighted GF/F filter, with the filters containing POM then being dried at 60°C for 24 h. Afterwards, the filters were stored in aluminium foil bags within a dessicator until further analysis. To determine sediment nutrient content, sediment samples (~ 8 g) were dried at 60°C for 48 h, ground to ensure homogenization and then acidified using 2% HCl solution to remove carbonates. Afterwards, sediment samples were rinsed with Milli-Q water and then dried again. Dissolved nitrogen determinations were conducted using a Skalar San++ Continuous-flow Analyser (Skalar Analytica BV, The Netherlands) according to the method of Mostert (1983).

After oven-drying at 60° for 48 h, zooplankton and macrobenthos samples were placed in 1.5 ml polypropylene micro centrifuge tubes. For the elemental composition analysis of zooplankton species, one replicate consisted of three up to several hundred individuals of each species depending of the size and weight of the organisms. For macrobenthos samples, three to a hundred individuals were pooled per replicate. Foot muscle tissue was preferably used for bivalves unless individuals were too small to make muscle tissue extraction possible. Macrobenthic species containing $CaCo_3$ were acidified (e.g. crabs) with 2% HCl to remove carbonates. The drop-by-drop acidification technique was used, 2% HCl was added to the samples until all $CaCo_3$ was removed and no more bubbles were evident (Jacob et al., 2005). Thereafter, the samples were washed in distilled water to eliminate acid presence and once again oven-dried at 60°C for 48h.

Ideally, three replicates (composed of several individuals) were analysed for each species and sampling events, except when a given species was not present on a particular sampling date or when the material available was insufficient for analysis. Thus, number and composition of species used for nutrient analysis differed among sampling events. All zooplankton and macrobenthos samples were crushed and homogenized with a glass rod within the centrifuge tubes to avoid loss of the reduced material available for some samples.

Between 5 and 150 mg was required for the elemental analysis of sediment samples, mainly because the nutrient content of some samples was too low to provide adequate nutrient determinations. From 0.5 to 1.2 mg was required for C and N analysis of zooplankton and macrobenthic species.

Carbon and nitrogen content (%), molar C:N ratios and isotope signatures were obtained for all samples using an ANCA-SL elemental analyser coupled to a Europa Scientific 20-20 isotope ratio mass spectrometer (IRMS) (Europa Scientific Limited, Crewe, England). Samples were analysed at Isoenvironmental cc, Rhodes University, Grahamstown. Beet sugar, ammonium sulphate and five certified protein standard casein were used as international standards and calibrated against International Atomic Energy Agency [IAEA] standards IAEA-CH-6 and IAEA-N-1. The analytical precision of the IRMS was 0.21 ‰ for $^{15}N/^{14}N$ and 0.17 ‰ for $^{13}C/^{12}C$. Results were calculated and expressed in the standard delta notation as:

$$\delta^{13}C\ (‰) = ([R_{sample}/Rs_{tandard}] - 1) \times 1000$$

$$\delta^{15}N\ (‰) = ([R_{sample}/R_{standard}] - 1) \times 1000$$

where R is the ratio of $^{15}N{:}^{14}N$ or $^{13}C{:}^{12}C$ in the sample (R_{sample}) and in the standard ($R_{standard}$), expressed relative to the international standard.

3.2.5. Data analysis

In order to determine carbon and nitrogen biomass pools at the Mlalazi and Mpenjati estuaries, %C and %N results from the elemental composition analysis were used. For dissolved inorganic carbon, phytoplankton and microphytobenthos biomass, Chl-a:C (60) and C:N (6.6) ratios from the literature were used (Groffman et al., 2006; Redfield, 1958; Wang et al., 2006). Masses of the abiotic and biotic pools were depth-integrated and expressed as mg C or $N \cdot m^{-2}$. Information of the %C and %N was available for most zooplankton and macrobenthic species for each sampling event. When there was not enough material to determine the elemental composition of a species in a given season, the mean %C and %N of that species among other sampling seasons was used to calculate the carbon and nitrogen biomass. Total suspended and sediment POM can include different quantities of bacteria and phytoplankton together with detrital POM, thus in order to provide a more realistic estimate of suspended and sediment detritus, phytoplankton and microphytobenthos biomass were subtracted from total suspended and sediment POM respectively.

In order to determine spatio-temporal variations in the %C, %N and C:N ratio of suspended and sediment detritus in each estuary, separate two-way ANOVAs were conducted using seasons and stations as factors. It was not possible to evaluate spatio-temporal variations in the elemental composition of zooplankton and macrobenthic species, since not enough replicates were available for each species to adequately test for spatial variations at both estuaries. Several one-way ANOVA were then conducted to test for significant seasonal differences in the %C, %N and C:N ratio of zooplankton and macrobenthic species. The species and the number of seasons compared for each species are listed in Table 3.1.

To determine if the elemental composition of zooplankton and macrobenthic species showed significant differences among taxa, separate one-way ANOVA using taxa as factor were conducted. Significant differences were tested separately for the zooplankton and macrobenthos communities. The total number of species compared for each community is listed in Table 3.1. All pair-wise comparisons were calculated using a Tukey HSD post-hoc test, with a significance level of 0.05. Statistical analyses were conducted using the software IBM SPSS Statistics 21. Carbon and nitrogen isotope signatures ($\delta^{13}C$ and $\delta^{15}N$) of each zooplankton and macrobenthos species were averaged through the study period to provide estimates of the trophic level and/or feeding preferences of the analysed species, since seasonal averages integrate long-term variations in the isotopes signatures of these species and possible time-lags between consumer and prey.

Table 3.1. List of components/species and the type of analyses conducted during this study.

Component/Species	**Estuary**	**Analysis**	**Factors**
Suspended and sediment detritus	Mlalazi Mpenjati	Seasonal Spatial	Five seasons Three (MP) and four stations (ML)
Acartia spp. (A)	Mlalazi	Among-taxa Seasonal	Six zooplankton species Four seasons
Pseudodiaptomus spp. (P)	Mlalazi	Among-taxa Seasonal	Six zooplankton species Three seasons
Rhopalophthalmus terranatalis (Rt)	Mlalazi	Among-taxa	Six zooplankton species
Acartia natalensis (An)	Mpenjati	Among-taxa Seasonal	Six zooplankton species Three seasons
Pseudodiaptomus hessei (Ph)	Mpenjati	Among-taxa Seasonal	Six zooplankton species Four seasons
Mesopodopsis africana (Ma)	Mpenjati	Among-taxa Seasonal	Six zooplankton species Three seasons
Apseudes digitalis (Ad)	Mlalazi	Among-taxa Seasonal	Twelve macrobenthos species Four seasons
Cirratulidae (Ci)	Mlalazi	Among-taxa Seasonal	Twelve macrobenthos species Three seasons
Dosinia hepatica (Dh)	Mlalazi	Among-taxa Seasonal	Twelve macrobenthos species Three seasons
Glycera spp. (G)	Mlalazi	Among-taxa Seasonal	Twelve macrobenthos species Three seasons
Macoma litoralis (Ml)	Mlalazi	Among-taxa Seasonal	Twelve macrobenthos species Four seasons
Spionidae (SP)	Mlalazi	Among-taxa Seasonal	Twelve macrobenthos species Three seasons
Ancistrosyllis parva (Ap)	Mlalazi	Among-taxa	Twelve macrobenthos species
Paratylodiplax blephariskios (Pb)	Mlalazi	Among-taxa	Twelve macrobenthos species
Ceratonereis keiskama (Ck)	Mpenjati	Among-taxa Seasonal	Twelve macrobenthos species Three seasons

Table 3.1 continued

Component/Species	Estuary	Analysis	Factors
Capitella capitata (Cc)	Mpenjati	Among-taxa Seasonal	Twelve macrobenthos species Three seasons
Dendronereis arborifera (Da)	Mpenjati	Among-taxa Seasonal	Twelve macrobenthos species Three seasons
Spionidae (SP1)	Mpenjati	Among-taxa Seasonal	Twelve macrobenthos species Four seasons

3.3. Results

3.3.1. Carbon and nitrogen mass of abiotic and biotic components

The biomass of planktonic and benthic communities, as well as the dissolved inorganic and detrital pools (mg C or N·m^{-2}) showed marked seasonal variations in the Mlalazi and Mpenjati estuaries. Dissolved inorganic nutrients and sediment detritus fluctuated between the lowest and highest carbon and nitrogen biomass during the study period at both estuaries (Tables 3.2-3.5). In terms of biotic components, the zooplankton and macrobenthic community had the lowest and highest C and N biomass (mg·m^{-2}) respectively at the Mlalazi Estuary (Table 3.2, 3.4). In the Mpenjati Estuary, zooplankton had the lowest C and N biomass and MPB the highest biomass (Table 3.3, 3.5).

DIC and DIN pools were highest during September 2010 (dry season) and lowest during February 2011 (wet season) at both estuaries. Highest suspended detritus carbon and nitrogen mass was also recorded during September 2010 at both systems, while lowest mass was recorded during November 2010 (wet season) at the Mlalazi Estuary and during February 2011 at the Mpenjati Estuary. Lowest sediment detritus mass was recorded during February 2011 at the Mpenjati Estuary and during May 2011 (dry season) at Mlalazi Estuary. In contrast, sediment detritus mass was highest during September 2010 and November 2010 at the Mlalazi and Mpenjati estuaries respectively.

Phytoplankton carbon and nitrogen biomass were lowest during May 2011 in both systems, whereas it was highest during November 2010 at the Mlalazi Estuary and during September 2010 at the Mpenjati Estuary. The highest zooplankton C and N biomass followed the trend reported for phytoplankton biomass, whereas it was lowest during February 2011 in both systems. MPB biomass in terms of carbon and nitrogen was lower during November 2010 and February 2011, but it was higher during September 2010 and May 2011 in both estuaries. Macrobenthic carbon and nitrogen biomass was lowest during September 2010 and highest during November 2010 in the Mlalazi Estuary. In contrast, macrobenthic carbon and nitrogen biomass was lowest during November 2010 and highest during May 2011 (abnormal dry season) in the Mpenjati Estuary.

Higher carbon and nitrogen biomass for planktonic and benthic communities was recorded during the dry season (closed phase) in the Mpenjati Estuary. In contrast, planktonic communities had a higher carbon and nitrogen biomass during the wet season in the Mlalazi Estuary but benthic communities did not show an identifiable pattern linked to season in this estuary.

Table 3.2. Mean carbon mass (mg C·m^{-2} ± SD) of abiotic and biotic components in the Mlalazi Estuary during September and November 2010 and February and May 2011.

Taxa	**September-2010**	**November- 2010**	**February-2011**	**May-2011**
DIC	4.24 ± 4.17	1.18 ± 0.24	0.61 ± 0.61	3.06 ± 2.92
Suspended POC	1107.7 ± 425.6	173.9 ± 523.4	377.8 ± 837.0	741.7 ± 242.6
Sediment POC	320215 ± 35138	257349 ± 86056	213322 ± 136719	153950 ± 72303
Picoplankton	21.23 ± 13.01	119.25 ± 22.53	90.90 ± 70.68	20.24 ± 11.73
Nanoplankton	44.03 ± 9.54	625.03 ± 295.15	415.27 ± 385.80	33.33 ± 30.66
Microplankton	7.73 ± 9.90	223.60 ± 236.73	93.39 ± 150.36	4.25 ± 3.34
MPB	1183.71 ± 988.24	530.97 ± 323.86	622.50 ± 868.04	1746.29 ± 1005.32
Acartia spp.	12.24 ± 10.49	5.85 ± 4.43	13.56 ± 19.12	12.65 ± 12.45
M. africana	7.05 ± 13.89	5.62 ± 10.96	0.45 ± 0.91	1.46 ± 1.14
Pseudodiaptomus sp.	0.27 ± 0.52	0.35 ± 0.45	2.57 ± 2.96	6.22 ± 11.85
R. terranatalis	5.42 ± 6.26	40.15 ± 49.48	0.00 ± 0.00	0.00 ± 0.00
A. parva	0.93 ± 1.13	29.42 ± 23.83	8.08 ± 11.40	9.56 ± 9.79
A.digitalis	15.71 ± 25.51	161.99 ± 172.49	0.63 ± 1.22	123.74 ± 138.69
B. virgiliae	1185.7 ± 2371.3	23360.9 ± 46721.9	309.7 ± 619.5	1070.5 ± 2140.9
C. capitata	0.11 ± 0.13	10.43 ± 3.88	0.97 ± 1.20	9.30 ± 16.45
Capitellidae	6.16 ± 4.07	0.00 ± 0.00	8.35 ± 16.70	12.25 ± 24.51
C. keiskama	35.32 ± 70.58	32.12 ± 64.24	0.00 ± 0.00	1.58 ± 2.36
Cirratulidae	1.04 ± 1.22	19.69 ± 22.75	1.35 ± 2.71	12.41 ± 15.82
C. triaenonyx	40.55 ± 65.54	0.31 ± 0.62	0.01 ± 0.03	10.50 ± 14.71
Cossura sp.	0.10 ± 0.13	0.51 ± 0.60	0.21 ± 0.28	0.29 ± 0.59
L. laevigata	2.41 ± 4.81	21.32 ± 42.64	28.07 ± 56.15	5.02 ± 10.04
D.arborifera	1.91 ± 1.86	5.21 ± 10.42	0.03 ± 0.06	64.98 ± 116.44
D. ornata	0.37 ± 0.55	0.03 ± 0.03	0.04 ± 0.08	0.19 ± 0.24
Donax sp.	0.00 ± 0.00	1076.15 ± 1290.27	0.00 ± 0.00	340.46 ± 680.93
D. hepatica	305.88± 526.21	1759.43 ± 2149.91	3864.18 ± 4664.14	553.10 ± 720.51
Glycera spp.	6.69 ± 13.38	31.60 ± 56.33	69.52 ± 97.77	33.18 ± 51.15
G. bonnieroides	0.61 ± 0.70	0.00 ± 0.00	0.00 ± 0.00	0.09 ± 0.19
Grandidierella spp.	2.49 ± 1.34	0.09 ± 0.19	0.00 ± 0.00	0.15 ± 0.31
H. orbiculare	24.59 ± 49.18	4.87 ± 9.73	22.01 ± 44.01	0.00 ± 0.00
I. truncata	0.31 ± 0.40	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
M. litoralis	27.53 ± 44.54	606.13 ± 786.48	597.10 ± 530.22	85.40 ± 101.35
P. blephariskios	0.00 ± 0.00	1479.44 ± 994.12	242.26 ± 296.56	0.00 ± 0.00
P. capensis	0.00 ± 0.00	7.99 ± 15.97	0.00 ± 0.00	0.83 ± 1.66
S. cylindraceus	47.56 ± 95.12	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00

Table 3.2 continued

Taxa	September-2010	November- 2010	February-2011	May-2011
Spionidae	22.51 ± 15.75	15.88 ± 21.90	2.12 ± 1.88	32.65 ± 27.66
Tarebia granifera	511.61 ± 1023.23	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00

Table 3.3. Mean carbon mass (mg C·m^{-2} ± SD) of abiotic and biotic components at the Mpenjati Estuary during September and November 2010 and February and May 2011. (- indicates no data available).

Taxa	September-2010	November- 2010	February-2011	May-2011
DIC	15.97 ± 1.31	8.87 ± 1.25	1.52 ± 0.32	12.32 ± 1.06
Suspended POC	4092.9 ± 2677.8	622.4 ± 338.1	332.6 ± 133.7	625.7 ± 137.0
Sediment POC	24834 ± 1261	71707 ± 65445	20659 ± 9264	36788 ± 17644
Picoplankton	172.37 ± 147.08	22.58 ± 10.07	70.21 ± 40.91	5.79 ± 2.99
Nanoplankton	1213.11 ± 755.32	45.52 ± 32.15	117.16 ± 57.10	4.15 ± 2.90
Microplankton	10.69 ± 6.18	12.24 ± 2.60	16.74 ± 13.58	0.55 ± 0.78
MPB	-	1791.6 ± 1791.6	1922.8 ± 1510.5	5843.8 ± 2154.5
A. natalensis	0.23 ± 0.11	1.87 ± 0.86	7.25 ± 7.03	1.96 ± 2.15
M. africana	202.44 ± 185.10	33.72 ± 22.91	0.00 ± 0.00	170.23 ± 163.35
P. hessei	2.82 ± 2.44	17.04 ± 7.54	8.54 ± 9.66	0.90 ± 1.15
R. terranatalis	0.00 ± 0.00	17.08 ± 14.79	0.00 ± 0.00	0.00 ± 0.00
A. digitalis	0.00 ± 0.00	0.00 ± 0.00	0.03 ± 0.05	0.02 ± 0.03
C. capitata	11.85 ± 20.52	1.88 ± 2.45	15.56 ± 19.40	94.14 ± 27.84
Capitellidae	3.48 ± 6.02	0.00 ± 0.00	2.53 ± 3.09	0.61 ± 1.05
C. keiskama	12.49 ± 20.27	1.47 ± 1.99	16.26 ± 18.78	102.82 ± 31.58
C. triaenonyx	0.00 ± 0.00	0.00 ± 0.00	0.65 ± 1.13	0.32 ± 0.38
D. arborifera	11.69 ± 17.10	3.24 ± 5.61	40.34 ± 29.86	78.87 ± 30.00
D. ornata	5.33 ± 9.17	1.44 ± 1.30	2.24 ± 1.79	5.57 ± 4.12
G. bonnieroides	0.00 ± 0.00	0.00 ± 0.00	1.24 ± 1.19	0.85 ± 1.01
G. lignorum	0.00 ± 0.00	0.00 ± 0.00	0.89 ± 0.78	0.00 ± 0.00
Grandidierella spp.	3.69 ± 5.80	0.00 ± 0.00	5.64 ± 4.70	3.94 ± 2.62
I. truncata	0.20 ± 0.34	0.00 ± 0.00	0.19 ± 0.32	1.96 ± 1.78
M. litoralis	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	494.94 ± 727.71
P. capensis	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	1.00 ± 1.72
Spionidae	19.92 ± 17.31	44.80 ± 40.78	11.52 ± 8.49	52.64 ± 29.48

Table 3.4. Mean nitrogen mass (mg N·m^{-2} ± SD) of planktonic and benthic taxa in the Mlalazi Estuary during September and November 2010 and February and May 2011.

Taxa	September-2010	November- 2010	February-2011	May-2011
DIN	0.64 ± 0.63	0.18 ± 0.04	0.09 ± 0.09	0.46 ± 0.44
Suspended PON	111.3 ± 46.2	23.00 ± 73.20	44.8 ± 102.80	104.5 ± 42.4
Sediment PON	20326 ± 2923	20675 ± 7202	16649 ± 10940	10503 ± 4911
Picoplankton	3.22 ± 1.97	18.07 ± 3.41	13.77 ± 10.71	3.07 ± 1.78
Nanoplankton	6.67 ± 1.45	94.70 ± 44.72	62.92 ± 58.45	5.05 ± 4.65
Microplankton	1.17 ± 1.50	33.88 ± 35.87	14.15 ± 22.78	0.64 ± 0.51

Table 3.4 continued

Taxa	September-2010	November- 2010	February-2011	May-2011
MPB	179.35 ± 149.73	80.45 ± 49.07	94.32 ± 131.52	264.59 ± 152.32
Acartia spp.	2.96 ± 2.55	1.23 ± 1.02	3.41 ± 4.78	3.07 ± 3.06
M. africana	1.92 ± 3.78	1.53 ± 2.98	0.12 ± 0.25	0.40 ± 0.31
Pseudodiaptomus sp.	0.06 ± 0.12	0.08 ± 0.10	0.58 ± 0.68	1.53 ± 2.91
R. terranatalis	0.77 ± 1.55	10.70 ± 13.11	0.00 ± 0.00	0.00 ± 0.00
A. parva	0.20 ± 0.24	5.77 ± 4.15	1.57 ± 2.13	2.25 ± 2.29
A .digitalis	3.93 ± 6.52	37.85 ± 40.44	0.15 ± 0.29	29.96 ± 33.62
B. virgiliae	186.63 ± 373.27	3889.32 ± 7778.65	64.13 ± 128.25	162.11 ± 324.22
C. capitata	0.02 ± 0.02	1.60 ± 0.92	0.16 ± 0.19	2.22 ± 4.10
Capitellidae	1.09 ± 0.59	0.00 ± 0.00	2.18 ± 4.35	3.06 ± 6.12
C. keiskama	5.75 ± 11.50	7.25 ± 14.49	0.00 ± 0.00	0.30 ± 0.45
Cirratulidae	0.19 ± 0.22	3.10 ± 3.60	0.31 ± 0.61	1.94 ± 2.35
C. triaenonyx	9.36 ± 15.05	0.07 ± 0.14	0.00 ± 0.01	2.46 ± 3.44
Cossura sp.	0.02 ± 0.03	0.12 ± 0.14	0.05 ± 0.07	0.07 ± 0.14
L. laevigata	0.58 ± 1.02	4.89 ± 9.77	6.61 ± 12.97	1.25 ± 2.49
D. arborifera	0.44 ± 0.43	1.05 ± 2.09	0.01 ± 0.01	17.20 ± 31.19
D. ornata	0.09 ± 0.13	0.01 ± 0.01	0.01 ± 0.02	0.04 ± 0.06
Donax sp.	0.00 ± 0.00	257.14 ± 306.31	0.00 ± 0.00	78.94 ± 157.88
D. hepatica	69.88 ± 119.20	422.17 ± 531.67	982.03 ± 1179.63	134.24 ± 180.06
Glycera spp.	1.45 ± 2.91	0.00 ± 0.00	13.72 ± 20.65	8.40 ± 13.15
G. bonnieroides	0.37 ± 0.59	0.00 ± 0.00	0.00 ± 0.00	0.02 ± 0.04
Grandidierella spp.	0.54 ± 0.29	0.021 ± 0.04	0.00 ± 0.00	0.03 ± 0.07
H. orbiculare	5.63 ± 11.26	0.920 ± 1.84	4.55 ± 9.10	0.00 ± 0.00
I. truncata	0.06 ± 0.08	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
M. litoralis	6.88 ± 11.19	148.29 ± 193.34	141.05 ± 126.41	21.01 ± 24.86
P. blephariskios	0.00 ± 0.00	322.56 ± 216.33	49.53 ± 61.69	0.00 ± 0.00
P. capensis	0.00 ± 0.00	1.96 ± 3.91	0.00 ± 0.00	0.20 ± 0.41
S. cylindraceus	11.83 ± 23.66	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Spionidae	2.82 ± 1.99	3.11 ± 4.17	0.39 ± 0.35	7.89 ± 6.85
Tarebia granifera	113.58 ± 227.17	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00

Table 3.5. Mean nitrogen mass (mg N·m^{-2} ± SD) of planktonic and benthic taxa in the Mpenjati Estuary during September and November 2010 and February and May 2011. (- indicates no data available).

Taxa	September-2010	November- 2010	February-2011	May-2011
DIN	2.41 ± 0.20	1.34 ± 0.19	0.23 ± 0.05	1.86 ± 0.16
Suspended PON	238.9 ± 234.1	60.4 ± 24.7	54.8 ± 31.2	61.5 ± 8.9
Sediment PON	642 ± 1372	4866 ± 5168	1511 ± 925	1734 ± 791
Picoplankton	26.12 ± 22.28	3.42 ± 1.53	10.64 ± 6.20	0.88 ± 0.45
Nanoplankton	183.80 ± 114.44	6.90 ± 4.87	17.75 ± 8.65	0.63 ± 0.44
Microplankton	1.62 ± 0.94	1.85 ± 0.39	2.54 ± 2.06	0.08 ± 0.12
MPB	-	271.46 ± 363.42	291.33 ± 228.87	885.43 ± 326.44
A. natalensis	0.06 ± 0.03	0.47 ± 0.24	1.80 ± 1.74	0.48 ± 0.52
M. africana	54.69 ± 49.78	8.46 ± 5.67	0.00 ± 0.00	45.28 ± 42.45

Table 3.5 continued.

Taxa	**September-2010**	**November- 2010**	**February-2011**	**May-2011**
P. hessei	0.65 ± 0.58	3.93 ± 1.67	2.04 ± 2.27	0.24 ± 0.31
R. terranatalis	0.00 ± 0.00	5.92 ± 5.13	0.00 ± 0.00	0.00 ± 0.00
A. digitalis	0.00 ± 0.00	0.00 ± 0.00	0.01 ± 0.01	0.003 ± 0.005
C. capitata	2.62 ± 4.54	0.40 ± 0.53	3.44 ± 4.29	19.38 ± 6.07
Capitellidae	0.59 ± 1.01	0.00 ± 0.00	0.50 ±0.64	0.11 ± 0.19
C. keiskama	2.89 ± 4.69	0.33 ± 0.45	3.90 ± 4.62	23.50 ± 8.15
C.triaenonyx	1.30 ± 2.26	0.00 ± 0.00	0.15 ± 0.26	0.07 ± 0.08
D. arborifera	2.77 ± 4.09	0.72 ± 1.25	8.27 ± 6.33	19.66 ± 7.61
D. ornata	1.29 ± 2.21	0.33 ± 0.30	0.52 ± 0.41	1.26 ± 0.93
G. bonnieroides	0.00 ± 0.00	0.00 ± 0.00	0.27 ± 0.26	0.18 ± 0.22
G. lignorum	0.00 ± 0.00	0.00 ± 0.00	0.19 ± 0.17	0.00 ± 0.00
Grandidierella spp.	0.79 ± 1.24	0.00 ± 0.00	1.20 ± 0.98	0.84 ± 0.56
I. truncata	0.04 ± 0.07	0.00 ± 0.00	0.04 ± 0.06	0.38 ± 0.34
M. litoralis	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	113.06 ± 167.80
P. capensis	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.19 ± 0.33
Spionidae	4.40 ± 3.79	9.96 ± 9.02	2.24 ± 1.74	12.06± 6.91

3.3.2. Spatio-temporal variations in elemental content of suspended and sediment detritus

Significant spatio-temporal differences were found for %C, %N and C:N ratio for the suspended detritus at the Mlalazi Estuary (Table 3.6, Fig. 3.2). The %C and %N in detritus were lowest during September 2010 (dry season) and highest during February 2011 (wet season) (Fig. 3.2). The C:N ratio of detritus was highest during September 2010 compared to all other sampling sessions. In terms of spatial differences, highest nutrient content was recorded in the upper-middle reaches at the Mlalazi Estuary (Fig. 3.2).

In the Mpenjati Estuary, significant spatio-temporal variations in the C:N ratio were recorded for suspended detritus, whereas the %C and %N responded to the main effects of seasons and stations separately (Table 3.6, Fig. 3.2). In this regard, %C, %N and the C:N ratio were highest during September 2010 in this estuary. The %C for detritus was significantly lower during November 2010 (wet season) and February 2011 (wet season). Similarly, the %N for detritus was lowest during November 2010, February and May 2011 (abnormal dry season). The C:N ratio of suspended detritus was highest during September 2010 (dry season). Finally, the %C and %N were significantly higher in the upper reaches when compared to the other reaches (Fig. 3.2).

Table 3.6. Two-way ANOVA results for %C, %N and C:N ratio of suspended and sediment detritus in the Mlalazi and Mpenjati estuaries. Abbreviations indicate seasons (Se), stations (St).

			Suspended		**Sediment**	
Estuary	**Component**	**Variables**	**Df**	**p**	**df**	**p**
Mlalazi	% C POM	Se	4, 58	<0.001	4, 58	<0.001
		St	3, 58	<0.001	3, 58	<0.001
		Se × St	12,58	<0.001	11, 58	<0.001
Mlalazi	% N POM	Se	4, 58	<0.001	4, 58	<0.001
		St	3, 58	<0.001	3, 58	<0.001
		Se × St	12, 58	<0.001	11, 58	<0.001
Mlalazi	C:N POM	Se	4, 58	<0.001	4, 58	<0.001
		St	3, 58	0.018	3, 58	0.051
		Se × St	12, 58	0.031	11, 58	<0.001
Mpenjati	% C POM	Se	2, 43	<0.001	3, 26	<0.001
		St	4, 43	<0.001	2, 26	0.006
		Se × St	8, 43	0.218	4, 26	<0.001
Mpenjati	% N POM	Se	2, 43	<0.001	3, 26	0.001
		St	4, 43	<0.001	2, 26	0.001
		Se × St	8, 43	0.224	4, 26	<0.001
Mpenjati	C:N POM	Se	2, 43	<0.001	3, 26	0.291
		St	4, 43	0.758	2, 26	0.001
		Se × St	8, 43	<0.001	4, 26	0.095

In terms of sediment detritus, significant spatio-temporal variations were recorded for %C, %N and the C:N ratio in the Mlalazi Estuary (Table 3.6, Fig. 3.3). The %C and %N in sediment detritus were lowest during May 2011 (Fig. 3.3) and the C:N ratio was highest during September 2010 and May 2011 than during November 2010 and February 2011. In terms of spatial variations, %C and %N of sediment detritus were highest at the upper reaches than at other stations (Fig. 3.3).

Significant spatio-temporal differences were also reported for %C and %N in sediment detritus at the Mpenjati Estuary and the C:N ratio vary significantly among stations (Table 3.6, Fig. 3.3). The %C and %N were higher during November 2010 than during other sessions, with the lowest %C recorded during February 2011 and the lowest %N during February and May 2011. The %N and C:N ratio of sediment detritus was lowest and highest respectively in the upper reaches of the Mpenjati Estuary.

In general, the peaks in carbon and nitrogen content of suspended detritus differed between the estuaries, with the highest nutrient content recorded during the wet and dry seasons in the Mlalazi and Mpenjati estuaries respectively. The C:N ratios of suspended detritus were highest during the dry season in both systems. No clear seasonal trends were indicated by the nutrient content of sediment detritus in both estuaries. Overall, the highest nutrient content of suspended and sediment detritus was recorded in the upper reaches of both estuaries.

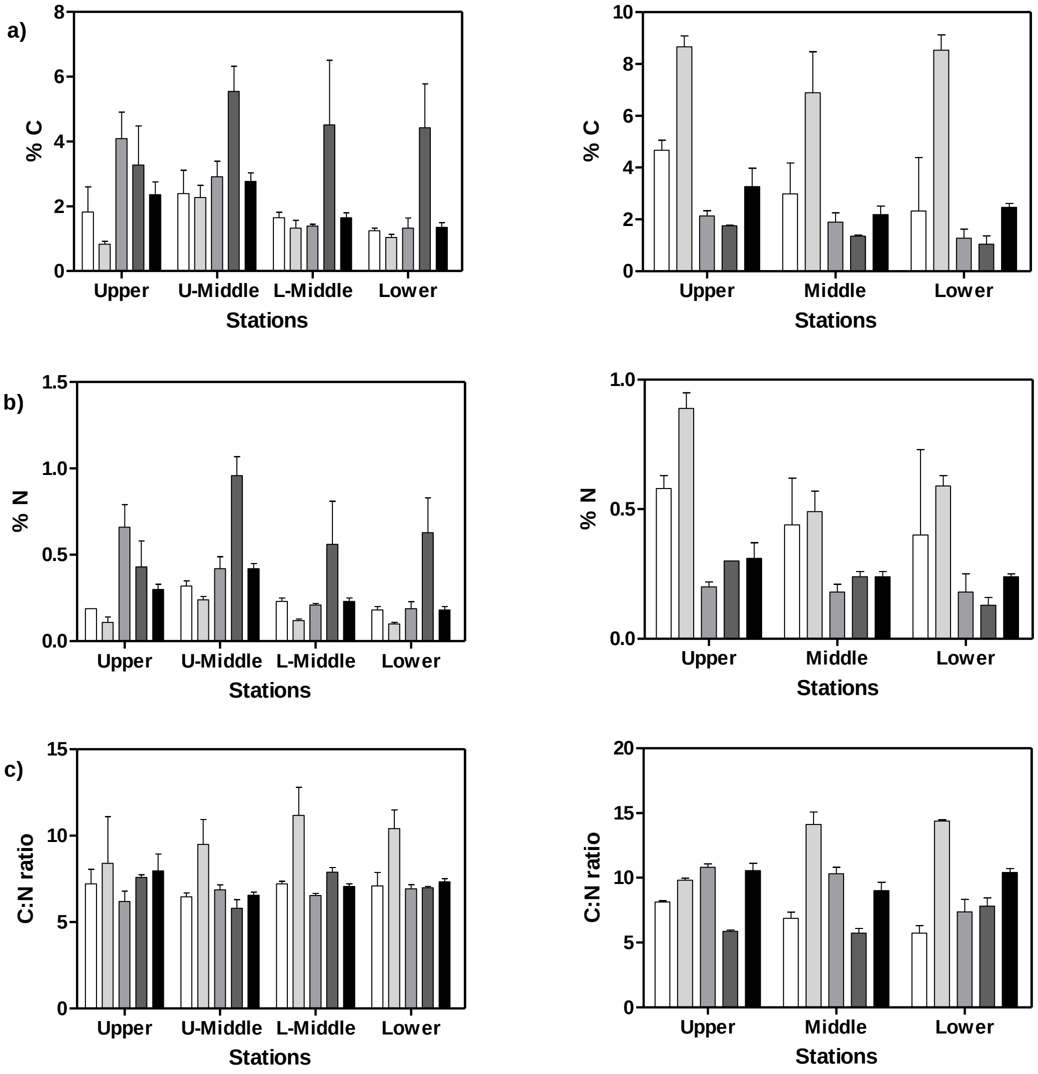

Figure 3.2. Carbon and nitrogen content, and C:N ratio (Mean ± SD) of suspended detritus in the Mlalazi (right) and Mpenjati (left) estuaries. Colours indicate seasons (white=June 2010, light grey=September 2010, grey=November 2010, dark grey=February 2011 and black=May 2011).

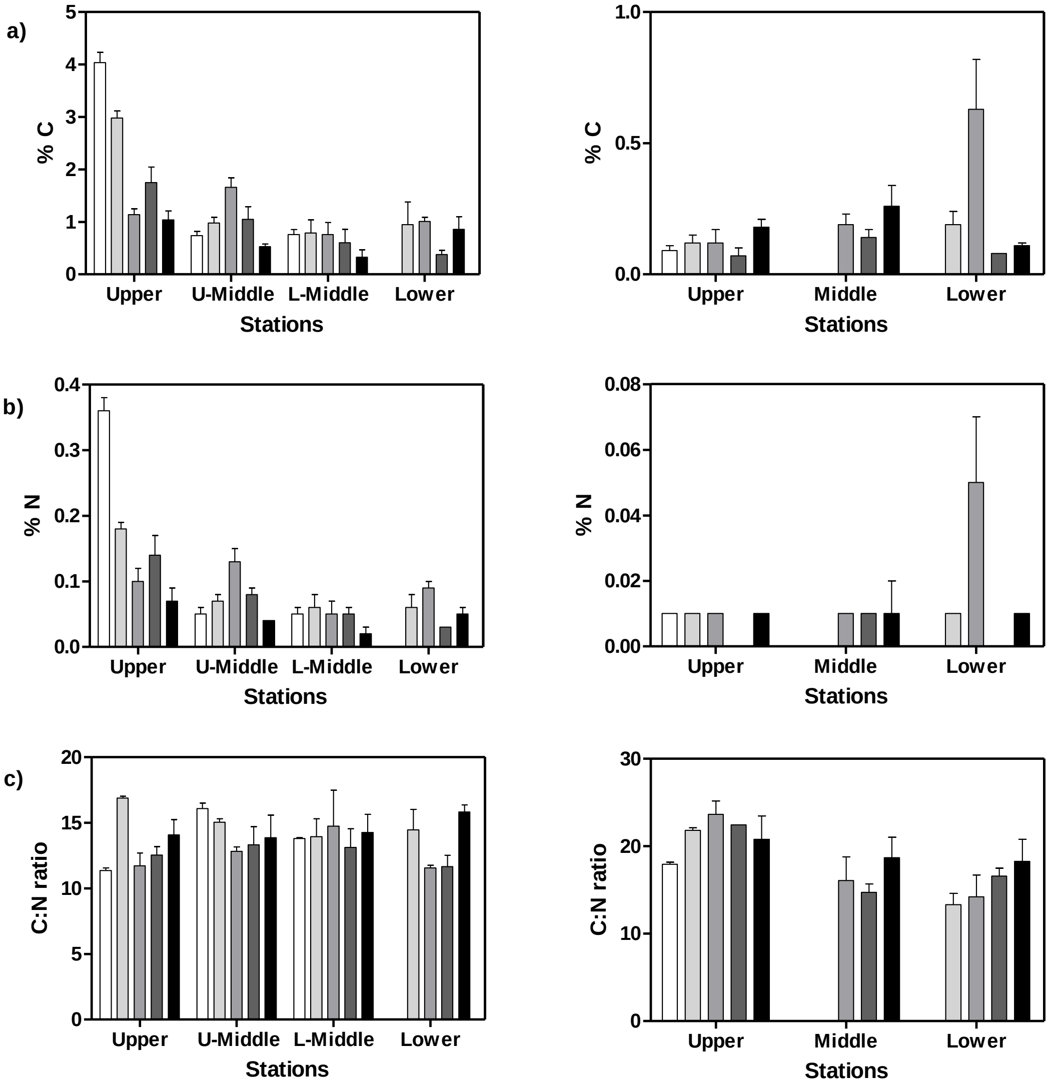

Figure 3.3. Carbon and nitrogen content, and C:N ratio (Mean ± SD) of sediment detritus in the Mlalazi (right) and Mpenjati (left) estuaries. Colours indicate seasons (white=June 2010, light grey=September 2010, grey=November 2010, dark grey=February 2011 and black= May 2011).

3.3.3. Seasonal variations in elemental content of planktonic and benthic species

Seasonal variations in the elemental composition of six zooplankton and ten macrobenthic taxa from both estuaries were analysed, with 10 taxa exhibiting significant seasonal differences in their elemental composition.

In terms of zooplankton taxa (Fig. 3.4), the copepod *Acartia* spp. showed significant seasonal differences in %C, %N and C:N ratio at the Mlalazi Estuary. The %C and %N of *Acartia* spp. was lowest during November 2010 (%C= 28.49 ± 6.78 SD, %N= 5.67 ± 1.46 SD), while the C:N ratio was highest during

November 2010 (C:N= 4.79 ± 0.77 SD) (Fig. 3.4). The copepod *Pseudodiaptomus* spp. showed significant differences in its C:N ratio, which was lowest during May 2011 (C:N= 3.97 ± 0.13 SD). In the Mpenjati Estuary, the copepod *A. natalensis* had highest %C values during November 2010 (%C= 44.74 ± 6.12 SD), while the copepod *P. hessei* had highest %N (%N= 47.26 ± 0.73 SD) and lowest C:N ratio (C:N= 3.72 ± 0.10 SD) during May 2011. The C:N ratio of the mysid *M. africana* was highest during November 2010 (C:N= 3.97 ± 0.10 SD).

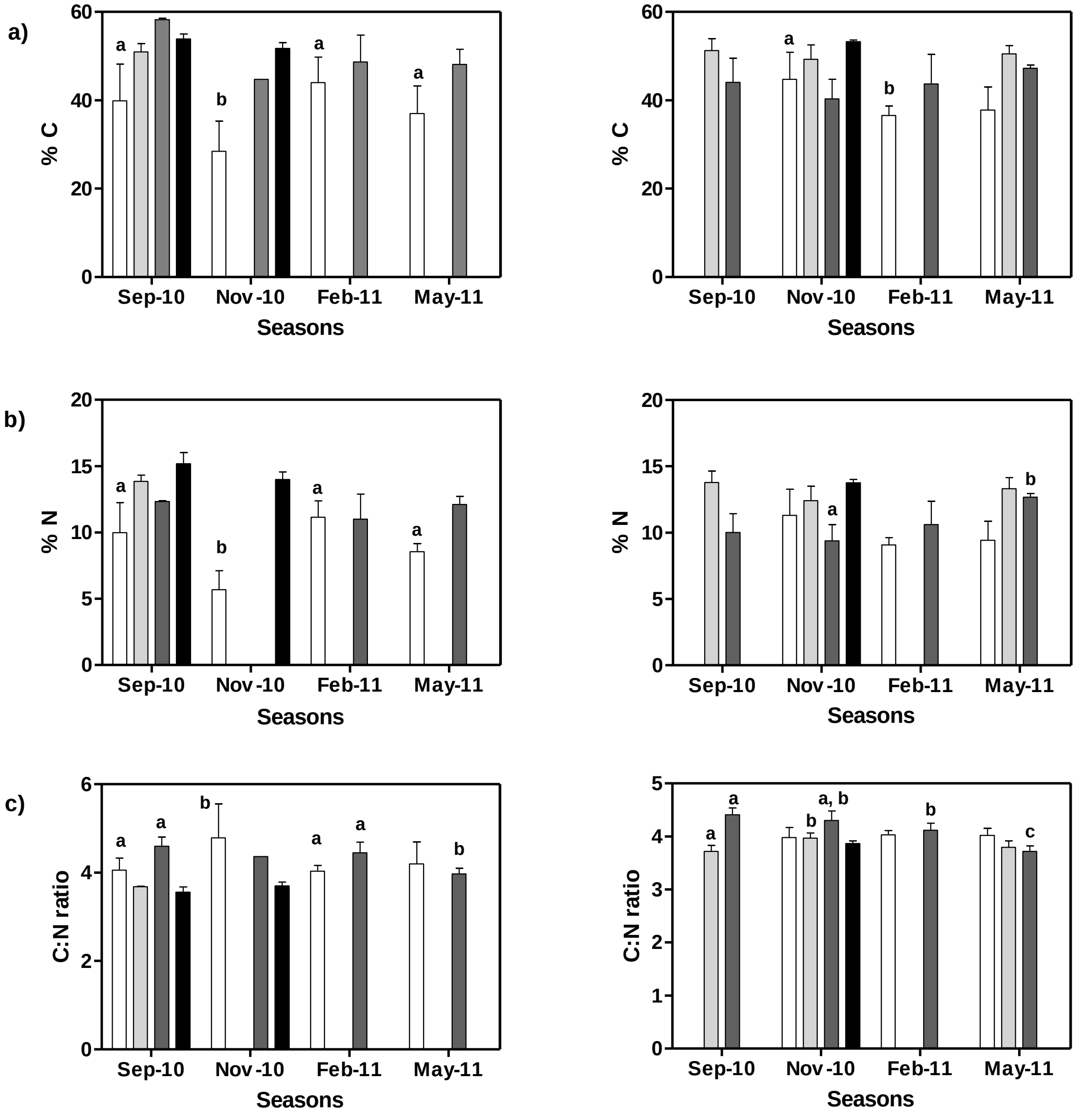

Figure 3.4. Carbon and nitrogen content, and C:N ratio (Mean ± SD) of zooplankton species in the Mlalazi (right) and Mpenjati (left) estuaries. Colours indicate taxa (white=*Acartia* spp., light grey=*Mesopodopsis africana*, dark grey=*Pseudodiaptomus* spp. and black= *Rhopalophthalamus terranatalis).*

In terms of macrobenthic taxa, the tanaid *A. digitalis* exhibited lowest carbon content during September 2010 (%C= 40.75 ± 6.49 SD) at the Mlalazi Estuary, whereas the bivalve *D. hepatica* had lowest carbon content during May 2011 (%C= 43.13 ± 2.08 SD) (Fig. 3.5). The polychaetes from the family Spionidae showed significant differences in %C, %N and C:N ratio with %C and %N highest during September 2010 (%C= 62.69 ± 6.21 SD) and May 2011 (%N= 12.24 ± 0.85 SD) respectively at the Malazi Estuary. Consequently, the C:N ratio of these polychaetes was lowest during May 2011 (C:N= 4.08 ± 0.16 SD). At the Mlalazi Estuary, the bivalve *Macoma litoralis*, and the polychaetes Cirratulidae and *Glycera* spp. showed no significant seasonal differences in %C, %N or C:N ratio throughout the study period. At the Mpenjati Estuary, the polychaetes *D. arborifera* and Spionidae had a significantly higher C:N ratio during February 2011 (C:N= 5.07 ± 0.78 SD and 5.58 ± 0.85 SD respectively) (Fig. 3.5). No significant seasonal differences in the %C, %N or C:N ratio of the polychaetes *Capitella capitata* and *Ceratonereis keiskama* were recorded during this study.

No consistent trends were observed in the seasonal variations of the analysed species. In addition, the variations did not resemble the seasonal changes recorded by the suspended and sediment detritus at both estuaries.

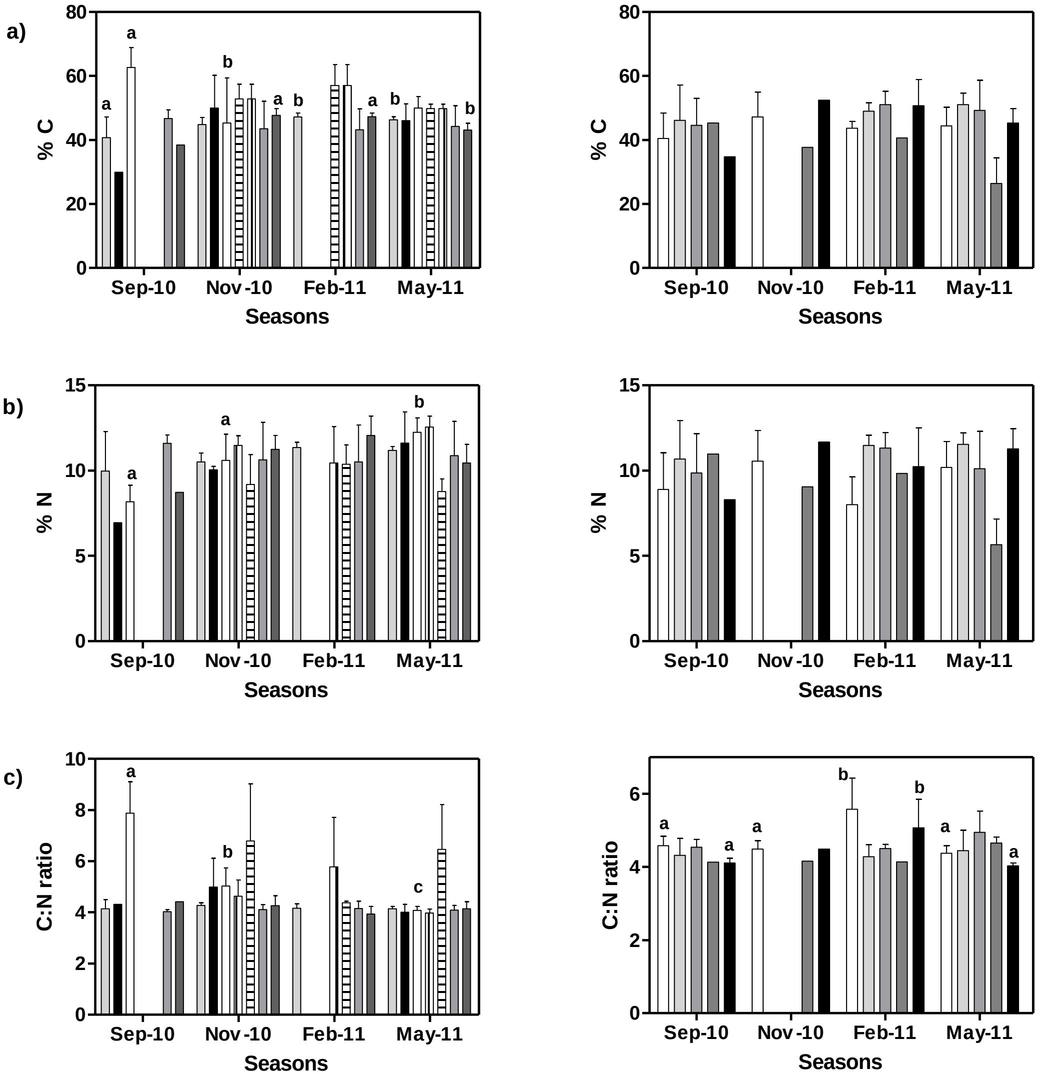

Figure 3.5. Carbon and nitrogen content, and C:N ratio (Mean ± SD) of macrobenthic species in the Mlalazi (right) and Mpenjati (left) estuaries. Colours indicate taxa (white=Spionidae and black= *Dendronereis arborifera*, for Mlalazi estuary: light grey=*Apseudes digitalis*, grey=*Macoma litoralis*, dark grey=*Dosinia hepatica*, horizontal bars=Cirratulidae and vertical bars=*Glycera* spp.; for Mpenjati estuary: light grey=*Ceratonereis keiskama*, grey=*Capitella capitata*, dark grey=*Desdemona ornata*).

3.3.4. Significant differences in elemental content among species

Marked variability was recorded in the %C, %N and C:N ratio among zooplankton and macrobenthic species (Table 3.7). The mysids *Rhopalophthalmus terranatalis* (%C= 52.25 ± 1.50 and %N= 14.25 ± 0.79) and *M. africana* (%C= 50.29 ± 3.02 and %N= 13.12 ± 1.09) had the highest carbon and nitrogen content among zooplankton species. In addition, the copepods *Acartia* spp. (%C= 36.77 ± 8.67 and %N=

8.71 ± 2.51) and *A. natalensis* (%C= 39.17 ± 5.46 and %N= 9.79 ± 1.56) had the lowest nutrient content. The C:N ratio of *Acartia* spp., *Pseudodiaptomus* spp. and *P. hessei* were significantly higher than that of the mysids *R. terranatalis* and *M. africana*.

In terms of macrobenthic taxa, the highest %C was recorded for the polychates *Glycera* spp. (%C= 53.55 ± 5.61), Cirratulidae (%C= 55.45 ± 8.56) and Spionidae (%C= 54.46 ± 8.56). The polychaetes *A. parva* (%N= 11.38 ± 1.11), *C. keiskama* (%N= 11.35 ± 1.08) and *Glycera* spp. (%N= 11.42 ± 1.68) exhibited the highest nitrogen content among macrobenthic taxa. Finally, the C:N ratio of the family Cirratulidae (C:N= 6.29 ± 1.89 SD) and Spionidae (C:N= 5.72 ± 2.15 SD) were the highest among macrobenthos.

Table 3.7. One-way ANOVA results for differences in %C, %N and C:N ratio among zooplankton and macrobenthic invertebrate species.

Components	**Df**	***P***	**Post-hoc comparison**[1]
Zooplankton			
C (%)	109	<0.001	Rt, P, Ph, Ma > A; P > An; Rt & Ma > An & Ph
N (%)	106	<0.001	Rt, P, Ph, Ma > A; Rt, Ma > An & Ph
C:N ratio	108	<0.001	A , P, Ph > Rt, Ma
Macrobenthos			
C (%)	229	<0.001	Ci , G > Ad; SP, G, Ci > Ml, Pb, SP1; Ap > Pb
N (%)	229	<0.001	G, Ck > Ci, Pb; Ap > Pb
C:N ratio	229	<0.001	Ci > Ad, G, Ml, Dh, Ap, Pb, SP1, Ck, Cc, Da; SP > Ad, Ml, Dh,SP1, Ck, Da

[1] Abbreviations are listed in Table 3.1.

3.3.5. Carbon and nitrogen signatures of abiotic and biotic components

Little variation was recorded between the mean suspended detritus δ^{13}C and δ^{15}N values of the Mlalazi (δ^{13}C= -24.02 ± 2.02 ‰ SD and δ^{15}N= 7.75 ± 1.22 ‰ SD) and Mpenjati estuaries (δ^{13}C= -21.51 ± 3.41 ‰ SD and δ^{15}N= 6.02 ± 0.77 ‰ SD). Mean sediment detritus δ^{13}C (Mlalazi= -21.40 ± 1.02 ‰ SD; Mpenjati= -22.73 ± 1.38 ‰ SD) and δ^{15}N were also similar in both estuaries (Mlalazi= 3.34 ± 1.35 ‰ SD; Mpenjati= 3.12 ± 1.34 ‰ SD). Suspended detritus had similar δ^{13}C signatures to sediment detritus, but the δ^{15}N of suspended detritus was considerably higher than sediment detritus in both estuaries (Fig. 3.6).

The δ^{13}C of zooplankton species in the Mlalazi Estuary was usually more depleted than of those in the Mpenjati Estuary. However, slightly more enriched δ^{15}N signatures were recorded by the zooplankton species of the Mlalazi Estuary when compared to those in the Mpenjati Estuary (Fig. 3.6). The most depleted δ^{13}C signature was that of *Acartia* spp. (δ^{13}C= -26.56 ± 2.12 ‰ SD) and the most enriched δ^{15}N signature recorded by *R. terranatalis* (δ^{15}N= 12.06 ± 0.83 ‰ SD). The δ^{13}C signatures among macrobenthic species showed relatively small variations, ranging from the polychaetes *D. arborifera* (-18.65 ± 1.82 ‰ SD) and *C. keiskama* (-18.68 ± 1.83 ‰ SD) to Spionidae (-24.29 ± 2.55 ‰ SD) (Fig. 3.6). The lowest δ^{15}N signatures were recorded for the bivalve *M. litoralis* (8.32 ± 0.88 ‰ SD) and crab *P. blephariskios* (8.37 ± 0.49 ‰ SD), and the highest δ^{15}N signatures for the polychaetes *A. parva* (12.35 ± 0.82 ‰ SD) and *Glycera* spp. (12.46 ± 0.57 ‰ SD). The δ^{15}N signatures of most macrobenthic species showed a range of 1.51 ‰, indicating that most species exhibit a similar trophic position.

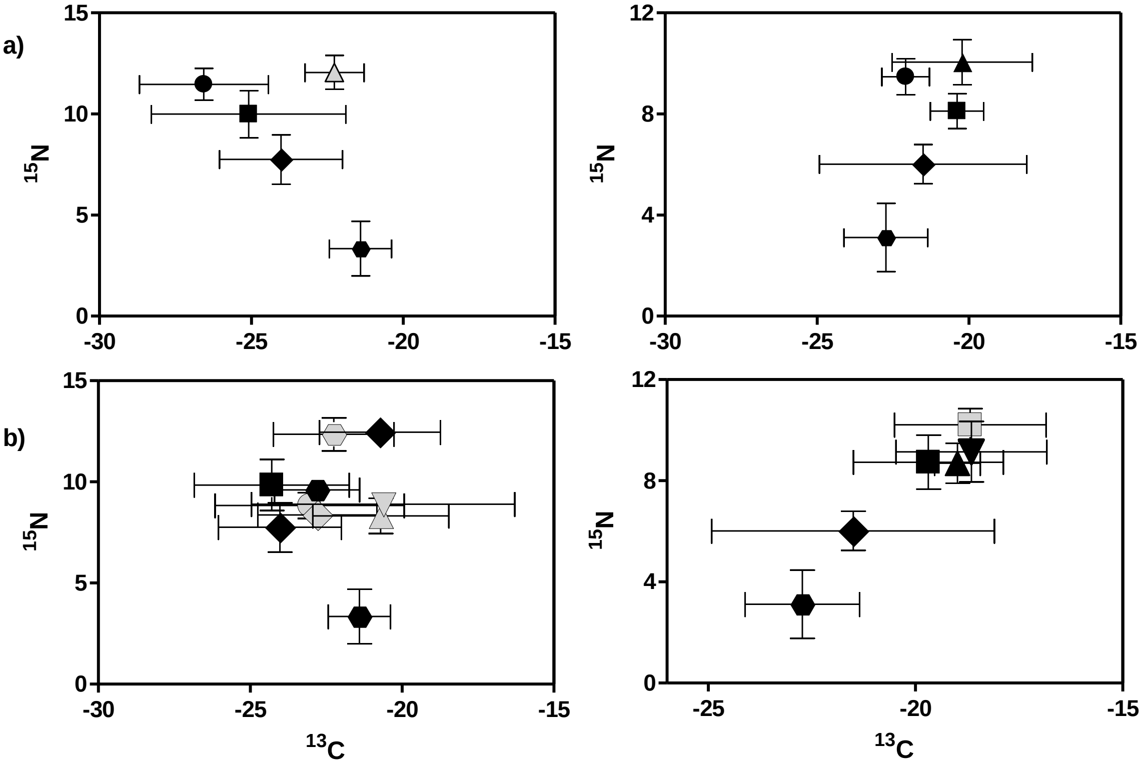

Figure 3.6. Mean (± SD) δ^{13}C and δ^{15}N of zooplankton and macrobenthic species in the Mlalazi (right) and Mpenjati (left) estuaries. Colour and shape indicate group or taxa (black diamond= suspended detritus, black rhombus= sediment detritus, in a) black circle= *Acartia* sp., black square= *Pseudodiaptomus* sp., black triangle= *M. africana*, grey triangle= *R. terranatalis*, in b) black square= Spionidae, grey diamond= *P. blephariskios*, grey rhombus= *A. parva*, black diamond= *Glycera* spp., black rhombus= Cirratulidae, grey triangle= *M. litoralis*, grey inverted triangle= *D. hepatica*, black inverted triangle= *D. arborifera*, grey square= *C. keiskama*, black triangle= *C. capitata*).

3.4. Discussion

This study evaluated the elemental composition of selected abiotic and biotic components of two KwaZulu-Natal estuaries, each influenced by marked seasonal changes in rainfall and river inflow. The elemental composition of suspended and sediment detritus differed significantly among seasons and stations, and also responded to the interaction effect of both factors. The stoichiometry of the suspended detritus revealed contrasting patterns between the estuaries, despite their biomasses showing similar seasonal trends. The permanently open Mlalazi Estuary had maximum C and N content during the wet season (February 2011) and the intermittently open Mpenjati during the dry season (September 2010). Previous studies have found that the nutrient content of suspended detritus decreased during the wet season in many global rivers (Meybeck, 1982), a mechanism known as organic dilution which can be attributed to the increase in inorganic particulate input during wet seasons (Ittekot, 1988). The higher nutrient content in suspended detritus at the Mpenjati Estuary during the dry season seems to follow the above-mentioned pattern but not those from the Mlalazi Estuary. Unfortunately, the lack of nutrient data of riverine input to these estuaries precludes us from making further conclusions.

The C:N ratio of suspended detritus was highest during the dry season months in both systems. These results are in agreement with previous findings, which reported significantly lower C:N, C:P and N:P ratios of suspended detritus during the wet season as a result of catchment wash-out from agriculturally derived N and P material or the increased nutrient input to estuaries arising from rainfall based catchment run-off (Albuquerque and Mozeto, 1997; Carmichael and Valiela, 2005). In the current study, higher carbon and nitrogen content were usually recorded in the upper reaches of both estuaries which suggests that there is a higher influence of nutrient-rich freshwater flow in this region of these systems. Similar findings were reported for the Great Ouse Estuary, England (Trimmer et al., 1998), Harney River Estuary, USA (Jaffé et al., 2001), Neuse and Pamlico estuaries, USA (Matson and Brinson, 1990), Pearl River Estuary, China (Yu et al., 2010) and Manko Estuary, Japan (Shilla et al., 2011).

Lower nutrient content of sediment detritus was expected during the wet season in both systems due to the increased river inflow, scouring and flushing of the more organically rich surface sediments to the sea. However, both the lowest and highest nutrient content was recorded during the wet season months in the Mpenjati Estuary and during the dry season months in the Mlalazi Estuary. This implies that the elemental composition of sediment nutrients was not influenced by seasonal changes in river inflow, and suggests that further studies are necessary to determine what factors influence the elemental composition of the sediment detritus in these systems. In addition, the C:N ratio of sediment detritus at the Mlalazi Estuary was significantly higher during the dry season months as expected due to the lower exchange of carbon and other nutrients between the estuary and adjacent environments (e.g. decreased riverine inputs) during this season (Cauwet and Mackenzie, 1993; Zhang et al., 2013). This pattern was not recorded at the Mpenjati Estuary despite the reduced freshwater and nutrient input to the system during the dry season (closed phase). The reason for the different patterns between estuaries is unclear; however the lack of seasonal trends in the elemental content of sediment detritus in these systems could partially explain these results. The C:N ratio of sediment detritus in this study fell within the range reported for sediments in the Shuangtaizi estuarine wetland, China (Zhang et al., 2013), Neuse, Pamlico and South Creek estuaries, USA (Matson and Brinson, 1990), Great Ouse Estuary, England (Trimmer et al., 1998), freshwater and brackish zones of the Pearl River Estuary, China (Yu et al., 2010) (Table 3.8).

The elemental composition and C:N ratio of some zooplankton and macrobenthic taxa showed significant seasonal differences, indicating that some species are not homeostatic (Hessen and Lyche, 1991). Previous studies have reported a marked variability in the elemental composition and ratios of certain benthic invertebrates (Cross et al., 2003; Evans-White et al., 2005; Frost and Elser, 2002; Liess and Hillebrand, 2005), but relatively few studies have found temporal variations in the elemental composition and ratios of zooplanktonic taxa (Ventura and Catalan, 2005; Villar-Argaiz et al., 2002). Overall, no consistent seasonal pattern in the nutrient content of estuarine invertebrates was found and the seasonal variations in the stoichiometry of zooplankton species did not correspond to the changes in suspended or sediment detritus composition. These findings are in agreement with Andersen and Hessen (1991) who reported no correspondence between the seasonal variations in nutrient content among zooplankton species in a Norwegian lake. The only similarity recorded between estuaries for zooplankton species was the lowest C:N ratio of the copepod *Pseudodiaptomus* spp. during May 2011. This may indicate that the seasonal variations in stoichiometry of *Pseudodiaptomus* spp. were species-specific and probably related to ontogenetic changes.

The nutrient content of the zooplankton species showed small differences between the two estuaries and fell within the range presented for zooplankton species in freshwater and marine systems (Beers, 1966; Gismervik, 1997; Walve and Larsson, 1999). The %C for the copepod *Acartia* spp. was within the lower range of values recorded for copepods in the oligotrophic Sargasso Sea, while the copepod *Pseudiaptomus* spp. had similar or slightly higher values than the ones reported for the same area (Beers, 1966). However, the %C and %N of *Acartia* spp. in the Mlalazi and *A. natalensis* in the Mpenjati estuaries was considerably lower than the nutrient content of *Acartia* sp. in the eutrophic Baltic Sea (Walve and Larsson, 1999). Moreover, the nutrient content of the mysids *M. africana* and *R. terranatalis* in the two KwaZulu-Natal estuaries was considerably higher than the nutrient content reported for euphausids-mysids in the oligotrophic Sargasso Sea (Beers, 1966) (Table 3.8).

In this study, few macrobenthic species showed significant seasonal variations in both their elemental content and ratio, with %C and the C:N ratio showing the highest variability among seasons. As with the zooplankton, the nutrient content of macrobenthic species did not show any clear seasonal trend or an association with the variations in nutrient content of detritus. Previous studies have found a relationship between macrobenthos stoichiometry and potential food sources (e.g. periphyton) (Cross et al., 2003; Liess and Hillebrand, 2005) but this was not the case in KwaZulu-Natal estuaries. Based on a trophic enrichment of ~ 3.4 ‰ (Post, 2002) and the $\delta^{15}N$ of most macrobenthic species (~ 8-10 ‰) in this study, it would appear that their food sources have a $\delta^{15}N$ signature of ~ 4.5-6.5 ‰. This indicates that macrobenthic species in the study estuaries are feeding on either a mixture of suspended and sediment detritus or on other more enriched food sources which were not appropriately represented through the measure of detritus nutrient content (e.g. benthic algae). Finally, macrobenthic elemental composition in this study was within the range reported for invertebrates in lakes (Liess and Hillebrand, 2005), streams in North America (Cross et al., 2003; Evans-White et al., 2005) and in the Antartic Sea (Clarke, 2008) (Table 3.8). No other published studies of C:N stoichiometry in estuarine macrobenthic organisms were available for comparison with this study.

Significant differences in elemental composition and C:N ratios among taxa were recorded. For zooplankton species, the lowest nutrient content was found with the copepods *Acartia* spp. in both estuaries and the highest nutrient content by the mysids *M. africana* and *R. terranatalis*. Consequently, these mysids had the lowest C:N ratio indicating that their food sources are less N-limited or they are very efficient in eliminating excess carbon (Elser et al., 1988). Mysids have been shown to feed on plankton, detritus and small copepods (e.g. *Acartia* spp. and *Pseudodiaptomus* spp. in this study). Stable isotope analysis revealed that these mysids had predatory feeding mode, indicating that they mainly feed on copepods in these systems. The lower C:N ratio of these mysids is thus explained by their higher N content typical of their predatory feeding mode as evidenced in the literature and the isotope analysis results from this study (Cross et al., 2003; Evans-White et al., 2005).

Variability in the elemental composition and stoichiometry of macrobenthic taxa have been mainly related to feeding guilds and phylogeny (e.g. Cross et al., 2003; Evans-White et al., 2005; Fagan et al., 2002; Martinson et al., 2008). Overall, the highest carbon content in this study was presented by the polychaetes *Glycera* spp., Cirratulidae and Spionidae. Very few studies have analysed the elemental composition of polychaetes but our results are comparable with the findings of Clarke (2008) who reported high nutrient content for two species of polychaetes in the Antarctic Sea (%C: ~40 - 60 and %N: ~ 8 – 14, Table 3.8). Lowest carbon and nitrogen content was recorded by the crab *P. blephariskios*. Similarly, Evans-White et al. (2005) reported a lower nutrient content for crustaceans when compared to insects and molluscs.

The polychaetes *A. parva*, *C. keiskama* and *Glycera* spp. had the highest nitrogen content among the studied zoobenthic taxa. Glyceridae and Pillargidae are primarily carnivores while the Nereidae can act as carnivore, filter feeder or surface deposit feeder (Fauchald and Jumars, 1979). The analysis of $\delta^{15}N$ signatures confirmed that *A. parva* and *Glycera* spp. are predators, since they exhibited the highest $\delta^{15}N$ signature among the macrobenthic taxa which is consistent with their higher trophic position. *C. keiskama* had intermediate $\delta^{15}N$ signatures suggesting that this species can be a carnivore or an omnivore. Previous studies have reported higher N content in predators than in herbivores (Clarke, 2008; Cross et al., 2003; Evans-White et al., 2005; Fagan et al., 2002). In this study, carnivores also had a higher N content than suspension or deposit feeders. Moreover, the crab *P. blephariskios* and the Cirratulidae polychaetes had the lowest %N, with the former being recorded as a grazer of benthic organic matter and the latter as a surface deposit feeder (Fauchald and Jumars, 1979). Fagan et al. (2002) suggested that herbivores may have a lower nitrogen content than carnivores because they evolved to a lower dependence on N due to their food source being N limited. In this sense, the low N content of detritivores (Martinson et al., 2008) could also be explained by the low nutrient quality of their food sources (Fagan et al., 2002).

Finally, the deposit feeders Cirratulidae and Spionidae had the highest C:N ratio among taxa; these results are in agreement with Clarke (2008) who found that suspension feeders have a tendency to exhibit high C:N ratios. It is probable that deposit feeders show a similar trend towards higher C:N ratios as has been reported for suspension feeders. The isotope analysis in this study indicated that the $\delta^{15}N$ signatures of most analysed taxa fell within the suspension or deposit feeders category. It is thus probably that the differences in elemental content and ratio found among taxa were related to other factors not evaluated in this study such as phylogeny.

This study has contributed to the understanding of the role of nutrients in two subtropical estuaries in South Africa. The elemental composition and ratios of the estuarine species analysed fell within the range reported for freshwater and marine environments. The zooplankton and macrobenthic species from the two estuaries exhibited significant seasonal variations in nutrient content and stoichiometry, thus confirming that some invertebrate species are not strictly homeostatic (as reported for freshwater and marine invertebrate species elsewhere). In this study, the among-taxa variability seems to be mainly related to feeding groups, although phylogeny also play a major role explaining the variability among taxa. Although preliminary, this study has provided a platform to explore stoichiometry of both abiotic and biotic components in South African estuaries. Future studies should include the analysis of other animal groups such as the meiofauna and fishes, as well as a wider range of potential food sources (e.g. benthic algae, bacteria).

Table 3.8. Elemental content and stoichiometry of taxa analysed during this study and in other global locations. Values represent mean ± SD, unless otherwise stated.

Taxa	**Location**	**% C**	**% N**	**C:N ratio**	**References**
Suspended POC	Baltic Sea,	-	-	6.6 - 9	Walve and Larsson (1999)
	Moji-Guaçu wetland, Brazil	-	-	6.6 - 11.0	Albuquerque and Mozeto (1997)
	C 53 and C 54, USA	-	-	29 – 34	Cross et al. (2003)
	Mlalazi Estuary, South Africa	2.38 ± 1.41	0.33 ± 0.23	7.56 ± 1.54	This study
	Mpenjati Estuary, South Africa	3.38 ± 2.59	0.35 ± 0.22	9.11 ± 2.64	This study
Sediment POC	Neuse, Pamlico and South Creek estuaries, USA	-	-	4.5 -20.2	Matson and Brinson (1990)
	Shuangtaizi wetland, China	-	-	16.15 ± 6.08	Zhang et al. (2013)
	Great Ouse Estuary, England	-	-	5 – 22	Trimmer et al. (1998)
	Pearl River Estuary, China	-	-	10.4 ± 1.3 - 15.2 ± 3.3	Yu et al. (2010)
	Mlalazi Estuary, South Africa	1.19 ± 0.91	0.09 ± 0.08	13.73 ± 1.81	This study
	Mpenjati Estuary, South Africa	0.19 ± 0.16	0.01 ± 0.01	17.70 ± 3.55	This study
Acartia spp.	Baltic Sea	~ 46 – 51	~ 12 – 13	4.5 - 4.7	Walve and Larsson (1999)
	Oslofjord, Norway	-	-	4.8 ± 0.7 - 8.7 ± 2.2	Gismervik (1997)
	Sargasso Sea (copepods)	35.2 – 47.6	8.16 – 11.17	~ 5.04	Beers (1966)
	Mlalazi Estuary, South Africa	36.77 ± 8.67	8.71 ± 2.51	4.29 ± 0.58	This study
	Mpenjati Estuary, South Africa	39.17 ± 5.46	9.79 ± 1.56	4.01 ± 0.12	This study
Mysids	Sargasso Sea (mysids-euphasids)	35.4 – 43.4	9.43 – 10.46	~ 4.77	Beers (1966)
	Mlalazi Estuary (*R. terranatalis*)	52.25 ± 1.50	14.25 ± 0.79	3.67 ± 0.11	This study
	Mpenjati Estuary (*M. africana*)	50.29 ± 3.02	13.12 ± 1.09	3.84 ± 0.15	This study
Other Crustaceans	35 streams, USA (5 taxa)	34.8 ± 1.7	7.4 ± 0.5	5.5 ± 0.3	Evans-White et al. (2005)
	8 lakes, Canada (amphipods)	36.2 ± 2.22	7.28 ± 0.68	5.84 ± 0.61	Frost et al. (2003)
	Lake Erken, Sweden (isopods)	-	-	5.4 ± 0.7	Liess and Hillebrand (2005)
	Mlalazi Estuary (*A.digitalis*)	45.15 ± 3.07	10.76 ± 0.90	4.20 ± 0.16	This study
	Mlalazi Estuary (*P. blephariskios*)	41.90 ± 4.89	9.00 ± 1.28	4.68 ± 0.36	This study
Molluscs	35 streams, USA (11 taxa)	42.2 ± 3.6	9.6 ± 1.6	5.2 ± 0.7	Evans-White et al. (2005)
	Lake Erken, Sweden (bivalves)	42.0 ± 1.3	10.3 ± 0.31	5.6 ± 0.9	Liess and Hillebrand (2005)

Table 3.8 continued.

Taxa	**Location**	**% C**	**% N**	**C:N ratio**	**References**
Molluscs	Antartic Sea (*Nacella concinna*)	48.8 ± 0.97 SE 49.4 ± 4.86 SE	10.6 ± 0.30 SE 12.6 ± 0.24 SE	4.53 ± 0.03 SE 5.37 ± 0.13 SE	Clarke (2008)
	Mlalazi Estuary (*D. hepatica*)	45.65 ± 3.36	11.11 ± 1.31	4.14 ± 0.33	This study
	Mlalazi Estuary (*M. litoralis*)	44.07 ± 6.60	10.80 ± 1.89	4.11 ± 0.20	This study
Polychaetes	Antartic Sea (2 species)	51.1 ± 2.71 SE 53.1 ± 0.75 SE	10.9 ± 0.15 SE 11.3 ± 0.61 SE	5.31 ± 0.11 SE 5.95 ± 0.09 SE	Clarke (2008)
	Mlalazi Estuary (4 taxa)	51.02 ± 4.78 55.45 ± 8.56	9.13 ± 1.34 11.42 ± 1.68	4.80 ± 1.23 6.29 ± 1.89	This study
	Mpenjati Estuary (4 taxa)	44.86 ± 6.45 49.66 ± 5.46	9.98 ± 1.77 10.40 ± 1.92	4.39 ± 0.48 4.77 ± 0.50	This study

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Comparative analysis of carbon and nitrogen dynamics of three estuaries on the east coast of South Africa using ecological network analysis

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4.1. Introduction

In South Africa, 280 estuaries have been recorded along the coastline (Whitfield and Baliwe, 2013). These systems have been classified into five types of estuaries named temporarily open/closed estuaries, permanently open estuaries, estuarine bays, estuarine lakes and river mouths (Whitfield, 1992). Temporarily open/closed estuaries are the dominant type of estuary, accounting for 65% of the total number of estuaries, while permanently open estuaries represent approximately 13% of the total number of estuaries. The number of permanently open estuaries has decreased in the last decades due to freshwater abstraction in the catchments of these systems. Catchment degradation constitutes one of the main threats to estuaries and approximately 15% of South African estuaries have been classified as degraded (van Niekerk and Turpie, 2012).

Temporarily open/closed estuaries (TOCEs) are separated from the sea by a sandbar at the estuary mouth during low rainfall and river flow periods (Whitfield, 1992). During high rainfall and river flow conditions, the connection between the estuary and the sea is re-established with an exchange of nutrients and biomass between these systems (Whitfield, 1992). The duration of the closed and open phases varies among systems and biogeographical regions, with the open phase lasting from days to weeks or months, while the closed phase can last from weeks to years (Perissinotto et al., 2010). Most South African TOCEs are characterized by small catchments (< 500 km^2), an absence of a tidal prism during the closed phase, and a prism of $< 1 \cdot 10^6$ m^3 during the open phase. On the other hand, permanently open estuaries (POEs) have a permanent connection to the sea and are characterized by catchments larger than 500 km^2 and a moderate tidal prism ($1 - 10 \cdot 10^6$ m^3) (Whitfield, 1992). The larger tidal prism in POEs helps maintain the permanent connection with the sea, although these systems are known to close to the sea during extreme drought conditions (Whitfield, 1998).

The South African coastline has been divided into three main biogeographical regions, the subtropical region from Kosi Bay to Mbashe, the warm temperate region from Mendwana Estuary to Cape Agulhas and the cool temperate region from the Uilkraals Estuary to the Orange River mouth (Whitfield and Baliwe, 2013). Most TOCEs are located in the subtropical (93) and warm temperate biogeographical regions (84), with only five TOCEs on the cool temperate region (Perissinotto et al., 2010). Because of the small number of permanently open estuaries in South Africa (approximately 37 estuaries), temporarily open/closed estuaries fulfil the ecosystem services provided by POEs (e.g. nursery areas, recreational zones, the export of nutrients to the sea) along the coastline where the latter are absent.

In South Africa, rainfall patterns exhibit marked variations among biogeographical regions, with a seasonal pattern (summer rainfall) in the subtropical region, mostly bi-modal rainfall (spring and autumn) in the warm temperate region, and winter rainfall in the cool temperate region (Davies and Day, 1998). Since fluctuations in rainfall and river inflow are one of the most important factors determining the mouth status of TOCEs, they exhibit a connection to the sea during summer in the subtropical region and during spring and autumn in the warm temperate region. Freshwater inflow to estuaries is a crucial factor controlling the species composition, abundance and biomass of estuarine communities in South Africa (e.g. Adams et al., 1999; Teske and Wooldridge, 2001; Wooldridge, 1999) and a number of authors have documented variations in the abiotic and biotic components of POEs and TOCEs as a result of changes in river flow (e.g. Chapter 1-3, Froneman, 2002b; Perissinotto et al., 2003; Schlacher and Wooldridge, 1996; Wooldridge, 1999). For instance, higher phytoplankton and microphytobenthic biomass have been recorded during the closed phase of TOCEs in South Africa (Perissinotto et al., 2002; Skinner et al., 2006; Walker et al., 2001). Similarly, increases in zooplankton abundance and biomass during the closed phase of TOCEs have been reported (Deale et al., 2013; Froneman, 2002b; Kibirige and Perissinotto, 2003b). Despite these findings, few studies have holistically analysed the effects of fluctuations in river inflow on the functioning of estuarine systems in South Africa (Baird and Heymans, 1996; Scharler, 2012; Scharler and Baird, 2005).

Macronutrients such as carbon, nitrogen and phosphorus are essential for growth and basic functions of aquatic organisms, therefore fluctuations in the availability of these nutrients have important implications for the structure and functioning of aquatic systems. The cycling of energy and matter is one of the most important features of ecosystem functioning, since it acts as a buffer during shortages in nutrient supply and enhances stability (Allesina and Ulanowicz, 2004 and references therein). Nutrient limitations may result in alterations in the community structure, primary productivity, quality of primary production, transfer efficiency of energy flows and food web dynamics of an ecosystem (Cross et al., 2003; Glibert et al., 2011; van de Waal et al., 2009). This has then repercussions in the consumer-driven recycling and impact the overall functioning and organization of a system. Few studies have investigated the role of nutrients in estuarine foodwebs, even though this analysis may provide important insights in the holistic understanding of ecosystem functioning in estuaries.

Ecological network analysis (ENA) constitutes one of the few methodologies that provide comprehensive information of whole ecosystem behaviour. The direct and indirect interactions among compartments of an entire food web are examined using a suite of network analyses indices, which provide information about the flow and cycling of energy and material through ecosystems (Kay et al., 1989). Ecological network analysis indices have been widely used to evaluate the impact of different environmental conditions in estuaries and other marine systems (as summarized by Christian et al., 2005). ENA indices have also been used for temporal (Borrett et al., 2006; Schramski et al., 2006) and inter-ecosystem comparisons within and between estuaries (Christian et al., 2005).

To date, most ecological networks have analysed the functioning of ecosystems using carbon trophic flows (e.g. Baird and Ulanowicz, 1993; Dame and Christian, 2008; Heymans et al., 2002), with considerably less studies analysing the behaviour of nitrogen and phosphorus in the functioning of ecosystems (e.g. Christian and Thomas, 2003; Forès et al., 1994; Kaufman and Borrett, 2010; Thomas and Christian, 2001). Similarly, very few studies have simultaneously analysed the dynamics of C, N and P in aquatic systems, with exceptions for the mesohaline community of Chesapeake Bay, USA (Ulanowicz and Baird, 1999), and nine sub-systems and the whole of the Sylt-Rømø Bight, German Wadden Sea (Baird et al., 2008, 2011).

The present study aims to build carbon and nitrogen ecosystem models based on quantified trophic links and use ecological network analysis to examine the structure and functioning of three estuaries on the east coast of South Africa. The specific objectives were to:

1) Quantify the contribution and dependency of primary producers and consumers in terms of carbon and nitrogen in each estuary;
2) Determine the number of trophic levels, efficiency of trophic transfers, and the amount of detritivory and herbivory in each estuary;
3) Quantify the structure and distribution of cycling within these estuaries;
4) Determine the ecosystem state of each estuary based on information analysis indices (e.g. development capacity, ascendency and overheads).

4.2. Materials and Methods

4.2.1. Study areas

The East Kleinemonde Estuary (33°32’ S; 27°03’ E) is a temporarily open/closed estuary (Whitfield, 1992) (Fig. 4.1), located in the warm temperate biogeographical region. The catchment area is approximately 46 km^2 (Badenhorst, 1988) and the estimated mean annual runoff is $2 \cdot 10^6$ m^3 (van Niekerk et al., 2008). The estuary area is 14.5 ha (van Niekerk and Turpie, 2012) and the system is in a good condition (Whitfield and Baliwe, 2013). The catchment area consists mainly of cattle and pineapple farms, with residential plots located in the lower reaches of the estuary (Whitfield et al., 2008). This system was closed to the sea for 90 % of the days from 1993 to 2006 (Whitfield et al., 2008).

The Mlalazi Estuary (28° 56'42" S; 31°48' 58" E) is classified as a permanently open system (Fig. 4.1), although the estuary mouth has been known to close during major droughts. The catchment area is approximately 492 km^2 (DEAT, 2001), with a river length of approximately 54 km. The estimated mean annual runoff is 122 · 10^6 m^3 (Jezewski et al., 1984) and the estuary area is 95.86 ha. Subsistence farming, sugar cane cultivation and commercial forestry account for around 46% of the catchment usage, with 53% of the catchment considered undegraded and 1% being urban (coastal village of Mtunzini and the town of Eshowe). The estuary is located in the subtropical region and is considered to be in good condition (Whitfield and Baliwe, 2013).

The Mpenjati Estuary (30°58'21"S, 30°17'02"E) is a temporarily open/closed system (Whitfield, 1992) (Fig. 4.1), also located in the subtropical region. The catchment area is approximately 101 km^2, with an axial length of 1.1 km. The estimated mean annual runoff is 20.7 · 10^6 m^{-3} (Jezewski et al., 1984) and the estuary area is 11.6 ha (van Niekerk and Turpie, 2012). The system is regarded as being in a good condition (Whitfield and Baliwe, 2013).

The Mlalazi and Mpenjati estuaries are both located within nature reserves and much of the East Kleinemonde Estuary surrounds comprise undisturbed natural vegetation; thus all three systems exhibit relatively unaltered conditions with respect to their original states.

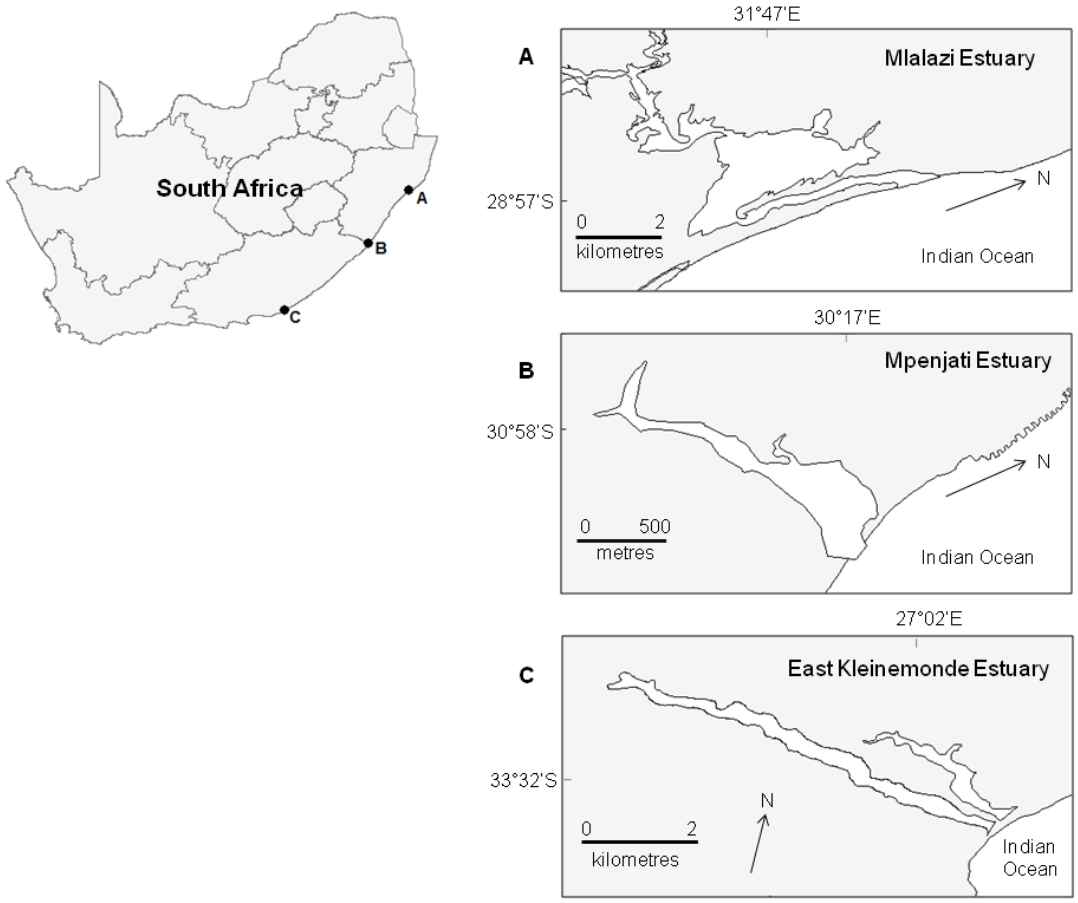

Figure 4.1. Map of the study areas (East Kleinemonde, Mlalazi and Mpenjati estuaries) on the east coast of South Africa.

4.2.2. Data sources and model construction

Ecological networks are representations of the flow of mass and energy in an ecosystem, they are structured by a collection of nodes (abiotic and biotic compartments) linked to each other by weighted trophic flows (Kay et al., 1989). Ecological networks of carbon and nitrogen were built for the East Kleinemonde, Mlalazi and Mpenjati estuaries representing four points in time for each estuary and nutrient. The number of compartments in each network varied among the estuaries and seasons, and reflected the species composition of these estuaries at a particular time (Table 4.1). The flows included in the system represent imports and exports across system boundaries, respiration and flows between nodes. For model construction, biomass of all nodes was expressed in mg $C{\cdot}m^{-2}$ or mg $N{\cdot}m^{-2}$ and the flows expressed in mg C or $N{\cdot}m^{-2}$ d^{-1}.

Table 4.1. List of study areas and information on the seasonal ecological networks for each system. Numbers in brackets indicates the number of compartments of aggregated networks.

Estuary	**Date**	**Mouth Status**	**Number of compartments**
East Kleinemonde	March 2006	Closed	49
	July 2006	Closed	43
	September 2006	Open	45
	November 2006	Open	44
Mlalazi	September 2010	Open	67 (64)
	November 2010	Open	66 (63)
	February 2011	Open	63 (61)
	May 2011	Open	65 (63)
Mpenjati	September 2010	Closed	41 (39)
	November 2010	Open	38 (35)
	February 2011	Open	45 (44)
	May 2011	Open	47 (45)

The ecological networks for the East Kleinemonde Estuary were built to represent the conditions during March, July, September and November 2006. The ecological networks for the Mlalazi and Mpenjati estuaries represented September and November 2010, February and May 2011. The biomasses for the planktonic and benthic compartments of the Mlalazi and Mpenjati networks were obtained from quarterly samplings at both systems from August 2010 to May 2011 (Chapter 2-3). Species composition and biomass of birds and fishes were not measured during the samplings at the Mlalazi and Mpenjati estuaries and were obtained from literature sources (Table 4.3). The biomass for the different compartments of the East Kleinemonde Estuary networks was measured during the “East Kleinemonde Intermediate Ecological Reserve Determination Study” (WRC Project No K5/1581). Despite the importance of bacterial groups, these were not included in the carbon and nitrogen networks due to a lack of appropriate data. Similarly, no dissolved inorganic nutrient pools were included in these networks.

A total of 24 seasonal carbon and nitrogen networks were built for the East Kleinemonde, Mlalazi and Mpenjati estuaries (3 systems, 4 seasons, 2 nutrients). Information on the biomass, consumption, respiration, production, egestion and diet for each compartment was necessary for network construction. Additionally, information of flows across the system boundaries were also required, e.g. imports from the river or sea to the estuary and exports to the sea. All networks were mass balanced, ensuring that:

$$\text{Consumption} = \text{Respiration} + \text{Production} + \text{Egestion}.$$

Carbon and nitrogen standing stocks for suspended and sediment detritus, phytoplankton, zooplankton, macrobenthos and meiobenthos were obtained from quarterly samplings at the Mlalazi and Mpenjati estuaries from September 2010 to May 2011 (methodological details in Chapter 3, section 3.2). Since fish and birds were not sampled at these estuaries, system-specific fish biomass was obtained from Harrison (2003) and bird abundance from CWAC counts (http://cwac.adu.org.za/, accessed on May 2012). Bird abundance was converted to wet weight using data from the literature for the same species (Hockey et al., 2005), then converted to dry weight, carbon (McLusky, 1981) and nitrogen (Cherel et al., 2008) biomass. Bird biomass was divided by the area of each estuarine system to express measurements as mg $C \cdot m^{-2}$. Fish biomass (wet weight) was converted to carbon biomass using a 9:1 ratio (Pauly and Christensen, 1995).

The information on fish biomass for the Mlalazi and Mpenjati estuaries was obtained from seine net catches, with details on the sampling method found in Harrison (2003, p.10). In the Mpenjati Estuary, no piscivorous fish species were captured through seine netting but *Caranx sexfasciatus* was caught in gill nets. Therefore this species was included in the networks for the Mpenjati Estuary to provide a more realistic representation of fish composition within this system. Since gill nets do not provide estimates of density or biomass per unit area, the biomass of this piscivore was estimated using the catch of a “standard” species which was adequately represented in both the gill net and seine net catches. It was then assumed that the biomass per unit area of the standard species (*Myxus capensis* in this case) from the seine net becomes the standard against which to calculate the biomass per unit area of other species in the gill net. Thus, the biomass per unit area of *C. sexfasciatus* was calculated by relating its biomass in the gill net catch to that of *M. capensis* and then scaling the result by the biomass per unit area conversion factor obtained for *M. capensis*.

For the East Kleinemonde, data on phytoplankton and microphytobenthos biomass was obtained from Gama (2008), zooplankton biomass from Froneman (2008), macrobenthos density from Wooldridge and Bezuidenhout (2008), fish density and lengths from (James, 2006) and birds abundance from Terörde (2008) (Table 4.2). Phytoplankton and microphytobenthos chlorophyll-a biomass (mg $Chl\text{-}a \cdot m^{-3}$ and mg $Chl\text{-}a \cdot m^{-2}$ respectively) were converted to carbon biomass using a ratio of 60 (Wang et al., 2006), phytoplankton biomass was then depth-integrated to express mg $C \cdot m^{-2}$. Zooplankton biomass (mg $DW \cdot m^{-3}$) was first depth-integrated and then converted to carbon biomass using mean %C for zooplankton species per dry or wet season obtained during this study (Chapter 3, section 3.3). Macrobenthos abundance was converted to dry weight using an abundance-biomass relationship for the same or similar species from data obtained for the Mlalazi and Mpenjati estuaries (Ortega-Cisneros, unpubl. data). Dry weight was converted to carbon biomass using mean %C data for the same or similar macrobenthic species obtained in this study for the dry and wet seasons (Chapter 3, section 3.3). Fish CPUE (number of individuals per haul) was converted to wet weight using length data (James, 2006) and length-weight relationships for South African estuarine fishes (Harrison, 2003). Fish biomass (wet weight) and bird abundance data were converted to carbon biomass as specified above for the Mlalazi and Mpenjati estuaries. Measurements of suspended and sediment detritus were available for the East Kleinemonde Estuary from Vorwerk (2006) and Vorwerk et al., (2001) respectively.

Information on production (P), respiration (R), consumption (C) and egestion (E) flows for each compartment was gathered from the literature (Table 4.2-4.3). These flows were estimated using P/B, C/B, R/B and P/C ratios and biomass measurements for each compartment in an estuary and season. Diet information was collected from different published and grey literature sources (Table 4.2-4.3). Isotope analyses of zooplankton and macrobenthic species from the Mlalazi and Mpenjati estuaries (Chapter 3) were also used to establish feeding guilds. Flows of unused production from the different living compartments were diverted to the suspended and sediment detrital pools. The unassimilated food for planktonic and pelagic components was assumed to contribute to the suspended detrital pool, while the unassimilated food of benthic components was assumed to contribute to the sediment detrital pool.

Imports and exports from across the system boundaries were included in the networks of the three estuaries. Riverine imports in the form of suspended detritus were estimated, as were marine imports and exports of suspended detritus, phytoplankton and zooplankton. Riverine imports of suspended detritus were determined using concentrations of suspended detritus from the upper reaches of the Mlalazi and Mpenjati estuaries measured quarterly from September 2010 to May 2011 and flow rates from their respective rivers. Flow rates for the Mlalazi River were obtained from the DWAF website (Station W1H004, www.dawf.co.za) No flow station was present on the Mpenjati River but river flow measurements were conducted at the head of the estuary during March 2013. Imports and exports to/from the sea were calculated using tidal flows and measurements of suspended detritus, phytoplankton and zooplankton from quarterly samplings at the mouth of these estuaries during high and low tides. For the East Kleinemonde Estuary, imports and exports were estimated using flow rates measured during 2006 obtained from van Niekerk et al., (2008) and concentrations of suspended detritus, phytoplankton and zooplankton determined for the Mpenjati Estuary during this study. Information on imports and exports from the Mpenjati Estuary were used for the calculations of the East Kleinemonde, since there are no available data of imports and exports for this system or other temporarily open/closed estuaries in South Africa.

Table 4.2. List of references used to estimate parameters for the East Kleinemonde Estuary. A= Abundance, B= Biomass, Chl-a:C= Chlorophyll-a to carbon, L-B= Length-weight relationships, NPP= Net primary production, P/B= Production/biomass, P/C= Production/consumption, P/R= Production/respiration and WW= Wet weight.

Model compartment	Parameter	References
Phytoplankton	Chl-a	Gama (2008)
	Chl-a:C	Wang et al., (2006)
	NPP	Adams et al., (1999), Froneman (2002b) (2004a)
	P/R	Scharler (2000)
Microphytobenthos	Chl-a	Gama (2008)
	Chl-a:C	Wang et al., (2006)
	NPP	Perissinotto et al. (2003), Scharler (2000)
	P/R	Scharler (2000)
Zooplankton	B	Froneman (2008)
	P/B, P/R	Scharler (2000)
	C	Froneman (2002a, b, 2004a, b)
	Diet	Froneman (2000, 2001a,b, 2002a, 2004b)
Macrobenthos	A A – B	Wooldridge and Bezuidenhout (2008) This study
	P/B	Brey (2012)
	R/B	Brey et al., (2010)
	P/C	Scharler (2000)
	Diet	Branch et al., (2005), de Villiers et al., (1999), Fauchald and Jumars (1979), Ross et al., (2000)
Fishes	A L-B	James (2006) Harrison (2001)
	WW:C WW:N	Pauly and Christensen (1995) Ramseyer (2002)
	P	Cowley and Whitfield (2002)
	P/R	Scharler (2000)
	C/B	Froese and Pauly (2012)
	Diet	Froese and Pauly (2012), Whitfield (1998)
Birds	Abundance	Terörde (2008)
	B B:C C:N	Hockey et al., (2005) McLusky (1981) Baird et al., (2008), Cherel et al., (2008)
	P, R	Castro et al., (1989), Tasker and Furness (1996)
	C	Nagy (1987)
	Diet	Hockey et al., (2005)
Suspended Detritus	Mass	Vorwerk (2006)
Sediment Detritus	Mass	Vorwerk et al., (2001)
Imports/ Exports	Flow/B	This study

Table 4.3. List of references used to estimate parameters for the Mlalazi and Mpenjati estuaries. A= Abundance, B= Biomass, Chl-a:C= Chlorophyll-a to carbon, L-B= Length-weight relationships, NPP= Net primary production, P/B= Production/biomass, P/C= Production/consumption, P/R= Production/respiration and WW= Wet weight.

Model compartment	Parameter	Mlalazi	Mpenjati
Phytoplankton	Chl-a	This study	This study
	Chl-a:C	Wang et al., (2006)	Wang et al., (2006)
	NPP	Perissinotto et al., (2003)	Perissinotto et al., (2003)
	P/R	Scharler (2000)	Scharler (2000)
Microphytobenthos	Chl-a	This study	This study
	Chl-a:C	Wang et al., (2006)	Wang et al., (2006)
	NPP	Perissinotto et al., (2003)	Perissinotto et al., (2003)
	P/R	Scharler (2000)	Scharler (2000)
Zooplankton	B	This study	This study
	P/B, P/R	Scharler (2000)	Scharler (2000)
	C	Carrasco and Perissinotto (2010), Perissinotto et al., (2003)	Carrasco and Perissinotto (2010), Perissinotto et al., (2003)
	Diet	Carrasco and Perissinotto (2011), Kibirige and Perissinotto (2003a), Kibirige et al., (2002), Wooldridge (1999)	Carrasco and Perissinotto (2011), Kibirige and Perissinotto (2003a), Kibirige et al., (2002), Wooldridge (1999)
Macrobenthos	B	This study	This study
	P/B	Brey (2012)	Brey (2012)
	R/B	Brey et al., (2010)	Brey et al., (2010)
	P/C	Scharler (2000)	Scharler (2000)
	Diet	Branch et al., (2005), de Villiers et al., (1999), Fauchald and Jumars (1979), Ross et al., (2000)	Branch et al., (2005), de Villiers et al., (1999), Fauchald and Jumars (1979), Ross et al., (2000)
Meiofauna	A	Ortega-Cisneros unpub. data	Ortega-Cisneros unpub. Data
	A:C	Dye and Furstenberg (1978)	Dye and Furstenberg (1978)
	C:N	Feller and Warwick (1988)	Feller and Warwick (1988)
	P/B	Scharler (2000)	Scharler (2000)
	P/R	Brey (2010), Dye et al., (1978)	Brey (2010), Dye et al., (1978)
	P/C	Scharler (2000)	Scharler (2000)
	Diet	Jensen (1987), Nozais et al., (2005)	Jensen (1987), Nozais et al., (2005)
Fishes	WW	Harrison (2003)	Harrison (2003)
	WW:C WW:N	Pauly and Christensen (1995) Ramseyer (2002)	Pauly and Christensen (1995) Ramseyer (2002)
	P/B	Scharler (2000)	Scharler (2000)
	P/R	Scharler (2000)	Scharler (2000)
	C/B	Froese and Pauly (2012)	Froese and Pauly (2012)
	Diet	Froese and Pauly (2012), Whitfield (1998)	Froese and Pauly (2012), Whitfield (1998)

Table 4.3 continued.

Model compartment	Parameter	Mlalazi	Mpenjati
Birds	A	CWAC counts	CWAC counts
	B	Hockey et al., (2005)	Hockey et al., (2005)
	B:C	McLusky (1981)	McLusky (1981)
	C:N	Baird et al., (2008), Cherel et al., (2008)	Baird et al., (2008), Cherel et al., (2008)
	P, R	Castro et al., (1989), Tasker and Furness (1996)	Castro et al., (1989), Tasker and Furness (1996)
	C	Nagy (1987)	Nagy (1987)
	Diet	Hockey et al., (2005)	Hockey et al., (2005)
Suspended Detritus	Mass	This study	This study
Sediment Detritus	Mass	This study	This study
Imports/ Exports	Flow/B	This study	This study

All compartments in the carbon networks were mass balanced, therefore all inputs to a compartment (Consumption) are equal to the outputs (Production + Respiration + Egestion) from the same compartment. The trophic flows between compartments were calculated using the least-inference method MATLOD, this method assumes that there is no difference among all flows in a network (Ulanowicz and Scharler, 2008). MATLOD operates by adding small uniform increments to all possible flows in a random sequence, this process is repeated until either the demand of a receiver is fulfilled or the availability of a donor is depleted. This method assures that both small and large links are maintained in the network (Ulanowicz and Scharler, 2008).

4.2.3. Nitrogen networks

Nitrogen networks were built based on the carbon networks. Information on biomass, imports, exports and flow topology (diet matrix) was needed to build the nitrogen networks. Biomass (mg $N \cdot m^{-2}$) were calculated using dry biomass and %N obtained for species in the Mlalazi and Mpenjati estuaries (Chapter 3, section 3.3) and also from the literature (Table 4.3). No information regarding elemental content or C:N ratios was available for the East Kleinemonde Estuary; thus the mean %N for the same or similar species from the Mlalazi and Mpenjati estuaries were used to determine nitrogen biomass. Nitrogen content was not measured for fish and bird groups during this study. Fish nitrogen biomass was calculated using regression analysis based on fish wet weight as proposed by Ramseyer (2002). Bird biomass was calculated using carbon biomass and C:N ratios for birds from the literature (Baird et al., 2008; Cherel et al., 2008). Imports and exports were calculated as specified above in section 4.2.2.

Using information of biomass, imports, exports (mg $N \cdot m^{-2}$) and carbon flows of each compartment, nitrogen trophic flows between compartments were quantified based on information of C:N ratios calculated from the carbon and nitrogen standing stocks of each donor compartment, then each carbon flow value was divided by the C:N ratio of its originating compartment to arrive at the flow value in N.

4.2.4. Balancing of networks

After the nitrogen trophic flows between compartments had been quantified, all carbon and nitrogen networks were balanced. The balancing procedure used in this study is an input or donor-based approach, balance is achieved by manipulating outputs (such as respiration and exports) and flow transfer coefficients, while imports are kept constant. Carbon and nitrogen networks were simultaneously balanced to ensure that the C:N stoichiometry between carbon and nitrogen flows was maintained. The outputs of the "balanced" networks were checked against the initial respiration estimates. If the respiration of a compartment in the balanced networks exceeded its consumption, the egestion of that compartment was modified to make respiration values plausible and achieve mass balance.

Carbon and nitrogen networks were mass balanced because some of the analyses (input – output and trophic level analyses) conducted in this study require strict mass balance around each compartment (Ulanowicz, 2012), e.g. any imbalances will invalidate the outputs of these analyses because these analyses require that the demands of all compartments equals the availability of resources in the system. The analyses of cycling and system information however do not require mass balance.

4.2.5. Ecological network analysis

The mass-balanced carbon and nitrogen networks were analysed using the software WAND (Allesina and Bondavalli, 2004) to examine the flow of energy and material in the seasonal networks of the East Kleinemonde, Mlalazi and Mpenjati estuaries through Ecological Network Analysis (ENA). Zooplankton biomass for the East Kleinemonde Estuary was available as total zooplankton biomass and not as biomass per species; therefore the biomass of the different zooplankton species at the Mlalazi and Mpenjati estuaries were aggregated into one compartment, "total zooplankton", to generate another set of networks that allowed for comparisons between estuaries. A total of 16 networks (two estuaries, two nutrients and four seasons) were also analysed, with the results from the aggregated and original networks being presented in the Results section below.

To quantify the contribution of detritus, primary producers (phytoplankton and microphytobenthos) and consumers (zooplankton, macrobenthos, fish) to the carbon and nitrogen requirements of the system, as well as the dependencies of each system on the above-mentioned groups, an input-output analysis was conducted (Szyrmer and Ulanowicz, 1987). The input-output analysis provides information about the influence that direct and indirect predator-prey interactions have on other compartments of the food web; the fractions of direct and indirect relationships between compartments are represented as components of the total contribution and total dependency matrix (Szyrmer and Ulanowicz, 1987). Input-output analysis is based on information of the matrix of dietary proportions (G), the elements in this matrix represent the fraction of compartment i in the diet of compartment j. By multiplying matrix (G) by itself $(G)^2$, one can determine all the pathways of length 2 connecting i to j. This procedure is repeated until the pathways of maximum length (m) are determined $(G)^m$. The series of matrix powers reach a limit:

$$\lim\{(I)+(G)+(G)^2+(G)^3+(G)^4+\ldots.\} \rightarrow (I-G)^{-1}$$

the matrix (I), which equals $(G)^0$, is named the identity matrix, and consists of 0's for $i\neq j$ and 1's for $i=j$. The limit or matrix inverse $(I-G)^{-1}$ is named the Leontief structure matrix (S), where S_{ij} represents the fraction of total input into j that left i and passed through all pathways to fulfil a final demand of j of one unit. The transformation of the (S) matrix calculate the intermediate transfers between compartments, this transformed matrix is named total dependency matrix (D). The elements of the (D) matrix represents the fraction of the total diet of j that passed through compartment i on its way to j over all direct and indirect pathways.

$$d_{ij=}\ (S_{ij}-\ \delta_{ij})\ (\frac{T_{i.}}{S_{ii}\,T_{.j}})$$

δ_{ij} are the elements of the identity matrix and d_{ij} describes the entries of the dependency matrix, which are termed dependency coefficients, $T_{i.}$ represents the flows from compartment i entering all other compartments in the system and $T_{.j}$ represents all flows entering j. The sum of the coefficients in the jth column of the dependency matrix represents its extended diet or dependency on the production of each compartment in the system via all direct and indirect pathways. Conversely, the row sum of the coefficients in the dependency matrix represents the dependencies of all the other compartments in the system on a particular compartment.

The contribution matrix (C) is calculated from the matrix of host coefficients (F), the output structure matrix (Σ) and the flows (T). The elements of the matrix of host coefficients f_{ij} represents the fraction of total activity of compartment i that flows directly to compartment j. The output structure matrix (Σ) equals $(I-F^T)^{-1}$

(Augustinovics, 1970), F^T is the transposed matrix of host coefficients indicating that the rows of this matrix were exchanged with its columns. The elements of the contribution matrix (*C*) represents the fraction of total flow that left compartment *i* and enters compartment *j* over all direct and indirect pathways.

$$c_{ij} = (\sigma_{ji} - f_{ji}) \left(\frac{T_{i.}}{\sigma_{ii} T_{.j}}\right)$$

σ_{ij} represents the amount of production of compartment *j* generated by one unit of input to *i*, $T_{i.}$ is the exports from compartment *i* entering all other compartments in the system, $T_{.j}$ represents all flows entering *j* and C_{ij} represents the entries of the contribution matrix or contribution coefficients. The row sum of the contribution coefficients determines the total contribution of each compartment to all other compartments in the system.

To determine the contribution of or dependency on a given compartment in the system, the contribution and dependency coefficients of each compartment were row summed. The total contribution of or dependency on the major groups in the system (phytoplankton, microphytobenthos, zooplankton, macrobenthos, fishes, suspended and sediment detritus) was then evaluated.

The Total System Throughput ($T_{..}$) is a measure of the size and activity of a system (Ulanowicz and Kay, 1991). The Total System Throughput equals the sum of all flows of energy or material through all the compartments in the food web.

In order to determine the trophic status of each system, indices such as the number of discrete trophic levels, efficiency of trophic transfers, and amount of detritivory and herbivory were calculated using the Lindeman trophic analysis. This analysis transformed each trophic interaction in a network into a food chain with discrete trophic levels (Ulanowicz and Kemp, 1979, Ulanowicz 1995a). Lindeman analysis first allocated a discrete trophic level to parts of each compartment in the network based on their feeding activities, detritus and primary producers were apportioned to the first trophic level (Ulanowicz and Kemp, 1979). Based on their discrete trophic levels, parts of compartments were grouped according to the trophic level they feed on. The efficiency of trophic transfer or the amount of energy and material passed from one discrete trophic level to another is quantified. The exports, respirations and returns to the detrital pool of each discrete trophic level, as well as the total amount of detritivory and herbivory in a system were also determined.

To examine the cycling of energy and material within these estuaries, a cycle analysis was conducted. This determines the topology of pathways over which energy or material is recycled within a system

(Ulanowicz, 1983). The Finn Cycling Index (FCI) represents the ratio of the total amount of energy or material cycled through the system to the system total throughput (Finn, 1980).

$$\text{FCI} = \quad {}_{i}\,T_i \;\; (S_{ii} - 1)/\,S_{ii}$$

where T_i is the total throughput through group *i* and (S_{ii}-1) is the throughput through group *i* resulting from cycling. The Average Path Length (APL) measures the average number of transfers that a unit of energy experiences since it enters to the system until it leaves the system (Kay et al., 1989). The Average Path Length is calculated as APL: (TST-Z)/Z, where TST is the total system throughput and Z is the sum of all the imports to the system, and

To determine the size, development and organization of each estuary, system information indices were calculated (Ulanowicz, 1986). The ascendency (*A*) provides an indication of the organization and specialization of the flows within a system, and is calculated by multiplying the TST by the average mutual information or measure of flow specialization in a system. Thus, *A* combines the total activity of a system ($T_{..}$) with the efficiency of flows in the system,

$$A = \quad {}_{i.j}\,T_{ij} \;\; \log\left(\frac{T_{ij}\,T_{..}}{T_{i.}T_{.j}}\right)$$

where T_{ij} represents a quantum of flow leaving compartment *i* and entering compartment *j*, $T_{..}$ is the total system throughput and represents the sum of flows over all combinations of T_{ij}, $T_{.j}$ represents all flows entering *j*. Higher *A* values indicate a well organized system, with higher internalization of resources and more specialized pathways (Ulanowicz, 1986). An increase in *A* is expected during the dry season or closed phase of TOCEs due to the lower disturbance caused by reduced freshwater inflow.

The development capacity (*DC*) determines the potential of a system to develop and is the maximum limit to the ascendency. The *DC* is calculated as the product of the total system throughput (TST) and the diversity of individual flows,

$$DC = - \quad {}_{i.j}\,T_{ij} \log\left(\frac{T_{ij}}{T_{..}}\right)$$

where $T_{ij}/T_{..}$ represents the actual flow from compartment *i* to *j*.

The system overhead (*O*) equals the difference between the development capacity and the ascendency, this represents the flexibility of a system to adapt to disturbances (Ulanowicz, 1986). The overhead is formed by the magnitudes and diversity of pathways of imports and exports to and from the system, dissipation of energy (or energy) and redundancy (*R*, parallel pathways). Since the overhead represents the capacity of a system to withstand perturbations, the integrity of an ecosystem depends on a balance between the ascendency and overhead. The overhead on imports and exports is expected to be higher during the wet season as a result of the increased rainfall and river inflow to these estuaries.

The ratio of the ascendency to the development capacity or relative ascendency (*A/DC*) is a measure of the degree of system order, where lower *A/DC* values are indicative of systems with low order (Ulanowicz, 2012). The relative redundancy (*R/DC*) is the ratio of the redundancy or flow on parallel pathways to the development capacity. *R/DC* represents the capacity of a system to adapt to novel perturbations, since parallel pathways of energy act as a buffer against perturbations (Ulanowicz, 2012). Higher *A/DC* values are expected during the dry season or closed phase of the TOCEs because of the reduced flushing and disturbance during this season (Scharler, 2012).

The above suite of indices was calculated for the carbon and nitrogen networks of the East Kleinemonde, Mlalazi and Mpenjati estuaries. The indices obtained are described, compared and related to the physical and biological conditions of the systems during a particular season.

4.3. Results

4.3.1. Total flows, contributions and dependencies

The total system throughput (TST) for carbon and nitrogen networks varied strongly among the three studied estuaries (Table 4.6). Highest values were recorded for the Mpenjati Estuary and lowest for the East Kleinemonde Estuary, with intermediate values for the Mlalazi Estuary (Table 4.6). No seasonal patterns were apparent between the dry and wet seasons for the TST of the TOC East Kleinemonde and Mpenjati estuaries. However, TST was clearly higher during the wet season at the Mlalazi Estuary probably due to the increased exports and imports from and to the estuary during this season.

At the Mlalazi Estuary, microphytobenthos, macrobenthos, suspended and sediment detritus compartments had the highest carbon and nitrogen throughput during the dry season, while higher throughput was recorded by phytoplankton, suspended and sediment detritus during the wet season. The TST reflected the trends reported for the carbon and nitrogen standing stocks in this estuary (Chapter 3). During the closed phase of the East Kleinemonde Estuary, phytoplankton, microphytobenthos and macrobenthos had the highest throughput. Similarly, microphytobenthos had the highest throughput among compartments, followed by detritus and macrobenthos during the closed phase at the Mpenjati Estuary.

Suspended and sediment detritus had the highest throughput among groups during the wet season of the TOC East Kleinemonde and Mpenjati estuaries due to the higher influence of imports and exports during the open phase.

The total contribution matrix provided information on what compartments contributed the most to the carbon and nitrogen requirements of the system via all direct and indirect pathways. The contribution coefficients of the major groups to the carbon networks varied among seasons at the three estuaries (Table 4.4). In the East Kleinemonde Estuary, the fish community contributed the most to the energy requirements of the system during all seasons. The macrobenthic invertebrate community was the second highest contributor to the carbon requirements of this system (Table 4.4). In the Mlalazi and Mpenjati estuaries, macrobenthic compartments contributed the most to the carbon requirement of these systems (Table 4.4). The fish compartments were the second most important contributor to the carbon requirements of these estuaries. In terms of nitrogen, fish and macrobenthic compartments contributed the most to the requirements in the Mlalazi Estuary among all seasons (Table 4.4). In the East Kleinemonde and Mpenjati estuaries, fish compartments contributed more to the nitrogen requirements of these systems during the closed phase, with macrobenthic compartments contributing more than fish during the open phase (Table 4.4).

While the relative contribution to the carbon and nitrogen requirements of the Mlalazi and Mpenjati estuaries varies slightly between aggregated and original networks, the same compartments (e.g. macrobenthos and fish) contributed the most to the requirements of these systems. However, zooplankton had a higher contribution of carbon and nitrogen on the complete networks of these two estuaries (Table 4.4).

The total dependency matrix provided information on what compartments the system depends on the most for nutrient requirements. The dependency coefficients evidenced that these three estuaries were mainly dependent on microphytobenthos, macrobenthos, suspended and sediment detritus. The East Kleinemonde Estuary was, however, mainly dependent on the fish compartments for the carbon requirements of the species in this system (Table 4.5). This system also depends on suspended detritus, macrobenthos and microphytobenthos but to a lesser extent. No clear seasonal pattern was observed in the relative dependencies of this estuary, except that the dependencies on detritus were relatively higher during the mouth open phase. The Mlalazi Estuary was dependent on suspended detritus, macrobenthos, microphytobenthos and sediment detritus in order of importance (Table 4.5). The Mpenjati Estuary was also highly dependent on suspended detritus and microphytobenthos (Table 4.5), the dependency on microphytobenthos was highest during the mouth closed phase of this system (September 2010).

In terms of nitrogen, the East Kleinemonde and Mpenjati estuaries were dependent on phytoplankton and microphytobenthos during the closed phase. During the open phase, the East Kleinemonde was also dependent on suspended detritus, macrobenthos and fish compartments (Table 4.5). Similarly, the Mpenjati Estuary depended on macrobenthos, suspended and sediment detritus during the open phase (Table 4.5). Finally, the Mlalazi Estuary was mainly dependent on microphytobenthos, macrobenthos and suspended detritus (Table 4.5). In addition, the dependency on suspended detritus was higher during the wet season in this estuary.

Small variations were recorded for the relative dependencies between the aggregated and original networks of the Mlalazi and Mpenjati estuaries, these differences were more pronounced for the carbon networks of the Mpenjati Estuary (Table 4.5).

Table 4.4 Contribution coefficients of the different compartments to the total carbon and nitrogen requirements of East Kleinemonde, Mlalazi and Mpenjati estuaries. Letters indicate compartments (PP=Phytoplankton, MPB=Microphytobenthos, D=Suspended detritus, SD=Sediment detritus, ZP=Zooplankton and MB=Macrobenthos).

Estuary	**PP**	**MPB**	**D**	**SD**	**ZP**	**MB**	**Fish**
Carbon							
East Kleinemonde (Mar-06)	5.14	1.90	3.34	2.55	5.05	13.18	32.46
East Kleinemonde (Jul-06)	7.68	1.34	4.45	2.77	0.88	18.85	38.16
East Kleinemonde (Sep-06)	2.97	1.69	2.13	2.70	0.70	17.09	24.94
East Kleinemonde (Nov-06)	3.54	1.53	2.24	1.63	0.66	11.06	23.45
Mlalazi (Aug-10)	0.00	1.26	1.46	2.67	2.88	33.54	24.30
Mlalazi (Nov-10)	0.07	1.57	0.96	2.77	2.97	33.72	21.09
Mlalazi (Feb-11)	0.00	1.58	0.85	2.36	1.70	27.36	20.28
Mlalazi (May-11)	0.02	0.11	0.28	2.58	0.14	21.21	3.06
Mpenjati (Aug-10)	5.34	1.30	2.23	1.78	3.10	14.69	8.65
Mpenjati (Nov-10)	0.24	1.92	1.44	1.94	1.20	12.12	3.55
Mpenjati (Feb-11)	4.00	2.31	2.00	2.31	3.28	22.46	12.77
Mpenjati (May-11)	0.05	1.35	1.02	1.89	0.17	14.07	2.07
Nitrogen							
East Kleinemonde (Mar-06)	7.67	2.16	1.24	3.97	4.64	19.05	38.70
East Kleinemonde (Jul-06)	7.75	1.91	1.55	5.41	2.07	27.40	31.42
East Kleinemonde (Sep-06)	5.03	2.37	0.90	4.82	2.01	33.31	26.95
East Kleinemonde (Nov-06)	5.41	2.32	1.15	2.96	2.21	29.98	28.06
Mlalazi (Aug-10)	4.53	2.15	1.29	2.90	8.15	34.17	52.27
Mlalazi (Nov-10)	0.31	2.72	1.02	3.10	7.03	42.69	52.21
Mlalazi (Feb-11)	0.57	2.40	0.74	2.75	4.59	30.17	46.16
Mlalazi (May-11)	2.75	1.09	0.28	2.40	3.78	23.59	33.08
Mpenjati (Aug-10)	6.81	1.49	1.34	3.77	6.90	20.49	29.48
Mpenjati (Nov-10)	3.54	4.11	0.60	3.76	10.32	24.40	32.43
Mpenjati (Feb-11)	4.62	3.33	1.12	3.75	4.57	39.87	33.21
Mpenjati (May-11)	4.00	2.03	0.48	3.00	4.52	28.49	22.96

Table 4.5. Dependency coefficients on the different compartments to the total carbon and nitrogen requirements of East Kleinemonde, Mlalazi and Mpenjati estuaries. Letters indicate compartments (listed in the Fig. 4.2 legend).

Estuary	**PP**	**MPB**	**D**	**SD**	**ZP**	**MB**	**Fish**
Carbon							
East Kleinemonde (Mar-06)	18.77	26.23	17.84	13.95	5.01	24.98	24.18
East Kleinemonde (Jul-06)	23.85	15.82	29.01	18.23	2.18	29.90	52.19
East Kleinemonde (Sep-06)	6.24	18.45	23.79	15.92	1.86	24.42	28.63
East Kleinemonde (Nov-06)	5.11	12.67	27.32	13.53	2.03	14.34	27.94
Mlalazi (Aug-10)	0.00	32.12	37.34	24.40	6.60	45.73	16.29
Mlalazi (Nov-10)	1.90	15.42	41.56	16.22	8.67	39.21	12.45
Mlalazi (Feb-11)	0.00	22.84	37.86	17.79	4.61	26.25	15.94
Mlalazi (May-11)	1.02	12.97	45.83	14.34	3.56	35.47	9.25
Mpenjati (Aug-10)	11.54	25.46	16.04	18.81	11.15	12.50	4.84
Mpenjati (Nov-10)	2.27	11.29	19.44	12.38	3.99	12.79	2.59
Mpenjati (Feb-11)	12.62	17.83	22.52	17.70	2.49	22.83	5.97
Mpenjati (May-11)	1.01	18.75	22.23	16.81	2.65	17.28	2.51
Nitrogen							
East Kleinemonde (Mar-06)	2.E+17	3.E+17	382.57	7.33	454.69	1.E+03	5.E+05
East Kleinemonde (Jul-06)	-1.E+18	-1.E+18	528.22	12.37	82.29	3.E+03	3.E+04
East Kleinemonde (Sep-06)	51.84	314.89	119.10	11.62	8.47	151.42	116.44
East Kleinemonde (Nov-06)	60.38	310.46	157.27	12.31	13.08	118.72	130.79
Mlalazi (Aug-10)	1.92	136.65	37.35	20.39	11.82	72.90	22.33
Mlalazi (Nov-10)	4.27	33.21	47.04	13.17	12.13	62.69	16.75
Mlalazi (Feb-11)	3.45	48.83	39.58	14.54	6.11	38.67	19.03
Mlalazi (May-11)	10.25	1264.56	206.60	10.68	8.97	92.42	23.65
Mpenjati (Aug-10)	-7.E+16	-5.E+17	46.91	18.02	4.E+04	4.E+02	8.E+02
Mpenjati (Nov-10)	11.85	15.71	18.45	15.04	8.22	16.73	4.35
Mpenjati (Feb-11)	25.90	27.29	29.45	17.90	3.38	32.41	9.53
Mpenjati (May-11)	1.21	88.57	23.89	16.67	7.80	26.57	5.73

4.3.2. Trophic structure

The Lindeman spine quantifies the number of discrete trophic levels in a system and the throughput assigned to each trophic level. Small seasonal variations were recorded in the number of trophic levels in these three estuaries, the East Kleinemonde Estuary had five to six trophic levels (March 2006), while the Mlalazi and Mpenjati estuaries had six trophic levels in all seasons. For the original networks, seven trophic levels were recorded during September and November 2010 in the Mlalazi Estuary and during November 2010 in the Mpenjati Estuary. Most of the throughput (mg C or N$\cdot$m^{-2} day^{-1}) of these three estuaries was concentrated on trophic levels I – III (Fig. 4.2a). Primary producers, suspended and sediment detritus occupy the first trophic level, while the second trophic level comprises mainly detritivorous and herbivorous taxa in the system.

The detritivory varied markedly among estuaries and seasons (Fig. 4.3a-c), the highest detritivory was recorded in the Mpenjati Estuary (277 – 408 mg C·m^{-2} day^{-1}). The detritivory ranged from 44 to 130 mg C·m^{-2} day^{-1} at the East Kleinemonde Estuary and from 41 to 329 mg C·m^{-2} day^{-1} in the Mlalazi Estuary. In terms of nitrogen, the East Kleinemonde Estuary had the lowest detritivory (1 to 6 mg N·m^{-2} day^{-1}) among the three systems (Fig. 4.3a). Detritivory was relatively similar for the Mlalazi (5 to 41 mg N·m^{-2} day^{-1}) and Mpenjati (7 to 33 mg N·m^{-2} day^{-1}) estuaries (Fig. 4.3b, c). A trend for higher detritivory values (in terms of carbon and nitrogen) was recorded at the Mlalazi Estuary during the wet season. Similarly, higher detritivory was reported for the East Kleinemonde and Mpenjati estuaries during the open phase, but this trend was only evident for detritivory in terms of nitrogen. The detritivory: herbivory ratio was also highest in the Mpenjati estuary, ranging from 14:1 to 38:1 and from 2:1 to 21:1 for carbon and nitrogen respectively (Fig. 4.3f). The detritivory: herbivory ratio was intermediate in the Mlalazi Estuary (carbon= 4:1 - 12:1, nitrogen= 2:1 - 10:1) and lowest in the East Kleinemonde Estuary (carbon= 2:1 - 9:1, nitrogen= 0.25:1 - 3:1) (Fig. 4.3d, e). The amount of detritivory and detritivory: herbivory ratio did not vary between aggregated and original networks in the Mlalazi and Mpenjati estuaries.

The trophic efficiencies represent the fraction of total energy passed from one trophic level (TL) to the next, overall, higher trophic efficiencies in terms of carbon were recorded at TL I in all three study systems. The trophic efficiency of the first TL showed small seasonal variations in the East Kleinemonde (68-78%) and Mpenjati (66-73 %) estuaries, but it was more variable in the Mlalazi Estuary (19-58 %) (Fig. 4.4a-c). The trophic efficiency in general decreased with increasing trophic levels, however, the trophic efficiency of TL III was higher than the TL II (14% versus 7%) during July 2006 in the East Kleinemonde Estuary. In terms of nitrogen, the second TL had the highest trophic efficiency in the East Kleinemonde and Mpenjati estuaries during all seasons (Fig. 4.4f, h). In the Mlalazi Estuary, the highest trophic efficiency was recorded at the first TL, with the exception of May 2011, when TL II had the highest trophic efficiency (Fig. 4.4g). At the East Kleinemonde and Mpenjati estuaries, the second highest trophic efficiency was at TL I. Among estuaries, highest trophic efficiency was shown by the East Kleinemonde Estuary, followed by the Mpenjati and then the Mlalazi estuaries (Fig. 4.4). Slightly higher mean trophic efficiencies were recorded for nitrogen than carbon networks, indicating that nitrogen is more efficiently transferred through the food web of all three estuaries. Overall, the highest trophic efficiency at TL I in the East Kleinemonde Estuary indicates that this estuary is more efficient at utilising primary production and transferring energy to higher trophic levels, this was supported by the low detritivory values and detritivory: herbivory ratio in this system.

Small variations were observed between the original and aggregated networks of the Mlalazi and Mpenjati estuaries (Fig. 4.4d, e, i, j), with the original networks of the Mpenjati Estuary having slightly higher carbon and nitrogen trophic efficiencies. The original networks of the Mlalazi Estuary recorded slightly lower trophic efficiencies from TL II to TL III, while the trophic efficiencies from TL III to TL V were slightly higher. Differences between the aggregated and original networks were more pronounced for the nitrogen networks in these two systems (Fig. 4.4i, j).

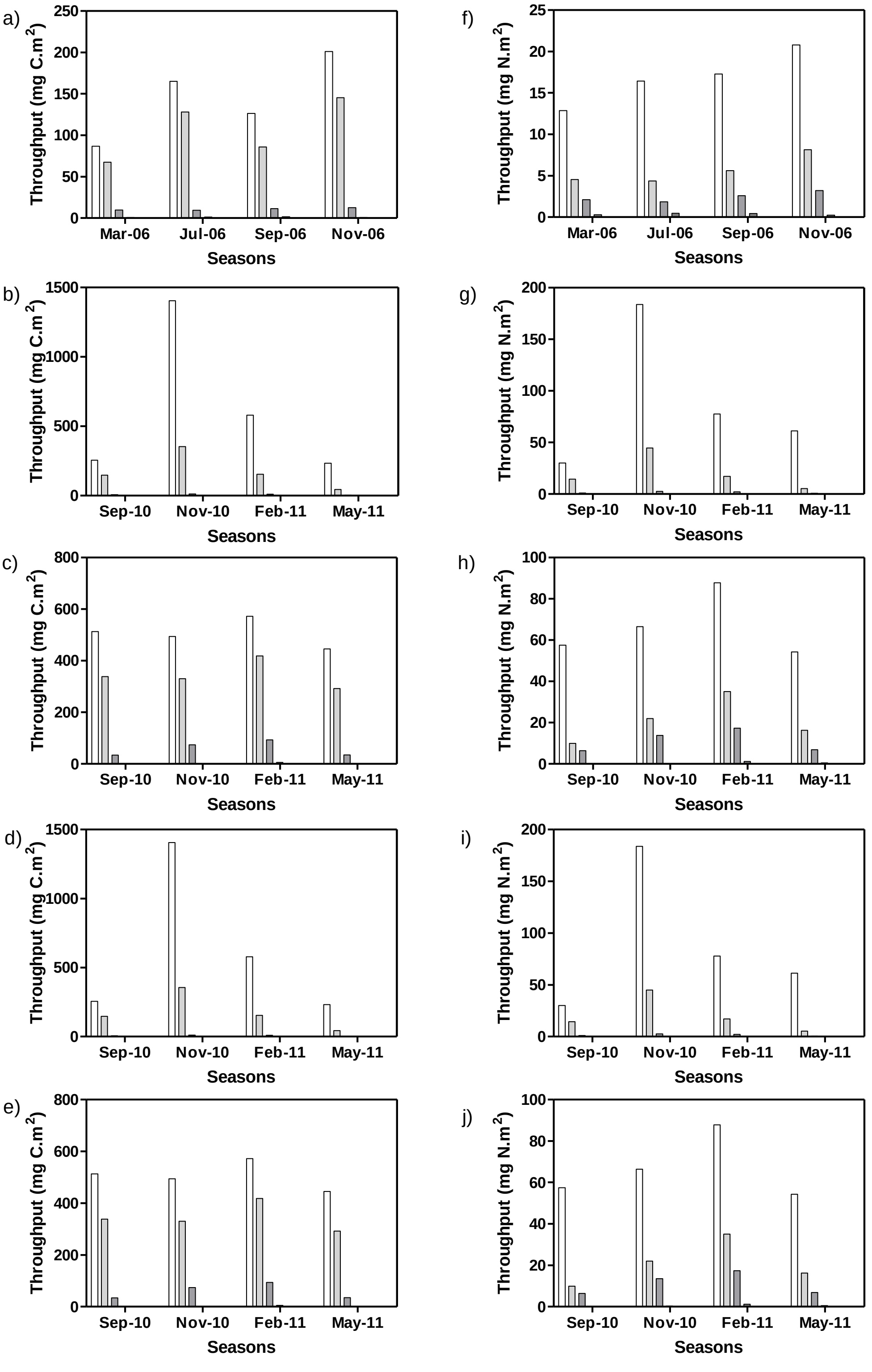

Figure 4.2. Throughput in terms of carbon (left) and nitrogen (right) per trophic level among seasons in the East Kleinemonde (a & f), Mlalazi (b & g) and Mpenjati (c & h) estuaries. Results of the original networks for the Mlalazi (d & i) and Mpenjati estuaries (e & j) are also shown. Colours indicate trophic levels (white= TL I, light grey= TL II, grey=TL III, dark grey= TL IV and black=TL VI).

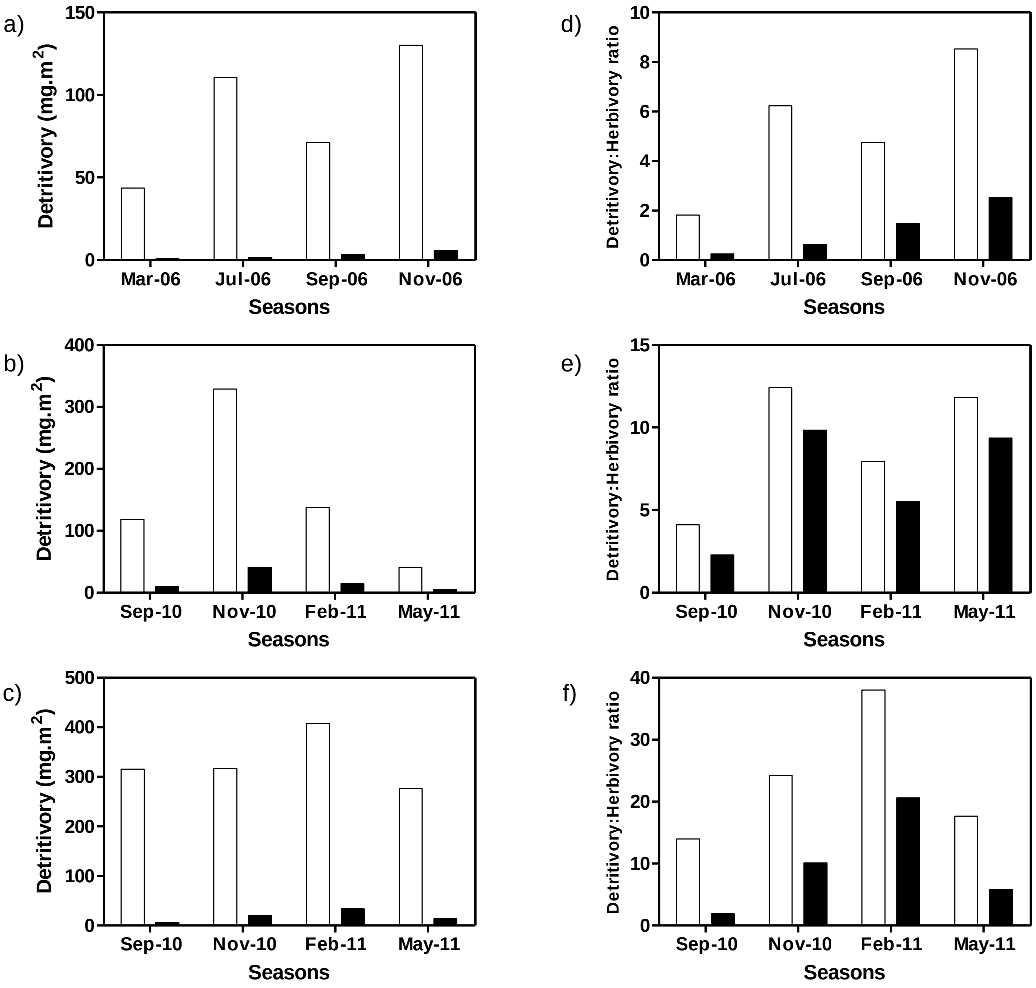

Figure 4.3. Detritivory (mg C or N·m^{-2} day^{-1}) and detritivory:herbivory ratio in the East Kleinemonde (a & f), Mlalazi (b & g) and Mpenjati (c & h) estuaries. Colours indicate the two elements studied (white= carbon and black= nitrogen).

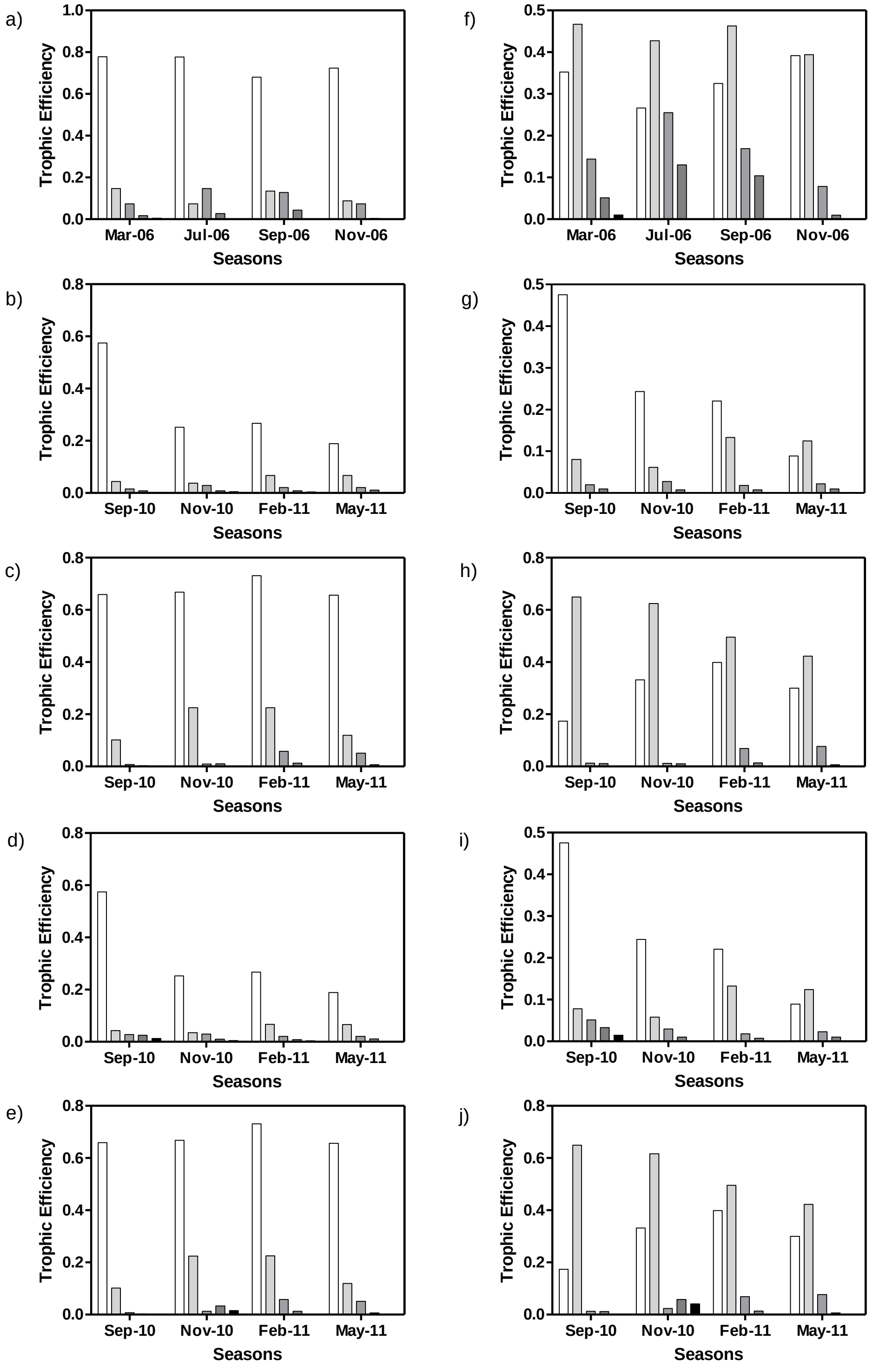

Figure 4.4. Trophic efficiency per trophic level for carbon (left) and nitrogen (right) networks of the East Kleinemonde (a & f), Mlalazi (b & g) and Mpenjati (c & h) estuaries. Results of the original networks for the Mlalazi (d & i) and Mpenjati estuaries (e & j) are also shown. Colours indicate trophic levels (white= from TL I, light grey= from TL II, grey= from TL III, dark grey= from TL IV and black= from TL VI).

4.3.3. Cycling structure and distribution

The total number of cycles in the East Kleinemonde Estuary varied from 43 800 (July 2006) to 260 094 (March 2006), whereas it ranged from 256 020 (May 2011) to 331 243 (November 2010) in the Mlalazi Estuary and from 40 697 (November 2010) to 119 119 (May 2011) in the Mpenjati Estuary. In the original networks, the total number of cycles ranged from 271 225 (May 2011) to 564 408 (November 2010) in the Mlalazi Estuary and from 75 998 (September 2010) to 140 344 (May 2011) in the Mpenjati Estuary. A marked reduction in the total number of cycles was caused by aggregating zooplankton species in the networks of these two estuaries, with the total number of cycles in the East Kleinemonde Estuary expected to be much higher if the zooplankton stock in this system was disaggregated. No seasonal trend was identified for the total number of cycles in the three study systems; with high and low values recorded during the dry and wet seasons (or open and closed phases).

The maximum path length of cycles was 10 in the East Kleinemonde and Mlalazi estuaries, while it was 11 in the Mpenjati Estuary. For the original networks, the maximum path length of cycles was 11 and 12 at the Mlalazi and Mpenjati estuaries respectively. The path length distribution of cycles was relatively stable among seasons in the three estuaries, most cycles took place through seven and eight compartments (66-89 %) in the East Kleinemonde Estuary, and eight and nine compartments at the Mlalazi (~ 72 %) and Mpenjati estuaries (64-67 %) respectively (Fig. 4.5a-c). In the original networks, most cycles also occurred through eight and nine compartments, which together accounted for ~ 68-73 % and 59-67 % at the Mlalazi and Mpenjati estuaries respectively (Fig. 4.5d, e). Cycles with a path length of two to five accounted for less than 2% of the total cycles at the three estuaries (Fig. 4.5a-e).

The average path length (APL) for carbon and nitrogen networks varied among systems. The East Kleinemonde Estuary had the highest carbon APL, the Mpenjati Estuary had intermediate values and the Mlalazi Estuary had the lowest (Fig. 4.6a-c). The nitrogen APL varied from 1.23 to 3.07 in the Mlalazi Estuary and from 1.78 to 3.33 in the Mpenjati Estuary, while nitrogen APL ranged from 2.48 to 2.67 at the East Kleinemonde Estuary (Fig. 4.6a-c). No seasonal pattern in carbon or nitrogen APL could be discerned for these three systems.

The Finn Cycling Index (FCI) in terms of carbon was lowest in the Mlalazi Estuary (3-18 %) and highest in the East Kleinemonde Estuary (18-37%) (Fig. 4.6d-f). No clear seasonal trend was observed for carbon FCI in these three estuaries. The FCI in terms of nitrogen was also lowest in the Mlalazi Estuary (16-27 %), intermediate in the Mpenjati Estuary (21-64 %) and highest in the East Kleinemonde Estuary (38-72 %). Higher nitrogen FCI values were recorded during the closed phase of the East Kleinemonde and Mpenjati estuaries, and during the dry season at the Mlalazi Estuary (Fig. 4.6d-f). The APL and FCI values did not vary between aggregated and original networks in the Mlalazi and Mpenjati estuaries.

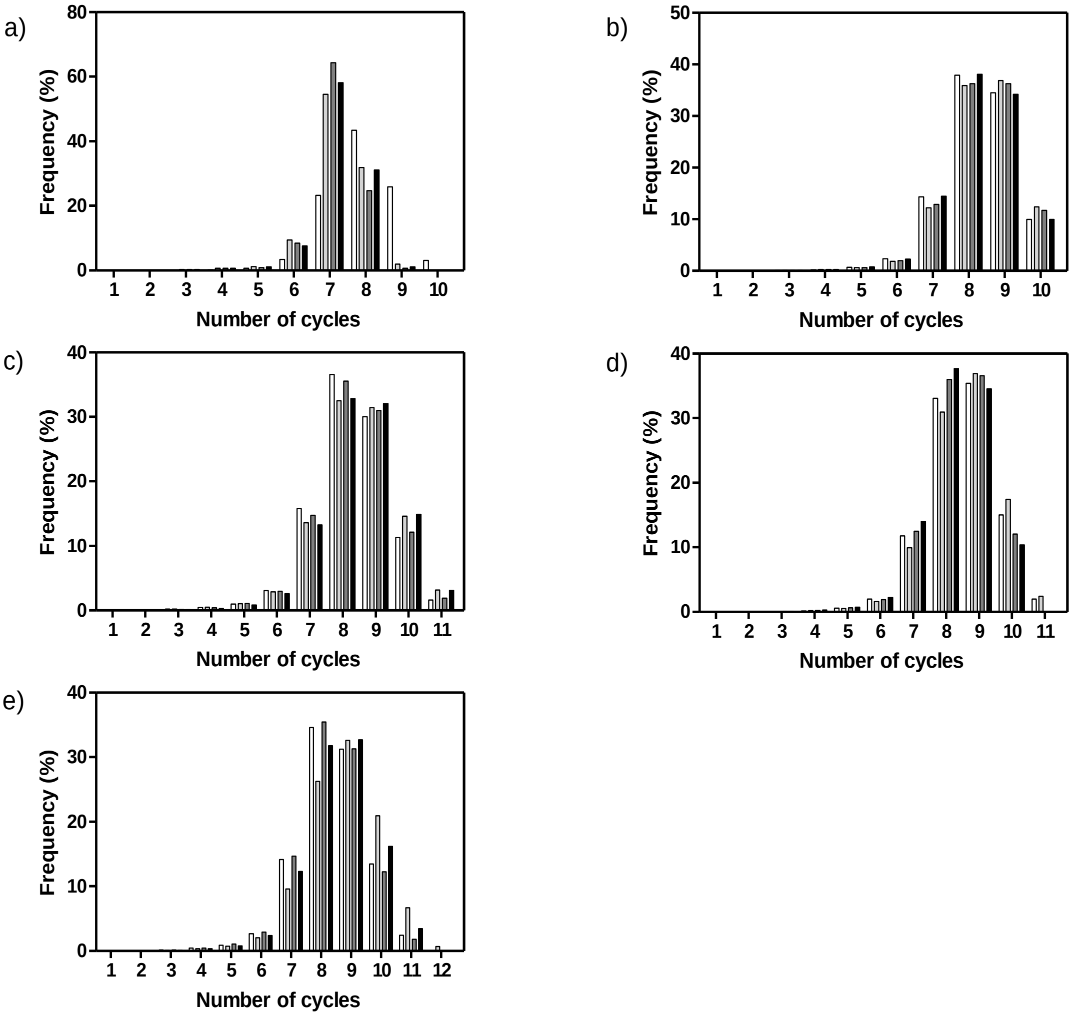

Figure 4.5. Cycles distribution (%) per path length in a) East Kleinemonde, b) Mlalazi and c) Mpenjati estuaries. Results of the original networks for d) Mlalazi and e) Mpenjati estuaries are also shown. Colours indicate dates (listed in the Fig. 4.2 legend).

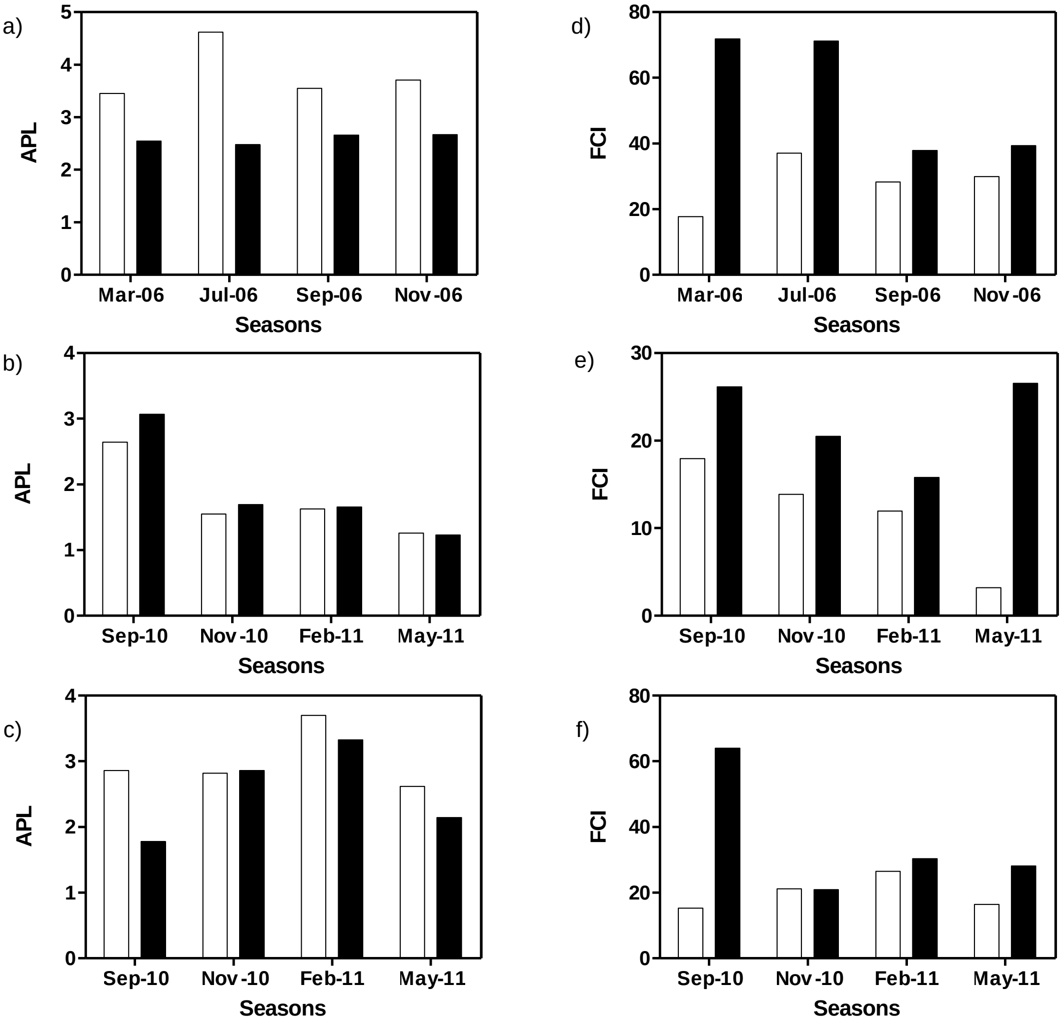

Figure 4.6. Average Path Length (left) and Finn's Cycling Index (right) in the East Kleinemonde (a & d), Mlalazi (b & e) and Mpenjati (c & f) estuaries. Colours indicate the two elements studied (white= carbon and black= nitrogen).

4.3.4. Ecosystem status

Information indices such as the development capacity (*DC*), ascendency (*A*) and overheads (*O*) describe the degree of development and organization of flows in a system (Kay et al., 1989; Ulanowicz, 1986), these indices varied strongly among the three studied estuaries (Table 4.6). For the carbon networks, highest *DC* and *A* were recorded for the Mpenjati Estuary and lowest for the East Kleinemonde Estuary, with intermediate values for the Mlalazi Estuary (Table 4.6). For the nitrogen networks, highest *DC* and *A* were recorded for the Mlalazi and Mpenjati estuaries respectively, the East Kleinemonde Estuary had the lowest *DC* and *A* (Table 4.6). No seasonal patterns were identified for the *A* and *DC* of the East Kleinemonde and

Mpenjati estuaries, however, these indices were higher during the wet season for the Mlalazi Estuary. Overall, the *A* and *DC* followed the trends reported for the TST in these estuaries (section 4.3.1).

The relative ascendency (*A/DC*) for carbon and nitrogen networks were highest in the Mpenjati Estuary and lowest in the East Kleinemonde Estuary (Table 4.6). No seasonal trends were recorded between dry and wet seasons for the *A/DC* from carbon networks. However, higher *A/DC* was identified during the dry season (or closed phase of TOCEs) for the nitrogen networks of the three estuaries indicating that these systems were more organized and specialized during the dry season (Table 4.6). The relative redundancy (*R/DC*) for carbon and nitrogen networks was highest in the East Kleinemonde Estuary, lowest for the carbon networks of the Mlalazi Estuary, while the *R/DC* was similar for the nitrogen networks of the Mlalazi and Mpenjati estuaries (Table 4.6). No differences in the *R/DC* between wet and dry seasons were recorded for these systems. The *R/DC* was higher than the *A/DC* among seasons in the East Kleinemonde, but not in the Mlalazi and Mpenjati estuaries. These results indicate that the East Kleinemonde Estuary has a higher proportion of parallel pathways, implying that this system has a higher resilience. The overhead on imports/*DC* and exports/*DC* ratio for the carbon and nitrogen networks were highest at the Mlalazi Estuary and lowest at the East Kleinemonde Estuary (Table 4.6). As predicted, overhead of imports and exports/*DC* were higher at the three study systems during the wet season or open phase of the TOCEs indicating a higher influence of external inputs during this season.

Table 4.6. System indices for carbon and nitrogen (right) aggregated networks of the East Kleinemonde, Mlalazi and Mpenjati estuaries. TST= Total System Throughput, DC= Development Capacity, A= Ascendency, A/DC= Relative Ascendency, R/DC= Relative Redundancy, OI/DC= Overhead on Imports/DC and OE/DC= Overhead on Exports/DC. (C) and (N) indicates results from carbon and nitrogen networks respectively.

	East Kleinemonde Estuary				**Mlalazi Estuary**				**Mpenjati Estuary**			
Indices	**Mar-06**	**Jul-06**	**Sep-06**	**Nov-06**	**Aug-10**	**Nov-10**	**Feb-11**	**May-11**	**Aug-10**	**Nov-10**	**Feb-11**	**May-11**
TST (C)	228.48	397.83	305.13	488.07	605.70	2963.69	1246.95	508.87	1499.67	1409.48	1655.91	1247.26
DC (C)	1335.53	2168.03	1700.32	2553.16	3058.09	12763.81	5683.65	1790.62	6121.55	6230.41	7976.63	5394.30
A (C)	437.51	850.46	573.75	850.63	1040.15	4410.73	1937.08	852.17	2695.85	2657.59	3126.48	2294.54
A/DC (C)	0.33	0.39	0.34	0.33	0.34	0.35	0.34	0.48	0.44	0.43	0.39	0.43
R/DC (C)	0.41	0.40	0.40	0.40	0.39	0.22	0.26	0.18	0.30	0.24	0.33	0.32
OI/DC (C)	0.06	0.05	0.10	0.10	0.09	0.20	0.18	0.14	0.05	0.11	0.10	0.07
OE/DC (C)	0.00	0.00	0.03	0.04	0.04	0.17	0.14	0.05	0.00	0.02	0.02	0.02
TST (N)	27.60	36.95	37.19	46.13	63.10	372.95	160.70	124.38	131.81	171.16	228.16	119.63
DC (N)	140.04	172.41	193.65	236.08	290.89	1560.46	698.23	379.85	394.07	722.68	1057.12	462.72
A (N)	52.56	68.86	69.01	83.08	118.58	532.59	237.35	179.39	235.89	319.45	459.95	230.57
A/DC (N)	0.38	0.40	0.36	0.35	0.41	0.34	0.34	0.47	0.60	0.44	0.44	0.50
R/DC (N)	0.53	0.50	0.49	0.48	0.48	0.28	0.31	0.29	0.33	0.32	0.37	0.38
OI/DC (N)	0.09	0.10	0.12	0.13	0.08	0.21	0.20	0.20	0.07	0.17	0.12	0.11
OE/DC (N)	0.00	0.00	0.03	0.04	0.03	0.18	0.15	0.04	0.00	0.07	0.08	0.02

4.4. Discussion

In this study, carbon and nitrogen networks of quantified trophic flows were constructed, representing four consecutive seasons for the East Kleinemonde, Mlalazi and Mpenjati estuaries. A total of 24 carbon and nitrogen networks were built and analysed using Ecological Network Analysis, thus contributing to the knowledge of ecosystem structure and functioning of these estuaries. This study represents the first attempt to holistically understand the dynamics of not only carbon, but also nitrogen flows in African estuaries.

The total system throughput (TST) did not reflect the seasonal variations in the carbon and nitrogen biomass of the pelagic and benthic communities in these estuaries. Seasonal differences in the TST between wet and dry seasons were only recorded at the permanently open Mlalazi Estuary due to the higher flows from imports and exports of sediment and suspended detritus, phytoplankton and zooplankton during this season. Despite the high biomass of planktonic and benthic communities at the TOCEs East Kleinemonde and Mpenjati during the closed phase, no variations were recorded in the TST between seasons. Similarly, the TST of the TOCEs Mdloti and Mhlanga did not reflect the seasonal trends reported for the biomass of selected biota (Scharler, 2012). The TST comprises all flows in the system, therefore the lack of differences in TST between closed and open phases is attributed to the higher flows from exchanges across the system boundaries during the wet season (Scharler, 2012).

In terms of the contribution of the different compartments to the energy requirements of the system, macrobenthic and fish compartments contributed more to the carbon requirements in the Mlalazi and Mpenjati estuaries, while fish compartments were most important in the East Kleinemonde Estuary. The differences in the relative contribution of macrobenthic and fish compartments to the carbon requirements of these estuaries were attributed to variations in the size of benthic and fish standing stocks, with very high macrobenthic standing stocks recorded in the Mlalazi and Mpenjati estuaries when compared to the East Kleinemonde Estuary where fish standing stocks were considerably higher than macrobenthic taxa. These results supported the findings of Scharler and Baird (2005), who found that the carbon requirements of the Kromme and Swartkops estuaries mostly relied on benthic compartments, however, benthic feeding fishes were included in the benthic compartments in that study.

Fish and macrobenthic compartments were also the highest contributors to the nitrogen requirements of the study systems. However, fish compartments contributed more to the nitrogen requirements of these systems than the macrobenthic ones. Interestingly, higher contribution of fish compartments was identified during the closed phase of the temporarily open/closed East Kleinemonde and Mpenjati estuaries, with a higher contribution of macrobenthos during the open phase. The differences in the contribution of fish and macrobenthos between carbon and nitrogen networks were attributed to the higher inputs from fish compartments to the nitrogen detrital pools of these systems. Fish excretions have been found to be a significant source of nitrogen and phosphorus to aquatic systems, and nutrient inputs by fish excretion can be a similar magnitude to watershed-derived nutrient runoff or nutrient release from sediments (Vanni, 1996; Vanni, 2002).

The study estuaries mainly depended on the production of suspended and sediment detritus, microphytobenthos and macrobenthos to fulfil the nutrient requirements of the compartments in these systems; although the East Kleinemonde Estuary mainly relied on fish compartments. Higher dependency on phytoplankton was reported during the closed phase in the East Kleinemonde and Mpenjati estuaries, and this was attributed to the very high throughput of phytoplankton during the closed phase of these systems (Gama, 2008; Chapter 1–3). In terms of nitrogen, the East Kleinemonde and Mpenjati estuaries were dependent on phytoplankton and microphytobenthos during the close phase. Similarly, high reliance on microphytobenthos was recorded in the Mlalazi Estuary during the dry season when microphytobenthos standing stocks were highest. These results highlighted the key role of primary production in sustaining these systems during the dry season or closed phase of TOCEs. These results also underline the importance of freshwater inflow in determining the functioning and dependencies of estuarine systems. Similarly, Scharler and Baird (2005) reported that freshwater and nutrient input were crucial in determining the magnitude of the dependencies of the Kromme, Swartkops and Sundays estuaries, South Africa. No other studies analysing contribution and dependencies in estuaries were available to this study, thus comparisons are limited to the above mentioned estuaries.

The detritivory and its ratio to herbivory were lowest in the East Kleinemonde Estuary and highest in the Mpenjati Estuary for the carbon networks. The Mlalazi and Mpenjati estuaries had similar amounts of detritivory in terms of nitrogen, but the detritivory: herbivory ratio was highest at the Mpenjati Estuary, thus confirming the relatively high reliance on detritus in this system. Higher detritivory was recorded for the carbon networks in the Mlalazi Estuary during the wet season and for nitrogen networks in all three study systems. Carbon and nitrogen detritivory: herbivory ratios were also higher during the wet season in the East Kleinemonde and Mpenjati estuaries. The higher availability of suspended and sediment detritus probably resulted from increased freshwater inflow into these systems during the wet season (or open phase of TOCEs), thus explaining the higher amounts of detritivory during these periods. Vosloo (2012) also recorded higher detritivory: herbivory in the TOCE Mngazi during the wet season, e.g. similar to that reported for the East Kleinemonde and Mpenjati estuaries during this study. Scharler (2012) analysed the dynamics of several network indices of two subtropical temporarily open/closed estuaries in relation to the duration of inlet closure and opening, and found that the carbon detritivory:herbivory ratio increased from mouth breaching to re-closure and decreased with the extent of closure. Therefore, the trends observed in this ratio in the two study TOCEs are strongly dependent on the mouth state and dynamics.

The detritivory: herbivory ratio reported for the East Kleinemonde, Mlalazi and Mpenjati estuaries were comparable to the ratios reported for other estuarine systems in the world, e.g. the Mngazana (11.9) and Mngazi (24.1) estuaries, South Africa (Vosloo, 2012), the Aiguillon Cove (4.9) and Brouage (6.6) mudflats, France (Leguerrier et al., 2007), Chesapeake Bay (4.8), USA (Baird et al., 1991). The ratios for the current study estuaries were higher than those for the Swartkops (1.5) and Ems (0.5) estuaries (Baird et al., 1991) but lower than that reported for the Kromme Estuary (57.0) by Scharler and Baird (2005). No detritivory: herbivory ratios in terms of nitrogen were available for comparisons with this study.

In terms of trophic efficiency, the East Kleinemonde Estuary had the highest trophic efficiency from trophic level (TL) I, suggesting that this system is more effective in transferring the primary production to primary consumers (TL II), whilst the Mlalazi Estuary had the lowest trophic efficiency from TL I. The latter is explained by the very high biomass and production of food sources (TL I) compared to the consumers, whereas in the East Kleinemonde and Mpenjati estuaries the relatively lower biomass of the food sources compared to consumers explained the higher trophic efficiencies. No seasonal trends in the trophic efficiency were recorded in the study systems, however, Scharler (2012) reported higher trophic efficiencies at TL I during the closed phase of the temporarily open/closed Mdloti and Mhlanga estuaries due to a simultaneous increase of TL II standing stocks during this phase.

In terms of nitrogen, the highest trophic efficiency was at TL II in the East Kleinemonde and Mpenjati estuaries, thus indicating that nitrogen is more efficiently transferred from TL II (e.g. zooplankton, some detritivores fishes) to TL III (e.g. proportions of predatory polychaetes, benthic invertebrate feeding fishes) in these systems. This implies that the consumption of TL III was high compared to the production rates of their prey at TL II. This trend was observed during May 2011 at the Mlalazi Estuary but the differences between the trophic efficiency at TL I and II were very small. This behaviour was not reported for the annual nitrogen networks in the Sylt-Rømø Bight, German Wadden Sea or Chesapeake Bay, USA (Baird et al., 2008; Baird et al., 1995), where the trophic efficiencies decreased with increasing trophic level. It is possible that this behaviour is characteristic of temporarily open/closed estuaries; however, the lack of nitrogen networks for this type of system precluded us from making comparisons or generalizations.

The trophic efficiencies from TL I to II reported in this study are similar to other permanently open and temporarily open/closed estuaries. For example, Chesapeake Bay, USA (52%), Swartkops, South Africa

(41%) and Ems, England (60%) estuaries (Baird et al., 1991), the Kromme (84%), Swartkops (72%) and Sundays (54%) estuaries, South Africa (Scharler and Baird, 2005), the Sylt-Rømø Bight (47%), German Wadden Sea (Baird et al., 2008), and the Mngazana (70%) and Mngazi (62%) estuaries, South Africa (Vosloo, 2012). The trophic efficiencies from TL I to II for the nitrogen networks of the East Kleinemonde, Mlalazi and Mpenjati estuaries (9–48%) were considerably lower than the ones reported for the Chesapeake Bay (77%) and Sylt-Rømø Bight (53%) (Baird et al., 2008; Baird et al., 1995).

The cycling structure and magnitude of flows in an ecosystem provides information on its functioning e.g. higher magnitude of cycling is characteristic of more mature systems, dependent on internal connections and better able to withstand perturbations (Odum, 1969). Cycling structure is represented by the total number of cycles and distribution, while the magnitude is expressed as the Finn cycling index and average path length. The number of cycles and its distribution is highly dependent on the number of compartments in a network and the degree of aggregation among them; therefore networks need to have the same number of compartments to make comparisons possible (Baird et al., 1991; Baird and Ulanowicz, 1993). Reductions of 40-50% in the total number of cycles were recorded between original and aggregated networks in the Mlalazi and Mpenjati estuaries respectively, thus confirming that the total number of cycles is sensitive to aggregation among compartments. The number of cycles and the maximum path length recorded for the study estuaries are not directly comparable to other studies due to the sensitivity of this analysis to network topology and degree of aggregation.

The Finn cycling index and average path length did not show differences between aggregated and original networks. The FCI of carbon and nitrogen networks was highest in the East Kleinemonde Estuary and lowest at the Mlalazi Estuary. Ulanowicz (2004) suggests that the probability of a nutrient being recycled in a system increases with its scarcity, indicating that possibly there was a shortage of food sources (e.g. primary producers, detritus) in the East Kleinemonde Estuary. This assumption is supported by the high trophic efficiencies from TL I to II recorded in this system. The nitrogen FCI was higher than the carbon index during all seasons in the three study estuaries, thus indicating that nitrogen is more limiting than carbon in these systems. Similarly, higher FCI for nitrogen networks were recorded in Chesapeake Bay (Baird et al., 1995), nine subsystems within the Sylt-Rømø Bight (Baird et al., 2011) and the whole bight (Baird et al., 2008).

The levels of carbon cycling in the study estuaries were similar to those from the Mngazana (11-13 %) and Mngazi (23-26 %) estuaries (Vosloo, 2012), Kromme (41%), Swartkops (26%) and Sundays (20%) estuaries (Scharler and Baird, 2005), Gironde (17%) (Lobry et al., 2008) and Seine (16%) estuaries, France (Rybarczyk and Elkäim, 2003), Chesapeake (24 %), Delaware (37%) and Narragansett (48%)

Bays, USA (Monaco and Ulanowicz, 1997). The nitrogen FCI reported here was in agreement with the indices reported for Chesapeake Bay (34-70%), USA (Baird et al., 1995), Sylt-Rømø Bight (43%) (Baird et al., 2008), Tancada Lagoon (52-62%), Spain (Forès et al., 1994) but lower than in the Neuse Estuary (74-98%), USA (Christian and Thomas, 2003).

Higher FCI was recorded for the nitrogen networks of the study systems during the dry season or closed phase of TOCEs, the higher levels of cycling were explained by the lower nutrient availability due to reduced freshwater and nutrient input during this season. Higher recycling was also found during the closed phase of the Mdloti and Mhlanga estuaries, with carbon recycling being strongly influenced by the fluctuations in inlet status of these TOCEs, e.g. FCI was lowest after mouth breaching, peaked during the closed phase and then decreased according to the duration of inlet closure (Scharler, 2012). Similarly, higher nitrogen cycling was recorded in Chesapeake Bay, USA, during the dry season (Baird et al., 1995). In contrast, the Mngazana and Mngazi estuaries, South Africa, showed slightly higher FCI values during the wet season (Vosloo, 2012).

The Average Path Length (APL) followed the trends recorded by the FCI; thus the APL was lowest in the Mlalazi Estuary and highest in the East Kleinemonde Estuary, thereby reflecting their levels of recycling activity. Despite the FCI being higher for all nitrogen networks, the APL for the nitrogen networks in the East Kleinemonde and Mpenjati estuaries were lower than for the carbon ones, thus indicating that nitrogen is cycled over fewer steps within these systems. A previous study reported an increase in the APL from carbon to nitrogen in the whole Sylt-Rømø Bight (Baird et al., 2008). However, this pattern was not recorded in three of the nine subsystems analysed in the same bight (Baird et al., 2011), the East Kleinemonde and Mpenjati estuaries. APLs are expected to increase in systems with high cycling rate and flow diversity (Christensen, 1995). It is thus suggested that although nitrogen recycling rate was higher at the East Kleinemonde and Mpenjati estuaries, the lower APLs of nitrogen could be explained by the lower flow diversity (lower evenness of the flows in a food web) of nitrogen when compared to carbon in these two estuaries.

System information indices quantify the development and organization of a system. These indices strongly fluctuated among seasons in the study estuaries, higher *A/DC* was recorded for the nitrogen networks of these estuaries during the dry season. Scharler (2012) predicted an increase in *A/DC* at the expense of redundancy with the duration of mouth closure, since the proportion of organized flows is expected to increase during the less disturbed closed phase of TOCEs. As predicted there was an increase in *A/DC* for the nitrogen networks of the TOCEs analysed in this study. The overhead on imports/*DC* and exports/*DC* increased during the wet season or open phase of the three study estuaries as expected due to the higher rainfall and freshwater inflow during this season.

The East Kleinemonde Estuary had the lowest *A/DC*, *OE/DC* and *OI/DC,* and the highest *R/DC* suggesting that this system is less organized than the other study estuaries, but it is also the most self-reliant and resilient. The overhead on parallel pathways (redundancy) comprised from 60-85% of the total overhead in this estuary. The East Kleinemonde Estuary is a predominantly closed system, which experiences closed mouth conditions for >90% of the time (Whitfield et al., 2008). The lower freshwater inflow and consequent less frequent opening phases in this system could thus explain the lower reliance on external inputs, while the redundancy or proportion of parallel pathways is high at the expense of the ascendency. The Mlalazi Estuary had intermediate *A/DC*, lowest *R/DC* and highest *OE/DC* and *OI/DC* among the systems indicating this system is influenced by high levels of imports and exports during the wet season. The Mpenjati Estuary showed the highest *A/DC* and intermediate *R/DC*, *OE/DC* and *OI/DC* implying that the Mpenjati is the most organized among the study estuaries. Overall, the three study estuaries showed attributes of organization and resilience which were mainly related to the magnitude and fluctuations in freshwater inflow to these systems. These results are in agreement with previous studies which have demonstrated the importance of freshwater inflow in determining the ecosystem functioning of estuaries (Heymans et al., 2002; Scharler, 2012; Scharler and Baird, 2005).

The main challenges faced during the construction of the models analyzed here were the sources of uncertainty from data inputs. Most models available in the literature are built using information from literature sources not specific to the study system or in a different time. A specific sampling program was conducted to build the networks analyzed here and most input data (i.e. biomass, diets) for this study was derived from the sampling program, however, there was still considerable uncertainty derived from the natural variability of input parameters such as biomass, diets and even flows. Decisions related to model construction such as degree of aggregation and assessment of input data quality were one of the most challenging parts of network construction. Similarly, data gaps are one of the main issues in data construction, because data from important groups such as bacteria are mainly scarce. Data is sometimes available for similar systems; however, the use of this information will only increase the uncertainty in the models. There are several limitations and sources of uncertainty for network analysis, which can preclude scientist from constructing network models. However, the outputs obtained from network analysis substantially contribute to the understanding of ecosystem structure and functioning.

The carbon and nitrogen networks built during this study provide evidence that the seasonal differences in rainfall and freshwater inflow do influence the functioning of the entire estuarine systems and not only compartments thereof. Certain indices such as the detritivory:herbivory ratio, FCI, APL, *A/DC*, *O/DC* were the most sensitive to the seasonal changes in river inflow and mouth status influencing these estuaries, since they reflected the strong seasonal changes in the standing stocks of the abiotic and biotic components reported for these systems. Similarly, the use of nitrogen networks allow for the determination of patterns in the behaviour of these systems, which were not evidenced through the use of individual carbon networks. Future studies, which include components such as dissolved inorganic and organic pools, bacteria and protozoa, are necessary in order to improve the analytical strength of current networks. This study highlights the need for constructing and analysing networks of other elements (e.g. phosphorus), since the simultaneous analysis of carbon, nitrogen and phosphorus networks will further the understanding of nutrient dynamics and limitations in estuaries. Nevertheless, the results from this study have already provided important information to aid the understanding of nutrient dynamics and the importance of nutrient sources on estuarine functioning on the east coast of South Africa, as well as for the management and conservation of these systems (e.g. threats posed by freshwater abstraction and catchment degradation).

GENERAL CONCLUSIONS

This study provided a comprehensive view of the influence of the seasonal variability in rainfall and river inflow on selected estuaries on the east coast of South Africa at the species-, community- and ecosystem-level. This study evaluated the spatio-temporal variations of the density, biomass, nutrient content and stoichiometry of planktonic and benthic communities in selected estuaries. The temporal stability of these community-level properties and its drivers were also analysed. Finally, ecosystem network models were built to provide an understanding of carbon and nitrogen dynamics in estuarine foodwebs and to examine the influence of the variability in river inflow in the functioning of selected estuaries.

Information was collated on dissolved inorganic nitrogen and phosphorus concentrations, phytoplankton biomass, zooplankton density and biomass from 16 TOCEs in South Africa to determine possible response patterns to inlet phase. This analysis detected a consistent pattern of higher phytoplankton chlorophyll-a concentrations, zooplankton density and biomass during the closed phase of the analysed estuaries, which was attributed to reduced flushing and stable water column conditions during this phase. An analysis of the temporal dynamics of five TOCEs showed that planktonic biomass can recover to pre-breaching levels within 10 days after mouth closure. Higher variability in the planktonic density and biomass was also recorded during the closed phase of these estuaries, mainly because this phase incorporates seasonal and other environmental changes (e.g. pollution events) that are usually not captured during the much shorter open phase. Additionally, estimates showed that planktonic standing stocks during the closed phase can be 26 to 10000 times higher than during the open phase. Therefore, this study has significant implications for the management and conservation of TOCEs, especially the regulation of freshwater inflow and artificial breaching of their mouths.

The influence of changing rainfall and river flow on the density and biomass of planktonic communities of selected estuaries on the east coast of South Africa was demonstrated in Chapter 1, this marked environmental variability may also influence the variability and temporal stability of estuarine communities. The variability and temporal stability of community-level properties such as phytoplankton biomass, zooplankton and macrobenthic density and biomass at the Mlalazi and Mpenjati estuaries was determined based on quarterly samplings from May 2010 to May 2011 (i.e. covering a complete wet/dry cycle). Maximum phytoplankton biomass, zooplankton density and biomass were recorded during the wet season in the permanently open Mlalazi Estuary. In contrast, a reduction in these community properties was recorded during the wet season at the temporarily open/closed Mpenjati Estuary as reported for other TOCEs on the east coast of South Africa in Chapter 1. Macrobenthic density and biomass were significantly higher during the dry season (or closed phase for the TOCE Mpenjati) in both estuaries.

The variability (as measured by the coefficient of variation, CV) of the different community-level properties did not vary significantly among seasons in these estuaries, despite the marked variations in the analysed community-level properties. This suggests that the seasonal fluctuations in river inflow did not influence the variability of the community-level properties. Significant differences were reported in variability at the community-level, with phytoplankton and macrobenthic biomass having the lowest and highest variability respectively. The temporal stability (*S*) was similar among community-level properties and systems, with species synchrony being the main driver of community stability in both estuaries, which has been reported for other systems with high prevalence of environmental forcing. The results of this study indicate that the temporal stability of the analysed community-level properties and its drivers were influenced by the marked seasonal changes in rainfall and river inflow in this region.

To determine the influence of the seasonal environmental variability on the standing stocks and stoichiometry of carbon and nitrogen in planktonic and benthic estuarine communities, this study analysed the elemental composition and stoichiometry of suspended and sediment detritus, zooplankton and benthic macroinvertebrates in two estuaries with contrasting mouth status over a dry/wet cycle. Carbon and nitrogen standing stocks of planktonic and benthic communities followed the seasonal trends recorded for the biomass in these two estuaries (Chapter 2). The C:N ratios of suspended detritus were highest during the dry season in both systems, and the highest nutrient content of suspended and sediment detritus was recorded in the upper reaches of both estuaries. Significant seasonal variations in the elemental content and stoichiometry of 10 zooplankton and macrobenthic taxa were reported, these variations were not related to the seasonal changes in suspended or sediment detritus in either estuary.

Significantly higher nitrogen content was reported for the mysids *Mesopodopsis africana* and *Rhopalophthalmus terranatalis*, and the polychaetes *Ancistrosyllis parva*, *Ceratonereis keiskama* and *Glycera* spp. These species were less N-limited due to their predatory feeding mode as evidenced in the literature and the isotope analysis results from this study. Stable isotope analysis reported that most macrobenthic species from the Mlalazi and Mpenjati estuaries are suspension or deposit feeders. In this study, the among-taxa variability in nutrient content seems to be mainly related to feeding mode. The seasonal variability in river inflow was key to determine the standing stocks of these two systems, however, they did not influence the nutrient content and stoichiometry of the organisms in these estuaries.

Seasonal carbon and nitrogen ecosystem models based on quantified trophic links were built to examine the flow and cycling of energy and material through three of the study estuaries. Information on standing stocks, carbon and nitrogen content were used to construct ecological networks for the East Kleinemonde, Mlalazi and Mpenjati estuaries. The analysis of the seasonal networks of these systems evidenced higher dependency on phytoplankton and microphytobenthos during the closed phase in the East Kleinemonde and Mpenjati estuaries, which was attributed to the very high phytoplankton carbon and nitrogen throughput during this phase in these systems (Gama, 2008; Chapter 1–3). Fish compartments contributed the most to the nutrient requirements of the temporarily open/closed East Kleinemonde and Mpenjati estuaries during the closed phase, and a higher contribution by macrobenthos was identified during the open phase.

The seasonal fluctuations in river inflow influenced the amount of detritivory and the detritivory: herbivory ratios in these systems, with higher values recorded during the wet season in these estuaries. Higher trophic efficiencies were recorded for nitrogen than carbon networks, indicating that nitrogen is more efficiently transferred through the food web of all three estuaries. Cycling indices showed that nitrogen was recycled to a higher degree than carbon in these systems and that more nitrogen was recycled during the dry season or closed phase of TOCEs. Seasonal differences in system indices were also recorded, which were explained by differences in the fluctuations in freshwater inputs to these systems. These results highlighted the importance of seasonal variations in rainfall and freshwater inflow to the functioning of the study estuaries.

This study allowed for the identification of data gaps in the knowledge of South African estuaries. Future research should therefore investigate the ecology of groups such as microplankton, bacteria and meiofauna. Similarly, comprehensive taxonomic and diversity analyses are necessary to understand the role of rare species and diversity in the stability and functioning of estuaries on the subcontinent.

Finally, the networks analysed in this study could be improved for further analyses by including data gaps such as dissolved inorganic and organic pools, bacteria and protozoa. It is also essential to construct and analyse networks of elements such as phosphorus in order to understand the role of macronutrient dynamics and their limitations in South African estuaries.

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APPENDIX

Appendix 2.1: Environmental variability

For the determination of Total Suspended Solids (TSS), triplicate water samples were collected in acid washed plastic bottles from ca. 50 cm depth at each station and subsequently stored in the dark on ice. In the laboratory, the water samples were filtered through a 0.72 µm pre-combusted and pre-weighted GF/F filter, which was then dried at 60°C for 24 h and re-weighed. Particulate Organic Carbon and Nitrogen (POC and PON) measurements were obtained from GF/F filters, on which POM had been collected. These were analyzed using a Europa Scientific Elemental Analyser 20-20 IRMS (Europa Scientific Limited, Crewe, England). Results for POC and PON were expressed as µg of carbon and nitrogen per liter. Three 100 ml samples of water filtrate from TSS determinations were collected in acid washed bottles and frozen at -20 °C for the determination of dissolved inorganic nitrogen (DIN: nitrate+nitrite) and phosphorus (DIP: orthophosphate) concentrations. Nutrient determinations were conducted using a Skalar San++ continuous-flow analyser (Skalar Analytica BV, The Netherlands).

For the determination of sediment nutrient concentrations (C, N), three sediment samples were collected at each station to a depth of 1 cm using a twin-corer (internal diameter 2 cm) to determine carbon and nitrogen content in the sediment. Nutrient content in the sediment was analyzed following the procedure described above for POC and PON content of TSS. Three sediment samples were collected at each station using a Zabalocki-type Ekman grab (September 2010) and a van Veen Grab (other sampling seasons) to determine grain size distributions and sediment organic content. Samples were dried at 60 °C for 48 h. For sediment organic content determinations, 5 g of oven-dried sediment was weighed and combusted at 450 °C for six hours before reweighing. Organic matter content was determined as the difference in sediment weights. For grain size analysis, the remaining dried sand was analysed to determine mud content (%) at Enviromap laboratory (Durban, South Africa).

Appendix 2.2: List of zooplankton and macrobenthos species at the Mlalazi and Mpenjati estuaries.

Zooplankton taxa recorded at the Mlalazi Estuary

Acartia sp.
Mesopodopsis africana
Pseudodiaptomus sp.
Rhopalophthalmus terranatalis
Bivalve veligers
Chaetognaths
Cumaceans
Medusae
Nauplii larvae
Polychaeta larvae
Zoea larvae

Zooplankton taxa recorded at the Mpenjati Estuary

Acartia natalensis
Mesopodopsis africana
Pseudodiaptomus hessei
Rhopalophthalmus terranatalis
Chaetognaths
Cumaceans
Medusae
Nauplii larvae
Ostracods
Polychaeta larvae
Zoea larvae

Macrobenthos taxa recorded at the Mlalazi Estuary

Crustacea

Apseudes digitalis
Balanus sp.
Corophium triaenonyx
Excirolana natalensis
Grandidierella sp.
Grandidierella bonnieroides
Hymenosoma orbiculare
Iphinoe truncata
Lepthanthura laevigata
Melita sp.
Munna shettoni
Paratylodiplax blephariskios
Sinelobus standfordi
Brachyuran larvae
Harpacticoidea
Isopod sp. 1

Polychaeta

Ancistrosyllis parva
Capitella capitata
Ceratonereis keiskama
Cossura sp.
Dendronereis arborifera
Desdemona ornata
Glycera spp.
Phyllodoce sp.
Polydora sp.
Tharyx sp.
Capitellidae
Cirratulidae
Spionidae

Mollusca

Assiminea ovata
Brachidontes virgilae
Dosinia hepatica
Eumarcia paupercula
Macoma litoralis
Nassarius kraussanis
Polinices sp.
Solen cylindraceus
Tarebia granifera
Tellina sp.

Ophiuroidea

Oligochaeta

Platyhelminthes

Macrobenthos taxa recorded at the Mpenjati Estuary

Crustacea

Apseudes digitalis
Callianassa kraussi
Corophium triaenonyx
Excirolana sp.
Grandidierella bonnieroides
Grandidierella lignorum
Grandidierella spp.
Hymenosoma orbiculare
Iphinoe truncata
Urothoe sp.
Sinelobus standfordi
Amphipod 1
Amphipod 2

Polychaeta

Capitella capitata
Ceratonereis keiskama
Dendronereis arborifera
Desdemona ornata
Capitellidae
Nereidae sp.1
Spionidae

Mollusca

Assiminea sp.
Macoma litoralis

Chironomidae larvae

Hirudinea

Nematoda

Oligochaeta

Platyhelminthes

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