

# Carbon and nitrogen system dynamics in three small South African estuaries, with particular emphasis on the influence of seasons, river flow and mouth state

K. Ortega-Cisneros<sup>1,3,\*</sup>, U. M. Scharler<sup>1</sup>, A. K. Whitfield<sup>2</sup>

<sup>1</sup>School of Life Sciences, University of KwaZulu-Natal, Durban 4041, South Africa

<sup>2</sup>South African Institute for Aquatic Biodiversity, Private Bag 1015, Grahamstown 6140, South Africa

<sup>3</sup>Present address: Department of Ichthyology and Fisheries Science, Rhodes University, Grahamstown 6140, South Africa

**ABSTRACT:** The importance of fluctuations in rainfall and riverine flow on ecosystem functioning in a permanently open estuary (POE), the Mlalazi, and 2 temporarily open/closed estuaries (TOCEs), the East Kleinemonde and Mpenjati, were investigated. These systems, located on the east coast of South Africa, are strongly influenced by seasonal changes in rainfall and river flow. Most TOCEs are open to the sea during the wet season and closed during the dry season. Ecosystem models based on ecological network analysis were applied to the data, using both carbon (C) and nitrogen (N) as model currencies. The analyses indicated that the dependency of consumers on primary producers was higher during the closed phase than the open phase in TOCEs, which was attributed to the higher phytoplankton and microphytobenthos standing stocks during the former phase. Higher trophic efficiencies at trophic level (TL) 1 (primary producers) were recorded for C and N networks in the TOCEs during the open phase, while in the Mlalazi Estuary the trophic efficiency at TL 1 was highest during the dry season. The detritivory:herbivory ratio and total system throughput (TST) reflected the seasonal variations in the phytoplankton and microphytobenthos standing stocks within the estuaries. Cycling indices suggested that more N was recycled during TOCE closed phases and during the POE dry season. The contrasting results between the dry and wet seasons in the 3 estuaries, as well as the opposite system-level responses within TOCEs and POE, clearly emphasised the importance of seasonal variations in river flow and mouth state to ecosystem level functioning.

**KEY WORDS:** Ecosystem function · Estuarine environments · Carbon · Nitrogen · Mouth status · Rainfall

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## INTRODUCTION

Temporarily open/closed estuaries (TOCEs) are the most common type of estuary in South Africa, accounting for approximately 65% of estuaries in the country (Whitfield & Baliwe 2013), and are usually separated from the sea by a sandbar at the mouth during low river-flow periods. During high river-flow conditions, the connection between the estuary and the sea is re-established, with an exchange of nutrients and biomass then occurring between these adja-

cent ecosystems (Whitfield et al. 2008). 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). Permanently open estuaries (POEs) have a permanent connection to the sea and are usually characterized by catchments larger than 500 km<sup>2</sup>, perennial river flow and a moderate tidal prism (from 1 × 10<sup>6</sup> to 10 × 10<sup>6</sup> m<sup>3</sup>) (Whitfield 1992). Changes in river flow arising from heavy rainfall and

\*Corresponding author: flypper5@hotmail.com

run-off events are one of the most important factors determining the mouth status of TOCEs (Whitfield et al. 2008). In the subtropical region of South Africa, heavy spring and summer precipitation results in river flooding and therefore frequent estuarine connections to the sea during summer, whereas in the warm temperate region, major rainfall events are less seasonal and can occur at any time of the year.

Several studies have documented variations in the abiotic and biotic components of POEs and TOCEs as a result of changes in rainfall and river flow (e.g. Schlacher & Wooldridge 1996, Wooldridge 1999, Perissinotto et al. 2003, Scharler & Baird 2003). For instance, higher phytoplankton and microphytobenthos biomass have been recorded during the closed phase of TOCEs in South Africa (Walker et al. 2001, Perissinotto et al. 2002, Skinner et al. 2006), while higher macronutrient concentrations have been reported during the open phase (Perissinotto et al. 2010, Ortega-Cisneros et al. 2014). Freshwater flow to estuaries is therefore a crucial factor controlling the nutrient concentrations, abundance and biomass of estuarine communities associated with TOCEs (e.g. Adams et al. 1999, Wooldridge 1999, Teske & Wooldridge 2001).

Carbon (C), nitrogen (N) and phosphorus (P) are essential elements for the growth and functioning of aquatic organisms; therefore fluctuations in the availability of these elements have important implications for the structure and functioning of aquatic systems. Large elemental imbalances (differences in elemental composition) 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, Sterner & Hessen 1994, Elser & Urabe 1999). Consumer-driven nutrient recycling, caused by imbalances in consumer-resource stoichiometry, may thus determine the overall nutrient limitation regime experienced by a system. For instance, a species with a low N:P ratio will release the surplus nutrient (P) through excretion into the environment, which is therefore available, usually after recycling, to be assimilated by primary producers once again (Elser et al. 1988, Elser & Urabe 1999). Under poor nutrient conditions, lower abundances of primary producers and herbivorous consumers have been recorded (Schoo et al. 2012). Nutrient limitations may thus result in long-term alterations in the primary productivity, quality of that production, transfer efficiency of energy flows, and food web dynamics of ecosystems, by constraining the growth of primary producers and consumers (Cross et al. 2003, van de Waal et al. 2010, Glibert et al. 2011).

A comprehensive understanding of system dynamics can be achieved by using a holistic approach such as ecological network analysis (ENA), a methodology that analyses the abiotic and biotic interactions of entire networks (Ulanowicz 1986). ENA indices have been used to evaluate the impact of different environmental conditions in estuarine and marine systems (Christian et al. 2005). To date, most ENA studies have focused on the functioning of ecosystems using C trophic flows (e.g. Baird & Ulanowicz 1993, Heymans et al. 2002, Dame & Christian 2008), with considerably fewer studies analysing the behaviour of N and P in the functioning of ecosystems (Forès et al. 1994, Thomas & Christian 2001, Kaufman & Borrett 2010). Similarly, very few studies have simultaneously analysed the dynamics of C, N and P in aquatic systems, with the exceptions of the mesohaline community in Chesapeake Bay, USA (Ulanowicz & Baird 1999), a mangrove system in Belize (Scharler et al. 2015), and the Sylt-Rømø Bight in the German Wadden Sea (Baird et al. 2008, 2011).

In this study C and N ecosystem models were built based on quantified trophic links, and used ENA to examine the structure and functioning of 3 estuaries on the east coast of South Africa during dry and wet seasons, and under different mouth states. The specific objectives were to (1) quantify the relative contribution of selected groups (e.g. phytoplankton, microphytobenthos, fish) to the C and N requirements of each estuary, and the relative dependency of each estuary on these communities; (2) determine the number of trophic levels (TLs), efficiency of trophic transfers between TLs, and the amount of detritivory and herbivory in each estuary; and (3) quantify the cycling structure of C and N within these estuaries.

## MATERIALS AND METHODS

### Study areas

Of the 3 estuaries studied, 2 are TOCEs (East Kleinemonde and Mpenjati) and the third is a POE (Mlalazi), with all 3 being in a 'good' ecological condition (Whitfield & 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.

The East Kleinemonde Estuary (33° 32' 28" S; 27° 03' 00" E) is located in the warm temperate biogeographical region of South Africa (Fig. 1) and has a catchment area of approximately 46 km<sup>2</sup> (Badenhorst 1988) with an estimated mean annual runoff of 2 ×

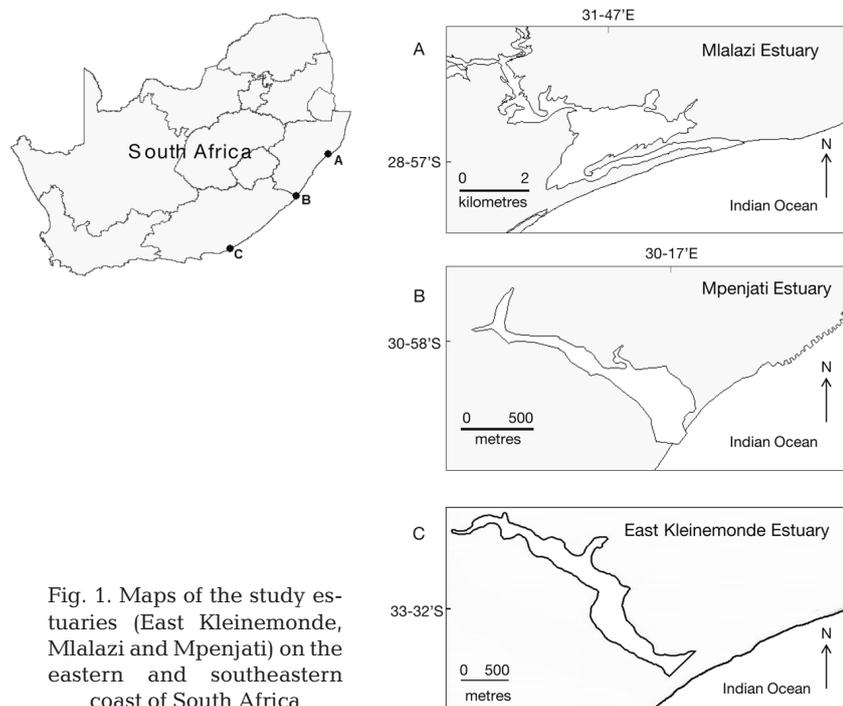


Fig. 1. Maps of the study estuaries (East Kleinemonde, Mlalazi and Mpenjati) on the eastern and southeastern coast of South Africa

$10^6 \text{ m}^3$  (van Niekerk et al. 2008). The estuary water area is 14.5 ha when the mouth is closed (van Niekerk & Turpie 2012). The catchment is mainly undeveloped and used for cattle grazing, with residential developments located adjacent to the lower reaches of the estuary (Whitfield et al. 2008). Monthly rainfall ranged from 12.0 mm in July 2006 to 229.0 mm in August 2006 during the study period March–November 2006 (Fig. S1 in Supplement 1 at [www.int-res.com/articles/suppl/m557p017\\_supp1.pdf](http://www.int-res.com/articles/suppl/m557p017_supp1.pdf)). Ecological networks were built for the East Kleinemonde Estuary covering this period.

The Mlalazi Estuary ( $28^{\circ} 56' 42'' \text{ S}$ ;  $31^{\circ} 48' 58'' \text{ E}$ ) is located in the subtropical region of South Africa and has a catchment area of approximately  $492 \text{ km}^2$  (DEAT 2001). The estimated mean annual runoff is  $122 \times 10^6 \text{ m}^3$  (Jezewski et al. 1984) and the estuary water area is 95.9 ha at high tide (van Niekerk & Turpie 2012). Subsistence farming, sugar cane cultivation and commercial forestry account for approximately 46% of the catchment usage, with 53% of the area considered undegraded and 1% being urban (DEAT 2001). Catchment monthly rainfall varied from a minimum of 11.0 mm in September 2010 to a maximum of 230.4 mm in January 2011 during the study period September 2010–May 2011 (Fig. S1 in Supplement 1). Ecological networks were built for the Mlalazi Estuary covering this period.

The Mpenjati Estuary ( $30^{\circ} 58' 21'' \text{ S}$ ,  $30^{\circ} 17' 02'' \text{ E}$ ) is also located in the subtropical region of South

Africa and has a catchment area of approximately  $101 \text{ km}^2$ . The estimated mean annual runoff is  $20.7 \times 10^6 \text{ m}^3$  (Jezewski et al. 1984) and the estuary water area is 11.6 ha when the mouth is closed (van Niekerk & Turpie 2012). Monthly rainfall varied from 18.2 mm in September 2010 to 223.5 mm in May 2011 during an abnormally wet winter for this region (Fig. S1 in Supplement 1). Ecological networks were built for the Mpenjati Estuary covering this period.

### Data sources and model construction

Ecological networks are representations of the flow of mass or energy in an ecosystem and are structured by a collection of nodes (abiotic and biotic compartments) linked to each other by weighted trophic flows (Kay et al. 1989). A total of 24 seasonal C and N networks were built for the East Kleinemonde, Mlalazi and Mpenjati estuaries (3 systems, 4 months, 2 elements). Ecological networks of C and N were built for the East Kleinemonde to represent the conditions during March, July, September and November 2006, with these months being chosen because of a comprehensive dataset available for this system over that period (van Niekerk et al. 2008). The ecological networks for the Mlalazi and Mpenjati estuaries represented September and November 2010, and February and May 2011, as for those months comprehensive datasets were available (Ortega-Cisneros et al. 2014, Ortega-Cisneros & Scharler 2015).

The months were assigned to dry and wet seasons according to the principal rainfall regime of the respective regions (Table 1, Fig. S1 in Supplement 1). The number of compartments (species or groups of species) in each network varied among the estuaries and seasons to reflect actual species composition of these estuaries at a particular time (Table 1). All networks included 2 detritus (suspended and sediment) and 4 primary producer compartments, whilst zooplankton was represented as a single compartment. The number of macrobenthos compartments varied from 6 to 22, while the number of fish compartments ranged from 14 to 28, and the number of bird compartments from 4 to 6 depending on seasons and estuaries (Supplement 2 at [www.int-res.com/articles/suppl/m557p017\\_supp2.xlsx](http://www.int-res.com/articles/suppl/m557p017_supp2.xlsx)). No bacterial groups or

Table 1. List of study areas and information on the seasonal ecological networks for each system. Dates are month-year

Estuary	Date	Mouth status	Season	Number of compartments
East Kleinemonde	Mar-06	Closed	Dry	49
	Jul-06	Closed	Dry	43
	Sep-06	Open	Wet	45
	Nov-06	Open	Wet	44
Mlalazi	Sep-10	Open	Dry	64
	Nov-10	Open	Wet	63
	Feb-11	Open	Wet	61
	May-11	Open	Dry	63
Mpenjati	Sep-10	Closed	Dry	39
	Nov-10	Open	Wet	35
	Feb-11	Open	Wet	44
	May-11	Open	Abnormally wet dry season	45

dissolved inorganic nutrients were included in the networks, despite their importance for estuarine functioning, primarily due to a lack of appropriate data for bacteria in general, and DIC in particular (see 'Data sources and model construction' in Supplement 1).

The flows included in the system represent imports and exports across system boundaries (i.e. imports from the river or sea to the estuary and exports to the sea), respiration and flows between nodes. For model construction, the biomass of all nodes was expressed in  $\text{mg C m}^{-2}$  or  $\text{mg N m}^{-2}$  and the flows were expressed in  $\text{mg C}$  or  $\text{mg N m}^{-2} \text{d}^{-1}$ . Information on the biomass, consumption, respiration, production, egestion and diet for each compartment is necessary for network construction (see Supplement 1).

All compartments in the C networks were mass balanced; therefore all inputs to a compartment (consumption) are equal to the outputs (production + respiration + egestion) from the same compartment. The diet composition was extracted from the literature and previous studies on each system (see Supplement 1), whereas the quantities of trophic flows between compartments were calculated using the MATLOD method (Ulanowicz & Scharler 2008). MATLOD is a least-inference method, because it makes no additional assumptions regarding the flows in the network except the availability and demand of the flow currency. 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 from a donor is depleted. This method ensures that both small and large links are maintained within the network (Ulanowicz & Scharler 2008) by starting

with the assumption that there is no difference among all possible flows in a network.

N networks were built, based on the C networks. Biomass ( $\text{mg N m}^{-2}$ ) was calculated using dry biomass and %N obtained for species in the Mlalazi and Mpenjati estuaries (Ortega-Cisneros & Scharler 2015), as well as from the literature (Table S2 in Supplement 1). No information regarding elemental content or C:N ratios was available for the East Kleinemonde Estuary, and the mean %N for the same or similar species from the Mlalazi and Mpenjati estuaries were used to obtain an estimate for the N biomass in the former system. Thus the magnitude of N biomass in a particular compartment is represented by the original biomass variability, rather than possible %N fluctuations in the East Kleinemonde Estuary.

Fish N biomass was calculated using regression analysis based on fish wet weight, as proposed by Ramseyer (2002) (see 'Data sources and model construction' in Supplement S1). Bird biomass was calculated using C biomass and C:N ratios for birds from the literature (Baird et al. 2008, Chereil et al. 2008). Imports and exports were calculated using measured tidal flows and simultaneous measurements of suspended detritus and plankton (See 'Data sources and model construction' in Supplement S1). Using N biomass ( $\text{mg N m}^{-2}$ ) data and C flows for each compartment, N trophic flows between compartments and across the system boundary were quantified based on the C:N ratios calculated from the C and N standing stocks of each donor compartment, with the C:N of a donor compartment's production flow being equivalent to its biomass C:N.

### Balancing of networks

After the N trophic flows between compartments had been quantified, all C and N networks were mass balanced, because some analyses (input–output and TL analyses) conducted in this study require mass balance around each compartment (Ulanowicz 2011). A 'layered' algorithm (Scharler et al. 2015) was used to balance the C and N networks simultaneously, while ensuring that the C:N stoichiometry of all compartments was maintained. In this approach, all flows of the same magnitude (magnitude to the power of 10) are assigned to a layer; the flows on that layer are gradually adjusted to achieve balance, while all flows 'outside' the layer are kept constant. This process is repeated among layers until the whole system is balanced. The vectors of the balanced networks were checked against the initial estimates, and val-

ues were adjusted if necessary; e.g. if the respiration of a compartment in the balanced networks exceeded the ranges provided in the literature, the flows from that compartment to suspended or sediment detritus were adjusted to ensure plausible respiration values and maintain mass balance.

### Ecological network analysis

The mass-balanced C and N networks were analysed using the software WAND (Allesina & Bondavalli 2004) to examine the flow of material in the monthly networks (replicated within seasons) of the 3 estuaries (Ulanowicz 1986). An input–output analysis was conducted (Szyrmer & Ulanowicz 1987) to quantify the relative contribution of detritus, primary producers (phytoplankton and microphytobenthos) and consumers (zooplankton, macrobenthos, fish) to the C and N requirements of the system, as well as the relative dependencies of each system on the above-mentioned groups. The input–output analysis provides information on the influence that direct and indirect predator–prey interactions have on other compartments in the food web; with the fractions of direct and indirect relationships between compartments being represented as components of the total contribution and total dependency matrix (Szyrmer & Ulanowicz 1987). The elements of the contribution matrix (i.e. the contribution coefficients) represent the fraction of total flow that exited donor compartment *i* and entered recipient compartment *j* over all direct and indirect pathways. The row sum of the contribution coefficients determines the relative degree of contribution by each compartment (*i*) to all other compartments in the system. The taxa with the highest relative contribution for each estuary and season were identified.

The elements of the dependency matrix represent the fraction of the total diet of *j* that passed through compartment *i* on its way to *j* over all direct and indirect pathways, and these are termed dependency coefficients. The row sum of these coefficients in the dependency matrix represents the relative degree of dependency of all the other compartments in the system on a particular compartment. The different nodes in each system were assigned to major groups such as phytoplankton, microphytobenthos, zooplankton, macrobenthos, fishes, suspended and sediment detritus (see column A of Tables S3 to S26 in Supplement 2). The dependency coefficients for each node in these major groups were then summed to determine the relative degree of dependency on these groups in the system.

In order to determine the trophic status of each system, indices such as the number of discrete TLs, efficiency of trophic transfers, and amount of detritivory and herbivory were calculated using the Lindeman trophic analysis (LTA). This analysis transforms all trophic interactions in a network into a food chain with discrete TLs (Ulanowicz & Kemp 1979, Ulanowicz 1995a). The LTA allocates discrete TLs proportional to each compartment in the network based on its feeding activities, with detritus and primary producers being apportioned to the first TL (Ulanowicz & Kemp 1979). Based on their discrete TLs, parts of compartments were grouped according to the TL at which they operate (e.g. a compartment may feed to 70% on TL 2, and to 30% on TL 3, thus placing this compartment at a TL of:  $(0.7 \times 2) + (0.3 \times 3) = 2.3$ ).

The total amount of detritus and primary production consumed in the system is represented as detritivory and herbivory respectively. The trophic efficiencies represent the fraction of total material from the input to one TL to the input of the next, and are influenced by the abundance, production and consumption of a specific TL. The trophic efficiency at TL 1 was calculated for primary producers only to track the seasonal differences in primary production in each estuary. This trophic efficiency reported for TL 1 therefore does not account for inputs of detritus from outside the system, returns into the detrital pool and detritivory. The efficiency of trophic transfer, or the amount of material passed from one discrete TL to another, was quantified to track differences over seasons, and between nutrients and estuaries.

In terms of system indices, the total system throughput (TST) is a measure of the size of a system's activity (Ulanowicz & Kay 1991) and equals the sum of all flows of material through all the compartments in the food web. It is expected that TST will reflect the variations in standing stocks, imports and export to/from the system during the dry and wet seasons. A cycle analysis was conducted to examine the magnitude and structure of cycling of material within these estuaries. The Finn cycling index (FCI) represents the ratio of the total amount of material cycled through the system to the TST (Finn 1980). Together with the FCI, the average path length (APL) measures the retention of a unit of C or N when 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 imports to the system.

The above suite of indices was calculated for the C and N networks of the 3 estuaries. The indices obtained are described, compared and related to the

physical and biological conditions of the systems during a particular season in order to gain a better understanding of the functioning and organization of these systems under different mouth states and river flow regimes.

## RESULTS

### Contributions and dependencies

The taxa with the highest relative degree of contribution to the C networks varied among seasons in all 3 estuaries (Table 2). Overall, the production from the macrobenthic community taxa (e.g. polychaete taxa of the families Capitellidae, Cirratulidae and Spionidae) had the highest relative contribution to the C and N requirements of all compartments in the

Mlalazi and Mpenjati estuaries during all seasons (Table 2). However, a few fish taxa from the family Mugilidae had the highest relative contribution to the food webs of the Mpenjati Estuary during the closed and onset of the open phase in terms of N (Table 2). In the East Kleinemonde, fish taxa from a number of families had the highest relative contribution to the C and N requirements of this estuary during all seasons (Table 2).

The relative dependency, on the other hand, showed that these 3 estuaries mainly relied on the production of microphytobenthos, macrobenthos and suspended detritus for both C and N requirements (Table 3). The East Kleinemonde Estuary, however, had a higher relative dependency on fish for C requirements. A higher relative dependency on microphytobenthos was recorded during the closed phase for both TOCEs. In terms of N, the food webs of the

Mlalazi and Mpenjati estuaries had a higher relative dependency on microphytobenthos production. The food web of the East Kleinemonde Estuary was mainly dependent on suspended detritus and phytoplankton, where the relative dependency on phytoplankton was higher during the closed phase.

Table 2. Contribution coefficients (carbon, C and nitrogen, N) of the 3 taxa with highest relative contribution at each date in the East Kleinemonde, Mlalazi and Mpenjati estuaries

Estuary and species	C	N	C	N	C	N	C	N
<b>East Kleinemonde</b>	Mar-06		Jul-06		Sep-06		Nov-06	
<i>Atherina breviceps</i>	-	-	-	2.85	-	-	-	-
<i>Ceratonereis keiskama</i>	-	-	-	-	2.58	-	-	-
<i>Gilchristella aesturia</i>	2.95	-	3.72	-	-	-	2.15	2.63
<i>Liza dumerilii</i>	3.00	3.02	3.80	-	-	2.93	2.32	2.62
<i>Myxus capensis</i>	3.54	2.96	3.84	2.81	2.60	2.92	2.42	2.61
<i>Oreochromis mossambicus</i>	-	3.02	-	2.96	-	2.93	-	-
<i>Solea bleekeri</i>	-	-	-	-	2.57	-	-	-
<b>Mlalazi</b>	Sep-10		Nov-10		Feb-11		May-11	
<i>Ancistrosyllis parva</i>	-	-	-	-	2.62	-	-	-
<i>Capitella capitata</i>	-	-	-	-	-	-	3.42	-
Capitellidae	3.58	-	3.18	-	2.64	2.00	3.43	-
<i>Ceratonereis keiskama</i>	3.62	-	3.21	2.56	-	-	-	-
Cirratulidae	-	-	3.20	-	-	-	3.43	-
<i>Cossura</i> sp.	-	-	-	2.57	-	-	-	-
<i>Desdemonia ornata</i>	-	2.66	-	-	-	-	-	-
<i>Glycera</i> spp.	-	-	-	-	2.64	-	-	-
<i>Hymenosoma orbiculare</i>	-	2.59	-	-	-	-	-	-
<i>Liza macrolepis</i>	-	-	-	-	-	-	-	1.57
<i>Paratyrodiplox blephariskios</i>	-	-	-	-	-	2.03	-	-
Spionidae	3.65	3.13	-	2.98	-	2.10	-	2.22
<i>Valamugil cunnesius</i>	-	-	-	-	-	-	-	1.57
<b>Mpenjati</b>	Sep-10		Nov-10		Feb-11		May-11	
<i>Callianassa kraussi</i>	-	-	-	2.79	-	-	-	-
<i>Capitella capitata</i>	2.24	-	1.60	-	1.99	-	2.03	2.44
<i>Ceratonereis keiskama</i>	2.24	-	-	-	1.98	2.14	-	2.46
<i>Ceratonereis</i> spp.	2.13	-	-	-	-	-	-	-
<i>Dendronereis arborifera</i>	-	-	-	-	-	2.24	-	-
<i>Desdemonia ornata</i>	-	-	1.94	-	-	-	2.08	-
<i>Liza dumerilii</i>	-	2.70	-	3.09	-	-	-	-
Spionidae	-	-	2.07	-	2.02	2.22	2.22	2.92
<i>Valamugil cunnesius</i>	-	2.32	-	-	-	-	-	-
<i>Valamugil robustus</i>	-	2.49	-	2.99	-	-	-	-

### Trophic structure

The Lindeman spine quantifies the number of canonical TLs in a system and the throughput assigned to each TL and flow (Ulanowicz & Kay 1991). The East Kleinemonde Estuary had 5 (July, September and November 2006) to 6 (March 2006) TLs, and the Mlalazi and Mpenjati estuaries had 5 TLs during all seasons. The difference in flows between TL 5 and 6 was small, this difference was lower than 0.001 mg C or mg N m<sup>-2</sup> d<sup>-1</sup> for most seasons. Most (~99%) of the throughput (mg C or mg N m<sup>-2</sup> d<sup>-1</sup>) at these 3 estuaries occurred at TL 1 to TL 3.

Highest trophic efficiencies were recorded in the Mpenjati

Table 3. Dependency coefficients (carbon and nitrogen) for the major groups in the East Kleinemonde, Mlalazi and Mpenjati estuaries. (PP = phytoplankton, MPB = microphytobenthos, SD = detritus, SED = sediment detritus, ZP = zooplankton, MB = macrobenthos and FI = fish)

Element and estuary	Date	PP	MPB	SD	SED	ZP	MB	FI
<b>Carbon</b>								
East Kleinemonde	Mar-06	37.91	7.09	33.56	18.33	3.78	30.61	19.15
	Jul-06	16.70	21.90	28.75	18.13	1.86	28.03	41.66
	Sep-06	7.95	7.58	32.36	16.47	1.91	27.43	31.21
	Nov-06	6.62	8.10	30.48	13.52	2.29	14.64	26.67
Mlalazi	Aug-10	<0.01	33.04	37.73	31.81	5.35	71.35	15.12
	Nov-10	1.00	19.07	37.26	21.31	9.49	40.47	10.17
	Feb-11	<0.01	26.98	33.49	19.91	4.36	22.32	13.57
	May-11	1.00	18.66	40.21	18.57	3.80	50.47	10.19
Mpenjati	Aug-10	7.26	29.73	13.81	19.90	9.24	16.98	4.54
	Nov-10	<0.01	17.05	15.95	15.49	3.48	11.05	2.68
	Feb-11	3.55	22.12	18.54	11.32	3.17	13.00	6.36
	May-11	1.00	15.94	25.05	17.04	4.20	18.19	2.82
<b>Nitrogen</b>								
East Kleinemonde	Mar-06	37.69	7.21	31.04	13.80	3.70	14.18	9.77
	July-06	27.11	11.82	27.52	14.41	1.75	9.87	11.89
	Sep-06	20.85	9.91	29.13	14.31	2.41	17.47	23.21
	Nov-06	21.08	10.62	26.91	11.28	2.90	12.33	17.22
Mlalazi	Aug-10	1.00	52.18	25.09	28.77	7.71	57.32	15.98
	Nov-10	2.19	28.01	27.42	17.76	11.06	33.02	10.02
	Feb-11	2.76	59.11	32.35	16.63	5.34	27.24	14.96
	May-11	2.19	31.31	31.57	15.58	5.99	27.22	16.03
Mpenjati	Aug-10	3.51	33.49	4.66	15.75	4.78	11.00	3.28
	Nov-10	0.82	23.02	13.31	14.34	6.22	11.69	4.39
	Feb-11	6.11	27.59	15.39	9.69	3.71	12.93	8.21
	May-11	1.12	31.83	15.71	17.49	7.99	18.61	5.20

Estuary, followed by the East Kleinemonde and then the Mlalazi (Fig. 2). The trophic efficiency in general decreased with increasing TLs but the efficiency from TL 4 to TL 5 was higher than TL 3 to TL 4 (4% versus 1%) during November 2010 in the Mpenjati Estuary. In terms of N, TL 2 had the highest trophic efficiency in the East Kleinemonde and Mpenjati estuaries during all seasons except May 2011 in the Mpenjati Estuary. The trophic efficiency at TL 1 (primary producers only) in terms of C and N showed marked seasonal variations in the 3 study estuaries, and it was the most variable in the Mlalazi Estuary (Fig. 2b,e). Overall, lower trophic efficiencies at TL 1 were recorded for both the C and N networks during the closed phase of the TOCEs East Kleinemonde and Mpenjati (Fig. 2a,c,d,f). Higher trophic efficiency at TL 1 was recorded for the C and N networks of the Mlalazi Estuary during the dry season.

The extent of detritivory in the networks varied markedly among the 3 estuaries and seasons (Fig. 3), with detritivory in terms of C and N being highest in

the East Kleinemonde system (Fig. 3a). Despite higher detritivory (C and N) in the East Kleinemonde and Mpenjati estuaries during the closed phase, the detritivory:herbivory ratio of these estuaries was lower during the closed phase due to a simultaneous increase in herbivory during this phase (Fig. 3d,f). In contrast, this ratio was lowest during the wet season in the Mlalazi Estuary when primary production was higher (Fig. 3e, Tables S12–S13 in Supplement 2). The consumption of detritus (detritivory) and primary producers (herbivory) clearly followed the seasonal variations in phytoplankton and microphytobenthos standing stocks, which are higher during the dry season (closed phase) in TOCEs and during the wet season in the POE (Supplement 2).

#### Total flows and cycling structure

The TST for C and N networks varied strongly among seasons in the 3 estuaries (Table 4). Highest TST occurred during the closed phase for the East Kleinemonde and Mpenjati estuaries; this is probably related to the high phytoplankton and microphytobenthos standing stocks recorded in these TOCEs

during the closed phase (winter). However, TST was highest during the wet season in the Mlalazi Estuary due to the increased exports and imports to and from this estuary during this season (summer).

Differences in the retention and recycling of C and N were observed among the study systems; higher APLs were associated with higher cycling activity since a unit of material is recycled over longer paths before it leaves a system. The APL for C and N networks varied among systems; the East Kleinemonde Estuary had the highest C and N APL, and the Mlalazi Estuary had the lowest values (Fig. 4a–c). The N APL was higher than that of C in the East Kleinemonde and Mpenjati estuaries during all seasons, and only during the dry season in the Mlalazi Estuary. No seasonal pattern in C or N APL could be discerned for these 3 systems.

The FCI in terms of C was lowest in the Mpenjati Estuary and highest in the East Kleinemonde Estuary (Fig. 4d–f). The C FCI varied among seasons in the 3 estuaries, but no clear seasonal trend was apparent.

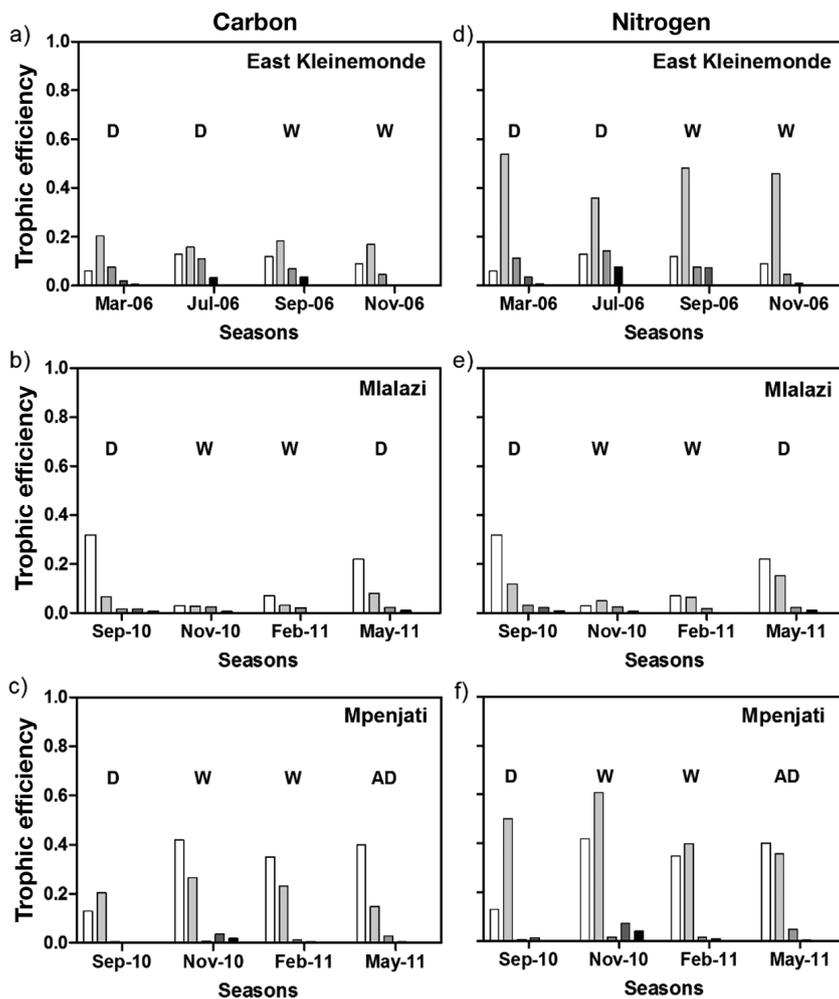


Fig. 2. Trophic efficiency per trophic level (TL) for (a–c) carbon and (d–f) nitrogen networks of the (a,d) East Kleinemonde, (b,e) Mlalazi and (c,f) Mpenjati estuaries. Trophic level: white = TL 1 (primary producers only); light grey = TL 2; mid-grey = TL 3; dark grey = TL 4; and black = TL 5. Seasons: D = dry; W = wet; AD = abnormally wet dry-season

However, higher N FCI values were recorded during the open phase of the East Kleinemonde and Mpenjati estuaries, and during the dry season in the Mlalazi Estuary (Fig. 4d–f). The N FCI followed the seasonal patterns observed by the detritivory:herbivory ratio and the trophic efficiencies at TL 1 (primary producers) in these systems (Figs. 2d–f, 3d–f, 4a–c).

## DISCUSSION

Seasonal C and N networks of quantified trophic flows were constructed for the East Kleinemonde, Mlalazi and Mpenjati estuaries, with the primary goal

of increasing our understanding of estuarine ecosystem structure and functioning in relation to seasonal changes in rainfall and therefore river flow. From the system attributes analysed in this study, the TST, dependencies, detritivory:herbivory ratio, trophic efficiencies at TL 1 (primary producers) and FCI, were all clearly influenced by the variations in rainfall and river flow between dry/wet seasons in the 3 estuaries.

Several patterns emerged from this study; for instance the lowest TST, highest trophic efficiency at TL 1 (primary producers), and highest detritivory:herbivory ratios were evident during the dry season in the permanently open Mlalazi Estuary. In terms of the TOCEs, higher relative dependencies on phytoplankton and microphytobenthos, the highest TST, lowest trophic efficiency at TL 1, lowest detritivory:herbivory ratio and lowest N FCI were all documented during the closed mouth phase. The system attributes of POE and TOCEs exhibited opposite responses to the dry and wet seasons. However, a high relative contribution by a few macrobenthic invertebrate and some fish taxa to the C and N requirements of the food webs, were common attributes to all 3 systems.

## Contribution and dependency

Several macrobenthic invertebrate and fish compartments had the highest relative contribution to the food webs of the 3 estuaries, with macrobenthic taxa being more important in terms of relative contribution in the Mlalazi and Mpenjati estuaries while fish taxa contributed the most to the East Kleinemonde food web. Very high invertebrate standing stocks were recorded in the Mlalazi and Mpenjati estuaries when compared to the East Kleinemonde Estuary where fish standing stocks were considerably higher than that of macrobenthic invertebrate taxa (see Tables S3–S10 in Supplement 2). However, the taxa (i.e. macrobenthic and fish taxa) with the higher relative contribution among seasons and estuaries were not necessarily the ones with the highest standing stocks, possibly due to the mobility of opportunistic fish and invertebrate species. The KwaZulu-Natal estuaries results from

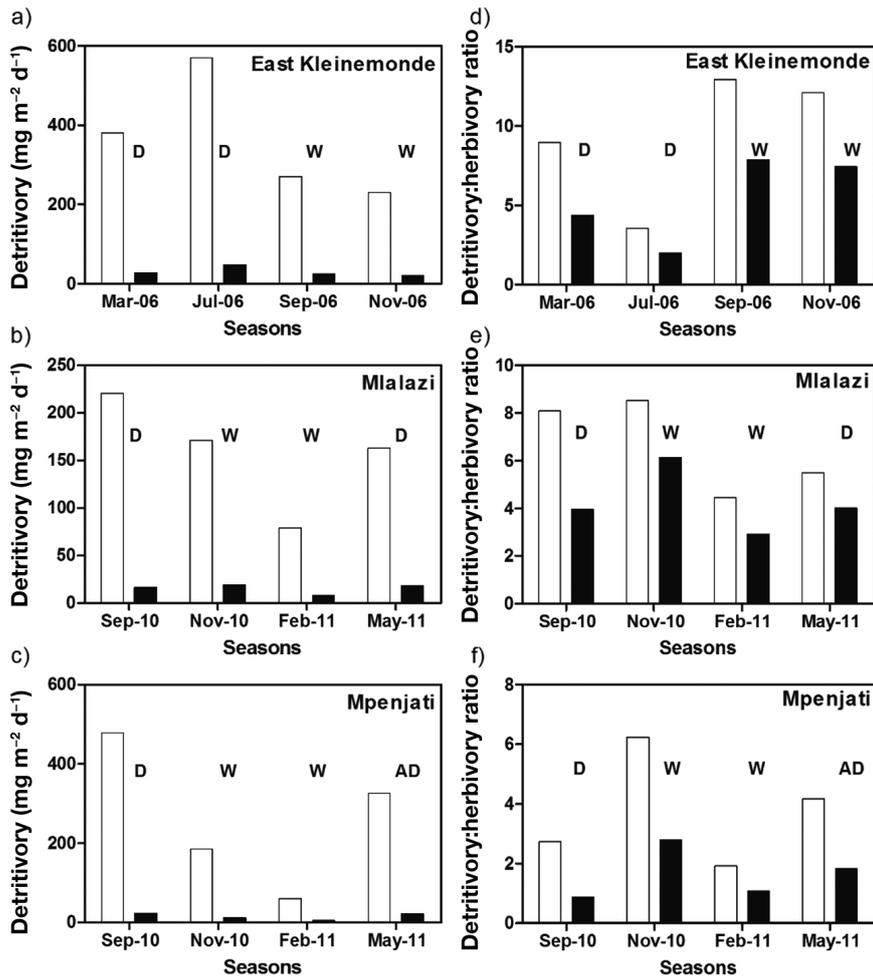


Fig. 3. (a–c) Detritivory ( $\text{mg C or N m}^{-2} \text{d}^{-1}$ ) and (d–f) detritivory:herbivory ratio in the (a,d) East Kleinemonde, (b,e) Mlalazi and (c,f) Mpenjati estuaries. Elements: white = carbon; black = nitrogen. Seasons: D = dry; W = wet; AD = abnormally wet dry-season

Table 4. Total system throughput ( $\text{mg C or N m}^{-2} \text{d}^{-1}$ ) of carbon and nitrogen for the East Kleinemonde, Mlalazi and Mpenjati estuaries

Estuary and date	Carbon	Nitrogen
<b>East Kleinemonde</b>		
Mar-06	2310	480
Jul-06	3912	846
Sep-06	1114	166
Nov-06	1119	178
<b>Mlalazi</b>		
Aug-10	813	86
Nov-10	2035	331
Feb-11	959	147
May-11	1130	177
<b>Mpenjati</b>		
Aug-10	3985	743
Nov-10	931	130
Feb-11	422	72
May-11	1488	196

this study are similar to findings from the Kromme and Swartkops estuaries in the Eastern Cape that also relied mainly on zoobenthic compartments (in terms of C) (Scharler & Baird 2005). However, demersal fish were included in the benthic compartment in that study, thereby biasing the system reliance on the benthos.

The differences in the relative contribution of fish and macrobenthos taxa between C and N networks of the 3 studied estuaries were attributed to the higher inputs from fish compartments to the N detrital pools of these systems. Fish faeces and other excretions have previously been found to constitute a significant source of N and P to aquatic systems, and nutrient inputs via fish excreta can be of a similar magnitude to watershed-derived nutrient runoff or nutrient release from sediments (Vanni 1996, 2002, Allgeier et al. 2015).

In terms of relative dependencies, higher proportional reliance on microphytobenthos was evident during the closed phase in the East Kleinemonde and Mpenjati estuaries, and this can be attributed to the very high productivity of microphytobenthos during the stable closed phase of these systems (Gama 2008, Ortega-Cisneros et al. 2014). In terms of N, the Mlalazi and

Mpenjati estuaries exhibited a higher relative dependency on microphytobenthos, while the East Kleinemonde relied more on suspended detritus and phytoplankton. Greater proportional reliance on microphytobenthos and phytoplankton was recorded in the Mpenjati and East Kleinemonde estuaries respectively during the closed phase, when phytoplankton and microphytobenthos standing stocks were highest.

The above results highlight the key role of primary production in sustaining the biota of TOCEs during the closed mouth phase and also underline the importance of river flow in driving the functioning and nutrient dependencies of these systems. Scharler & Baird (2005) also reported that freshwater and nutrient inputs were crucial in determining the magnitude of the compartmental dependencies in the Kromme, Swartkops and Sundays estuaries.

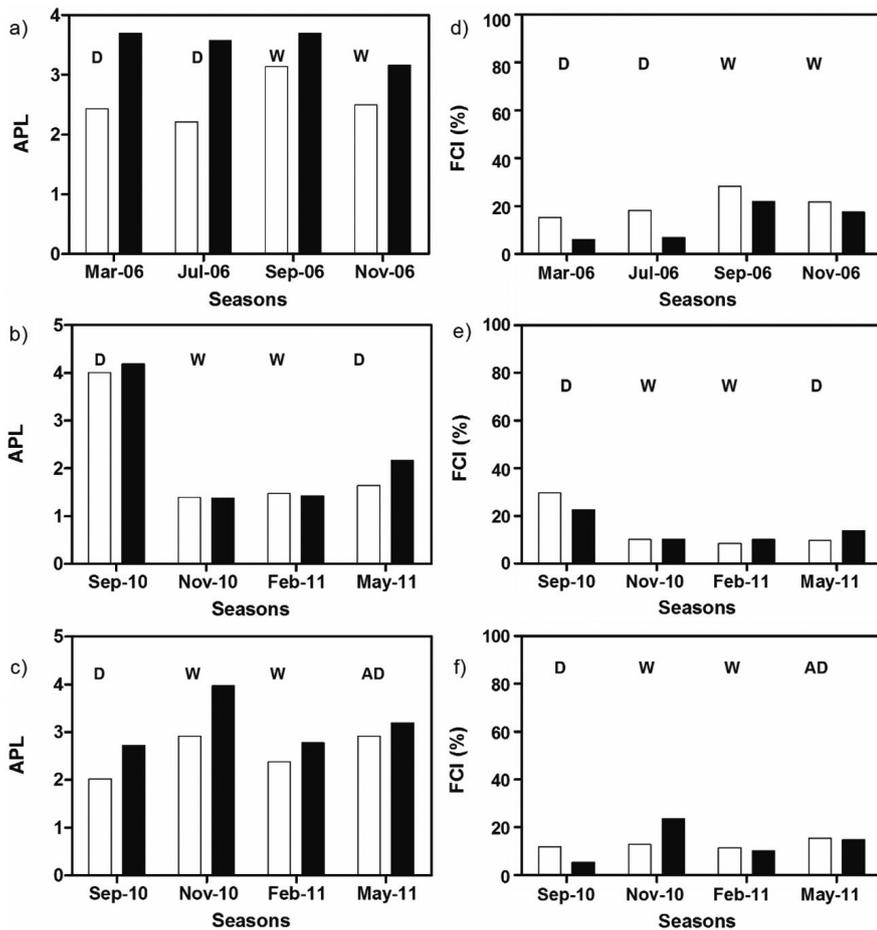


Fig. 4. (a–c) Average path length, APL, and (d–f) Finn cycling index, FCI, for the (a,d) East Kleinemonde, (b,e) Mlalazi and (c,f) Mpenjati estuaries. Elements: white = carbon; black = nitrogen. Seasons: D = dry; W = wet; AD = abnormally wet dry-season

### Trophic structure

As expected, the trophic efficiency decreased with increasing TL for the C networks. In terms of N, the highest trophic efficiency was at TL 2 in the East Kleinemonde and Mpenjati estuaries. This suggests that N is more efficiently transferred between TL 2 (e.g. herbivorous, detritivores) and TL 3 (e.g. secondary consumers) in TOCEs, and that the consumption at TL 3 was high compared to the production rates of their prey at TL 2. The latter is attributed to the higher C:N ratios recorded at TL 2 in both TOCEs compared to the values observed in the POE Mlalazi. N seems to be more limiting than C in both TOCEs at TL 2, and this can possibly explain why N is more efficiently transferred from TL 2 to 3 in these systems.

The above trends were not reflected in the annual N networks calculated for the Sylt-Rømø Bight, Wad-

den Sea or Chesapeake Bay (Baird et al. 1995, Baird et al. 2008), where the trophic efficiencies decreased with increasing TL. Higher trophic efficiencies from TL 1 (primary producers) were recorded for the C and N networks of both TOCEs during the open phase, and this can be explained by a shortage of food sources documented in these systems during this phase, i.e. low standing stocks of phytoplankton, microphytobenthos and detritus during open mouth conditions. The opposite pattern was observed in the Mlalazi Estuary, where trophic efficiencies from TL 1 (primary producers) were higher during the dry season when phytoplankton standing stocks are lowest due to reduced riverine flow and nutrient input.

Based on C and N networks, the detritivory:herbivory ratio was lowest in the 2 TOCEs during the closed phase and during the wet season in the Mlalazi Estuary. The high phytoplankton and microphytobenthos standing stocks recorded during the closed phase and wet season of TOCEs and POEs respectively explains the higher levels of herbivory during these periods. These results are supported by the relative contribution and dependency analyses,

which reported higher relative dependency on microphytobenthos and phytoplankton in both TOCEs during their closed phase. In the TOCE Mngazi, lowest C detritivory: herbivory was also recorded during the dry season (Vosloo 2012).

Similarly, in 2 subtropical TOCEs the C detritivory:herbivory ratio was found to increase from mouth breaching to re-closure, and to decrease with increasing duration of the closed phase (Scharler 2012). Therefore, the trends observed for this ratio in the 2 study TOCEs are strongly dependent on mouth state and length of mouth closure. The detritivory:herbivory ratio reported for the East Kleinemonde, Mlalazi and Mpenjati estuaries fell within the lower range of the ratios reported for other global estuarine systems (Table 5). No detritivory:herbivory ratios in terms of N were available for comparisons with this study.

Table 5. Selected system attributes for carbon networks in a range of global estuaries. ('-' means no data available). FCI: Finn cycling index

System	Detritivory: herbivory ratio	FCI (%)	Source
Aiguillon Cove, France	4.9	15	Leguerrier et al. (2007)
Brouage, France	6.6	19	Leguerrier et al. (2007)
Chesapeake Bay, USA	4.8	24	Baird et al. (1991)
Delaware, USA	3.4	37.3	Monaco & Ulanowicz (1997)
East Kleinemonde, South Africa	3.6–13	15–28	This study
Ems, Germany	0.5	30	Baird et al. (1991)
Gironde, France	–	17	Lobry et al. (2008)
Kromme, South Africa	57.0	41	Scharler and Baird (2005)
Mlalazi, South Africa	4.5–8.5	8–30	This study
Mngazana, South Africa	11.9	11–13	Vosloo (2012)
Mngazi, South Africa	24.1	23–26	Vosloo (2012)
Mpenjati, South Africa	1.9–6.2	11–15	This study
Narrangasett, USA	8.1	48.2	Monaco & Ulanowicz (1997)
Seine, France	2.5	16	Rybarczyk & Elkäim (2003)
Sundays, South Africa	10	20	Scharler & Baird (2005)
Swartkpos, South Africa	1.5, 32	26	Baird et al. (1991), Scharler & Baird (2005)
Sylt-Rømø Bight, German Wadden Sea	–	17.2	Baird et al. (2008)

### Total flows and cycling structure

The biological activity within these estuaries, represented as TST, largely reflected seasonal variations in the C and N biomass of the pelagic and benthic communities. Seasonal differences in the TST between wet and dry seasons were recorded in the permanently open Mlalazi Estuary due to the higher flows through detritus, phytoplankton and zooplankton compartments during the wet season. Similarly, the high biomass of pelagic and benthic communities in the East Kleinemonde and Mpenjati estuaries during the closed phase reflected an increased TST when compared to the open mouth phase.

The cycling structure and magnitude of flows in an ecosystem provides information on overall functioning, e.g. a higher magnitude of cycling is characteristic of systems less dependent on external connections and better able to withstand perturbations (Odum 1969). Ulanowicz (2004) suggests that the probability of a nutrient being recycled in a system increases with increasing scarcity. Contrasting results were recorded by the FCI and APL; higher FCI was recorded by some of the C networks representing the study estuaries suggesting that C may be more limiting (to consumption of primary consumers) than N in specific seasons. In most cases, low standing stocks of primary producers and detritus were recorded in these particular seasons. However, the higher APL for the N networks of the TOCEs suggests that N may be more limiting in these systems (as supported by

the mean trophic efficiencies) or that N dissipation may not occur (e.g. N flows visit several compartments before exiting the system).

It is possible that the contrasting results reported here (higher C FCI versus higher N APL) are an artefact of our network structure. Our networks assumed that the requirements of primary producers (dissolved inorganic N; DIN) were imported from outside the estuary boundary, and therefore our networks do not account for the cycling associated with DIN. Christian & Thomas (2003) reported that imported nitrate and nitrite in the Neuse Estuary was utilised by phytoplankton up to 35 times before being exported. It is also possible that N is indeed not a limiting nutrient in our study estuaries based on the high standing stocks of primary producers recorded in these systems during specific seasons. N has been found to be more limiting than C in many systems such as Chesapeake Bay (Baird et al. 1995, Ulanowicz & Baird 1999), the Neuse River Estuary (Christian & Thomas 2003) and the Sylt-Rømø Bight (Baird et al. 2011); however constraints in energy transfer (as C) have previously been reported for some coastal food webs (e.g. Howarth & Marino 2006, Scharler et al. 2015).

The N FCI clearly reflected the variations in the phytoplankton and microphytobenthos standing stocks between wet/dry seasons in the study systems. A higher N FCI was recorded at the Mlalazi Estuary during the dry season, similar to that documented for the KwaZulu-Natal Bight, South Africa (Ayers 2013). However, a higher N FCI was recorded during the

open phase of both TOCEs. The FCI is a relative measure of cycling and represents the proportion of material cycled with respect to TST. The total amount of material cycled in the TOCEs was actually higher during the closed phase than the open one, with the higher FCI observed during the open phase being attributed to the lower overall activity (TST) in these systems during the open phase. The Mngazana and Mngazi estuaries also showed slightly higher FCI values during the wet season (Vosloo 2012). In contrast, higher recycling was found during the closed phase of the Mdloti and Mhlanga estuaries, with C recycling being strongly influenced by the fluctuations in mouth status of these TOCEs (Scharler 2012). Evidence suggests that the levels of cycling in TOCEs are directly influenced by mouth status, with the duration of the open or closed phase determining the cycling efficiency and thus making general seasonal patterns difficult to discern.

### Caveats and conclusions

The networks presented here are based on data collected in each system, with information from the Mlalazi and Mpenjati estuaries being based on a sampling programme specifically geared to building ecological networks, e.g. biomass, stoichiometry and isotope data targeting the plankton and macrobenthic invertebrate communities in these 2 systems (Ortega-Cisneros & Scharler 2014, 2015). For the East Kleinemonde Estuary, isotope data were not available and average C:N ratios from the other 2 estuaries were used. Therefore, the variations in standing stocks observed in the East Kleinemonde Estuary only reflect biomass changes and not actual seasonal variations in stoichiometry ratios.

Due to the large amount of data required to build ecological networks, networks are usually built by including some proportion of the data from different sources/studies collected during different periods. The biomass data used to build our networks was system specific and collected within the same time frame (but not fish data for the Mlalazi and Mpenjati estuaries). Information on biomass exchanges through the mouth of the East Kleinemonde was not available; thus it is probable that the actual imports or exports calculated for this system are underestimated. Similarly, average biomass values (per station and sampling season) were used for network construction and not minimum or maximum biomass values, thus not accounting for the natural variability in biomass observations.

While most C and N flows were calculated based on system-specific data, flows were modified during the mass-balance process (e.g. flows changed up to 15%), thus adding another source of uncertainty to our networks. These networks have different numbers of compartments to reflect the differences in species composition in each system between months and seasons. ENA indices such as FCI, APL and others are influenced to some extent by trophic aggregation, or by having a different number of compartments. However, the difference in magnitude of such an effect originating from variations in link weight has yet to be determined for weighted networks. Finally, not accounting for important groups in a system, such as bacteria and inorganic nutrients in this study, due to lack of available data can have important implications on model outputs and bias the results.

In conclusion, our results provide evidence that the differences in rainfall and river flow between wet and dry seasons do influence the functioning of estuaries at the system level and not only at the population or community level. High TST, herbivory and relative dependency on phytoplankton and microphytobenthos were characteristic of the TOCEs during the closed phase, with these patterns reflecting the high standing stocks of primary producers during this phase. Similarly, the lowest detritivory: herbivory ratio, lowest trophic efficiencies at TL 1 (primary producers) and N FCI were recorded in TOCEs during the closed phase. Opposite patterns were documented during the open phase for these TOCEs and during the dry season in the Mlalazi Estuary. These results have therefore provided important insights in terms of understanding the nutrient dynamics and trophic functioning of small estuarine systems on the east coast of South Africa. In order to increase our understanding of the above, further elements (e.g. P networks) and components (e.g. bacteria) need to be included in future estuarine ecosystem analyses.

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