Spatial and temporal variations in trophic connectivity within an estuarine environment: benthic-pelagic and terrestrial-aquatic linkages via invertebrates and fishes

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Leandro Bergamino Roman

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Abstract

Estuarine ecosystems are among the most biologically productive areas and they provide important ecosystem services such as erosion control, habitat and refugia for several species. These environments are characterized by the presence of a variety of organic matter sources due to their transitional position between rivers and the sea. The biotic compositions can undergo spatial and seasonal changes along the estuary due to the spatial and temporal fluctuations of environmental factors such as salinity, temperature and seston loads. Therefore, the different combinations of biotic and abiotic factors make each estuary a unique ecosystem. Because of this spatial and temporal complexity, the understanding of estuarine food web structure and which factors affect the trophic relationships within the ecosystem through space and time represent challenging tasks. Furthermore, estuaries are under an increasing number of anthropogenic perturbations because of the growing concentration of human populations in coastal areas. Knowledge of ecosystem structure and functioning is essential for effective conservation and management planning of coastal areas.

In this dissertation, I combine the utilization of biological tracers to examine spatial and temporal variability in the food web structure within a small temperate and microtidal estuary located in South Africa. To this end, fatty acid profiles and stable isotope signatures were measured in several primary organic matter sources and consumers (including zooplankton, fishes and benthic invertebrates) during four consecutive seasons and in three different estuarine regions: upper, middle, and lower reaches. The three reaches had distinct habitat features of vegetation type and morphology, and in particular the lower reaches were colonized by the marsh grass *Spartina maritima*. Isotopic mixing models were used to estimate the relative contribution of each food source to the diets of invertebrates and fishes within the estuarine food web. The isotopic and fatty acid data showed similar results. In general, the lower reaches of the estuary were characterized by a higher deposition and assimilation by brachyuran crabs of carbon derived from marsh grass detritus, while towards the upper reaches a mixture of microphytobenthos and particulate organic matter (phytoplankton and detritus) was deposited and sustained the pelagic and benthic fauna. The highest deposition and assimilation of marsh grass detritus in the lower reaches of the estuary occurred during periods of low freshwater discharge (autumn and winter). In the upper reaches, microphytobenthos and suspended particulate organic matter were dominant basal food resources for the food web during all seasons. These results indicated that benthic consumers incorporated mainly local carbon sources from their local habitat.

To clarify isotopic and fatty acid patterns I examined the trophic behaviour of the sesarmid crab *Sesarma catenata* through laboratory feeding experiments. Results from these experiments validated that decomposed leaves of riparian trees and the salt marsh plant *S. maritima* were the preferred food of the sesarmid crabs, potentially due to high bacterial loads. The remaining leaf material not assimilated by crabs, together with faecal material, are likely important subsidies for adjacent environments, hence representing an important energy pathway involving the microbial food chain.

Furthermore, this dissertation showed the importance of mobile top predators as vectors energetically connecting distinct food chains within the estuary (i.e. littoral, benthic and pelagic). I concluded that a combination of physical (i.e. patterns of freshwater discharge and estuary morphology) and biological factors (i.e. organism feeding behaviour, mobility, primary productivity, the local vegetation type) influence the pattern of dominant primary organic matter sources, and therefore the food web structure along the estuarine environment. In particular, marsh grass detritus contributed substantially to the diets of estuarine fauna during periods of low freshwater discharge. Given the importance of the salt marsh habitat in providing trophic resources, it is important to preserve this environment to sustain the natural biota and ecosystem functioning.

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Thesis declaration

I declare that this thesis submitted for a degree at Rhodes University (Grahamstown, South Africa) has not been submitted to any other university. The work presented here is that of the author unless otherwise stated.

Leandro Bergamino
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Chapter 1

General introduction

1.1 The estuarine environment

Estuaries are coastal ecosystems where terrestrial, aquatic, and marine habitats are linked. Different definitions of estuaries appear in the literature. In general, estuaries are considered as transitional zones between marine and terrestrial systems with an intermittent or permanent connection with the sea (Kennish 2002; McLusky and Elliot 2004). Therefore estuaries are characterized by a spatial variation of salinity due to the mixture of sea water with fresh water derived from land drainage. Different regions can be distinguished within the estuary, including the tidal river zone with no influence of ocean waters and affected by tidal rise, the mixing zone where freshwater is diluted with ocean water producing a spatial gradient of physico-chemical parameters (e.g. salinity, temperature), and a nearshore turbid zone dominated by ocean water (Kjerfve 1989).

Estuaries are among the most biologically productive systems given the influence of various forms of primary and microbial production and also provide important ecological services such as recreation, food production, erosion control, and habitat for fauna (Costanza et al. 1997; McLusky and Elliot 2004). They receive and process a large amount of land-based nutrients and pollutants entering via surface runoff, atmospheric deposition and groundwater discharge (Paerl 2006). The high productivity results mainly from the input of new nutrients, especially nitrogen and phosphorous, from the adjacent land, sea and river (McLusky and Elliot 2004). The organic matter is derived from many sources including emergent macrophytes, submerged grasses (*Zostera*, etc.), macroalgae (green, brown and red), epiphytic microalgae, benthic microalgae, phytoplankton, marsh grass (e.g. *Spartina*), dead macrophytes, submerged macrophytes, and mangrove litter (Knox 1986; Peterson et al. 1994; Heip et al. 1995). Populations of bacteria play a key role in consuming dissolved organic matter (derived mainly from plant exudation and animal excretion), and become part of the particulate matter in estuaries (Heip et al. 1995). In addition, allochthonous organic matter (dissolved and particulate) derived from the river flow enters estuarine waters (Polis et al. 1997; Abrantes and Sheaves 2010; Howe and Simenstad 2011). A previous work by Boynton et al. (1982) examining 63 estuaries including fjords, lagoons, embayments and river dominated systems found that there was an overlap in primary production along the different estuaries that ranged between 40 and 550 $gCm^{-2}yr^{-1}$. Multiple factors are important in controlling the algal production including phytoplankton biomass, incident irradiance, turbidity, nutrient availability, grazing, temperature, daylength and transport processes (Heip et al. 1995). Estimates of annual phytoplankton productivity in estuaries range between 6.8 gCm^{-2} in very turbid water to 530 gCm^{-2} in a clear system (Cole and Cloern 1984). Phytoplankton production contributes between 2 and 43%, while epiphytes and macroalgae contribute to less than 8.5% (McLusky and Elliot 2004). In the Seine Estuary located in Eastern Channel, Normandy, France, with an area of 34.64 x 10^6 km^2 and a volume of 231 x 10^6 m^3, the production of phytoplankton (572 $gCm^{-2}y^{-1}$) was higher in the upstream section and was rapidly consumed by zooplankton and suprabenthos; however, the phytobenthos production dominated towards the downstream section (281 $gCm^{-2}y^{-1}$) together with the lowest consumption and major export of detritus (548 $gCm^{-2}y^{-1}$) (Rybarczyk and Elkaïm 2003). These results from the Seine Estuary indicated a role of ‘‘consumer’’ for the upstream region and ‘‘exporter’’ for downstream region, highlighting the complementary roles of the different sections within an estuarine environment. Another ecosystem model derived from the Barataria Bay in Louisiana showed that of the net production of marsh plants (1546 g dry wt m^{-2}), only 5% was consumed by herbivores and the rest eventually passed to the detritus-microbial system (Knox 1986). In a mangrove area of the Upper Watemata Harbor in New Zeland, a carbon flow model showed that of the mangrove net production, 13% becomes available to the pool of dead organic matter via litter fall, and of the benthic microflora net production, 59% is estimated to be consumed by benthic fauna (Knox 1986). In estuaries with mudflats, epibenthic algae contributes to over 30% of the total primary production, and values of net production range between 30 and 300 $gCm^{-2}year^{-1}$ (Heip et al. 1995; McLusky and Elliot 2004). Different physical and biological properties among estuaries contribute to a large amount of differentiation in the dominant carbon sources from one system to the next (McLusky and Elliot 2004). As such, it is important not to generalise results from one estuary to another before these basic characteristics are thoroughly investigated in each system.

1.2 The estuarine food web

A food web describes feeding interactions among species that co-occur within particular habitats. Our understanding of how several carbon sources flow through the estuarine food web has grown substantially during the last several decades. Previous work showed that estuarine food webs are supported mainly by fresh marsh plants such as *Spartina* sp., estuarine phytoplankton and benthic microalgae (Deegan and Garritt 1997; Kwak and Zedler 1997; Paterson and Whitfield 1997; Kang et al. 2003; Richoux and Froneman 2007). In estuaries with extensive intertidal flats, benthic microalgae and phytoplankton have been reported as the most important diet component for benthic detritivores (Riera and Richard 1996; Kang et al. 2003; Page and Lastra 2003). However, some studies have stressed the importance of terrestrial organic matter at the base of estuarine food webs (Chanton and Lewis 2002; Darnaude et al. 2004; Wissel and Fry 2005; Darnaude 2005; Abrantes and Sheaves 2010). In addition, the organic detritus that results from decomposition by fungi and bacteria of vascular plants also supports food webs formed mainly by detritivores including clams *Corbicula* spp. and *Macoma* sp., mussels *Mytilus* sp., and amphipods *Corophium* sp. (Kennish 2002; McLusky and Elliot 2004; Howe and Simenstad 2011). For example, in estuaries dominated by marsh systems, *Spartina* detritus can support estuarine fauna such as crab species (Haines and Montague 1979; Currin et al. 1995), fishes (Winemiller et al. 2007) harpacticoid copepods, nematodes, and ostracods (Carman and Fry 2002) and also can be assimilated by the benthic infauna (Galván et al. 2008). In this context, all the different types of organic matter (terrestrial and aquatic) deposited by sedimentation processes sustain pelagic and benthic communities (Fig. 1.1). In particular, estuarine food webs contain a rich fauna including suspension feeders, herbivores and deposit/detritivorous feeders, all potential food for predators such as fishes (McClelland and Valiela 1998), marine mammals, marine reptiles, swimming molluscs, crustaceans (e.g. swimming crabs), wading birds and shorebirds (Kennish 2002). Furthermore, heterotrophic bacteria and protozoans play a key role as a link between dissolved organic matter and nutrients and higher trophic levels, when protozoans are preyed upon by larger zooplankton (Heip et al. 1995) (Fig. 1.2). Despite this information, little is know about how the importance of alternative food sources (benthic and terrestrial) for consumers changes with season and spatially along the estuarine gradient.

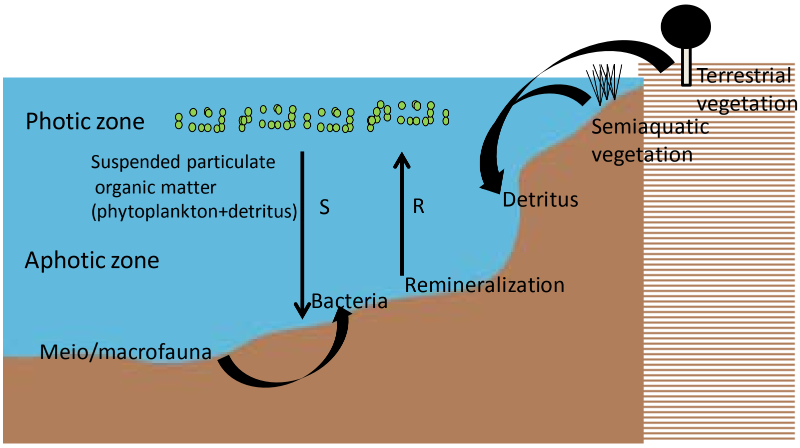

Figure 1.1. Schematic diagram of the major energy flows in an estuarine environment. S: sedimentation of the suspended organic matter, which is utilized by the benthic fauna; and R: resuspension of nutrients and decomposed organic matter to the photic zone.

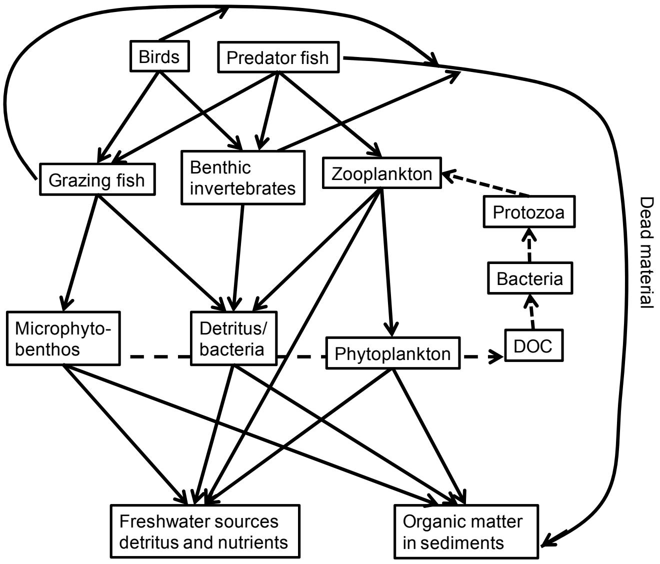

Figure 1.2. Schematic representation of an estuarine food web. The dotted lines highlight the microbial loop with assimilation of dissolved organic carbon by bacteria.

Movement of nutrients and consumers within and among adjacent habitats can have major effects on the food web structure and productivity, and therefore has become an important issue to understand (Polis et al. 1997; Nakano and Murakami 2001; Guest et al. 2004; Howe and Simenstad 2007; Heck et al. 2008). Guest et al. (2004) suggested a model that potentially explained the movement and assimilation of carbon by resident animals in estuarine habitats based on three different spatial scales: 1) the coarse scale model (>30 m) that comprises large scale movement of carbon; 2) the intermediate scale model (2 to 30 m) that considers more limited carbon movement and assimilation across the habitat boundary; and 3) the fine scale movement of carbon (<2 m) that predicts no movement or assimilation of carbon across the habitat boundary. In estuaries from South Australia located on a sheltered embayment, movements of particulate organic matter over a few kilometers and its assimilation by invertebrates have been detected through analyzing stable carbon isotopes of invertebrates (Connolly et al. 2005). Furthermore, drifting phytoplankton from a dam reservoir in Japan has been observed to subsidize estuarine food web over a 10 km below the dam (Doi et al. 2008). Carbon movements also occur at small scales (m to km) and consumers assimilate carbon mainly derived from local food sources (Deegan and Garritt 1997; Guest and Connolly 2004; Guest et al. 2004; Bouillon et al. 2004; Richoux and Froneman 2007; Olsen et al. 2013). These results highlight the notion that carbon sources and energy pathways within estuarine environments may change across very small spatial scales over a few meters (Olsen et al. 2013).

The variation in the productivity of plants and other carbon sources among sites within an estuarine environment is affected by a combination of physical and biological factors. In particular, environmental features such as tidal amplitude, pattern of freshwater discharge, the vegetation type and hydrogeomorphic features have been suggested as main forces driving spatial and temporal changes in the food web structure along estuaries (Deegan and Garritt 1997; Kwak and Zedler 1997; Bouillon et al. 2004; Hoeinghaus et al. 2007; McLeod et al. 2010; Hoeinghaus et al. 2011). These habitat characteristics affect patterns of dominant primary producers and therefore the resultant food web structure. For example, primary production of microphytobenthos depends largely on the position in the tidal frame (emersion duration) and sediment type (Van der Wal et al. 2010; Orvain et al. 2012). Muddy sediments accumulate in areas with low hydrodynamic condition, which is associated with an increase in the content of organic matter. Also, large emersion durations are associated with the high presence of photo-autotrophs and less turbid water (Van der Wal et al. 2010). For these reasons, emersion duration and muddy content seem to be positively related with microphytobenthos production. Furthermore, periods of low river flow and wind speed enhance localized resuspension and fluxes of particles from the estuarine bed including total suspended sediment concentration, particulate organic carbon and particulate organic nitrogen (Goñi et al. 2009). Different patterns of freshwater discharge may also affect several food web properties in estuarine ecosystems such as the cycling of carbon, and the trophic structure including the extent of detritivores in relation to planktonic organisms with low freshwater inflow leading to high detritivory/herbivory ratio (Scharler and Baird 2005). Concerning the effects of vegetation on the estuarine food web structure, in a temperate estuary located in Waquoit Bay on the south coast of Cape Cod, vegetated areas (i.e. seagrass and macroalgae) were associated with higher faunal abundance compared with bare sediments given the heterogeneity and higher food availability in vegetated areas (Olsen et al. 2013). Furthermore, the importance of terrestrial subsidies for estuarine food webs depend on several factors including the hydrology and the vegetation type (Hoeinghaus et al. 2011; Abrantes et al. 2013). Rodríguez-Graña et al. (2008) compared the food web structure between sites with contrasting hydrodynamic conditions within a coastal lagoon in the Southwest Atlantic, and found no differences in faunal and floral isotopic signatures between sites. This result suggested that differences in the physical environment may not necessarily translate into spatial differentiation in trophic structure. In addition to physical factors, biological processes also affect spatial changes in food web structure in estuaries. The movements of organisms, gravity, water flow and air flow generate fluxes of predators, prey, detritus and nutrients among spatially distinct habitats and promote the connection of discontinuous habitats within a ecosystem (Schindler and Scheuerell 2002). For example, large mobile predators can provide cross-habitat linkages in food webs by predation and excretion of nutrients (habitat coupling) (Polis et al. 1997; Schindler and Scheuerell 2002). Such movement can transport fecal matter rich in fertilizing nutrients across the conceptual boundaries of habitats within the water column, between benthic and pelagic waters, between onshore and offshore waters and to refuge areas (Polis et al. 1997). For example, piscivorous fish species in lakes utilize littoral and pelagic habitats due to their mobility, and they strengthen top-down control of pelagic and littoral food chains (Vadeboncoeur et al. 2005). In estuaries with little fluvial discharge, feeding behavior by organisms may provide an important vector of food web connectivity in estuaries (Howe 2012). As a result, spatially distant food chains within an estuarine environment may be energetically connected at higher trophic levels (McCann et al. 2005; Rooney et al. 2006; Abrantes and Sheaves 2009).

Despite the advances in our knowledge of estuarine trophic dynamics, we still have much to learn about which potential food sources are the most important for a variety of estuarine fauna. One of the difficulties is the large number of potential food sources available that can be morphologically similar. As such, the estimation of the relative importance of different food sources within an estuarine food web represents a challenging task. The development of new techniques to analyze trophic interactions gives us the opportunity to obtain more detailed information about food web structure.

1.3 Stable isotopes and fatty acids as tracers

Traditional methods for studying trophic relationships among organisms include gut content and direct observation techniques. However, the food sources of many organisms, particularly benthic invertebrates, are difficult to determine because of their small size, and the gut contents are often amorphous (Layman et al. 2007). Gut content analysis can provide detailed information on species' diet, but it does not reflect an individual's long term diet as it reflects only recent ingestion. Alternatively, stable isotope (SI) analysis has been used as one of the primary means to analyze the structure of food webs in aquatic and terrestrial ecosystems (Rubenstein and Hobson 2004; Boecklen et al. 2011).

Isotopes are forms of the same chemical element that contain the same number of protons but a different number of neutrons, and they can be divided into stable and unstable (radioactive) species. Stable isotopes differ from radioactive isotopes in that they do not decay over time. The SI of the elements hydrogen, carbon, nitrogen, oxygen and sulfur have been used extensively to study animal foraging ecology and migration (Peterson and Fry 1987; Hobson 1999; Newsome et al. 2007).

Chemical and physical differences between the isotopes of an element cause light isotopes to react slightly differently than heavy isotopes. As a consequence, the reaction product tends to be depleted in heavy isotopes with respect to its source. This process is called fractionation and it results in isotopic shifts in consumers in relation to their food sources (Vander Zanden and Rasmussen 2001). The estimation of this trophic fractionation allows us to use SI signatures in animals to indicate feeding preferences and carbon flow pathways within an ecosystem (Peterson and Fry 1987; Fry 2006). The $\delta^{13}C$ values in tissues of consumers are typically stable with little enrichment (increase in the $\delta^{13}C$ value) near ~1‰ relative to those of their food, therefore indicating the original sources of energy or carbon (Peterson and Fry 1987). Furthermore, in natural environments marine primary producers have higher values compared to terrestrial producers (Fry and Sherr 1984; Hobson 1999; Rubenstein and Hobson 2004). This is because the control of the carbon signatures in aquatic plants is whether the plant utilises bicarbonate (HCO_3) or dissolved CO_2 (Lamb et al. 2006). Dissolved CO_2 has lower $\delta^{13}C$ values (−8‰) than HCO_3 (~0‰), and phytoplankton tend to preferentially assimilate CO_2. Because of the pH of the environment, there is less CO_2 available in the marine environment than in the freshwater system, and marine phytoplankton have higher $\delta^{13}C$ values than freshwater phytoplankton since they will rely on HCO_3 (Lamb et al. 2006). Therefore isotopic signatures of primary organic matter sources and consumers provide a robust model to identify the origin of carbon assimilated by the resident fauna in estuarine and coastal environments (Riera and Richard 1996; Darnaude et al. 2004; Antonio et al. 2012). Signatures of $\delta^{15}N$ increase by ~3‰ in consumers relative to their food, and this provides an estimate of their trophic position (Post 2002; McCutchan et al. 2003). The trophic position of an organism represents the number of energy transfers or trophic links that have occurred before passing to the consumer population in question (Vander Zanden and Fetzer 2007), and the number of trophic levels is an important characteristic mediating the relationship between species diversity and ecosystem function (Duffy et al. 2005). For example, higher diversity of grazers can both enhance grazer pressure on resources below, and dampen responses to predators above them in the food web (Duffy et al. 2005).

SI in conjunction with fatty acids (FAs) have successfully been applied to assess trophic interactions and give new insights into food web structure (Boecklen et al. 2011). Fatty acids constitute the main part of the lipids in organisms, and they are distributed in all living cells (animals, plants and microbes) (Ratledge 1997). They play a vital role as structural components in cell membranes (i.e. phospholipids) and also as energy sources (i.e. neutral lipids such as triacylglycerols and wax esters), so FAs are needed for growth and reproduction (Arts et al. 2009). Different taxonomic groups of primary producers can exhibit distinct FA profiles, and the lipid pattern of consumers can reflect the lipid profiles of their food sources (Dalsgaard et al. 2003; Budge et al. 2006; Kelly and Scheibling 2012). Fatty acid profiles can discriminate among some macrophytes, and among coarse groupings of phytoplankton sources (Volkman et al. 1989; Dalsgaard et al. 2003). Essential fatty acids, which are obtained primarily from consumer diets, are often preferentially conserved in trophic webs and are important biomarkers in aquatic systems (Arts et al. 2001). The prerequisite for an ideal biomarker FA is that the molecule is assimilated without degradation and is unique to a specific food source. However, these prerequisites are seldom achieved and multivariate methods are often used (e.g. Crawley et al. 2009; Allan et al. 2010; Gonçalves et al. 2012; Kelly and Scheibling 2012) to examine similarity patterns and to identify FAs that are important in separating groups of animals or primary producers from different study sites or seasons. The use of SI together with FAs provides informative data towards improving our understanding of complex trophic relationships in dynamic environments and minimizes the limitations of each technique (e.g. Kharlamenko et al. 2001; Jaschinski et al. 2008; Budge et al. 2008; Allan et al. 2010; Dubois et al. 2014).

1.4 Rationale and thesis overview

In this dissertation I present new ecological information on estuarine food web structure from a spatial and temporal approach. To this end, a detailed food web was examined in different estuarine reaches (i.e. upper, middle and lower) and across seasons within the microtidal Kowie Estuary located in South Africa. This analysis considered the dominant food sources and consumers: phytoplankton, microphytobenthos (also called benthic microalgae), terrestrial and aquatic vascular plant food sources (live and senescent), invertebrates, zooplankton and fishes from different trophic guilds. The hypothesis is that differences in the food web structure among reaches and seasons correspond to local physical and biological features (i.e. patterns of freshwater discharge, vegetation type, primary productivity and estuarine morphology) that affect patterns of dominance of organic matter sources along the estuary. A second hypothesis is that top predators provide vertical and lateral linkages between different food chains from distinct habitats in the estuary ecosystem. In addition, since physical, biological and physiological processes (i.e. fractionation) influence SI patterns in the flora and fauna, I included laboratory feeding experiments to clarify and validate conclusions based on isotopic and FA analyses (Gannes et al. 1997).

This dissertation focuses first on the spatial and temporal variability in the structure of the benthic estuarine food web (Chapters 2, 3 and 4) and second on the functioning of the pelagic food web within the estuary (Chapter 5) to address the following questions:

- What are the major sources of organic matter deposited along the Kowie Estuary?
- To what extent does each food source contribute to different benthic consumers along the estuary?
- What factors influence spatial and temporal variations in the food web structure within the Kowie Estuary?
- Do the crabs *Sesarma catenata* prefer a particular plant material or leaf stage as a food source under laboratory conditions?
- Does a mobile predator link spatially distinct habitats within the estuary through its feeding habits?

All the individual chapters of this dissertation were written with the intention for publication in peer-reviewed journals. Versions of chapters 2, 3 and 5 have been accepted for publication. Co-authors provided constructive comments that helped to improve the work, but I am responsible for the bulk of the research (design, sampling, analysis and writing).

Articles accepted for publication:

Bergamino L, Dalu T, Richoux NB. 2014. Evidence of spatial and temporal changes in sources of organic matter in estuarine sediments: stable isotope and fatty acid analyses. Hydrobiologia 732: 133-145.

Bergamino L, Richoux NB. 2014. Spatial and temporal changes in estuarine food web structure: differential contributions of marsh grass detritus. Estuaries and Coasts, doi: 10.1007/s12237-014-9814-5.

Bergamino L, Dalu T, Whitfield AK, Carassou L, Richoux NB. 2014. Stable isotope evidence of food web connectivity by a top predatory fish (*Argyrosomus japonicus*: Sciaenidae: Teleostei) in the Kowie Estuary, South Africa. African Journal of Marine Science, doi:10.2989/1814232X.2014.923782.

Chapter 2

Evidence of spatial and temporal changes in sources of organic matter in estuarine sediments: stable isotope and fatty acid analyses

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2.1 Introduction

In aquatic ecosystems, vast amounts of organic matter sources suspended in the water column reach the benthic system through sedimentation events (Graf 1992). In estuaries, the benthic environment is influenced by a diversity of organic matter sources including bacteria, benthic microalgae, phytoplankton, terrestrial organic matter and mixed detritus (Canuel et al. 1997; Hu et al. 2006). The relative importance of the diverse food sources within an estuary changes spatially and seasonally from shifts in freshwater discharge, together with local differences in habitat characteristics including vegetation type and tidal amplitude (Deegan and Garritt 1997; Riera and Richard 1997; Bouillon et al. 2004; Olin et al. 2013). The high complexity of estuarine ecosystems arising from these temporal and spatial fluctuations in physical, chemical and biological factors (McLusky and Elliott 2004) creates a challenging platform from which it is possible to assess organic matter dynamics.

Physical and biological processes are involved in the distribution and preservation of organic matter in estuarine sediments including primary productivity, oxygen exposure time, patterns of freshwater discharge and winds (Hedges and Keil 1995; Arzayus and Canuel 2005; Goñi et al. 2009; Palomo and Canuel 2010). In particular, freshwater flow and algal bloom events in the overlying surface water are important factors affecting the type and amount of organic matter that is deposited (Carrie et al. 1998; Zimmerman and Canuel 2001; Gogou and Stephanou 2004). Despite the major influx of terrestrial organic matter from rivers, terrestrially-derived organic matter seems to contribute relatively small amounts to estuarine sediments due to remineralization processes (Hedges et al. 1997), although contributions are strongly linked to freshwater flow rates (Zimmerman and Canuel

2001; Dai and Sun 2007; Palomo and Canuel 2010). Intertidal flats are important areas that receive and accumulate plant material transported through estuaries (Meziane et al. 1997). In turn, the quality and quantity of organic matter delivery to surface sediments influences the biomass and biodiversity of the benthic fauna (Rowe et al. 1991; Herman et al. 1999). In this context, and given the spatial and temporal dynamics of estuarine ecosystems, it is important to include temporal and spatial approaches to assess the organic matter composition in sediments so that we may enhance our understanding of ecosystem functioning.

Given the trophic complexity associated with the high diversity of organic matter sources that characterize estuarine ecosystems, a variety of analytical techniques have been used to increase the amount of information that can be extracted from sediments. In particular, fatty acid (FA) profiles and stable isotope (SI) ratios have been successfully used in different aquatic ecosystems (Dalsgaard et al. 2003; Fry 2006). Both techniques provide time-integrated information on the composition of complex materials such as sediments. Stable isotope mixing models can also inform us about carbon sources when they have distinct isotopic signatures (e.g. Phillips and Gregg 2003; Parnell et al. 2010). Previous analyses have shown that FA and SI composition in marine and estuarine sediments can provide useful information about the major sources of organic matter such as bacteria, diatoms and terrestrial plants (e.g. LeBlanc and Bourbonniere 1989; Canuel et al. 1997; Colombo et al. 1997; Carrie et al. 1998; Budge et al. 2001), and they have also demonstrated the transfer of specific FAs from sediments to predators in the food web (Meziane and Tsuchiya 2000). Therefore, the combination of FA and SI presents an ideal complementary approach for identifying sources of organic matter across different sites and seasons within an estuarine environment.

In this study FA profiles and SI values in sediments and diverse sources of organic matter were evaluated to examine the spatial and seasonal changes within a temperate estuarine environment. According to previous studies where the organic matter in sediments depended on hydrodynamic conditions, local carbon sources and productivity pulses (Zimmerman and Canuel 2001; Palomo and Canuel 2010), I expected differences in the FA profiles and SI signatures in sediments to occur among estuarine reaches and seasons owing to shifts in primary productivity and freshwater discharge. Furthermore, given that low discharge periods promote the deposition of marsh plants in upper intertidal zones (Dai and Sun 2007), it was expected that differences in FA and SI signatures in sediments along the estuary correspond with the deposition of marsh plants in intertidal zones from the lower reaches, particularly during times of low river discharge.

2.2 Methods

2.2.1 Study area and sample collection

The study was carried out in the Kowie Estuary (33°36'11'S, 26°54'10'E) located on the southeast coast of South Africa (Fig. 2.1). This is a permanently open, warm temperate and semidiurnal microtidal estuary with a spatial extent of 21 km (Whitfield et al. 1994; Schumann et al. 2001). The estuary channel is 2–3 m deep, depending on the tide, although deep scour holes of up to 8 m have been recorded in the lower reaches (Whitfield et al. 1994). The estuary is tidal for 20.8 km and the spring tidal range in the upper reaches is about 1.1 m, middle reaches 1.5 m and lower reaches 1.7 m (Whitfield et al. 1994). Temperature increases towards the upper reaches and seasonal water temperatures in the whole system range from 20–28°C in summer and 12–16°C in winter (James and Harrison 2010). The width of the estuary is approximately 10 m at the head, broadening to more than 50 m in the lower reaches. Shallow salt marsh areas and large mud flats occur in the lower reaches, with intertidal mud banks exceeding 100 m in width. The Kowie catchment area is between 576 and 769 km^2, and the mean annual freshwater run-off ranges from approximately 23 to 46 x 10^6 m^3 (Heinecken and Grindley 1982). An increasing salinity gradient from the head to the mouth (1 – ~35) typically occurs along the estuary, and this gradient is accentuated during periods of high river flow (Whitfield et al. 1994).

Different types of vegetation occur along the length of the system. The emergent vegetation in the upper reaches is dominated by *Phragmites australis* and *Schoenoplectus brachyceras*, and *Stukenia pectinatus* is the most widespread submerged macrophyte under oligohaline conditions (Whitfield et al. 1994). Mud flats and intertidal salt marshes occur in some areas of the middle and lower reaches. The middle portion of the estuary is inhabited by the salt marsh plants *Chenolea diffusa* and *Sarcocornia perennis* (upper intertidal zone). In the lower reaches, the upper intertidal zone is colonized by the marsh grass *Spartina maritima* (Whitfield et al. 1994). No macroalgae inhabited the estuary throughout this study.

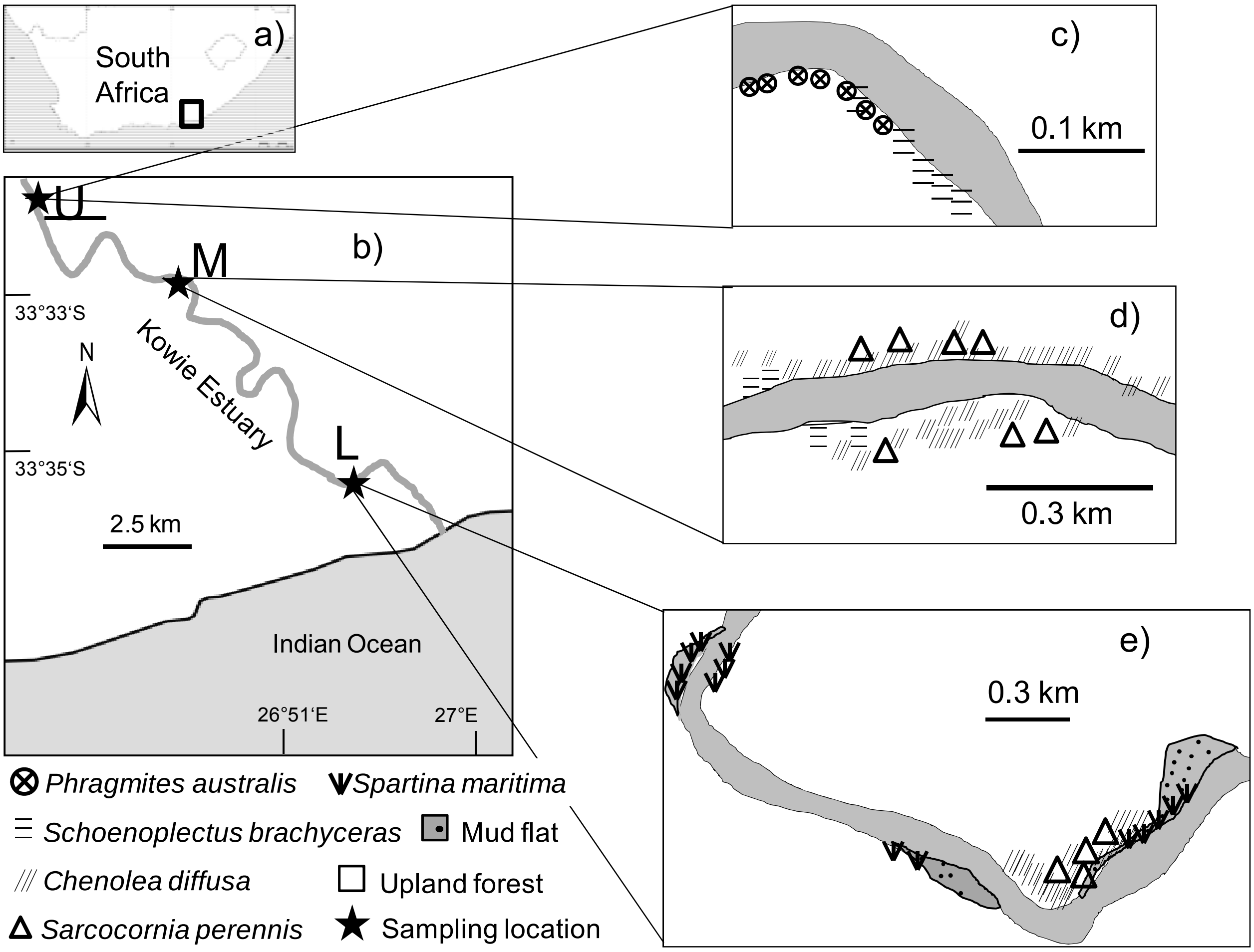

Figure 2.1. Location of the study site (a), the Kowie Estuary (b) in the southeast coast of South Africa and the sampling sites for all the sources of organic matter are indicated: c) upper reaches (U); d) middle reaches (M); e) lower reaches (L).

Sediment samples together with the sources of organic matter including macrophytes and senescent leaves were collected concurrently in four consecutive seasons: September 2012 (spring), December 2012 (summer), March 2013 (autumn) and June 2013 (winter). Three sites were sampled along the estuary: (1) upper reaches near the head of the estuary, which are dominated by river influences (salinity annual mean = ~3; range tidal of 1.1 m); (2) middle reaches located at approximately 11 km from the mouth (salinity annual mean = ~17; tidal range = 1.5), and (3) lower reaches, near the ocean (river mouth) where extensive marine influence occurs (salinity annual mean = ~30; tidal range of 1.7; Whitfield et al. 1994). Monthly average discharges (m^3/s) of the Kowie River were 0.29 in September 2012, 0.54 in December 2012, 0.55 in February 213 and 1.10 in June 2013 (Department of Water Affairs and Forestry database, http://www.dwaf.gov.za/hydrology; Fig. 2.2). In October 2012, the Kowie Estuary experienced flooding conditions showing the highest water discharge with a monthly average of 94.1 m^3/s, followed by November 2012 with an average of 2.2 m^3/s (Fig. 2.2).

At each estuarine reach per season, surface sediment organic matter (SOM) samples were collected from the upper 1 cm and then frozen. The dominant emergent macrophytes were collected by hand and washed using estuarine water. All samples were collected in triplicate during the day at low tide. After collection sediment and plant samples were stored on ice in a cooler box for transportation to the laboratory where they were processed within a day or two of collection.

In addition, total chlorophyll-*a* concentrations (Chl-a) and tracer data for additional aquatic end-members from the upper, middle and lower reaches of the Kowie Estuary [i.e. particulate organic matter (POM), and microphytobenthos (MPB)] were sourced from a separate study (T. Dalu, unpublished data). Water samples for POM analysis and samples of surface sediment for MPB were collected following the methodology described in Antonio et al. (2012). Samplings of all the organic matter sources including SOM, POM, MPB and plant material were collected concurrently and from the same sites within the Kowie Estuary.

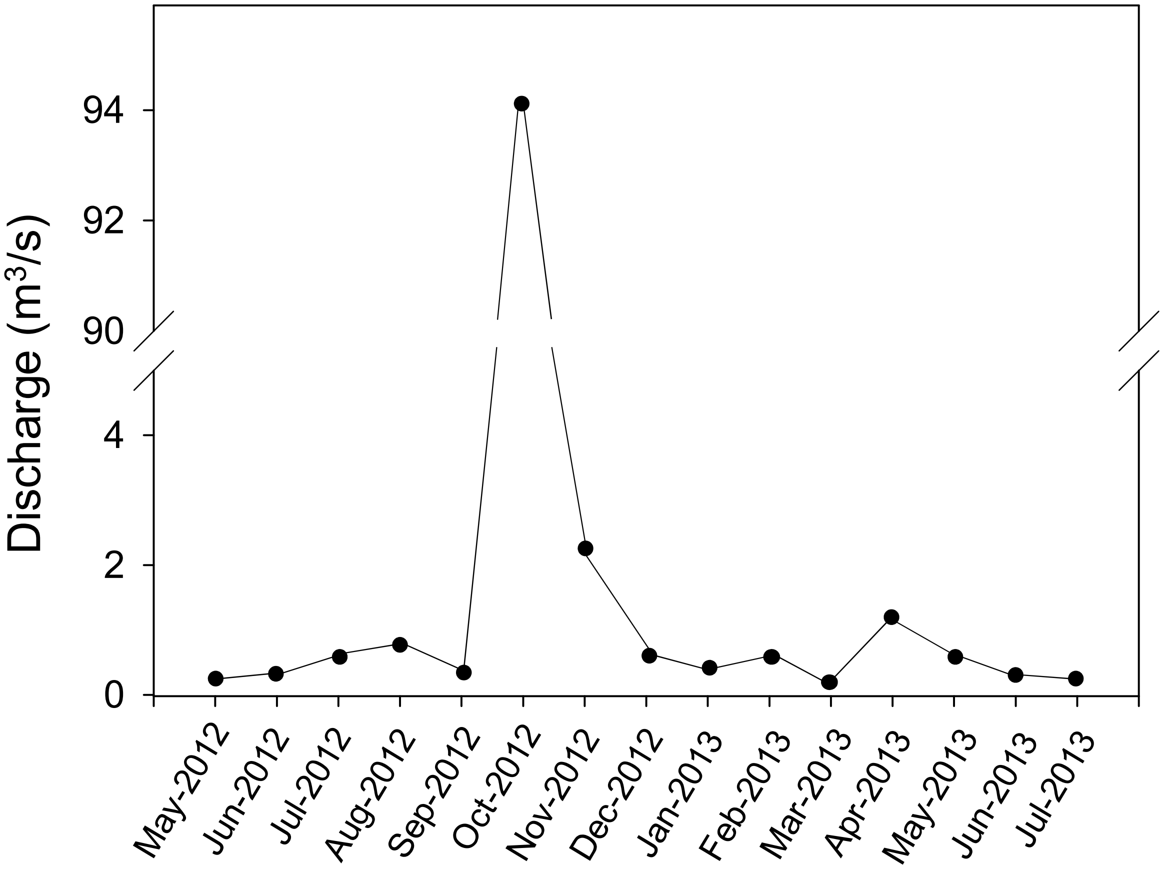

Figure 2.2. Monthly average values of the Kowie River discharge (m^3/s) during the period of this study from May 2012 to July 2013. Data were obtained from the Department of Water Affairs and Forestry database South Africa, http://www.dwaf.gov.za/hydrology.

2.2.2 Stable isotope analysis

The sediment surface samples were treated to remove carbonates by soaking sub-samples of 0.3 g in 1 M HCl for several hours using the drop-by-drop technique until bubbling stopped, rinsed in distilled water, and dried at 60°C (Jacob et al. 2005). Since this acid treatment can alter $\delta^{15}N$ signatures of sediments, these values for SOM were not included in statistical analyses (Ryba and Burgess 2002).

All samples including sediments and plants were placed in aluminum foil envelopes, lyophilized in a VirTis Benchtop 2K, and ground to a fine homogeneous powder using a mortar and pestle. For sediments and plants, sub-samples were weighed (25 to 30 mg for sediments, and approximately 3 mg for plants) into tin capsules. All samples were analyzed for stable carbon and nitrogen isotope composition at IsoEnvironmental cc, Rhodes University, South Africa, using a Europa Scientific 20-20 IRMS linked to an ANCA SL Prep Unit. Beet sugar, ammonium sulphate and casein were the internal standards, calibrated against International Atomic Energy reference materials (Vienna Pee Dee Belemnite for carbon and atmospheric N_2 for nitrogen). The analytical precision of the measurements was ±0.1‰ for both nitrogen and carbon. Results are expressed as per mil (‰) using the δ notation defined by the equation: $\delta^{13}C$ or $\delta^{15}N$ (‰) = [(Rsample/Rstandard) −1)] x 1000, where R is $^{13}C/^{12}C$ for carbon and $^{15}N/^{14}N$ for nitrogen of the sample and the standard respectively.

2.2.3 Fatty acid extraction

Lipid extractions were done using a one-step method modified from Indarti et al. (2005). Each sample, represented by 1000-1200 mg of dry sediments or 100 mg dry mass of plant material, was placed in 2 ml chloroform with 0.01 % of the antioxidant butylated hydroxytoluene, topped with nitrogen gas and stored at -20 °C. Two ml of an anhydrous methanol-sulfuric acid mixture were added to each sample and placed at 100 ºC for 30 minutes. One ml of MilliQ-distilled water was added to each sample, and after centrifugation the lower phase containing the FA methyl esters (FAMEs) was transferred through anhydrous Na_2SO_4 into a clean vial and concentrated to dryness. FAMEs were topped with hexane and injected into an Agilent 7890 gas chromatograph fitted with a ZB-Waxplus 320 column and a flame ionization detector, and helium as the carrier gas. FAME peaks were visualized using ChemStation chromatography software, identified using external standards (marine PUFA no. 1, 37 component FAMEs mix; Supelco) and confirmed using mass spectrometry on an Agilent 7000A GC/MS-QQQ coupled with a NIST 08 MS library (column and methods as per the GC runs). Each FA was measured as a proportion of the total fatty acids (% TFA). FAs are named according to a:bωx, where a is the number of carbon atoms, b gives the number of double bonds and x is the position of the first double bond from the methyl end of the molecule.

2.2.4 Data analyses

Two-way ANOVA were used to test for differences in $\delta^{13}C$ and $\delta^{15}N$ signatures of sediments among the estuarine reaches, using ‘reach’ and ‘season’ as the main fixed factors. No transformations were needed to fulfill statistical assumptions.

To analyze possible spatial and temporal changes (separately) in the non-transformed FA profiles of sediments, I used non-parametric multi-dimensional scaling (nMDS: Kruskal and Wish 1978) based on Euclidean distance matrices. A one-way ANOSIM was used to test for differences in the FA profiles among reaches and seasons within each reach, and the similarity percentages routine (SIMPER) was used to determine the FAs contributing to any differences. In addition, SIMPER results were compared with the loadings results following principal components analysis (PCA) using the same data as outlined above. This comparison was performed as a visual aid only, and the PCA loadings served as guideline estimates to superimpose the SIMPER results onto the nMDS plots. Furthermore, the potential relationship between chl-*a* and proportions of diatom-associated FAs were examined using Pearson correlation analysis. Statistical analyses were completed using PAST 1.42 (Hammer et al., 2001) and Statistica 7 (StatSoft, Tulsa, OK).

Bayesian SIAR models (Stable Isotope Analysis in R; Parnell et al., 2010) were used to assess the relative contributions of the different organic matter sources to the sediment mixtures. This Bayesian model incorporates uncertainty and variation in parameters such as trophic enrichment factor. This model was run for each season and each estuarine reach to assess spatial and seasonal differences in the sources of organic matter in sediments. I assumed small carbon and nitrogen fractionation factors (0.5 for both) based on short-term degradation experiments (e.g. Zieman et al. 1984; Wedin et al. 1995; Schweizer et al. 1999; Dehairs et al. 2000).

2.3 Results

2.3.1 Isotopic values for organic matter sources and sediments

In the upper reaches, the macrophytes *Phragmites australis* and *Schoenoplectus brachyceras* had the lowest $\delta^{13}C$ signatures in all seasons and signatures ranged from (mean ± SD) -27.6 ± 0.1‰ to -26.7 ± 0.1‰ and -27.8 ± 0.1‰ to -30.3 ± 0.1‰, respectively (Table 2.1). In the middle reaches of the Kowie Estuary, the salt marsh plants *Chenolea diffusa* and *Sarcocornia perennis* exhibited the lowest $\delta^{13}C$ signatures in all seasons (ranged from -27.8 ± 0.1‰ and -29 ± 0.2‰). For the lower reaches of the estuary, terrestrial leaves had the lowest $\delta^{13}C$ signatures (range of -29.2 ± 0.3‰ to -27.8 ± 0.7‰), whereas the marsh grass *Spartina maritima* had the highest $\delta^{13}C$ signatures in all seasons with values ranging from -13.8 ± 0.3‰ to -14.1 ± 0.1‰ (Table 2.1). In the lower reaches, MPB had intermediate $\delta^{13}C$ values between those of terrestrial leaves and the marsh grass *S. maritima* (Table 2.1).

The $\delta^{13}C$ signatures of sediments were distinct among estuarine reaches and seasons (Table 2.1). $\delta^{13}C$ values were significantly lower in the upper reaches (range from -21.1 ± 0.3‰ and -24 ± 0.2‰) and middle reaches (ranged from -19.5 ± 0.3‰ to -21.7 ± 0.1‰) in comparison with the lower reaches of the estuary during all seasons (ranged from -10.5 ± 1.3‰ to -21.9 ± 0.3‰; two way ANOVA $F = 336.6$, df = 55, $p < 0.001$), with autumn and winter $\delta^{13}C$ values in sediments higher than those in spring and summer at all estuarine reaches (two-way ANOVA $F = 283.6$, df = 55, $p < 0.001$). Regarding $\delta^{15}N$ values in sediments, the mean $\delta^{15}N$ values in the upper reaches ranged from 5.0 to 8.1‰ in winter, in the middle reaches ranged from 7.2 to 8.3‰, and in the lower reaches ranged from 5.5 to 7.5‰ (Table 2.1). Significantly higher $\delta^{15}N$ values were found in the middle reach sediments than in the lower and upper reaches (two-way ANOVA $F = 7.2$, df = 55, $p < 0.01$), but no significant variation among seasons (two-way ANOVA $F = 1.2$, df = 55, $p > 0.05$).

Table 2.1. Seasonal values (mean ± SD) of δ^{13}C and δ^{15}N (‰) for sources of organic matter in each estuarine reach: upper, middle and lower reaches. POM: particulate organic matter; SOM: sediment organic matter; MPB: microphytobenthos; PHR: *Phragmites australis*; SCH: *Schoenoplectus brachyceras*; SPA: *Spartina maritima; d*: detritus; CHE: *Chenolea diffusa*; SAR: *Sarcocornia perennis*; LEA: terrestrial leaves; SEED: terrestrial seeds. Isotope values of POM and MPB from the Kowie Estuary were collected by T. Dalu (unpublished data).

	Spring		Summer		Autumn		Winter	
Sources	δ^{13}C	δ^{15}N	δ^{13}C	δ^{15}N	δ^{13}C	δ^{15}N	δ^{13}C	δ^{15}N
Upper								
PHR	-27.6 (0.1)	5.0 (0.3)	-27.2 (0.3)	12.2 (0.1)	-26.7 (0.1)	6.6 (0.5)	-26.8 (0.05)	8.5 (0.2)
PHR d	-26.5 (0.5)	9.4 (0.3)						
SCH	-28.8 (0.2)	8.2 (0.1)	-27.8 (0.1)	7.4 (0.4)	-30.3 (0.1)	7.9 (0.3)		
SOM	-24.1 (0.2)		-21.6 (0.2)		-21.1 (0.3)		-22.0 (0.2)	
POM	-22.5 (0.2)	6.8 (0.2)	-25.0 (0.5)	11.0 (0.4)	-26.4 (0.8)	12.5 (0.8)	-27.6 (0.4)	11.3 (0.3)
MPB	-27.1 (0.3)	12.3 (0.2)	-20.0 (1.3)	11.0 (0.05)	-20.8 (1.0)	6.0 (0.2)	-21.0 (0.1)	11.3 (0.1)
LEA			-27.3 (0.5)	9.0 (0.3)			-27.8 (0.1)	6.3 (0.2)
SEED					-28.7 (0.5)	9.6 (1.0)		
Middle								
CHE	-27.8 (0.1)	1.8 (0.0)			-29.0 (0.2)	11.8 (0.1)	-28.8 (0.07)	11.4 (0.5)
SAR	-28.8 (0.1	10.1 (0.2)						
POM	-23.8 (0.1)	11.8 (0.0	-25.5 (0.4)	11.1 (0.3)	-28.0 (0.04)	14.0 (0.1)	-26.9 (0.03)	11.8 (0.1)
MPB	-26.0 (0.0)	11.9 (0.0)	-19.8 (1.2)	10.0 (0.2)	-21.0 (2.7)	4.5 (1.4)	-21.1 (0.15)	9.0 (0.2)
SOM	-21.7 (0.1)		-22.1 (0.08)		-19.5 (0.3)		-20.0 (0.47)	
LEA					-27.4 (0.7)	6.8 (0.8)		
PHR d					-26.4 (0.1)	8.8 (0.8)		
SAR					-30.2 (0.1)	8.3 (0.4)		
Lower								
LEA	-29.9 (0.3)	7.3 (0.2)	-27.8 (0.7)	5.4 (1.5)	-29.2 (0.1)	8.5 (0.6)		
SOM	-21.6 (0.8)		-21.9 (0.08)		-15.5 (0.2)		-10.5 (1.3)	
SPA	-13.8 (0.3)	12.4 (0.3)	-14.1 (0.1	10.3 (0.2)	-13.78 (0.16)	8.6 (0.1)	-13.8 (0.05)	8.5 (0.1)
POM	-23.4 (0.7)	11.3 (0.3)	-22.4 (0.4)	7.3 (0.04)	-24.0 (0.4)	10.1 (0.4)	-21.28 (1.1)	8.0 (0.4)
MPB	-24.7 (0.06)	12.2 (0.1)	-24.2 (0.8)	13.6 (0.5)	-23.6 (0.7)	5.4 (0.9)	-21.3 (0.5)	6.2 (0.3)
SPA d					-13.9 (0.1)	7.9 (0.2)	-13.6 (0.1)	8.8 (0.7)

2.3.2 Contributions of organic matter sources to sediments

The SIAR outputs suggested that in the upper and middle reaches of the estuary, MPB was the main organic matter source contributing to sediments in all the seasons except in spring, when POM was the dominant contributor to sediments (Fig. 2.3). In the lower reaches during winter and autumn, the marsh grass *Spartina maritima* was the main contributor to the sediments, while in summer POM was the most important source of organic material for sediments in the lower reaches (Fig. 2.3; see also Table 2.2 for the 95% credibility intervals).

2.3.3 Fatty acid composition of organic matter sources and sediments

Large proportions of 18:2ω6 and 18:3ω3 (>40% TFA) were present in the vascular plants, with the exception of detritus from *S. maritima* where lower proportions of these PUFAs were found (annual means of 9.2% and 8.4%, respectively, Table 2.3). Detritus from *S. maritima* had enhanced proportions of 24:0 (~6% TFA) compared with the other organic matter sources (one-way ANOVA, $F = 19.6$, df = 24, $p < 0.001$).

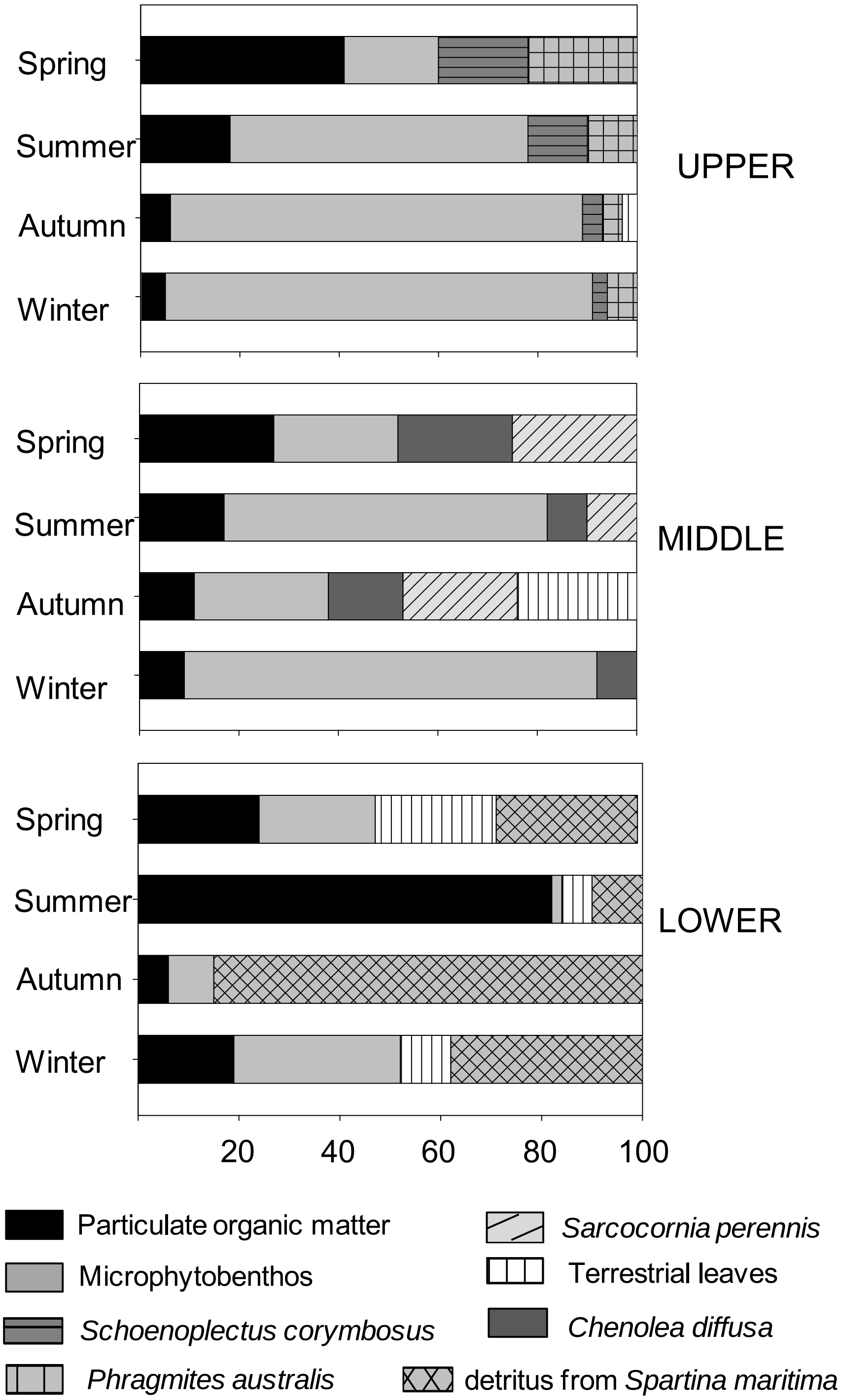

Figure 2.3. Results of the isotopic mixing model determined by SIAR showing the mean percentage contributions of several organic matter sources to the sediment organic matter composition in all the three reaches of the Kowie Estuary and in the four seasons.

Table 2.2. Mixing model results determined by SIAR showing the mean percentage contribution (95% credibility interval) of several organic matter sources to the sediment organic matter composition in all the three reaches of the Kowie Estuary and in the four seasons. Abbreviations of species/groups are shown in Table 2.1.

	POM	MPB	SCH	PHR	CHE	SAR	LEA	SPA
Upper								
Spring	**40 (7-76)**	18 (0-41)	18 (0-42)	22 (0-45)				
Summer	18 (0-40)	**59 (26-81)**	12 (0-31)	10 (0-27)				
Autumn	6 (0-17)	**83 (61-96)**	3 (0-8)	4 (0-13)			3 (0-10)	
Winter	5 (0-10)	**86 (82-89)**	3 (0-8)	6 (0-13)				
Middle								
Spring	**27 (0-51)**	25 (0-49)			23 (0-46)	25 (0-48)		
Summer	17 (0-36)	**65 (53-77)**			8 (0-20)	10 (0-23)		
Autumn	11 (0-23)	**27 (9-45)**			15 (0-30)	23 (0-43)	24 (2-43)	
Winter	9 (0-26)	**83 (59-100)**			8 (0-24)			
Lower								
Spring	24 (0-50)	23 (0-45)					24 (0-45)	**28 (3-33)**
Summer	**82 (59-95)**	2 (0-3)					6 (0-20)	10 (2-16)
Autumn	6 (0-13)	9 (0-15)						**85 (82-88)**
Winter	19 (0-43)	33 (0-64)					10 (0-27)	**38 (0-77)**

Table 2.3. Annual mean values (±SD) of fatty acids in different plants from the Kowie Estuary. In the upper reaches: SCH: *Schoenoplectus corymbosus*, PHR: *Phragmites australis*, the middle reaches: CHE: *Chenolea diffusa*; LEA: Terrestrial leaves; and from the lower reaches: SPA: *Spartina maritima,* SPAd: detritus from *S. maritima.* POM: particulate organic matter; MPB: microphytobenthos. Nd = not detected. The fatty acid profiles of POM and MPB from the Kowie Estuary were collected by T. Dalu (unpublished. data).

	UPPER		MIDDLE		LOWER		ALL REACHES	
	SCH	PHR	CHE	LEA	SPAd	SPA	POM	MPB
	Mean (SD)	Mean (SD)	Mean (SD)	Mean (SD)	Mean (SD)	Mean (SD)	Mean (SD)	Mean (SD)
14:0	0.7 (0.4)	2.0 (0.06)	1.2 (0.2)	7.6 (0.2)	3.8 (0.2)	1.2 (0.9)	8.0 (2.4)	5.6 (1.6)
i-15:0	0.1 (0.1)	0.13 (0.04)	0.2 (0.06)	0.1 (0.14)	0.0 (0.0)	0.14 (0.14)	0.7 (0.5)	0.8 (0.7)
15:0	0.8 (0.03)	0.21 (0.03)	0.4 (0.03)	0.3 (0.01)	0.6 (0.02)	0.08 (0.07)	1.6 (1.6)	7.3 (6.2)
16:0	20.1 (0.1)	15.6 (0.2)	19.3 (0.01)	25.3 (0.06)	26.0 (1.2)	16.5 (0.9)	29.5 (4.4)	21.2 (5.8)
16:1ω7	1.1 (0.3)	1.9 (0.3)	0.3 (0.0)	0.21 (0.1)	9.0 (0.4)	1.0 (1.1)	12 (5.2)	13.3 (2.3)
i-17:0	0.9 (0.03)	Nd	1.0 (0.1)	0.2 (0.02)	0.0 (0.0)	0.8 (0.80)	0.2 (0.4)	0.3 (0.3)
17:1ω7	0.2 (0.13)	Nd	0.12 (0.0)	0.1 (0.01)	0.6 (0.04)	0 (0.0)	Nd	0.75 (1.8)
18:0	1.3 (0.03)	1.72 (0.05)	2.9 (0.04)	9.4 (0.1)	6.5 (0.3)	1.5 (0.2)	9.1 (4.4)	3.5 (2.2)
18:1ω9	4.7 (0.1)	1.8 (0.06)	14.0 (0.2)	5.2 (0.04)	5.5 (0.1)	2.1 (0.74)	3.6 (1.7)	2.7 (2.0)
18:1ω7	0.3 (0.0)	0.1 (0.02)	0.8 (0.01)	0.4 (0.06)	5.1 (0.1)	0.5 (0.02)	2.1 (1.3)	3.1 (1.6)
18:2ω6	22.9 (0.3)	11.1 (0.04)	19.3 (0.3)	11.9 (0.1)	9.2 (0.3)	16.2 (1.4)	2.0 (0.8)	2.5 (1.6)
18:3ω3	42.1 (0.7)	62.8 (0.3)	32.6 (0.2)	30.3 (0.5)	8.4 (0.3)	55 (1.0)	5.6 (5.9)	3.3 (5.5)
20:0	Nd	0.6 (0.03)	1.4 (0.02)	3.0 (0.03)	0.7 (0.03)	0.0 (0.07)	0.5 (0.7)	0.7 (0.6)
20:4ω6	0.05 (0.05)	0.08 (0.07)	0.1 (0.01)	0.4 (0.01)	2.0 (0.13)	Nd	0. 3 (0.3)	1.6 (0.9)
20:5ω3	0.02 (0.04)	0.1 (0.2)	0.3 (0.0)	0.3 (0.03)	6.1 (0.3)	Nd	5.4 (2.5)	10.8 (4.3)
22:0	1.4 (0.1)	0.5 (0.03)	2.9 (0.03)	1.3 (0.04)	2.7 (0.6)	1.0 (0.1)	0.7 (0.7)	0.6 (0.4)
24:0	2.4 (0.7)	0.5 (0.03)	1.7 (0.2)	1.8 (0.1)	6.1 (2.2)	2.2 (2.2)	1.1 (1.2)	1.6 (1.3)

The sediments from the upper, middle and lower reaches were dominated by the FA 16:0 (>23% TFA), followed by 16:1ω7 (~10% TFA; Table 2.4). These FAs were dominant in all seasons (Table 2.5). The presence of higher plant fatty acids (HPFAs) in sediments from all the three estuarine reaches was relatively low (range of 1-4.7% TFA), with no significant differences among reaches (Table 2.4). Furthermore the HPFAs were more prevalent during summer and winter due to the presence of the FA 18:3ω3 (Table 2.5). The proportions of bacterial fatty acids (BAFAs) in sediments from the three estuarine regions showed a range of 13.3-15.4% TFA, with no differences among reaches (Table 2.4) and small temporal variations (Table 2.5). Among the eighteen FAs identified at proportions >1% in sediments, certain FAs were significantly different among reaches (Table 2.4). Proportions of the diatom-associated fatty acids 16:1ω7 and 20:5ω3 increased from the lower reaches (range of ~3 to 9% TFA) towards the middle (range of ~6−8% TFA) and upper reaches (range of ~6−12% TFA). Post-hoc multiple comparisons (Tukey test) showed that the proportions of 16:1ω7 in sediments were significantly higher in the upper compared with the middle and lower reaches ($p < 0.001$). The essential fatty acid (EFA) 22:6ω3 occurred in all three regions with a range between 1.5 and 2.8% TFA. The levels of 24:0 markedly decreased from the lower reaches (annual mean of 4.4 ± 0.6% TFA) towards the upper reaches (middle = 1.6 ± 0.2% TFA, upper = 1.7 ± 0.6% TFA). Post-hoc multiple comparisons (Tukey test) showed that the levels of 24:0 did not differ between middle and upper reaches, but both differed significantly from the lower reaches (one-way ANOVA, $F = 3.8$, df = 27, $p < 0.01$). The ratios 16:1/16:0 and 22:6ω3/20:5ω3 showed no significant differences among reaches (Table 2.4). In particular, the FA 24:0 increase during summer in the lower reaches of the estuary (Table 2.5).

The nMDS ordination based on FA profiles of sediments (all dates pooled) discriminated among estuarine regions (stress values <0.1 indicated a useful two dimensional representation of the groups; Fig. 2.4). The ANOSIM results revealed dissimilarity between the upper and lower reaches (R = 0.3; $p < 0.05$), and these differences were driven mainly by four FAs discriminating ~60% of the cumulative profiles across sites: 16:0 (31%), 16:1ω7 (12%), 22:6ω3 (10% TFA) and 24:0 (7%; Fig. 2.4). Furthermore, in the middle and lower reaches, the nMDS ordination showed that FA profiles in sediments were not separable on the basis of the seasons. However, in the upper estuary, the FA profiles of sediments in winter were significantly dissimilar to those in autumn (R = 0.9; $p < 0.05$) and summer (R = 0.9; $p < 0.05$), but not with those in spring (R = 0.8; $p > 0.05$). Proportions of diatom-associated FAs (20:5ω3 + 16:1ω7) in sediments (all seasons and reaches pooled) were positively and significantly correlated with chl-*a* (Pearson's $r = 0.6$, $p < 0.005$; Fig. 2.5).

Table 2.4. Annual mean values (±SD) of fatty acids in sediments from the lower, middle and upper reaches of the Kowie Estuary. Different letters indicate significant differences within the same fatty acid (row) (one-way ANOVA, Tukey test, $p < 0.05$). BAFA: bacterial fatty acids; HPFA: higher plant fatty acids.

	LOWER Mean (±SD)	MIDDLE Mean (±SD)	UPPER Mean (±SD)
14:0	7.1^{A} (1.2)	6.91^{A} (1.7)	7.5^{A} (1.2)
i-15:0	4.3^{A} (0.5)	4.1^{A} (0.6)	3.2^{A} (1.9)
15:0	4.1^{A} (0.9)	5.3^{A} (1.7)	5.7^{A} (2.8)
16:0	30.1^{A} (7.7)	23.3^{A} (2.0)	32.1^{A} (11.4)
16:1ω7	9.2^{A} (1.8)	8.2^{A} (3.4)	12.4^{B} (2.0)
i-17:0	1.4^{A} (1.3)	2.4^{B} (0.42)	1.7^{A} (0.7)
17:1ω7	0.2^{A}(0.5)	1.5^{B} (0.52)	1.4^{B} (0.5)
18:0	7.1^{A} (3.6)	6.9^{A} (0.5)	6.5^{A} (1.2)
18:1ω9	4.4^{A} (2.5)	5.0^{B} (1.6)	3.0^{A} (1.9)
18:1ω7	4.8^{A} (2.8)	5.9^{A} (0.4)	4.0^{A} (1.7)
18:2ω6	0.7^{A} (0.8)	2.7^{A} (2.7)	1.5^{A} (0.7)
18:3ω3	0.4^{A} (0.8)	0.8^{A} (0.8)	0.5^{A} (0.5)
20:0	3.8^{A} (0.3)	2.4^{AB} (1.6)	0.6^{B} (0.5)
20:1ω9	0.5^{A} (1.2)	0.6^{A} (1.1)	0.9^{A} (1.1)
20:5ω3	3.3^{A} (1.7)	6.6^{A} (0.6)	6.0^{A} (3.3)
22:0	5.8^{A} (1.4)	4.0^{B} (0.6)	3.8^{B} (1.4)
24:0	4.4^{A} (0.6)	1.6^{B} (0.2)	1.7^{B} (0.6)
22:6ω3	1.5^{A} (1.9)	1.9^{A} (3.1)	2.8^{A} (3.6)
ΣBAFA	13.3^{A} (2.1)	15.4^{A} (3.2)	13.5^{A} (1.1)
ΣHPFA	1.1^{A} (1.2)	4.7^{A} (7.3)	1.8^{A} (1.3)
16:1/16:0	0.3^{A} (0.1)	0.4^{A} (0.2)	0.5^{A} (0.3)
22:6ω3/20:5ω3	0.3^{A} (0.4)	0.2^{A} (0.4)	0.9^{A} (1.3)

Table 2.5. Seasonal mean values (±SD) of fatty acids in sediments from the lower, middle and upper reaches of the Kowie Estuary. BAFA: bacterial fatty acids; HPFA: higher plant fatty acids.

	SPRING			SUMMER			AUTUMN			WINTER		
	Lower	Middle	Upper	Lower	Middle	Upper	Lower	Middle	Upper	Lower	Middle	Upper
	Mean (±SD)	Mean (±SD)	Mean (±SD)	Mean (±SD)	Mean (±SD)	Mean (±SD)	Mean (±SD)	Mean (±SD)	Mean (±SD)	Mean (±SD)	Mean (±SD)	Mean (±SD)
14:0	5.63 (0.7)	5.8 (0.6)	6.85 (2.2)	7.3 (0.07)	6.5 (0.6)	8.88 (0.21)	7.76 (1.3)	9.46 (0.40)	7.71 (0.86)	8.1 (0.07)	5.6 (0.9)	6.7 (0.3)
i-15:0	3.60 (0.1)	3.3 (0.8)	4.6 (1.9)	4.5 (0.09)	3.8 (0.30)	1.4 (1.2)	5.6 (0.6)	5.40 (0.04)	3.26 (0.41)	4.7 (0.2)	3.4 (0.6)	3.7 (0.2)
15:00	3.0 (0.4)	4.6 (0.5)	6.7 (1.2)	4.3 (0.1)	5.7 (0.2)	8.8 (0.32)	4.6 (0.9)	8.1 (0.42)	7.6 (0.9)	5.1 (0.4)	3.1 (0.5)	3.5 (0.17)
16:00	23.5 (1.5)	13.8 (6.1)	11.2 (1.3)	28.9 (0.5)	25.1 (0.6)	28.4 (0.70)	38.80 (8.80)	30.2 (1.2)	47.3 (8.4)	32.1 (0.7)	22.5 (2.1)	24.3 (1.5)
16:1ω7	10.97 (1.5)	7.9 (4.3)	14.6 (0.6)	6.8 (0.25)	10.1 (0.4)	14.1 (1.00)	9.6 (1.65)	4.5 (5..0)	12.1 (1.0)	10.8 (0.8)	10.3 (2.7)	10.3 (0.24)
i-17:0	1.4 (0.2)	1.1 (0.1)	11.4 (13.6)	2.2 (0.28)	2.3 (0.37)	1.0 (0.03)	0.76 (1.3)	3.0 (0.12)	2.6 (0.77)	2.1 (0.07)	2.0 (0.4)	1.51 (0.07)
17:1ω7	0.00	0.8 (1.1)	1.5 (2.1)	1.77 (0.014)	1.4 (0.05)	1.4 (0.12)	0.0000	2.1 (0.03)	0.45 (0.80)	1.7 (0.2)	0.9 (0.2)	1.4 (0.1)
18:0	6.65 (0.1)	8.1 (0.5)	2.6 (3.7)	10.0 (0.44)	6.5 (0.45)	7.4 (1.9)	7.60 (7.0)	7.82 (0.80)	8.4 (2.3)	6.5 (0.6)	5.6 (0.4)	6.4 (0.02)
18:1ω9	3.6 (0.04)	4.4 (0.6)	1.9 (2.6)	7.26 (0.55)	4.35 (0.20)	3.7 (1.1)	4.15 (4.12	4.25 (0.30)	1.20 (2.0)	5.0 (0.09)	6.8 (2.0)	4.3 (0.05)
18:1ω7	5.83 (0.52)	6.1 (0.9)	4.5 (0.5)	5.4 (0.23)	5.51 (0.05)	4.2 (0.2)	2.0 (3.4)	6.23 (0.21)	1.5 (1.6)	5.8 (0.3)	5.8 (0.7)	5.55 (0.66)
18:2ω6	0.00	0.2 (0.3)	1.7 (0.4)	0.9 (0.006)	1.8 (0.30)	0.93 (0.07)	0.6 (1.0)	0.70 (0.63)	0.5 (0.88)	0.7 (0.88)	11.6 (10.0)	2.0 (0.26)
18:3ω3	0.00	0.00	0.00	0.8 (1.2)	1.78 (0.20)	0.42 (0.08)	0.0000	0.0000	0.0000	1.7 (1.6)	1.2 (0.08)	1.2 (0.15)
20:0	0.00	0.4 (0.5)	0.00	0.3 (0.43)	1.0 (0.07)	1.0 (0.17)	0.0000	0.30 (0.50)	0.0000	0.0000	0.7 (0.08)	1.16 (0.09)
20:1ω9	4.4 (1.1)	2.6 (0.5)	2.8 (0.5)	4.7 (0.28)	3.6 (0.21)	1.66 (0.04)	3.0 (2.77)	3.72 (0.62)	0.0000	0.0000	0.0000	0.00000
20:5ω3	4.3 (1.1)	9.3 (1.9)	3.1 (0.2)	2.9 (0.15)	6.27 (0.40)	6.20 (0.50)	3.2 (3.0)	5.4 (0.22)	2.8 (3.0)	4.8 (0.9)	6.3 (0.90)	9.8 (0.33)
22:0	4.44 (2.1)	3.9 (0.8)	5.6 (0.9)	5.9 (0.6)	4.22 (0.63)	2.15 (0.15)	8.1 (0.94)	4.7 (0.60)	3.0 (0.27)	4.9 (0.02)	3.4 (0.70)	4.7 (0.10)
24:0	4.9 (0.9)	3.4 (3.1)	6.2 (0.9)	5.4 (0.65)	3.66 (0.80)	2.54 (0.40)	2.34 (4.05)	0.0000	0.0000	0.0000	0.0000	0.00000
22:6ω3	2.8 (0.6)	1.7 (0.6)	2.8 (0.6)	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	4.2 (0.04)	5.2 (1.41)	7.2 (2.6)
ΣBAFA	12.4 (1.0)	14. (2.3)	15.7 (3.6)	14.3 (0.45)	15.14 (0.53)	14.5 (1.8)	11.2 (4.9)	19.76 (0.70)	12.3 (3.0)	15.5 (0.9)	12.3 (1.9)	12.8 (0.45)
ΣHPFA	0	0.24 (0.34)	1.77 (0.44)	1.80 (1.21)	3.60 (0.50)	1.36 (0.15)	0.62 (1.1)	0.7 (0.63)	0.51 (0.88)	2.5 (1.0)	12.8 (10.1)	3.1 (0.42)
16:1/16:0	0.4 (0.9)	0.57 (0.7)	1.3 (0.440	0.23 (0.50)	0.40 (0.73)	0.50 (1.46)	0.24 (0.20)	0.15 (4.15)	0.25 (0.12)	0.3 (1.1)	0.45 (1.27)	0.45 (0.16)
22:6ω3/20:5ω3	0.65 (0.55)	0.2 (0.3)	0.9 (2.2)	0	0	0	0	0	0	0.86 (0.04)	0.83 (1.6)	0.73 (7.8)

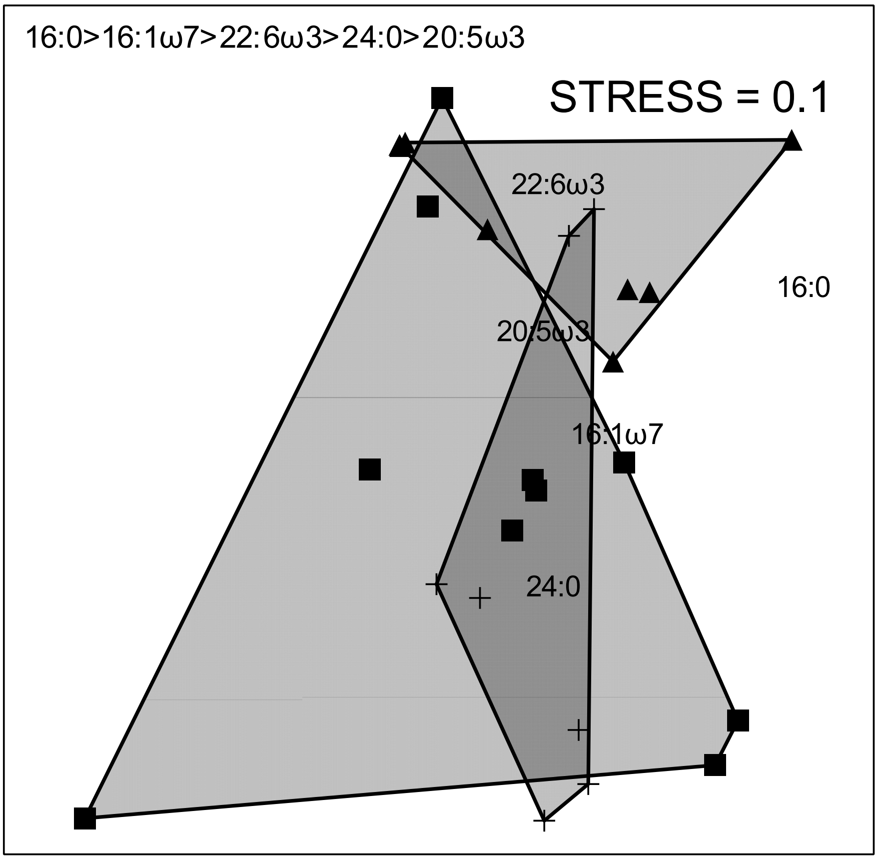

Figure 2.4. Two-dimensional non-metric multi-dimensional scaling (nMDS) ordination of the fatty acids in sediments (dates pooled) from three different reaches within the Kowie Estuary: ■, Middle Reaches; +, Lower reaches; ▲, Upper reaches. Axes are dimensionless. Major fatty acids contributing to the separation across reaches (derived from SIMPER and PCA) are shown in decreasing order and also superimposed in the plot.

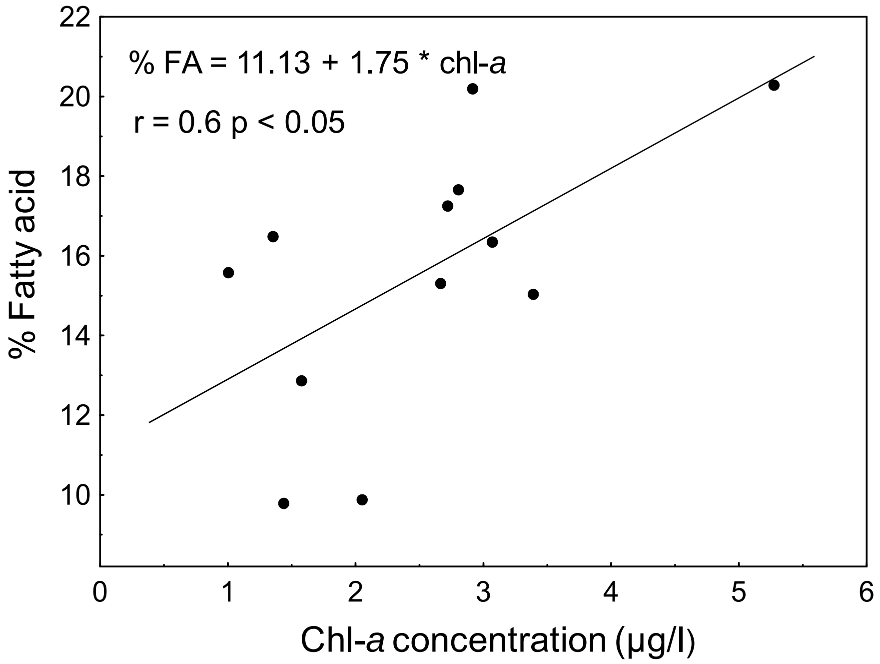

Figure 2.5. Proportions of diatom-associated fatty acids (20:5ω3 + 16:1ω7) in relation to pelagic chl-*a*. Data from all reaches and all seasons are pooled. Chl-a data from the Kowie Estuary were collected by T. Dalu (unpublished data).

2.4 Discussion

Fatty acid profiles and isotopic signatures in sediments were spatially and temporally variable within the Kowie Estuary, revealing changes in the organic matter deposited. In general, both approaches suggested a higher presence of MPB in sediments towards the upper reaches, while organic matter from plants tended to be more dominant in the lower reach sediments. Previous studies showed that sediment organic matter in estuaries and coastal environments is derived mainly from autochthonous sources including a mixture of fresh and detrital phytoplankton, zooplankton and bacteria (Harvey and Johnston 1995; Canuel and Zimmerman 1999; Zimmerman and Canuel 2001; Budge et al. 2001). In addition, allochthonous organic matter sources derived from plant material influenced sediments in upper portions of other estuarine environments and in regions surrounded by forests (LeBlanc and Bourbonniere 1989; Zimmerman and Canuel 2001; Budge et al. 2001; Palomo and Canuel 2010). Because biotic and abiotic characteristics such as chl-*a*, changes of freshwater discharge, and sources of organic matter all influence the FA and SI composition of sediments (Zimmerman and Canuel 2001; Gogou and Stephanou 2004; Palomo and Canuel 2010), the variability of chl-*a*, and freshwater discharge, together with the local vegetation type within the Kowie Estuary may help explain the spatial and temporal variations in the sediment organic matter composition. These spatial and temporal differences in FA and SI signatures along the estuary provide information regarding the relative importance of different food sources available to primary consumers along an estuarine gradient and over time.

Spatial and temporal variations in the sources of organic matter deposited within the Kowie Estuary were clearly represented by the SI signatures. Lower $\delta^{13}C$ signatures were found in sediments in the upper reaches, with the lowest values occurring during spring and summer (Table 2.1). A possible explanation for this trend is the large influence of river water in the upper reaches, where carbon sources with low $\delta^{13}C$ signatures such as terrestrial matter and freshwater phytoplankton dominate (ranging from -24 to -30‰; Fry and Sherr 1984; France 1995a). The higher freshwater discharge during November 2012 (Fig. 2.2) may explain the lowest $\delta^{13}C$ values in POM during the following period in autumn, reflecting a greater presence of terrestrial matter in the water column after the period of high freshwater discharge. Terrestrial seeds delivered from river waters were detected in the water column of the upper reaches only during autumn, suggesting they potentially form a component in sediments of the upper reaches (Table 2.1). The higher $\delta^{13}C$ values found in sediments from the lower reaches may be explained by a greater influence of ^{13}C-enriched sources including marine phytoplankton, marsh grass detritus and dissolved inorganic carbon (Chanton and Lewis 1999) in this section of the Kowie Estuary. These results are congruent with the mixing model outputs that suggested greater influences of POM and marsh grass detritus in sediments of the lower estuarine reaches. The greater plant contributions into the sediments in the lower reaches could be explained by differences in local habitat characteristics such as the shallow intertidal flats together with the vegetation type. Intertidal flat areas have been associated with the accumulation of salt marsh material in other regions of the world (Meziane et al. 1997). In these flattened areas, calm physical conditions dominate due to the presence of canopies from saltmarsh plants that reduce hydrodynamic forces and enhance deposition (Bouma et al. 2005). Furthermore, the deposition of marsh grass was more pronounced during autumn and winter, when freshwater discharge was low. This finding is consistent with that of a previous study that identified elevated concentrations of salt marsh plants during periods of low river discharge (Dai and Sun 2007). Therefore, hydrodynamic regimes together with local environmental characteristics such as vegetation type and the presence of intertidal flats seem to affect the organic matter found in sediments within estuarine ecosystems (e.g. Zimmerman and Canuel 2001; Goñi et al. 2009; Palomo and Canuel 2010). The combination of these environmental characteristics (i.e high presence of saltmarsh plants, the hydrology patterns and shallow areas) may hide the influence of MPB in the food web structure of the lower reaches within the Kowie Estuary.

The predominance of the saturated FAs 16:0 and 18:0 in the sediment profiles of the Kowie Estuary was consistent with reports from other estuaries and marine areas (Perry et al. 1979; Volkman et al. 1980), and these FAs are recognized as being ubiquitous (Volkman et al. 1998). The FAs 16:1ω7 and 20:5ω3 were dominant in the MPB and estuarine POM, so it was possible to evaluate the influence of these carbon sources within the Kowie Estuary. These FAs increased significantly towards the upper reaches, suggesting a greater influence of living diatoms in those sediments (since these FAs are degraded at high rates in aquatic environments; Canuel and Martens, 1996). Moreover, higher ratios of 16:1ω7/16:0 were found in the upper reaches (0.5) relative to the lower reaches (0.3), and this index represents an additional indicator of relative diatom dominance (Napolitano et al. 1997; Kharlamenko et al. 2001). Furthermore, the ratio of 22:6ω3/20:5ω3 reflects the predominance of dinoflagellates versus diatoms (Budge and Parrish 1998), and is used as dinoflagellate marker in some ecosystems. In this study, the ratio of 22:6ω3/20:5ω3 showed little variation among estuarine reaches and was always below 1.0, suggesting that dinoflagellates were not an important input to sediments from the Kowie Estuary. Furthermore, the upper reaches had significantly higher chl-*a* biomasses (3.7 ± 1.1 µg/l; Bergamino et al. 2014; T. Dalu unpublished data) together with the highest occurrence of diatom-associated FAs in the sediments. This positive correlation showed a direct coupling between microalgae in the water column settling to the sediments, and other researchers have shown that this coupling can change both spatially and temporally (Carrie et al. 1998; Zimmerman and Canuel 2001; Gogou and Stephanou 2004). Freshwater inputs may contribute new nutrients that result in increased phytoplankton production, particularly during summer and autumn, as has been shown in the nearby Kariega Estuary in South Africa (Froneman 2001a). However, the lack of temporal variability in FA profiles of sediments suggests that any seasonal pelagic blooms did not significantly influence the sediments during my study.

Spartina maritima had large proportions of the long-chain 24:0, and this FA was useful for tracking the fate of marsh grass detritus in the Kowie Estuary (Table 2.2). The FA 24:0 is diagenetically stable over long periods (i.e. up to 144 days), as it shows little or no changes in concentration after its deposition in sediments (Canuel and Martens 1996). This FA was generally more pronounced in the sediments of the lower reaches (Table 2.3), although other plant-associated FAs were not particularly abundant in sediments from any of the estuarine reaches. The PUFAs 18:2ω6 and 18:3ω3 were dominant in the vascular plants from the Kowie Estuary, and therefore they can be use to evaluate the role of these plants within the food web, as has been showed in other estuarine ecosystems (Meziane and Tsuchiya 2000; Richoux and Froneman 2008). Furthermore, the generally small contents of FAs associated with terrestrial material in the sediments could be further attributed to high rates of photochemical degradation or decomposition by heterotrophic behaviour (Canuel 2001). High levels of BAFAs (between 12 and 15 % TFA) were found in the sediments from all three estuarine reaches, suggesting the existence of active populations of bacteria responsible for the degradation and remineralization of organic material (Haddad et al. 1992). Levels of BAFAs in the Kowie Estuary (range of 12−15% TFA) were comparable to levels found in other estuarine and coastal ecosystems (e.g. Rajendran et al. 1993; Harvey and Johnston 1995; Meziane and Tsuchiya 2000; Budge et al. 2001), and this sedimentary bacteria might be supported by organic matter inputs from phytoplankton (Dai and Sun 2007; Palomo and Canuel 2010).

Using FA profiles and SI signatures, spatial and temporal variations in organic matter delivered to sediments were evident along a temperate estuary in South Africa.

Microalgal inputs to the sediments were generally greater in the upper reaches, whereas organic matter derived from vascular plants increased in the lower reaches. Moreover, variations in local primary productivity, freshwater discharge, and structural habitat features including vegetation type and depth could all contribute to the spatial and seasonal changes found in the sediment composition. These findings highlight the importance of adopting a spatial and seasonal approach to address questions about food webs in estuarine environments. Future research should be focussed towards increasing our understanding of which factors affect the relative importance of terrigenous material within estuarine environments given the potential influence of these carbon sources in the food web structure (Polis et al. 1997; Abrantes et al. 2013).

Chapter 3

Spatial and temporal changes in estuarine food web structure: differential contributions of marsh grass detritus

3.1 Introduction

Estuaries are characterized by gradients of physical and chemical parameters (e.g. salinity, nutrients, primary productivity), and hence the structure of biological communities can shift along these systems. Variability in an estuary also arises from the large potential diversity of organic matter inputs (McLusky and Elliott 2004), and seasonal variations in environmental factors such as salinity, temperature and the hydrology of the estuary may influence species distribution and abundance andthe structure of food webs (Akin et al. 2003; Hoeinghaus et al. 2011; Antonio et al. 2012). Since factors that influence estuarine ecosystem function can change through space and time, I was interested in exploring the extent of spatial and temporal variations in food web pathways within a temperate system.

The diversity and biomass of estuarine benthic fauna are highly structured by the food availability and quality in the overlying water column (Herman et al. 1999). Benthic organisms depend partially on sedimentation processes in the water column to transfer organic matter from the pelagic to the benthic zones. As such, benthic fauna can utilize a variety of food sources including marine phytoplankton, bacteria, benthic microalgae and littoral plants (Currin et al. 1995; Deegan and Garritt 1997; Kang et al. 2003). The identification of carbon sources in estuaries is typically difficult since these habitats are characterised by potentially extensive movements of suspended materials over long distances or time periods (Connolly et al. 2005). Depending on the hydrodynamics, locally suspended food sources can also be highly influential in consumer diets at relatively small spatial (m to km) or temporal (weeks to months) scales (Deegan and Garritt 1997; Guest et al. 2004; Richoux and Froneman 2007). Previous research has shown that the diets of benthic consumers can be affected by spatial differences in the primary organic matter sources of detritus (Keats et al. 2004), the relative abundance of seagrasses or macroalgae (McClelland and Valiela 1998; Olsen et al. 2011), and the quality and availability of animal prey (Fox et al. 2009). However, there is still much to learn about which potential food sources are the most important for a variety of estuarine fauna, and how these contributions to consumer diets change along an estuarine gradient and through time. Since benthic organisms have limited motility, they represent excellent models with which we can study the importance of energy pathways in different locations within an estuary.

In this study, the food web in three regions of an estuary was assessed over four seasons to examine how organic matter sources of benthic consumers change. I have utilised both SI and FA tracers to create new data towards answering questions about the spatial and temporal variations in estuarine food web structure. Based on previous studies that showed the influence of local habitat characteristics (i.e. vegetation type and hydrology) on primary producers and consumers (Deegan and Garritt 1997; Bouillon et al. 2004; Richoux and Froneman 2007; Hoeinghaus et al. 2011; Dubois et al. 2014), the hypothesis tested was that consumers from different areas within the estuary have distinct SI and FA signatures owing to differential food availability along the spatial gradient. I also tested the hypothesis that the consumer diets shift through time owing to seasonal shifts in local primary production and detrital inputs that correspond to distinct changes in freshwater discharge.

3.2 Methods

3.2.1 Study site and sample collection

The study area and sampling times has been described elsewhere (Chapter 2). Briefly, sampling was done for organic matter sources and benthic consumers over four consecutive seasons (spring, summer, autumn, and winter) between September 2012 and June 2013 (Table 3.1). Samples were collected from three different areas encompassing a spatial gradient of environmental conditions (Bok 1983): (1) upper reaches, near the head of the estuary and dominated by river influences; (2) middle reaches (approximately 11 km from the mouth); and (3) lower reaches, near the river mouth where extensive marine influence occurs (see Fig. 2.1).

All samples (fauna and basal sources) were collected in replicates of three from each estuarine region during each season. Portions of the dominant plants and surface sediments were collected during the day at low tide using a scalpel, and material was stored in ziplock bags inside an insulated cooler. Vegetation sampled from the upper reaches included *Phragmites australis* and *Schoenoplectus brachyceras,* from the middle reaches included salt marsh plants *Chenolea diffusa and Sarcocornia perennis,* and from the lower reaches included the marsh grass *Spartina maritima* and associated detritus. Senescent leaves were also collected by hand from each estuarine area. Benthic invertebrates (Table 3.1) were collected using a dip net (mouth area 0.3 m x 0.3 m, mesh size = 1.0 mm) pushed perpendicular to the shore over a distance of 1-2 m following the method of Froneman and Henninger (2009), and in replicates of three in each estuarine region. In the field, invertebrates were washed of debris and kept alive in estuarine water for several hours to allow for their guts to clear. For the smallest invertebrates, 3 to 5 similar-sized individuals of the same species were pooled to obtain adequate biomasses. Zooplankton samples were obtained during the night by towing a 200-μm mesh net for two minutes. All samples were stored at -80 ^{o}C until they were processed in the laboratory.

Tracer data for additional aquatic end-members from the upper, middle and lower reaches of the Kowie Estuary [i.e. particulate organic matter (POM), epiphytic algae and microphytobenthos (MPB)] were sourced from a separate study (T. Dalu, unpublished data; Bergamino et al. 2014).

Table 3.1. Abundance (ind.m^2; ±1 SD) and presence/absence data for fauna and flora collected from the Kowie Estuary. U: upper reaches; M: middle reaches; L: Lower reaches (•, species present, abundance data not available; x, species absent). Descriptions were taken from Whitfield (1998), or Day (1969). Fish size is given in total length. Isotope values of particulate organic matter, epiphyton and microphytobenthos from the Kowie Estuary were collected by T. Dalu (unpubl. data).

Species/Group	Acronyms	Description/Size (cm)	Spring			Summer			Autumn			Winter		
			U	M	L	U	M	L	U	M	L	U	M	L
ORGANIC MATTER SOURCES														
Phragmites australis	PHR	Macrophytes	•	•	x	•	•	x	•	•	x	•	•	x
Schoenoplectus brachyceras	SCH	Macrophytes	•	•	x	•	•	x	•	•	x	•	•	x
Chenolea diffusa	CHE	Saltmarsh plant	x	•	x	x	•	x	x	•	x	x	•	x
Sarcocornia perennis	SAR	Saltmarsh plant	x	•	x	x	•	x	x	•	x	x	•	x
Spartina maritima	SPA	Marsh grass	x	x	•	x	x	•	x	x	•	x	x	•
Particulate organic matter	POM	detritus/phytoplankton	•	•	•	•	•	•	•	•	•	•	•	•
Epiphyton	EPI	Algae attached on plants	•	•	•	•	•	•	•	•	•	•	•	•
Terrestrial leaves	LEA	Senescent leaves	•	•	•	•	•	•	•	•	•	•	•	•
Sediment organic matter	SOM	Microalgae/detritus/bacteria	•	•	•	•	•	•	•	•	•	•	•	•
Microphytobenthos	MPB	Micoralgae	•	•	•	•	•	•	•	•	•	•	•	•
CONSUMERS														
Pseudodiaptomus hessei	COP	Copepod (<0.02)	•	•	•	•	•	•	•	•	•	•	•	•
Acartia longipatella	COP	Copepod (<0.02)	•	•	•	•	•	•	•	•	•	•	•	•
Myscidacea	MYS	<2	x	x	x	x	x	•	x	x	•	x	x	x
Polychaeta	POL	4-6 cm	x	x	x	•	x	x	•	x	x	x	x	x
Exosphaeroma hylecoetes	EXO	Isopoda (1-2)	•	•	•	•	•	•	•	•	•	•	•	•
Grandidierella lignorum	GRA	Amphipoda (1-2)	•	•	•	•	•	•	•	•	•	•	•	•
Hymenosoma sp.	HYM	Brachyura (0.5-1.5)	x	4.0 (1.1)	2.9 (1.1)	3.7	3.7	x	2.2 (1.0)	x	x	3.0	2.2	x
Scylla serrata	SCY	Brachyura (13- 10)	x	x	x	x	x	x	x	1.4	1.6	x	x	x
Sesarma catenata	SES	Brachyura (1-3)	x	x	x	x	x	2.9	x	•	•	x	x	7.4
Cleistostoma edwardsii	CLE	Brachyura (1-2)	x	x	•	x	x	•	x	x	x	x	x	x
Crab zoea	CRA	Crustacean larvae	x	•	•	x	•	•	•	•	•	x	•	•
Palaemon peringueyi	PAL	Caridean shrimp (2-3)	2.2	x	16.6 (2.7)	x	x	2.5 (0.4)	x	x	x	3.7	4.4	4.8 (0.6)
Penaeus monodon	PEN	Tiger prawn (4-6)	x	x	x	x	x	x	•	1.5	x	•	•	x
Upogebia africana	UPO	Mudprawn (6.5-7.5)	x	x	x	x	x	x	x	x	•	x	x	6.6
Diogenes brevirostris	DIO	Hermit crab (4-6)	x	x	x	x	x	x	x	x	•	x	x	•
Glossogobius callidus	GLO	Gobiidae (2-4)	2.5 (0.5)	2.3 (0.2)	1.4 (1.0)	2.9 (1.1)	3.0 (1.1)		7.4 (1.1)	4.1 (2.6)	1.8 (0.5)	2.2	1.8 (0.5)	5.6 (0.5)
Liza dumerilii	DUM	Mugilidae (19-22)	•	•	•	•	•	•	•	•	•	•	•	•
Liza richardsonii	RIC	Mugilidae (23-27)	•	•	•	•	•	•	•	•	•	•	•	•
Mugil cephalus	CEP	Mugilidae 18	•	•	•	•	•	•	•	•	•	•	•	•
Solea bleekeri	SOL	Soleidae (5-8)	x	x	2.2 (1.0)	x	x	0.7	1.8 (0.5)	1.8 (0.5)	1.5	1.5	x	2.2
Syngnathus acus	SYN	Pipefish (12-14)	x	x	1.1 (0.5)	x	x	x	x	x	x	x	x	x
Gilchristella aestuaria	GIL	Clupeidae (1.5-2.5)	x	x	x	x	•	x	•	x	x	x	x	x

3.2.3 Laboratory procedures

In the laboratory, all individuals were identified to species using Day (1969) or Whitfield (1998). For the zooplankton samples, individuals of the two dominant copepod species (*Pseudodiaptomus hessei* and *Acartia longipatella)* were isolated under a dissecting microscope and transferred to filtered estuarine water to allow for gut clearance overnight. Approximately 50 individuals per copepod species were pooled to represent each sample. Crab zoea and mysids were similarly isolated from the mixed zooplankton sample. All samples were processed within a day or two of each collection. Animal and plant samples intended for SI and FA analyses were rinsed with distilled water to remove salts, freeze dried in foil envelopes, and ground to a fine homogeneous powder using a mortar and pestle. For invertebrates and fishes I did not perform a lipid extraction given the low C/N ratios that varied between 3-5 indicative of low lipid content (Table 3.2; Post et al. 2007).

Table 3.2. Range values of the C/N ratios of organisms considered in this study.

Species	C/N ratio
Pseudodiaptomus hessei	4.5-5.2
Acartia longipatella	4.5-5.3
Myscidacea	3.6-4.1
Polychaeta	3.8-4.0
Exosphaeroma hylecoetes	4.1-5.1
Grandidierella lignorum	3.7-4.8
Hymenosoma sp.	3.6-4.7
Scylla serrata	3.2-3.4
Sesarma catenata	3.3-4.0
Cleistostoma edwardsii	4.6-5.3
Crab zoea	4.2-5.0
Palaemon peringueyi	3.5-4.2
Penaeus monodon	3.5-4.6
Upogebia africana	3.4-4.3
Diogenes brevirostris	4.4-4.7
Glossogobius callidus	3.4-3.9
Liza dumerilii	3.4-3.5
Liza richardsonii	3.4-3.5
Mugil cephalus	3.4-3.5
Solea bleekeri	3.5-3.7
Syngnathus acus	3.2-3.4
Gilchristella aestuaria	3.3-4.0

3.2.4 Stable isotope analysis

All isotopic analyses were completed as described in Chapter 2. Animal tissues were not acidified to avoid unwanted alterations in isotopic measurements (Serrano et al. 2008).

To examine the trophic structure of the different estuarine regions and seasons, $\delta^{15}N$ signatures of the consumers were converted to trophic positions (TP) using the equation:

$$TP_i = \left[\left(\delta^{15}N_i - \delta^{15}N_{pc}\right) / 2.9\right] + 2$$

where TP_i is the average trophic position of species i, $\delta^{15}N_i$ represents the average $\delta^{15}N$ of species i, $\delta^{15}N_b$ represents the average $\delta^{15}N$ of the organism selected for baseline, 2.9 is the mean $\delta^{15}N$ trophic enrichment occurring per trophic level suggested by McCutchan et al. (2003) for animal muscle tissue, and λ is the TP (= 2) of the baseline organism. To select a baseline organism, two criteria were considered (Post 2002); the baseline organism should: (1) have limited mobility to adequately capture spatial and temporal variations at the food web base in a particular location; and (2) reflect spatial variations in primary producers and detrital energy sources. The amphipod *Grandidierella lignorum* was selected as the baseline since it is a surface detritivore with very limited mobility, and so it should best reflect the base of the food web in each area of the estuary.

3.2.5 Fatty acid analyses

Sub-samples of the same specimens used to derive isotopic data were processed for FA analysis whenever possible (i.e. this sub-sampling was possible with relatively large specimens). Twenty to forty mg dry mass (DM) of animal tissues were placed in 2 ml chloroform with 0.01% of the antioxidant butylated hydroxytoluene, topped with nitrogen gas and stored at -20 °C. Fatty acid methyl esters (FAMEs) were analyzed using a one-step method modified from Indarti et al. (2005) as described in Chapter 2. Each FA was measured as a proportion of the total fatty acids (% TFA). FAs are named according to a:bωx, where a is the number of carbon atoms, b gives the number of double bonds and x is the position of the first double bond from the methyl end of the molecule.

3.2.6 Data analysis

Spatial and temporal differences in the isotopic signatures of species occurring at all three reaches were identified using a two-way analysis of variance (ANOVA; main fixed factors were 'reach' with 3 levels, and 'season' with 4 levels) followed with a Tukey's test at the 5% significance. This analysis was performed for the pool of benthic species at each estuarine region and season including: *Hymenosoma* sp., *Exosphaeroma hylecoetes, Grandidierella lignorum*, *Palaemon peringueyi*, *Glossogobius callidus*, *Solea bleekeri*, *Sesarma catenata*, *Diogenes brevirostris* and *Upogebia africana*. This approach enabled me to make a community-based comparison, as every species was not present at each reach and during every season. Data were tested for normality and homogeneity of variances using Cochran's test prior to analysis.

The Bayesian stable isotope mixing model in the SIAR package (Stable Isotope Analysis in R; Parnell et al. 2010) was used to estimated the source contributions to the diets of benthic consumers. This model was run using each of the benthic consumers found in all three areas of the Kowie Estuary so that I could assess intra-specific spatial variability in diets. I used the fractionation factors of 2.9 ± 0.3‰ for δ^{15}N and 1.3 ± 0.3‰ for δ^{13}C since these values were derived from muscle tissues of consumers (McCutchan et al. 2003). Time-averaged values of SI were used of each potential food resource because the time integration of isotopes depends on organism growth and hence on body size, protein turnover and temperature (McCutchan et al. 2003; Power et al. 2003; Martínez del Rio et al. 2009). As such, smoothing isotopic data of food sources collected over time prevents direct comparisons of SI values between animal tissues and food sources at the same time. Potential food sources for each consumer were included based on literature reports of gut contents. The potential prey for the epibenthic crab *Hymenosoma* sp. included plant detritus, amphipods, isopods and copepods (Lucas 1980; Whitfield 1989b). For the gobiid *Glossogobius callidus*, copepods, amphipods and POM were selected as potential food sources (Wasserman 2012), and for the soleidae *Solea bleekeri* I considered benthic crustaceans as potential prey (Cyrus 1988). For the brachyuran crabs, POM, plant detritus and small crustaceans were included as potential prey (Vorsatz 2009), and in the case of the mugilids *Liza dumerilii*, *Liza richardsonii* and *Mugil cephalus* I considered MPB, POM and plant material as potential food sources (Whitfield 1988). The potential diet sources considered for the amphipod and isopod species were selected from detailed stomach contents data reported in Whitfield (1989) and included detrital aggregates represented by POM, SOM and marsh grass detritus in my study.

Isotopic niche width was estimated for the dominant benthic consumers within each reach of the Kowie Estuary. This concept of isotopic niche suggests that the isotopic variation in consumer tissues reflects variability in resource use both within and among individuals in a population (Bolnick et al. 2003; Bearhop et al. 2004; Newsome 2007). This parameter is correlated with trophic niche (Layman et al. 2012). The isotopic niche was examined using the Stable Isotope Bayesian Ellipses in R (SIBER) routine in SIAR, a package in the R programming environment (Jackson et al. 2011). SIBER is based on Bayesian inference techniques that provide a measure of the niche space occupied by a species using estimations of convex hulls, standard ellipse areas (SEA) and standard ellipse areas corrected for small sample sizes (SEAc). The estimations of SEA represent robust measures of the isotopic niche width for comparison among consumers because this model is unbiased with respect to sample size (Jackson et al. 2011). The niche space and area were compared for selected species (e.g. those present in most sampling occasions within each estuarine reach): the gobiid *Glossogobius callidus*, the epibenthic crab *Hymenosoma* sp., the caridean shrimp *Palaemon peringueyi,* the soleidae *Solea bleekeri* and the salt marsh crab *Sesarma catenata*.

To examine spatial and temporal differences in the non-transformed FA profiles of the consumers common among the three sites, a non-metric multidimensional scaling (nMDS: Kruskal and Wish 1978) was used based on Euclidean distance matrices. Differences in FA profiles among estuarine reaches and seasons within each reach were tested using analysis of similarity (ANOSIM), and similarity percentages (SIMPER) among profiles were investigated to identify the influential FA. A SIMPER-PCA comparison was performed as described in Chapter 2, and significant differences in the proportions of specific FAs in benthic consumers among estuarine reaches were tested using one-way ANOVA at the 95% significance level, followed with a Tukey's test at 5% significance. For this ANOVA, the pooled data from the nMDS analysis were used. Statistical analyses were completed using Statistica version7 (StatSoft, Tulsa, OK) and PAST 3.01 (Hammer et al. 2001).

3.3 Results

3.3.1 Community composition

Within the three areas sampled, 405 animals were identified (represented by 21 species: 2 species of copepod, 1 mysid, 1 shrimp, 2 prawns, 7 fish, 1 amphipod, 1 isopod, 1 polychaete; 5 crabs, and 1 larval crab component that included several species; Table 3.1). Brachyuran crabs and mugilid fishes were the most represented taxa comprising 3 and 4 species, respectively. Species richness and abundance of taxa varied among area and season, although some groups were abundant in all areas during all sample times (Table 3.1). Common taxa in the upper, middle and lower reaches of the estuary included the goby *Glossogobius callidus,* the soleidae *Solea bleekeri*, the epibenthic crab *Hymenosoma* sp.*,* the estuarine isopod *Exosphaeroma hylecoetes*, and the amphipod *Grandidierella lignorum*. The middle and lower reaches were dominated in abundance by salt marsh crabs *Sesarma catenata* (abundance peak during summer) and *Scylla serrata,* and the caridean shrimp *Palaemon peringueyi* (peak during spring). Mudprawns *Upogebia africana* and the hermit crab *Diogenes brevirostris* were numerically abundant during summer in the lower section of the estuary. Crab larvae contributed significantly to the zooplankton community in the lower estuary.

3.3.2 Isotopic composition of consumers

Invertebrate $\delta^{13}C$ ratios showed clear differentiation among reaches and seasons: consumers in the lower reaches had significantly higher $\delta^{13}C$ values than those in the middle and upper reaches during all four seasons, with the lowest $\delta^{13}C$ values observed in autumn at the three reaches, and the 'reach x season' interaction was significant (Table 3.3). Furthermore, the pool of benthic consumers in different reaches of the estuary differed in their change in isotopic signatures across seasons (Fig. 3.1). For example, in the upper reaches generally higher mean $\delta^{13}C$ values of the benthic fauna occurred in summer than in the other seasons, whereas in the middle and lower reaches $\delta^{13}C$ values were generally higher in winter. The $\delta^{15}N$ values of the benthic community in the lower reaches were lower during winter than in the rest of the seasons. However, in the upper and middle reaches, $\delta^{15}N$ values were lower during autumn in comparison with the other seasons (Fig. 3.1).

Table 3.3. Results for a two-way factorial ANOVA of isotopic data for benthic species pooled (*Hymenosoma* sp., *Exosphaeroma hylecoetes*; *Grandidierella lignorum*, *Palaemon peringueyi*, *Glossogobius callidus*, *Solea bleekeri*, and *Sesarma catenata*) in the upper (U), middle (M) and lower (L) reaches, with reach and season as main fixed factors. Significant Tukey's post hoc tests are shown. Spring (Sp), Summer (Su), Autumn (A), and Winter (W).

	df	MS	*F*	*p*	Tukey's post hoc
δ^{13}C					
Reach	2	327.34	100.00	<0.001	U > M > L
Season	3	16.17	4.94	<0.01	W > Au; Sp > Au
Interaction	6	15.12	4.62	<0.001	
Residual	84	3.27			
δ^{15}N					
Reach	2	92.86	55.23	<0.001	U > L; M > L
Season	3	18.72	11.14	<0.001	Sp > W, Au; Su> W, A
Interaction	6	6.77	4.03	<0.01	
Residual	141.24	1.68			

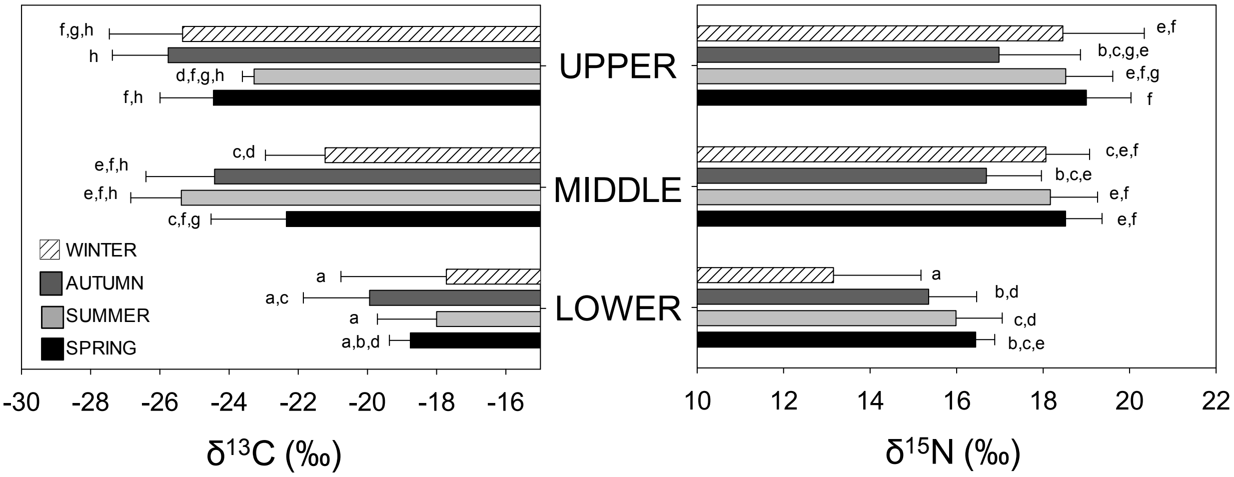

Figure 3.1. Mean (±SD) seasonal variability of δ^{13}C and δ^{15}N for all benthic consumers (species were pooled within each season and site; see methods for more details) in the upper, middle and lower reaches within the Kowie Estuary. Different letters indicate significant differences between each season and reach combination (two-way ANOVA, Tukey test, $p < 0.05$).

Annual means (±SD) in the upper reaches ranged from -27 ± 0.1‰ in the amphipod *G. lignorum* to -23 ± 0.1‰ in the prawn *Penaeus monodon* (Fig. 3.2). In the middle reaches, the mugilid *Liza dumerilii* had the highest carbon values (-18.8 ± 0.1‰), whereas copepods had the lowest carbon values (-27.2 ± 1.2‰), showing the wider range of $\delta^{13}C$ signatures (Fig. 3.2). In the lower reaches, copepods had the lowest $\delta^{13}C$ signatures (-24.6 ± 0.2‰), whereas the anomuran prawn *U. africana* and the hermit crab *D. brevirostris*, together with the flathead mullet *Mugil cephalus*, had the highest $\delta^{13}C$ values among consumers (range from -12 to -15‰; Fig. 3.2).

The $\delta^{15}N$ ratios for benthic consumers differed significantly among estuarine reaches and seasons, as well as in the corresponding interaction 'reach x season' (Table 3.3). The upper and middle reaches had benthic consumers with significantly higher $\delta^{15}N$ values compared with the lower reaches in all four seasons, with the highest values observed in summer and autumn. In the upper reaches of the estuary $\delta^{15}N$ values ranged from 20.2 ± 0.7‰ (*Glossogobius callidus*) to 19.6 ± 1.2‰ (isopod *Exosphaeroma hylecoetes*) (Fig 3.2). In the middle reaches, the goby *G. callidus* had the highest $\delta^{15}N$ values of 19.0 ± 0.4‰, while the crabs *Sesarma catenata* had the lowest $\delta^{15}N$ values of 16.0 ± 1.1‰ (Fig. 3.2). In the lower reaches values ranged from 16.3 ± 0.7‰ in *Solea bleekeri* to 12.5 ± 1.0‰ in the crab *S. catenata* and crab zoea (Fig. 3.2).

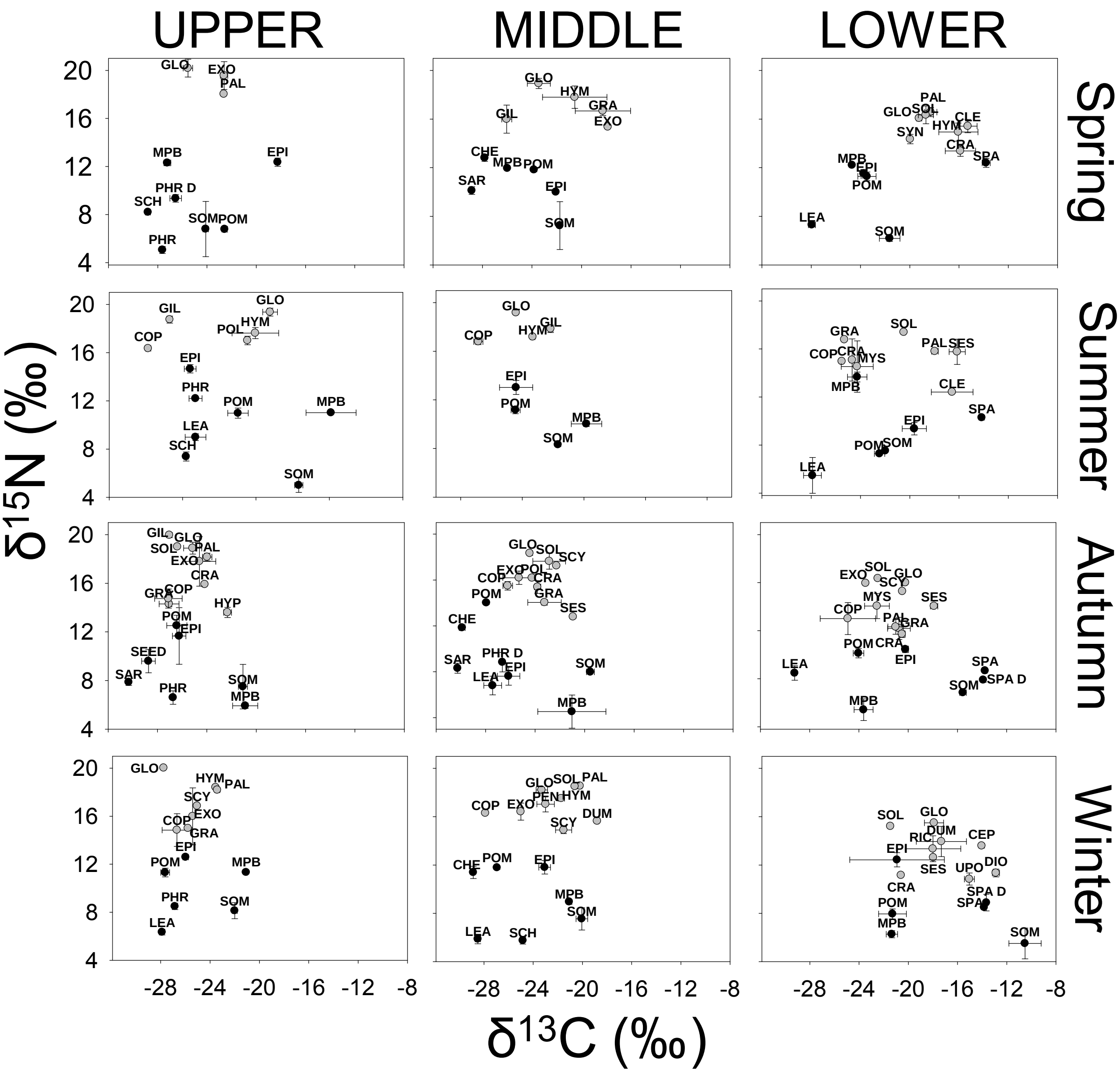

Figure 3.2. Mean (±SD) seasonal variability of $\delta^{13}C$ and $\delta^{15}N$ for the organic matter sources (black circles), and consumers (grey circles) in the upper, middle and lower reaches within the Kowie Estuary. Abbreviations of species/groups are shown in Table 3.1. Isotope values of particulate organic matter (POM), epibenthic material (EPI) and microphytobenthos (MPB) from the Kowie Estuary were collected by T. Dalu (unpubl. data).

3.3.3 Trophic position (TP)

The benthic fauna in the upper, middle and lower reaches of the Kowie Estuary were represented by three trophic levels (Fig. 3.3). In the upper reaches, the highest TP was occupied by the goby *G. callidus* in all seasons, together with the clupeid fish *Gilchristella aestuaria* and the sole *S. bleekeri* in autumn (Fig. 3.3). In the middle reaches the top consumer was *G. callidus* in all seasons, together with the sole in autumn and winter (Fig 3.3). In the lower reaches, *S. bleekeri* and *G. callidus* had the highest TP in all seasons, and the shrimp *P. peringueyi* in spring*.* The TP between 2 and 3 was comprised of brachyuran crabs *Hymenosoma* sp., the amphipod *G. lignorum*, the isopod *E. hylecoetes* and copepods in the three reaches. The lower reaches of the estuary had more primary consumers than the upper reaches, including the mudprawn *U. africana*, the hermit crab *D. brevirostris,* and the crabs *Cleistostoma edwardsii* and *S. catenata,* in autumn and winter (Fig. 3.3).

3.3.4 Food source contributions to benthic consumers using SIAR

In the upper, middle and lower sections of the estuary, the goby *G. callidus* utilized copepods as a principal food source (Table 3.4). In addition, the amphipod *G. lignorum* contributed significantly to goby diets in the middle and lower reaches. *G. lignorum* and copepods showed high contributions to the diet of the sole *S. bleekeri* in the upper and middle reaches, whereas mysids were the main prey in the lower reaches. The crab *Hymenosoma* sp*.* in the upper and middle reaches depended mainly on POM, whereas *Spartina maritima* together with benthic invertebrates made important dietary contributions to the crabs in the lower reaches. Detritus from the marsh grass *S. maritima* contributed significantly to diets of the mudprawn *U. africana,* the hermit crab *D. brevirostris* and the brachyuran crab *S. catenata* in the lower reaches (Table 3.4). This marsh grass food source combined with POM occurred in high proportions in the diets of the mugillids *Liza dumerilii, Liza richardsonii* and *Mugil cephalus* (Table 3.4).

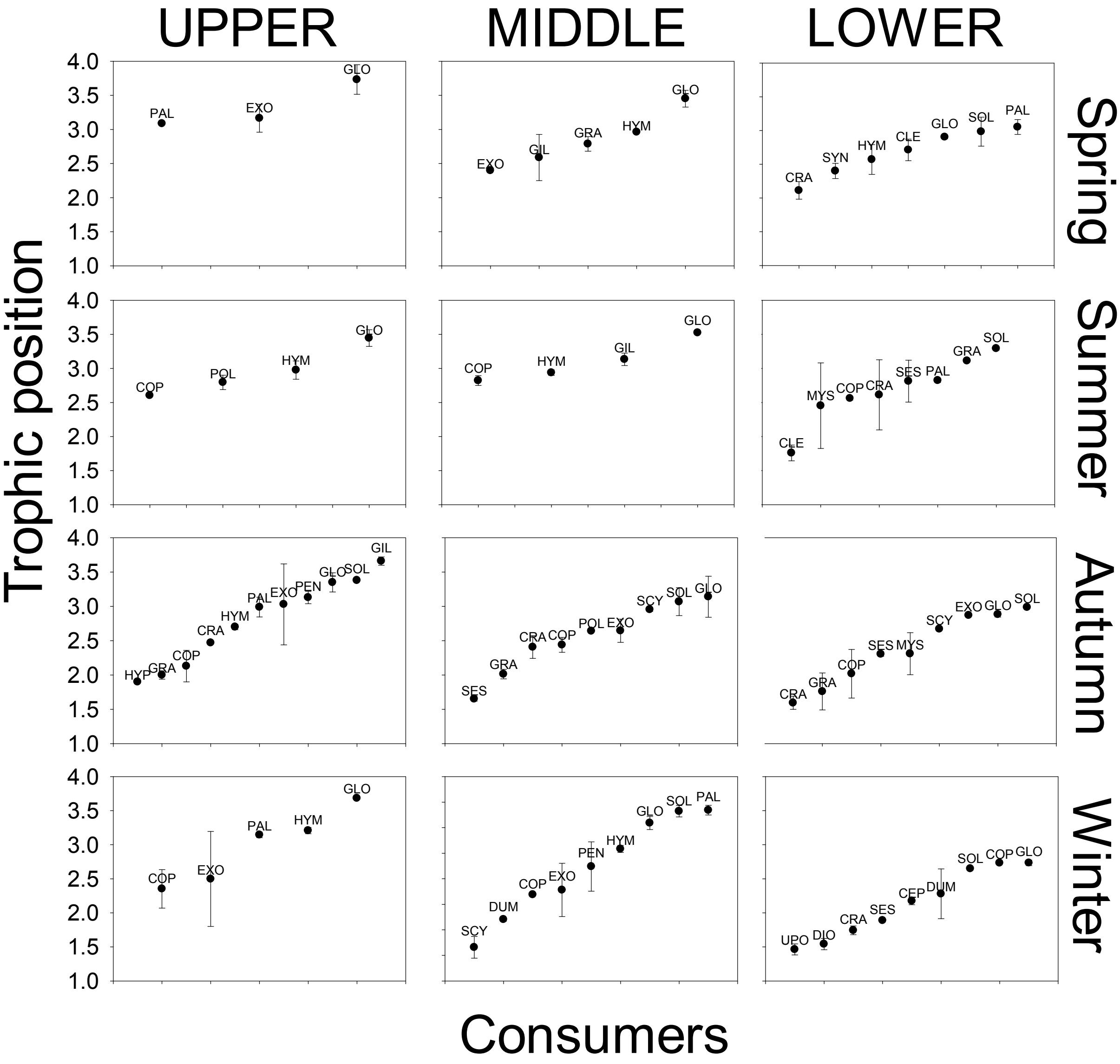

Figure 3.3. Trophic positions of consumers from the upper, middle and lower section of the Kowie Estuary, according to their $\delta^{15}N$ values. Abbreviations of species/groups are shown in Table 3.1.

Table 3.4. Mean percentage contributions (95% credibility interval) of organic matter sources to the diets of benthic consumers in 3 reaches of the Kowie Estuary. These results were determined by SIAR v. 4.0. Abbreviations of species/groups are shown in Table 3.1.

Consumers	**Food sources**							
	POM	COP	EXO	GRA	MPB	SPA	CRA	SOM
Upper								
GRA	**57 (14-83)**				21 (0-48)			25 (0-53)
EXO	**83 (68-98)**				8 (0-20)			8 (0-20)
HYM	**16 (7-25)**	12 (0-32)	**29 (13-44)**	11 (0-31)				
GLO	5 (0-16)	**75 (45-100)**		22 (0-48)				
SOL		30 (0-56)	10 (0-28)	**43 (13-83)**			12 (0-32)	
Middle								
GRA	**44 (8-82)**				30 (0-57)			28 (0-60)
EXO	**83 (68-95)**				8 (0-21)			9 (0-23)
HYM	**36 (18-36)**	1 (0-27)	16 (0-34)	**36 (8-64)**				
GLO	6 (1-11)	**52 (41-61)**		41 (27-54)				
SOL		8 (0-21)	11 (0-31)	**57 (27-77)**			27 (0-52)	
SCY	33 (9-53)		22 (4-42)	**52 (26-80)**				
SES	17 (0-35)		12 (0-30)	20 (0-40)	**50 (30-70)**			
Lower								
GRA	**64 (30-97)**				23 (0-54)	12 (0-30)		
HYM	18 (0-38)	23 (0-45)	21 (0-39)	22 (0-44)		**37 (11-63)**		
GLO	22 (12-32)	29 (0-51)		**48 (24-77)**				
SOL		14 (0-32)		**40 (14-64)**			**45 (22-70)**	
SES	41 (13-63)				7 (0-23)	**41 (33-49)**		
UPO	10 (0-27)				17 (0-42)	**66 (56-74)**		
DIO	2 (0-7)				4 (0-13)	**92 (80-99)**		
SCY	**30 (12-47)**		31 (12-48)	20 (0-40)		**18 (6-30)**		
DUM	**37 (7-60)**				13 (0-36)	**50 (31-67)**		
CEP	10 (0-22)				7 (0-16)	**82 (63-64)**		
RIC	**31 (0-61)**				**32 (0-64)**	**36 (0-68)**		

3.3.5 Isotopic niche of dominant species

The isotopic niche space estimated with Bayesian procedures clearly differed among benthic species co-occurring in the upper, middle and lower reaches of the estuary (Fig. 3.4a). In the upper reaches, the gobiid *Glossogobius callidus* had a different isotopic niche compared to those of shrimp *Palaemon peringueyi* and the epibenthic crab *Hymenosoma* sp. (Fig. 3.4a). However, the niche space of *Hymenosoma* sp. overlapped with that of *P. peringueyi* in the upper reaches*.* In addition, *G. callidus from* the upper reaches had a greater niche area than *P. perengueyi* and *Hymenosoma* sp. (Fig 3.4b.). In the middle reaches, the goby *G. callidus*, the epibenthic crab *Hymenosoma* sp. and the sole *Solea bleekeri* had distinct isotopic niche spaces with a small overlap only between *S. bleekeri* and *Hymenosoma* sp. (Fig. 3.4a). In the middle reaches, the dominant benthic consumers had similar isotopic niche areas with low values (Fig. 3.4b). In the lower reaches, the goby *G. callidus*, shrimps *P. peringueyi*, sole fish *S. bleekeri* and sesarmid crabs *Sesarma catenata* showed distinct isotopic niche spaces, with overlap only between *G. callidus* and sole S*. bleekeri*. Moreover, *S. catenata* together with *S. bleekeri* exhibited a larger overall niche area (Fig. 3.4b).

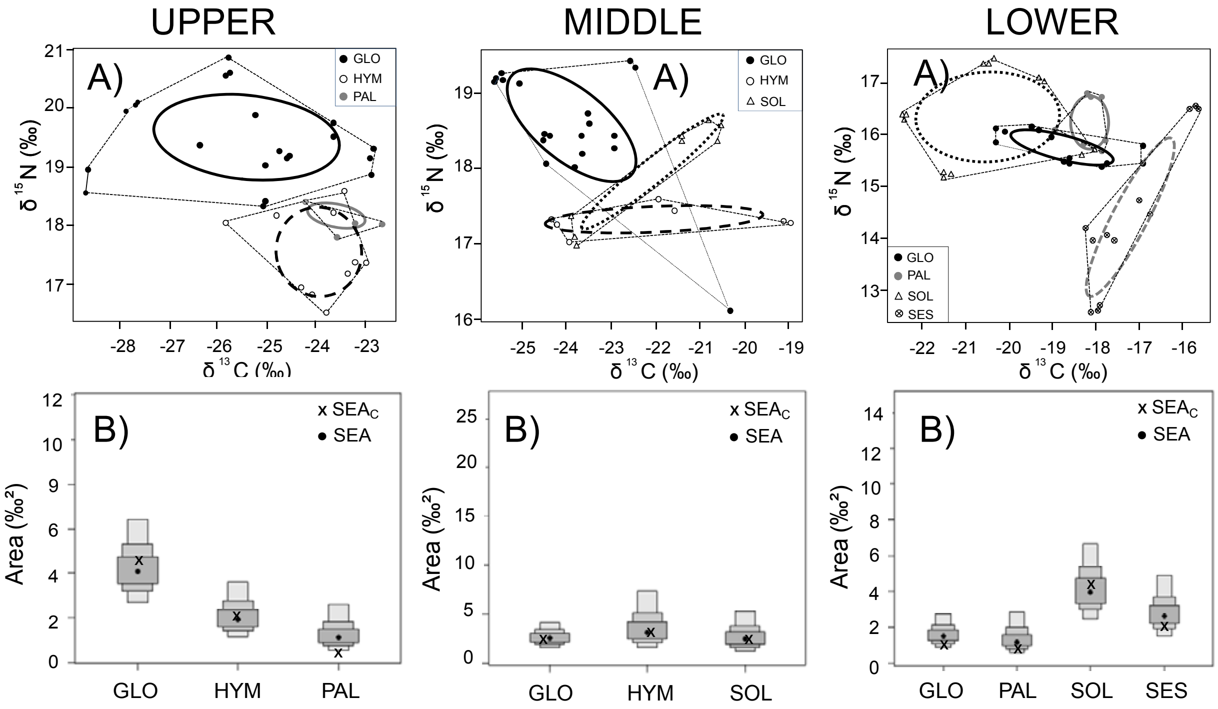

Figure 3.4. A) δ^{13}C and δ^{15}N values for the dominant benthic consumers in the upper, middle and lower reaches of the Kowie Estuary from September 2012 to July 2013 (all dates pooled). The outermost thick line represents the convex hull area, and the inner circles represent the bayesian standard ellipse area (SEA). B) Resultant uncertainty of standard ellipse areas after small sample size correction (SEAc). Abbreviations of species are shown in Table 3.1.

3.3.6 Fatty acid compositions of consumers

The FA profiles of the dominant benthic consumers showed differences among the upper, middle and lower sections of the estuary. Seventeen FAs were detected at proportions >1% of the total FAs in these consumers (Table 3.5). The consumers were characterized by high levels of 16:0, 18:0 (considered as ubiquitous; Volkman et al. 1998) and the polyunsaturated FAs 20:5ω3 and 22:6ω3. In addition, the brachyuran crabs *S. catenata and S. serrata,* the mudprawn *U. africana,* and the hermit crab *D. brevirostris* had high levels of 18:2ω6 and 18:3ω3 in the lower reaches. Furthermore, among consumers from the upper, middle and lower reaches monounsaturated fatty acids (MUFAs) were the least dominant overall compared with PUFAs and saturated fatty acids (SFAs), with the most abundant MUFAs 18:1ω9, 18:1ω7 and 16:1ω7 (Table 3.5).

The nMDS ordination (all dates pooled) showed that FA profiles in the dominant benthic consumers were separable on the basis of the estuarine region (stress values <0.1 indicated a useful 2D representation of the groups; Fig. 3.5). ANOSIM indicated that the FA profiles of these consumers were significantly different among the estuarine reaches (Global R 0.12; $p < 0.05$). Analysis of similarity percentage (SIMPER) together with PCA loadings showed that the polyunsaturated FAs 20:5ω3 (41%); 22:6ω3 (40%), 22:5ω3 (6%) and 18:2ω6 (5%) contributed significantly to distinguishing benthic invertebrates among the lower, middle and upper reaches.

Additional nMDS ordinations based on FA profiles of the co-ocurring species (species pooled) discriminated among seasons within each estuarine region (Fig. 3.6). In the upper reaches, the FA profiles for consumers in spring were fairly similar to those in winter, but they differed significantly from summer and autumn data (Global R 0.33; $p < 0.05$). Three FA contributed ~60% to the discrimination among seasons in the upper reaches: 22:6ω3 (26%), 20:5ω3 (25%) and 16:0 (10%). In the middle reaches, the ANOSIM results showed that the FA profiles differed among seasons (Global R 0.31; $p < 0.05$). Pairwise comparisons revealed significant differences between summer and the rest of the seasons, and winter showed significant differences with all other seasons ($p < 0.05$). The differences observed between seasons were driven mainly by three FA: 16:0 (36%), 20:5ω3 (22%) and 18:0 (13%; Fig. 3.6). In the lower reaches, the FA composition of consumers in winter differed from the other sampling times (Global R 0.562; $p < 0.05$; Fig. 3.6), and the same pattern was identified for summer (Global R 0.562; $p < 0.05$), but no differences were identified between autumn and spring profiles ($p > 0.05$). SIMPER (and PCA loadings) showed that the FA responsible for discriminating among seasons (up to ~70% of the cumulative profiles) in the lower reaches were 16:0 (39%), 22:6ω3 (15%) and 20:5ω3 (12%; Fig. 3.6).

Table 3.5. Annual mean percent of total fatty acids composition (± SD) of dominant benthic consumers from 3 sites in the Kowie Estuary: upper, middle and lower reaches. Data represent all the fatty acids at concentration >1% of the total fatty acids. SFAs: saturated fatty acids; MUFAs: monounsaturated fatty acids; PUFAs: polyunsaturated fatty acids. Abbreviations of species are shown in Table 3.1.

	UPPER			**MIDDLE**					**LOWER**									
	HYM	GLO	SOL	HYM	GLO	SOL	SES	SCY	HYM	GLO	SOL	SES	DIO	UPO	SCY	DUM	CEP	RIC
14:0	1.7 (0.4)	5.3 (5.5)	4.43	1.3 (1.0)	3.2 (0.6)	2.6 (0.7)	1.02	0.6 (5.6)	4.55	1.9 (1.5)	2.5 (1.1)	2.4 (1.22)	7.5 (12)	1.7 0.6)	1.2 (0.4)	8.8	5.7	5.6
16:0	16.5 (2.4)	21.2 (3.6)	19.72	14.9 (4.8)	19.7 (8.7)	19.3(1.7)	44.60	14.5 (10.7)	28.62	23.2 (9.7)	16.4 (1.0)	30 (13.7)	18.3 (0.30)	17.45 (1.6)	15 (1.6)	38.6	46.8	10.8
17:0	1.9 (0.5)	1.6 (0.1)	1.34	2.4 (1.3)	1.3 (0.4)	3.7 (0.7	6.01	1.2 (0.8)	5.42	1.2 (0.5)	1.0 (1.0)	1.4 (1.3)	2.2 (0.2)	2.4 (0.3)	1.6 (0.0)	3.6	3.8	0.8
15:0	1.0 (0.17)	1.5 (0.6)	1.63	1.1 (0.2)	2.5 (2.1)	1.6 (0.9)	2.72	0.7 (1.2)	1.31	1.0 (0.3)	2.5 (2.3)	1.3 (0.4)	1.7 (0.3)	0.9 (0.1)	0.9 (0.4)	0.0	0.0	1.2
18:0	11.9 (0.9)	10.4 (3.0)	8.35	8.9 (1.2)	12.1 (6.1)	13.1 (0.7)	30.91	10.4 (3.5)	16.13	15.2 (6.4)	11.4 (2.4)	13.3 (7.9)	9.8 (1.6)	13.4 (2.5)	9.6 (2.1)	11.8	15.4	3.5
SFAS	34.1 (2.0)	38 (6.5)	36.25	34.7 (2.3)	40.5 (15.1)	41.3 (3.3)	86.68	28.3 (22)	57.8	42.8 (16)	36.4 (7.7)	50.8 (24)	40.3 (14.8)	38 (5.4)	28.8 (0.6)	62.7	71.7	22.0
16:1ω7	3.9 (1.3)	7.3 (2.2)	7.44	6.6 (2.1)	9.0 (5.2)	6.0 (0.9)	0.00	5.3 (25.8)	0.00	5.0 (2.7)	3.6 (2.3)	5.2 (5.7)	6.2 (2.6)	5.9 (1.8)	4.5 (1.0)	0.0	0.0	25.8
17:1ω7	0.6 (1.1)	0.8 (0.9)	1.35	1.3 (0.5)	0.7 (0.5)	1.6 (0.7)	0.00	3.5 (0.3)	2.87	0.2 (0.3)	1.8 (2.6)	0.3 (0.6)	1.4 (1.0)	0.0 (0.00	1.7 (2.5)	0.0	0.0	0.4
18:1ω9	6.8 (1.2)	8.0 (2.3)	10.21	7.8 (2.2)	7.9 (2.5)	7.4 (2.8)	0.00	11.2 (10.4)	2.87	6.5 (2.6)	6.1 (2.5)	6.0 (3.0)	5.0 (1.1)	6.0 (0.2)	10.5 (3.5)	0.0	0.0	10.4
18:1ω7	7.2 (2.4)	6.2 (2.4)	4.05	5.8 (0.1)	6.4 (3.7)	6.6 (4.4)	0.00	3.4 (0.8)	0.00	6.2 (3.9)	2.4 (0.9)	3.8 (2.9)	6.4 (1.6)	4.7 (0.2)	3.8 (1.6)	0.0	0.0	0.9
MUFAS	18.8 (2.8)	26.2 (12.3)	23.89	22.2 (0.9)	25.0 (7.9)	24.5 (5.6)	0.00	24.5 (37.4)	7.08	19.5 (6.2)	16.4 (1.6)	15.5 (10.6)	19. (6.3)	19 (2.6)	22.3 (3.7)	0.0	0.0	37.4
18:2ω6	1.3 (0.2)	1.7 (1.4)	0.96	1.4 (0.2)	1.2 (0.7)	1.0 (0.1)	0.00	0.9 (1.5)	0.00	0.8 (0.6)	0.5 (0.4)	3.8 (2.9)	2.0 (1.6)	2.6 (0.8)	4.1 (4.1)	0.0	0.0	1.6
18:3ω3	05 (0.5)	0.6 (0.8)	0.78	0.9 (0.9)	0.9 (0.7)	0.3 (0.4)	0.00	0.3 (0.9)	0.00	0.3 (0.3)	0.7 (0.6)	2.8 (2.5)	4.3 (3.3)	1.7 (0.7)	1.1 (0.6)	0.0	0.0	0.9
18:4ω3	0.6 (0.4)	0.9 (0.7)	1.59	0.3 (0.5)	0.9 (1.0)	0.6 (0.3)	0.00	0.5 (1.5)	0.00	0.1 (0.2)	1.3 (1.0)	0.3 (0.5)	0.6 (0.3)	0.6 (0.4)	0.5 (0.1)	2.5	1.9	1.6
20:4ω6	5.9 (0.4)	3.2 (2.2)	3.33	2.1 (3.0)	3.3 (1.4)	3.0 (0.05)	0.24	6.8 (2.3)	1.62	4.7 (3.0)	3.8 (0.7)	3.1 (3.0)	4.7 (1.3)	4.9 (1.0)	6.8 (0.9)	3.7	2.4	2.3
20:5ω3	23.5 (4.0)	12.2 (4.6)	7.89	21.5 (1.8)	11.2 (7.1)	8.5 (3.0)	0.00	21.1 (11.9)	9.40	11.5 (2.5)	6.1 (3.1)	12.4 (8.5)	19 (4.4)	23 (0.9)	19.0 (6.8)	3.1	6.3	12.0
22:4ω6	0.2 (0.4)	0.3 (0.4)	0.93	0.3 (0.3)	0.4 (0.4)	0.6(0.2)	1.09	0.7 (0.2)	0.00	1.0 (0.9)	2.7 (2.6)	0.07 (0.13)	0.00	0.0 (0.)	0.9 (0.6)	2.9	0.0	0.2
22:5ω3	1.3 (0.3)	3.9 (2.0)	7.98	1.4 (0.4)	3.0 (2.3)	7.4 (0.8)	0.00	1.7 (3.2)	3.65	4.6 (3.2)	5.8 (2.9)	0.2 (0.4)	1.7 (0.6)	0.8 (0.0)	2.3 (1.0)	5.9	6.7	3.2
22:6ω3	11.3 (1.5)	10.9 (10.6)	13.70	10.2 (1.9)	11.0 (8.4)	10.6 (2.5)	8.02	0.0	14.48	12.5 (9.7)	19.8 (11.4)	7.3 (3.6)	10.7 (1.6)	0.0	10.7 (3.6)	13.7	9.6	15.4
PUFAS	45.6 (4.5)	34.5 (18.6)	38.27	38.5 (5.2)	33.0 (19)	32.4 (0.07)	9.35	32.1 (38)	29.48	36.9 (20.7)	46.0 (8.2)	30.4 (17.25)	42.3 (13)	26.4 (4.0)	46.7 (5.0)	33.9	26.9	38

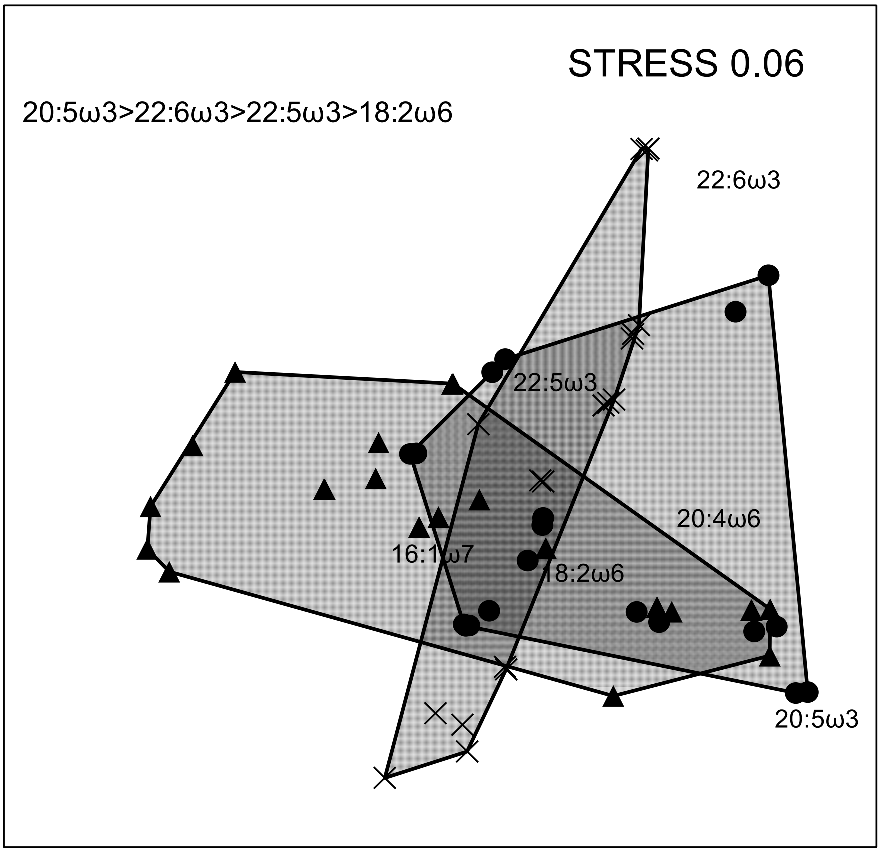

Figure 3.5. Two-dimensional non-metric multi-dimensional scaling (nMDS) ordination of the fatty acid profiles for the dominant benthic consumers from 3 sites in the Kowie Estuary (all dates pooled): Upper reaches (●); Middle reaches (▲); Lower reaches (x). Abbreviations of species are shown in Table 1. Axes are dimensionless. Major fatty acids contributing to the separation across reaches (derived from SIMPER and PCA) are shown in decreasing order and also superimposed in the plot.

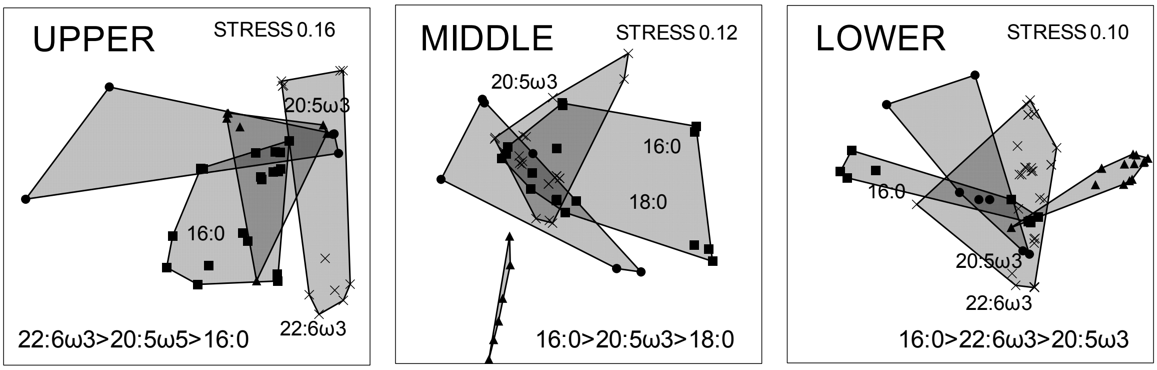

Figure 3.6. Two-dimensional non-metric multi-dimensional scaling (nMDS) ordination for fatty acid profiles for benthic consumers among four different seasons based on the normalised Euclidean distance similarity matrix (●, spring; ▲, summer; x, winter; ■ autumn). Axes are dimensionless. Major fatty acids contributing to the separation among seasons (derived from SIMPER and PCA) are shown in decreasing order and also superimposed in the plot.

3.4 Discussion

The benthic food webs had measurable distinctions among the upper, middle and lower portions within the small temperate Kowie Estuary. Moreover, the three different estuarine reaches differed in their patterns of isotopic change over seasons for the benthic fauna (all data pooled within each season and reach). A variety of potential primary carbon sources were considered including phytoplankton, epiphyton, microphytobenthos, sediment organic matter, live and senescent plants for diverse consumers including invertebrates and fishes. This approach allowed for robust comparisons of the feeding habits of benthic consumers among different regions of the estuary.

The $\delta^{13}C$ values in consumers showed significant differences among areas within the estuary, suggesting that different carbon sources supported the food web in different reaches. In this way, consumers seemed to assimilate local carbon sources given that these consumers had similar $\delta^{13}C$ values to the local resources where they resided (such as POM, MPB and plant material). The SIAR mixing model demonstrated that POM was highly utilised by consumers in the upper estuary, whereas larger contributions of detritus from marsh grasses were detected in the diets of lower estuary benthic consumers (Table 3.4). These results were supported by FA and SI data of sediment organic matter in the system, which indicated that the marsh grass *Spartina maritima* contributed substantially to the sediment material in the lower reaches of the Kowie Estuary (see Bergamino et al. 2014).

In estuarine ecosystems, differences in habitat characteristics such as local vegetation type (Bouillon et al. 2004; Richoux and Froneman 2007) and the estuary hydrogeomorpholgy (Hoeinghaus et al. 2011) can be important factors determining variation in the carbon source contributions to consumers. In the Kowie Estuary, in contrast with the upper and middle reaches, the lower reaches are colonised by extensive beds of marsh grasses that may contribute largely to the production and accumulation of detritus in the region. These dense patches of saltmarsh plants and other vegetation in the shallow waters that characterize the lower reaches may promote the deposition of detritus by reducing hydrodynamic forces and leading to calm physical conditions (Bouma et al. 2005). These general physical differences in upper and lower estuarine reaches support the suggestion that food webs based on detritus are dominant in the downstream shallow sections having extensive plant communities, contrasting with deeper upstream regions where phytoplankton supports the estuarine food web (see also Odum and Heald 1972). In addition to these hydrodynamic differences along an estuarine gradient, absolute salinity and salinity variability both affect the spatial distribution of organisms having differential salinity tolerances (Attrill 2002; Whitfield et al. 2012). The salinity gradient along the length of an estuary may similarly affect the prey availability within the estuary (Gning et al. 2010). With these concepts in mind, a combination of environmental conditions (i.e. shallow waters, local vegetation type and salinity regime) and biological features (i.e. feeding behaviour) can all contribute to the spatial changes in community and food web dynamics within the Kowie Estuary.

Greater assimilation of detritus from the marsh grass *Spartina maritima* by consumers in the lower reaches is well supported by the SI and FA signatures. Assuming an increase in $\delta^{13}C$ in consumers of ~1‰ compared to that of their food, in the middle and upper reaches of the estuary the epibenthic crab *Hymenosoma* sp. utilized large proportions of POM and small invertebrates. However, in the lower reaches, plant detritus was identified as an additional component in the diet of *Hymenosoma* sp. (Table 3.4). Gut content analysis of *Hymenosoma* sp., which identified detritus and benthic invertebrates as key food items (Whitfield 1989), supported the SI results from this work. The nitrogen values for *Hymenosoma* sp. confirmed that this species occupies a primary consumer trophic position (Fig. 3.3). A clear trophic association with detritus from the marsh grass *S. maritima* was also indicated for the salt marsh crab *S. catenata,* small mud crab *S. serrata,* the mudprawn *U. africana,* and the hermit crab *D. brevirostris* that inhabited the lower estuary in autumn and winter. In addition, the enriched $\delta^{13}C$ values in the mugilids *L. dumerilii*, *L. richardsonii* and *M. cephalus* suggested that their dietary carbon was derived partially from detritus of *S. maritima*. This contribution of detritus from *S. maritima* in mugilid fishes in the lower estuary was expected since stomach content analysis of these species suggested that they feed predominantly on POM, unicellular algae and benthic plant material (Whitfield 1988). Furthermore, given the high mobility of mugilid fishes together with their ability to utilize different sources including detritus from *Spartina*, these species may play a role as habitat couplers, as has been observed in other aquatic ecosystems (Schindler and Scheuerell 2002). In this context, the dominance of detritivores in the lower reaches suggests a donor control dynamic in which the rate of detrital input is a major factor influencing the trophic interactions within the macrobenthic community (Pimm 2002). It should be noted that SIAR model might have overestimated the importance of marsh grass because of missing potential food sources such as filamentous algae colonizing *Ruppia cirrhosa* and *Zostera capensis* that are periodically abundant in littoral areas of the Kowie Estuary (Whitfield et al. 1994). Such epiphytes have been noted in the guts of some estuarine benthic invertebrates such as amphipods, brachyuran crabs and isopods (Whitfield 1989).

SIBER results showed distinct isotopic niches among dominant benthic species within each estuarine reach (Fig 3.4a). This resource partitioning may result from differences in the trophic behaviour and may prevent strong competition among the populations that co-occur in each reach of the Kowie Estuary (Huxel et al. 2002). These results are congruent with previous studies on food web structure within the nearby Kariega Estuary in South Africa using isotopic data, which showed the existence of different trophic groups including detritivores/deposit feeders (e.g. *S. catenata, Hymenosoma* sp.), zoobenthivores (e.g. *G. callidus*), and omnivores (*P. peringueyi)* (Paterson and Whitfield 1997; Richoux and Froneman 2007, 2008). However, in the upper reaches of the Kowie Estuary, where steep banks and a narrow intertidal area occurs, food sources such as detritus may be concentrated and detrital feeders may overlap in the foraging habitat. This may explain the overlapping of the isotopic niches between the epibenthic crab *Hymenosoma* sp. and the shrimp *P. peringueyi* in the upper reaches of the Kowie Estuary.

In agreement with the SI results, the FA profiles of the dominant benthic consumers were distinct among estuarine regions. Differences in the ω3 and ω6 PUFAs (particularly 20:5ω3 and 22:6ω3) in the gobies and sole collected from different sections of the estuary confirmed that the quality of their food sources differed spatially (Fig 3.5). These FAs are dominant in benthic consumers from other temperate estuaries (Alfaro et al. 2006; Richoux and Froneman 2008). Primary producers are the sources of 20:5ω3 and 22:6ω3, which are essential FAs since invertebrates and fishes depend on their supply from feeding, and they are key components necessary for consumer growth, reproduction and survival (Arts et al. 2001). Furthermore, the FAs 20:5ω3 and 16:1ω7 are major components in the MPB and POM (phytoplankton and detritus) in the Kowie Estuary (Bergamino et al. 2014; T. Dalu unpublished data), and they can therefore be used as indicators of these components. These FAs have been identified as diatom-associated components in marine environments (Dunstan et al. 1993; Parrish et al. 2000), so the relative high levels of 20:5ω3 and 16:1ω7 in the profiles of the benthic consumers may indicate a dominance of diatoms (benthic and pelagic) at the base of the Kowie Estuary food web during all seasons. The PUFAs 18:2ω6 and 18:3ω3 are dominant in estuarine vascular plants like *S. maritima* (see Chapter 2; Richoux and Froneman 2008; Bergamino et al. 2014) and they can be incorporated into consumer tissues (Richoux and Froneman 2008). These FAs were present in significant amounts only in the brachyuran crabs (mostly in the *S. catenata* and the small mud crab *S. serrata*), mudprawns *U. africana* and hermit crabs from the lower reaches where marsh grasses occur. Like the SI results, these FA results provide evidence of the assimilation of plant detritus by local consumers. However, the FA profiles in *Hymenosoma* sp., the mugilid species *L. dumerilii* and *M. cephalus* differed from the other primary consumers in the lower estuary and did not reflect assimilation of marsh grasses, whereas Richoux and Froneman (2008) reported small amounts of the PUFAs 18:2ω6 and 18:3ω3 in *Hymenosoma* sp. from the nearby Kariega Estuary. The lack of congruence between our FA and SI signatures in these species from the Kowie Estuary may suggest that increased sample sizes are needed for the lipid analysis to accommodate large among-individual variability in consumer diets.

The spatial variations in the relative importance of organic matter sources supporting estuarine consumers within the Kowie Estuary is consistent with other studies in estuarine environments using SI as trophic tracers (e.g. Deegan and Garritt 1997; Richoux and Froneman 2007; Hoeinghaus et al. 2011; Claudino et al. 2013; but see Rodríguez-Graña et al. 2008). These works showed that consumers tend to assimilate carbon sources from the habitats where they reside. The contributions of saltmarsh grasses to the diets of estuarine animals vary with the region studied and the local environmental conditions (Peterson et al. 1985; Deegan and Garritt 1997; Kwak and Zedler 1997; Riera et al. 1999; Kang et al. 2003; Hoeinghaus and Davis 2007). Furthermore, the relative trophic contribution of saltmarsh material to consumers is associated with the size of saltmarsh patches (Guest and Connolly 2006). Thus, the saltmarshes should be a subject of further research to determine how extensive (at large scales) are the contributions of this carbon source to food webs within different estuaries. Given this important role of the marsh habitat in providing trophic resources (Deegan et al. 2012), the loss of marsh habitats could negatively affect estuarine biodiversity in many regions.

The data from this work represents evidence of different seasonal changes in the isotopic signatures of consumers within each estuarine region (Fig. 3.1). These seasonal isotopic changes may be induced by annual variability in environmental features, perhaps mainly determined by seasonal pulses of freshwater discharge (Riera and Richard 1997; McLeod et al. 2010; Antonio et al. 2012; Olin et al. 2013). The highest freshwater discharge in October and November 2012 through the Kowie River (see Fig 2.2.) invariably brought copious amounts of nutrients and resulted in increased production of phytoplankton (e.g. Froneman 2001a). Increased inputs of terrestrial detritus likely follow the high rainfall periods. River phytoplankton and terrestrial C_3 plants are characterized by low $\delta^{13}C$ signatures that range from -23 to -30‰ (Fry and Sherr 1984). Therefore, the low $\delta^{13}C$ signatures in estuarine benthic consumers relative to POM during autumn in the three estuarine regions likely resulted from increased assimilation of low $\delta^{13}C$ components during periods of high freshwater discharge. On the other hand, during periods of low freshwater discharge, the estuary may be dominated by marine phytoplankton, resulting in higher $\delta^{13}C$ signatures that were in turn reflected in consumers. Regarding $\delta^{15}N$, the higher values during summer and spring could be explained by high rates of denitrification processes promoted by the elevated temperatures during summer that lead to loss of isotopically light ^{14}N, and result in an enrichment of the remaining N pools (Baeta et al. 2009). As such, a combination of changes in abiotic variables such as river discharge, and biochemical processes such as denitrification, may all contribute to the seasonal variability in the isotopic values in consumers and food sources in the Kowie Estuary.

In summary, these results provide evidence of spatial and temporal variability in an estuarine food web. Relatively high $\delta^{13}C$ values and increased FAs from higher plants were reflected in the benthic fauna inhabiting the lower reaches of the estuary. These tracer data indicated a greater utilization of marsh grass detritus as a food source for detritivores in the lower estuary. This pattern contrasted with consumers in the upper and middle reaches, which relied primarily on suspended POM together with small invertebrates as their main food sources. I suggest that the input of detrital material from marsh grasses to the estuarine system represents an important carbon pathway, particularly in intertidal zones and shallow waters, and it affects the faunal community structure. My results also highlighted the importance of considering spatial and temporal approaches in food web studies of dynamic environments such as estuaries.

Chapter 4

Food preferences of the estuarine crab *Sesarma catenata* estimated through laboratory experiments

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4.1 Introduction

Salt marshes are among the most productive ecosystems in the world, and this high productivity is partially associated with the contribution of dissolved nutrients by salt marsh vegetation (e.g. *Spartina* spp.; McLusky and Elliott 2004). This ecosystem provides habitat for birds (Smith and Odum 1981), gastropods (Slim et al. 1997; Bouillon et al. 2002) and crabs (Krebs and Valiela 1978; Taylor and Allanson 1993). Sesarmid crabs play a key ecological role by processing deposited leaf litter, a process which enhances nutrient retention within the system (Robertson 1986; Camilleri 1989; Emmerson and McGwynne 1992; Ashton 2002; Chen and Ye 2008). Data derived in previous studies indicated that sesarmid crabs feed on leaves, including fruits and propagules, and prefer decomposed to senescent or fresh leaves (Giddins et al. 1986; Micheli 1993; Chen and Ye 2008). Their feeding activities influence the composition of particulate organic matter in the adjacent aquatic environment by contributing small particles of leaf litter (Camilleri 1992). Furthermore, crab faeces provide habitat and fuel for bacterial productivity, which in turn provides food sources for small detritivores (Lee 1997). Thus, feeding activities by crabs contribute substantially to higher trophic levels.

The importance of salt marsh vegetation as a food source varies among estuaries (McLusky and Elliott 2004). Reports of daily leaf removal by crabs in different mangrove habitats vary between 10% (Slim et al. 1997) and about 80% (Robertson 1986; Robertson and Daniel 1989; Chen and Ye 2008). Riera et al. (1999) studied a salt marsh area along the Atlantic coast of France, and they suggested that although the marsh grass *Spartina* sp. was available to the fauna, this marsh grass did not contribute significantly as a food source. Further assessment of the trophic role that sesarmid crabs play within different salt marsh areas could provide valuable insights into the variable interactions between plants and animals that potentially affect ecosystem functioning and food web structure.

The crabs *Sesarma catenata* (Crustacea: Decapoda: Sesarmidae) are conspicuous members of salt marsh ecosystems in certain estuaries of South Africa (Alexander and Ewer 1969; Taylor and Allanson 1993). Using fatty acid and stable isotope analyses, researchers studying various estuarine areas in South Africa have confirmed that *S. catenata* utilizes salt marsh plants as a component of its diet (Chapter 3; Paterson and Whitfield 1997; Richoux and Froneman 2007, 2008; Bergamino and Richoux 2014). However, there is a lack of information concerning consumption rates and food preferences of *S. catenata*.

Gut content analysis of the crabs *S. catenata* have revealed the presence of varied amounts of mud particles and plant material (Alexander and Ewer 1969), suggesting that these crabs can use their chelipeds to collect detritus deposited on the surface. As such, *S. catenata* is considered a detritivore that can potentially feed on local marsh vegetation. Sesarmid crabs can show preferetial utilization of certain plant species (Camilleri 1989; Emmerson and McGwynne 1992; Micheli 1993; Thongtham and Kristensen 2005; Chen and Ye 2008), so I tested the hypothesis that *S. catenata* feeds preferentially on plant material with higher nutritional value. I performed laboratory experiments to determine the food preferences of *S. catenata* among the different plants and leaf stages available in a temperate salt marsh area of South Africa. Carbon/nitrogen ratios (C/N ratios) and water content for each plant material and leaf stages were taken into consideration.

4.2 Methods

4.2.1Study area and sample collection

Sampling of crabs and leaves was carried out in the salt marsh area located in the lower reaches of the Kowie Estuary, South Africa, on 11 February 2014 (Fig. 4.1). A detailed description of this study area is provided in Chapter 2 (Fig. 2.1).

Figure 4.1. Salt marsh area at the lower reaches of the Kowie Estuary (South Africa) at low tide showing salt marsh vegetation.

Crabs were collected by hand and were all of approximately the same size to remove size as an influential factor for the comparisons among experimental treatments. Twenty individuals of mixed sex with a carapace width of 17−25 mm and a wet weight range of 5.1-8.4 g were chosen for the experiments (Fig 4.2). Fresh (green colour) and decomposed leaves (brown colour) of the marsh grass *Spartina maritima*, terrestrial leaves from riparian trees and the marsh plants *Chenolea diffusa* and *Sarcocornia perennis* were collected by hand for the feeding experiments. These plant species and leaf stages were selected because they were the most common in the lower reaches of the Kowie Estuary during this study (Bergamino pers. obs.). Collected leaves were cleaned with distilled water to remove any feeding deterrents and stored in plastic bags within an insulated cooler box at 18°C until the experiment began.

Figure 4.2 *Sesarma catenata* collected from the salt marsh area in the lower reaches of the Kowie Estuary in South Africa.

4.2.2 Feeding preference experiments

In the laboratory, each aquarium for a feeding assay (16 cm diameter, 17 cm depth) contained a 3 cm layer of quartz silica sand The sand had been rinsed with tap water, dried for 48 h at 65 °C, and then ashed (500 °C for 5 h) to remove any organic matter. One side of each container was elevated so that 2 cm of sand surface was above 2 cm of filtered estuarine water to provide a dry refuge for the crabs (water had been filtered through Whatman GF/F filters that were precombusted at 450°C for 4 h; salinity was 30 at 25°C; aquarium design as per Mchenga and Tsuchiya (2010) (Fig. 4.3). The water from each aquarium was replaced every day. A total of 20 aquaria were set up.

The crabs were acclimated to the aquarium conditions and allowed to empty their gut contents for three days, as faecal production ceased after this period of time. Each aquarium included one crab for one feeding trial, and they were kept between 23 to 25°C at a natural photoperiod of approximately 12 h light/12 h dark. After the starvation period, five types of feeding choice experiments were conducted, and each experiment type was replicated 5 times. The food choices offered in each type of feeding choice experiments were: 1) fresh leaves of *S. maritima*, *C. diffusa* and *S. perennis*; 2) decomposed leaves of *S. maritima*, *C. diffusa* and terrestrial leaves from riparian trees; 3) mature and decomposed leaves of *S. maritima*; (4) mature and decomposed terrestrial leaves; (5) mature and decomposed leaves of *S. perennis*. All leaves were weighed before each experiment (aprox. 1 gram of each), and after 24 h the remaining leaf materials were dried for 48 h at 65°C and weighed. I estimated the consumption rate for each crab as the difference between the initial dry weight and final dry weight for each food source offered expressed as mg dry weight of leaves consumed per crab per day. Twenty leaves of each plant type and leaf stage were weighed (mg) and then dried for 48 h at 65°C to determine the initial dry mass and water content of leaves offered as food to crabs. Controls consisted of plant material with crabs absent, and otherwise all conditions were identical to those trials involving crabs. Controls were used to adjust consumption rate estimations to account for differences in the initial and final plant weight due to leaching. Finally, to estimate leaf consumption rates by *S. catenata*, single choice experiments were conducted in the laboratory. Crabs were starved for three days to empty their guts before they were offered one leaf type during a single choice experimen. All feeding experiments were conducted from 11 to 28 of February 2014.

Figure 4.3 Aquarium containing a sediment layer and the crab *Sesarma catenata* in preparation for a feeding experiment.

To estimate faecal production by *S. catenata*, the faeces from the multiple choice experiments involving decomposed and mature leaves were removed every day from each aquarium using forceps. Faeces were placed on Whatman GF/F filters (pre-ashed at 500°C for 5 h and weighed), dried at 65°C for 48 h and weighed. Faecal production was expressed as mg dry weight of faeces produced per crab per day. Faecal pellets were measured (length n=30) and photographed for description.

Carbon and nitrogen data for each plant and leaf stage were reported in Chapter 2. Briefly, the different plant materials were lyophilized in a VirTis Benchtop 2K, ground to a fine homogeneous powder and analyzed for content of carbon, nitrogen content.

4.2.3 Data analysis

Significant differences in C/N ratios and water content among the different plants and leaf stages were evaluated through one-way ANOVA, followed with a Tukey's test at the 5% significance. This test was also used to evaluate significant differences in consumption rates among treatments of single choice food. To evaluate whether crabs were preferentially choosing a particular food item, the multivariate Hotelling's T^2 test (Hotelling 1931) was used for specific pair-wise comparisons of mean consumption rates given the lack of independence of the different plant materials in the multiple choice feeding experiments (Roa 1992).

4.3 Results

4.3.1 Leaf characteristics

Mature and decomposed leaves from the marsh grass *S. maritima* had significantly lower C/N ratios than the rest of the plant materials examined (Table 4.1). The highest C/N ratio was in decomposed terrestrial leaves (63.4). Mature leaves from marsh plants *S. perennis* and *C. diffusa* had significantly higher water contents than all the other plant species and leaf states (Table 4.1). Water content in decomposed leaves from *S. maritima* was significantly higher than that in mature leaves. In contrast, terrestrial leaves and marsh plants *S. perennis* had significantly higher water content in mature leaves than in decomposed leaves (Table 4.1).

Table 4.1. Mean (±SD) of C/N ratios and water content (%) for each plant type and leaf stage considered in the feeding experiments. d: decomposed; m: mature. Different letters indicate significant differences at the 5% level within each column (one-way ANOVA, Tukey test, $p < 0.05$). (n): numbers of leaf samples analysed Na: data not available.

			C/N ratio		Water content (%)
	Acronyms	n	Mean	n	Mean
Spartina maritima (m)	SPAm	7	16.6 (2.2) a	20	55.1 (5.0) e
Spartina maritima (d)	SPAd	7	25.2 (1.6) b	20	67.9 (5.3) a
Terrestrial leaves (d)	LEAd	7	63.4 (3.9) c	20	37.2 (9.5) b
Terrestrial leaves (m)	LEAm	7	32.86 (6.6) d	20	55.6 (3.0) e
Sarcocornia perennis (m)	SARm	7	43.3 (2.5) e	20	84.7 (1.8)d
Sarcocornia perennis (d)	SARd		Na	20	27.9 (7.2) c
Chenolea diffusa (m)	CHEm	7	36.9 (4.0) d,e	20	82.2 (2.6) d

4.3.2 Laboratory feeding preference experiments

The multiple choice feeding experiments showed that *S. catenata* had food preferences (Fig. 4.4). When mature leaves of different plants were offered simultaneously, *S. catenata* had a preference for the marsh grass S. *maritima* with a mean consumption rate, after adjusting for leaching loss, of 65 ± 41 mgDW ind^{-1} d^{-1} (mean ± SD; Fig. 4.4a). The mature leaves of the marsh plants *C. diffusa* and *S. perennis* were under-utilized by *S. catenata* as food sources in these multiple choice experiments (Fig. 4.4a). When I offered decomposed leaves simultaneously, terrestrial leaves were consumed in significantly greater amounts than decomposed leaves from marsh plants, with a mean consumption rate for terrestrial leaves of 83.5 ± 50.2 mgDW ind^{-1} d^{-1} (Fig. 4.4b). When I offered mature and decomposed terrestrial leaves or *S. maritima* leaves simultaneously, crabs preferred decomposed leaves (Fig. 4.5). Crabs did not feed on mature and decomposed leaves of the marsh plant *S. perennis* (Fig. 4.5).

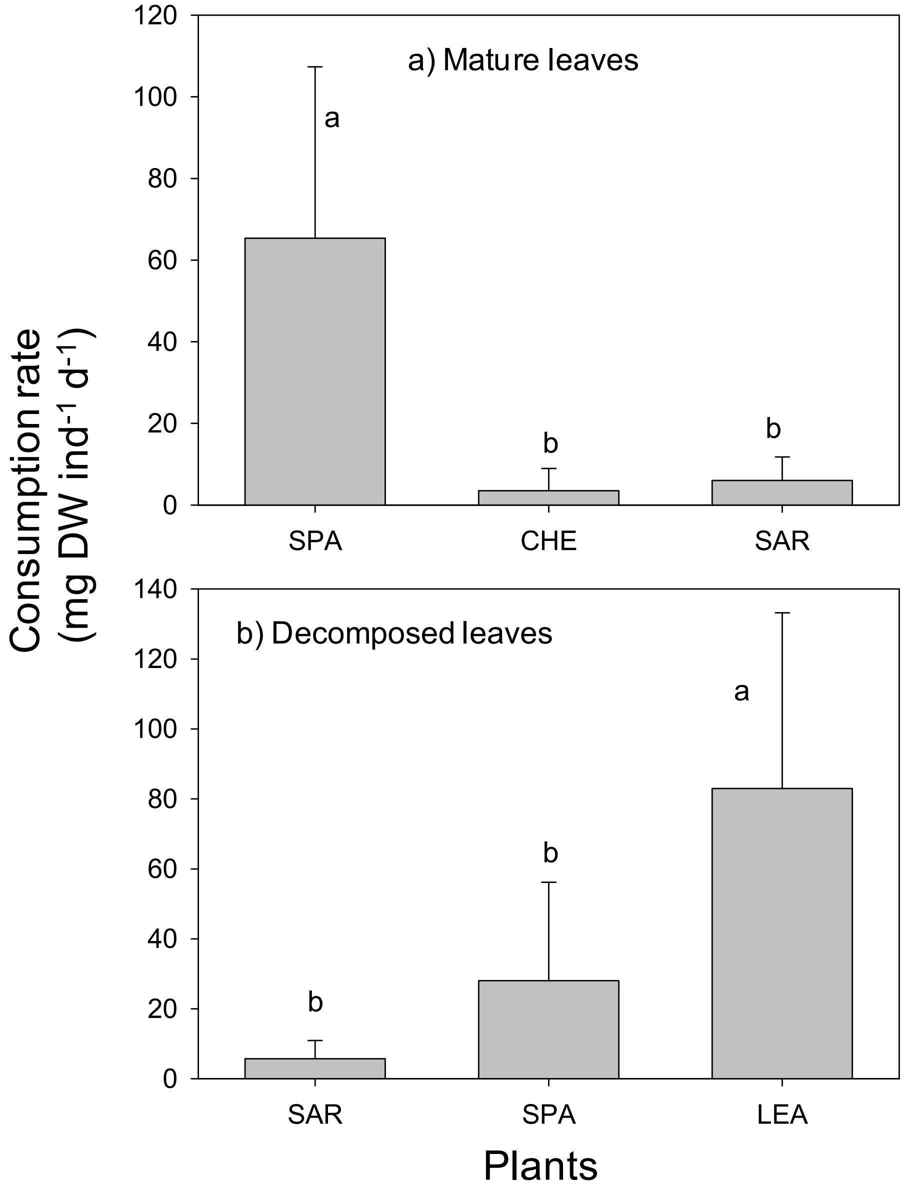

Figure 4.4. Consumption rates (mean ± SD) of *Sesarma catenata* when simultaneously offered three food sources of different plants and leaf stages in the laboratory: a) mature leaves and b) decomposed leaves. Different letters indicate significant differences among plants (Hotelling's T^2 for pair wise comparisons: $p < 0.05$). Abbreviations of plant species/group are shown in Table 4.1.

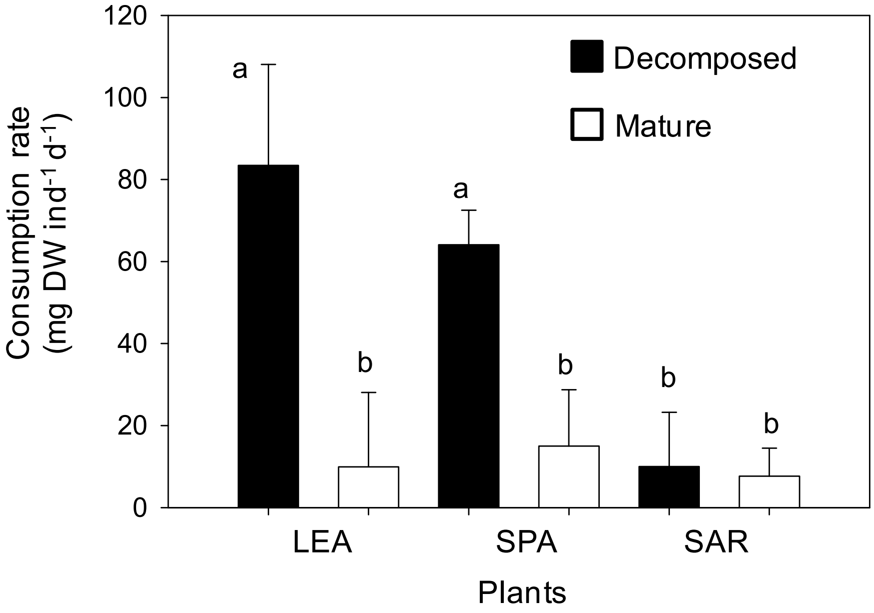

Figure 4.5. Consumption rates (mean ± SD) of *Sesarma catenata* when simultaneously offered mature and decomposed leaves of particular plant materials in the laboratory. Different letters indicate significant differences among plants (one-way ANOVA, Tukey test, $F_{5,12} = 13.15$, $p < 0.05$). Abbreviations of plant material are shown in Table 4.1.

In the single choice feeding trials, the consumption rate by crabs of terrestrial leaves was the highest (mean ± SD: 141 ± 67 mgDW ind^{-1} d^{-1}), followed by decomposed and mature leaves of *S. maritima* (67 ± 36 and 57 ± 23 mgDW ind^{-1} d^{-1} respectively; Fig 4.6). The post hoc Tukey test showed that sesarmid crabs consumed decomposed terrestrial leaves in significantly greater amounts than marsh plants (Fig. 4.6). However, consumption rate of terrestrial decomposed leaves by crabs did not differ with those of marsh grass leaves *S. maritima* (decomposed and matures; Fig. 4.6).There were no significant differences in the crab consumption rates between decomposed and mature leaves of the marsh grass *S. maritima* when they were offered independently (Fig. 4.6).

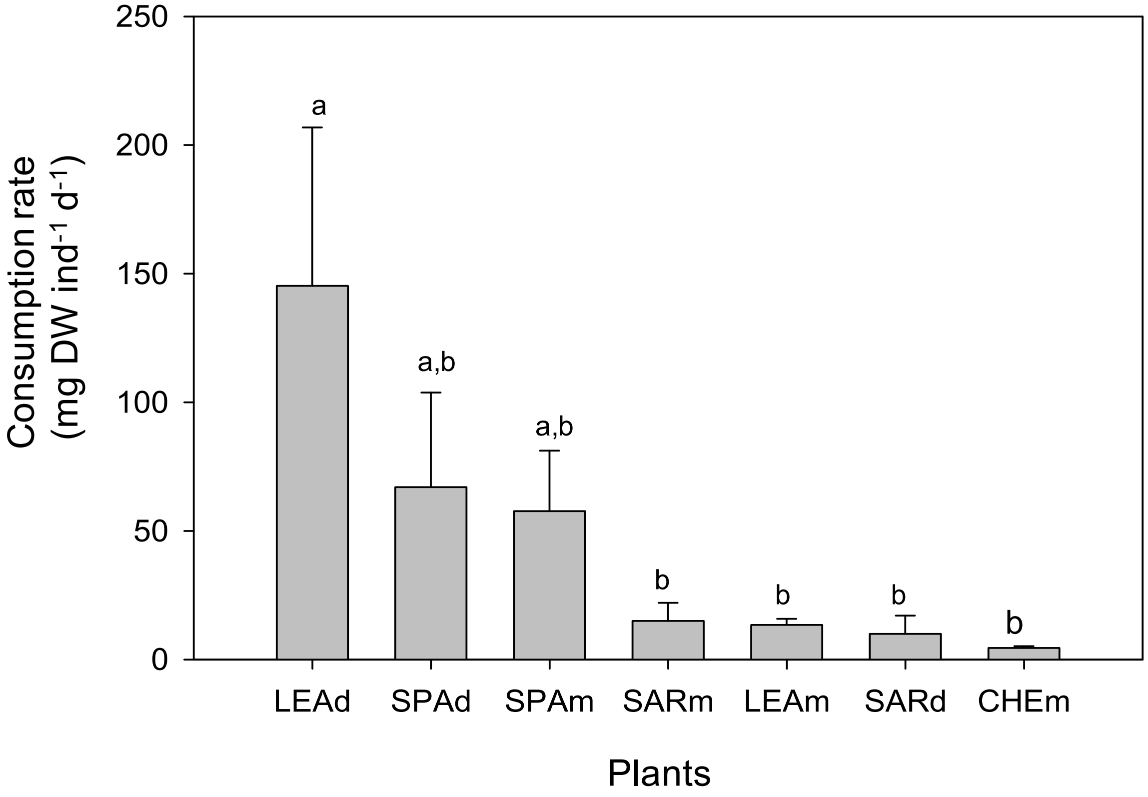

Figure 4.6. Consumption rates (mean ± SD) of *Sesarma catenata* when offered a single choice of plant material and leaf stage in the laboratory. Different letters indicate significant differences among plants (one-way ANOVA, Tukey test, $F_{6,11} = 6.68$, $p < 0.05$). Abbreviations of plant species/group are shown in Table 4.1.

The faecal production rates of crabs were greatest during multiple choice experiments that included decomposed leaves (mean ± SD: 14.3 ± 5.1 mg DW ind^{-1} d^{-1}), and lower when crabs were fed mature leaves of different plant types simultaneously (6.0 ± 3.8 mgDW ind^{-1} d^{-1}; Fig. 4.7). Size of faeces ranged between 1 and 5 mm (Fig. 4.8).

Figure 4.7. Faecal production rates (mean ± SD) in *Sesarma catenata* when simultaneously offerred different plant material and leaf stages in the laboratory. Different letters indicate significant differences among leaf stages (one-way ANOVA, Tukey test, $F_{1,10} = 10.2$, $p < 0.05$). Fresh and mature leaves considered in each treatment are described in Table 4.1

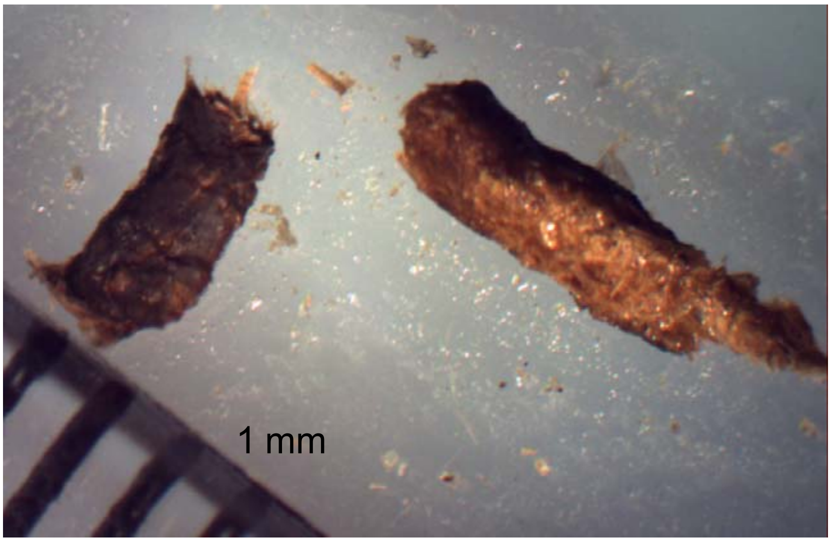

Figure 4.8. Faecal pellets produced by the estuarine crab *Sesarma catenata* in the laboratory.

4.4 Discussion

The data derived from this laboratory study indicates that *Sesarma catenata* was selective and preferred decomposed terrestrial leaves, followed by decomposed and mature leaves of the marsh grass *Spartina maritima.* Leaf consumption by *S. catenata* (range of 14 – 90 mg DW ind^{-1} d^{-1}) fell within the range of estimations previously reported for similar-sized sesarmid crabs (Emmerson and McGwynne 1992; Machiwa and Hallberg 2002). In particular, Emmerson and McGwynne (1992) working in a temperate southern Africa mangrove provided ingestion rates of litter (73 to 461 mg DW per day) by crabs *Sesarma meinerti* ranging between 4.1 and 66.6 g (fresh mass). Machiwa and Hallberg (2002) reported consumption rates between 10.7 and 1189 mgDW crab^{-1} day^{-1} in the crab *Neosarmatium meinerti* (carapace sizes ranged from 10 to 60 mm) feeding on *Avicennia marina*. Given that these experiments were conducted under laboratory conditions that considered no predators but unlimited food supply, these results represented overestimations of leaf consumption, especially since less preferred leaves could normally be consumed when preferred leaves were absent. Some field-based experiments revealed no pattern of food selectivity by the sesarmid crabs *Sesarma messa, Sesarma smithii* (Micheli 1993), and *Neoepisesarma versicolor* (Thongtham et al. 2008), suggesting that other mechanisms such as competition for space may affect food selection. However, these field experiments were carried out during dry seasons with low forest productivity and therefore may have underestimated food selectivity by crabs (Micheli 1993). In this context, results from both field and laboratory experiments are extremely useful for comparisons.

The food preference by sesarmid crabs is associated with different leaf characteristics such as C/N ratio, water content, crude fibers and tannin content (Neilson et al. 1986; Camilleri 1989; Chen and Ye 2008). For example, for mature leaves, preferences by crabs are negatively related to C/N ratio, but for decomposed leaves preference is positively associated with water content (Chen and Ye 2008). The C/N ratio represents a good indicator of the nutritional value of the plant material, with low ratio indicating higher nitrogen concentrations and high nutritional quality (Poovachiranon et al. 1986; Seneviratne 2000). In this study, the low C/N values in the marsh grass *S. maritima* indicated that it was a potentially high quality nitrogen source for consumersinhabiting the salt marsh habitat. As such, the lower C/N ratio of marsh grasses compared with the rest of the plant materials considered in our feeding trials helped to explain the feeding preference by S*. catenata* for *S. maritima*. This result is congruent with field-derived stable isotope and fatty acid tracer data that indicated that *S. catenata* was assimilating salt marsh material (Richoux and Froneman 2007, 2008; Chapter 3; Bergamino and Richoux 2014). However, Alexander and Ewer (1969) collected *S. catenata* during summer from the lower reaches of the Kowie Estuary and found no evidence of consumption of *S. maritima* by crabs when they were offered fresh leaves of *S. maritima* in the laboratory, and there was no evidence of leaves in the burrows of *S. catenata*. These contrasting results highlight potential intra-population variation in food sources utilized by *S. catenata*, or possibly just the effects of different experimentation protocols between studies. Additional studies in the laboratory and the field would help to reveal the underlying mechanisms behind contrasting results derived among studies.

In addition to marsh grasses, *S. catenata* showed high consumption rates of terrestrial decomposed leaves, despite their relative high C/N ratios and low water content. However, the process of leaf fragmentation by crabs during feeding may speed microbial decay, increasing the content of nitrogen and therefore increasing the nutritional quality of this detrital material (Cundell et al. 1979; Rice and Tenore 1981). As plant fragments become smaller and decomposition occurs, the protein content increases (McLusky and Elliott 2004). Nordhaus et al. (2011) analysed 21 hydrolysable amino acids and 2 hexosamines (glucosamine, and galactosamine) in leaves and suggested that the biochemical composition and particular nitrogen compound composition may determine the bioavailability of leaves in an ecosystem by increasing the N fraction and bacterial activity on plants. The work of Nordhaus et al. (2011) showed that for certain plants, decomposed leaves had higher amounts of nitrogenous compounds (indicated by the highest reactivity index) than mature leaves. Furthermore, the bacterial productivity reported in estuarine sediments (see Chapter 2; Alongi 1988; Bergamino et al. 2014) may be explained by the utilization of leaves by crabs (Micheli

1993). In this context, higher bacterial productivity in decomposed terrestrial leaves could enhance the nutritional value of this material and explain the preference by the crab *S. catenata* for this material in the feeding trials. It should be noted that the high C/N ratios that I found for decomposed terrestrial leaves may be because they were partly decomposed and the crabs were feeding on more decayed material in the laboratory experiments compared with the terrestrial leaf detritus that I measured C/N ratios for in the field. Further investigation regarding the molecular composition of salt marsh plants at different stages of decomposition is needed to better understand the mechanisms behind feeding preference.

Based on crab consumption rates of terrestrial leaves determined in these experiments, and a mean density of 7.4 ind m^{-2} of *S. catenata* occurring in the lower reaches of the Kowie Estuary (Chapter 3; Bergamino and Richoux 2014), this crab population could potentially remove an average of 701 mgDW m^{-2} d^{-1} of leaf litter. This removal represents approximately 34% of the leaf litter that is processed by crabs daily, considering a mean litter fall of 1790 mg m^{-2} d^{-1} estimated by Emmerson and McGwynne (1992) in a warm-temperate mangrove in South Africa. This calculation of leaf litter processed by *S. catenata* is significantly lower than those estimated for other sesarmid crabs in mangrove forests, which can reach 80% removal of the daily leaf litter in those ecosystems (Robertson 1986; Robertson and Daniel 1989; Ashton 2002; Chen and Ye 2008), but similar to the 43% removal rate reported by Emmerson and McGwynne (1992). Either way, most estimates of removal, including the present calculations based on crabs in the Kowie Estuary, suggest that although large proportions of the leaf material can be consumed by the crabs, large amounts of leaf litter may also be exported from the salt marsh area to adjacent aquatic areas. Differences in leaf litter removal by crabs among sites are expected, particularly since physical and biological factors such as hydrodynamic conditions, tidal elevation, faunal composition and density of crabs can be extremely variable among locations (Twilley 1985; Taylor and Allanson 1993; Kristensen et al. 2008). Furthermore, sesarmid crabs can assimilate sediment organic matter that includes benthic algae and bacteria as additional food sources that can help to fulfil their N requirements (Bouillon et al. 2002;

Skov and Hartnoll 2002; Thongtham and Kristensen 2005; Mchenga and Tsuchiya 2010). Therefore, the importance of plant material in the diets of sesarmid crabs may be partially explained by the availability of other food sources and the relative connectivity to adjacent habitats that could contribute additional sources of organic matter to a particular location (Cannicci et al. 2008).

The estimation of faecal production by *S. catenata* in this study (range of 14.3 – 6.0 mg DW ind^{-1} d^{-1}) was comparable to that estimated for *Sesarma meinerti* (10 mg d^{-1} for a crab of 4.96 g) in South Africa (Emmerson and McGwynne 1992). The higher faecal production by crabs feeding on decomposed material (14.3 ± 5.1 mg DW ind^{-1} d^{-1}) than those feeding on fresh plants (6.0 ± 3.8 mgDW ind^{-1} d^{-1}) confirmed that *S. catenata* preferentially selected and assimilated decomposed plants. Faecal production by these crabs could provide an important carbon pathway within the ecosystem for coprophagous invertebrates (Giddins 1986; Lee 1997). Furthermore, by processing plant materials, *S. catenata* contributes significantly to the production of detritus that can further subsidize adjacent environments. As such, the crab *S. catenata* potentially has an extremely important role in linking primary producers and other estuarine or marine consumers (Camilleri 1989, 1992; Emmerson and McGwynne 1992).

In summary, this study identified decomposed terrestrial leaves as a preferred dietary component for the crabs *S. catenata,* followed by decomposed and mature leaves of *S. maritima*. The low C/N ratios for *S. maritima* and the high bacterial production associated with decomposed terrestrial leaves may explain the trophic behaviour of *S. catenata*. Furthermore, the crabs seem to play an important role in the estuarine food web by processing leaf material and contributing to the production of plant detritus and faeces available for other consumers. Finally, this work showed that laboratory feeding experiments can help to validate feeding patterns identified using stable isotope and fatty acid data, and hance can improve food web models. Further investigations are needed to thoroughly describe the variability in plant–crab interactions as a result of changes in physical and other habitat features (e.g. tidal regimes).

Chapter 5

Stable isotope evidence of food web connectivity by a top predatory fish (*Argyrosomus japonicus*: Sciaenidae: Teleostei) in the Kowie Estuary, South Africa

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5.1 Introduction

In estuarine ecosystems, the determination of trophic interactions among species is difficult to evaluate due to the presence of diverse sources of organic matter (Deegan and Garritt 1997). Previous studies have indicated that estuarine food webs are primarily supported by aquatic and emergent macrophytes, phytoplankton and benthic microalgae (Kwak and Zedler 1997; Deegan and Garritt 1997; Richoux and Froneman 2007; Choy et al. 2008). Researchers have also identified terrestrial organic matter (Chanton and Lewis 2002; Darnaude et al. 2004; Wissel and Fry 2005) and detritus derived from emergent marsh plants as major food sources in marsh systems (e.g. Peterson and Howard 1987; Currin et al. 1995). Because of this complexity, studies that evaluate species interactions within estuarine ecosystems are increasingly important for gaining new insights into estuarine trophic ecology.

In estuaries, fishes consume food from a wide variety of sources including plants, zooplankton, benthic invertebrates and other fishes (Deegan and Garritt 1997; Litvin and Weinstein 2003; Alfaro et al. 2006). The fish community should be considered as a trophic entity (Deegan and Garritt 1997; Paterson and Whitfield 1997; Abrantes and Sheaves 2009), and this holistic approach is important to provide a reliable representation of the energy flow within a system.

The inclusion of top predators in estuarine trophic studies is critical given the key role they play in influencing ecosystem structure and dynamics (Duffy 2003). In particular, the role of large mobile predators in connecting different food chains has been explored in different aquatic ecosystems (Schindler and Scheuerell 2002; Duffy et al. 2007; Abrantes and Sheaves 2009). On way in which top predators can connect food webs in space is through their movements and feeding preferences (McCann et al. 2005). The assessment of trophic linkages through top predators is important for improving our understanding of ecosystem structure and functioning in dynamic environments (Pasquaud et al. 2010).

Despite the wide use of SI to assess trophic relationships in estuarine environments, fewer researchers have applied this technique towards answering questions about habitat connectivity through trophic interactions. In this study, SI ratios are used to examine the role of an abundant mobile predator, the dusky kob *Argyrosomus japonicus*, in providing trophic connectivity among habitats (i.e. littoral, channel and benthic) in the Kowie Estuary of the Eastern Cape Province. The dusky kob is found from coastal waters around South Africa, Moçambique, Australia, Pakistan, India, China, Korea and Japan (Griffiths 1997). Adult dusky kob occurs mainly in the near shore zone at depths up to approximately 100 m, with early juveniles (20–30 mm total length) recruit into estuaries (Griffiths 1996). Juveniles of this predatory fish do not move long distances from their nursery grounds (Cowley et al. 2008) and are most abundant in the middle and upper regions of estuaries (Griffiths 1996). Hence this species is a useful model organism with which to trace organic matter linkages in estuaries without the added complications of long distance migrations.

Major dietary components of early juveniles (<50 mm total length) include calanoid copepods, mysids, insects, amphipods and swimming prawns (Griffiths 1996), and juveniles >170 mm are primarily piscivorous (Whitfield 1998). Prey availability seems to be a major determinant governing the spatial distribution of early juveniles within estuaries (Griffiths 1997).

Authors of a study in the nearby Kariega Estuary suggested that aquatic macrophytes and epiphytes supported the littoral biotic community dominated by benthic invertebrates and fishes, whereas the channel community of zooplankton and associated fish species sourced their carbon from phytoplankton, terrestrial plant debris and macrophyte detritus (Paterson and Whitfield 1997). The results derived in that study implied a lateral separation of food chains within a single estuary, whereas my hypothesis is that because of the mobility of *A. japonicus,* this species provides strong lateral and vertical links between different food chains. To test this hypothesis, the isotopic composition of the main food sources and consumers were evaluated in the middle and upper reaches of the Kowie Estuary, where *A. japonicus* is the most abundant piscivorous fish species (Whitfield et al. 1994). Trophic interactions of the dusky kob were explored using both carbon and nitrogen stable isotopes, as well as the Bayesian stable isotope mixing model approach (SIAR, Parnell et al. 2010).

5.2 Methods

5.2.1 Study system

The study area comprises the middle and upper reaches of the Kowie Estuary. These estuarine reaches have been described elsewhere (Chapter 2).

5.2.2 Sample collection for stable isotope analysis

Consumers (zooplankton, benthic invertebrates and fishes) were collected during February and March 2013 in the upper and middle reaches of the Kowie Estuary (Table 5.1). The samples collected represent ‘snapshots’ of the food web connections in the estuary during late summer.

Fishes were collected using a purse seine net (50 m long x 2 m deep with a 3 cm stretch mesh in the wings and 1 cm stretch mesh in the bag) in the upper and middle reaches. Littoral invertebrates were collected using a dip net (mouth area 0.3 m x 0.3 m, mesh size = 1.0 mm) pushed perpendicular to the shore over a distance of 1-2 m following Froneman and Henninger (2009). Zooplankton were collected at night by towing a 200 μm mesh plankton net from a slowly moving boat. The live zooplankton samples (Table 5.1) were sorted in the laboratory under a dissecting microscope and then placed in filtered estuarine water to allow gut clearance overnight. Submerged and emergent macrophytes were collected by hand and washed with estuarine water. In the field, invertebrates were rinsed free of sediment and debris using filtered estuarine water and kept alive for several hours to allow gut clearance. All samples were stored at -80 °C. In the case of consumers, five replicates consisting of individuals of the same species and similar size were considered for SI analysis.

Table 5.1. Food sources and consumers (zooplankton, fishes and invertebrates) collected from the Kowie Estuary and feeding mode information as per Whitfield (1989; 1998), or Day (1969). Fish size is given in total length.

Component	Size (cm)	Description/feeding mode
Organic matter sources		
Microphytobenthos		Dense algae from the intertidal sediments
Particulate organic matter		Phytoplankton and suspended detritus
Epiphyton		Algae attached on *Stukenia pectinatus*
		Algae attached on *Schoenoplectus corymbosus*
Stukenia pectinatus		Submerge macrophyte
Consumers		
Zooplankton	<0.02	Copepods: *Acartia longipatella, Pseudidiaptomus hessei*
Exosphaeroma hylecoetes	0.5-1	Isopoda (omnivore)
Grandidierella lignorum	0.5-1	Amphipoda (detritivore/omnivore)
Hymenosoma sp.	1-2	Brachyuran crab (detritivore)
Palaemon peringueyi	2-3	Caridean shrimp (detritivore/omnivore)
Penaeus monodon	10-12	Tiger prawn (herbivore)
Hyporhamphus capensis	9.5-11	Cape halfbeak (herbivore)
Liza dumerilii	12-18	Groovy mullet (detritivore)
Gilchristella aestuaria	1.5-2.5	Estuarine roundherring (zooplanktivore)
Rhabdosargus holubi	5-11	Cape stumpnose (omnivore)
Glossogobius callidus	2-3.5	River goby (zoobenthivore)
Argyrosomus japonicus	15-19	Dusky kob (piscivore)

Tracer data for additional aquatic end-members from the upper reaches of the Kowie Estuary [i.e. particulate organic matter (POM), epiphytic algae and microphytobenthos (MPB)] were sourced from a separate study (T. Dalu, unpublished data).

5.2.3 Laboratory processing and stable isotopes analysis

All isotopic analyses were completed as described in Chapter 3 for the fauna and in Chapter 2 for plant material. Dorsal muscle tissues of fishes were selected for the isotope analysis. In the laboratory, all animals and plants were identified to the lowest possible taxonomic level using Day (1969) and Whitfield (1998). Lipid removal was not performed on fishes given the low C/N ratios which indicated low lipid contents (C/N < 4; Post et al. 2007). Trophic position estimations were completed as described in Chapter 3.

5.2.4 Data analysis

To estimate source contributions to *A. japonicus*, the SIAR package (Stable Isotope Analysis in R; Parnell et al. 2010) was used. Fractionation values from the literature were applied to the data, namely 2.9 ± 0.4‰ for $\delta^{15}N$ and 1.1 ± 0.4‰ for $\delta^{13}C$ (McCutchan et al. 2003). The potential prey for *A. japonicus* were selected from detailed stomach content analyses in Eastern Cape estuaries (e.g. Marais 1984; Griffiths 1997), and included both fishes (*Rhabdosargus holubi, Gilchristella aestuaria*, *Liza dumerilii, Glossogobius callidus*) and invertebrates (*Penaeus monodon; Palaemon peringueyi; Hymenosoma* sp.).

5.3 Results

5.3.1 Isotopic composition of consumers

The clupeid *Gilchristella aestuaria*, together with the amphipod *Grandidierella lignorum*, had the lowest $\delta^{13}C$ values among the consumers (-27.03 ± 0.9‰ and -27.06 ± 0.8‰, respectively; Fig. 5.1). The mugilid *Liza dumerilii* was the most ^{13}C-enriched consumer (-17.3 ± 2.0‰), followed by the hemirhamphid *Hyporhamphus capensis* (-22.3 ± 0.3‰). The isopod *Exosphaeroma hylecoetes*, together with the gobiid

Glossogobius callidus, sciaenid *Argyrosomus japonicus,* shrimp *Palaemon peringueyi* and prawn *Penaeus monodon,* had similar intermediate $\delta^{13}C$ values ranging from -24.8 to -23.6‰ (Fig. 5.1).

Regarding $\delta^{15}N$ signatures, the predatory fish *A. japonicus* had the highest value (20.8 ± 1.1‰,), whereas among the primary consumers *G. lignorum,* copepods, *H. capensis* and *L. dumerilii* had the lowest $\delta^{15}N$ values, ranging from 13.9 to 14.3‰. The brachyuran crab *Hymenosoma* sp., together with the prawn *P. monodon*, isopod *E. hylecoetes*, shrimp *P. peringueyi* and ominivorous fish *Rhabdosargus holubi,* presented intermediate $\delta^{15}N$ signatures (Fig. 5.1).

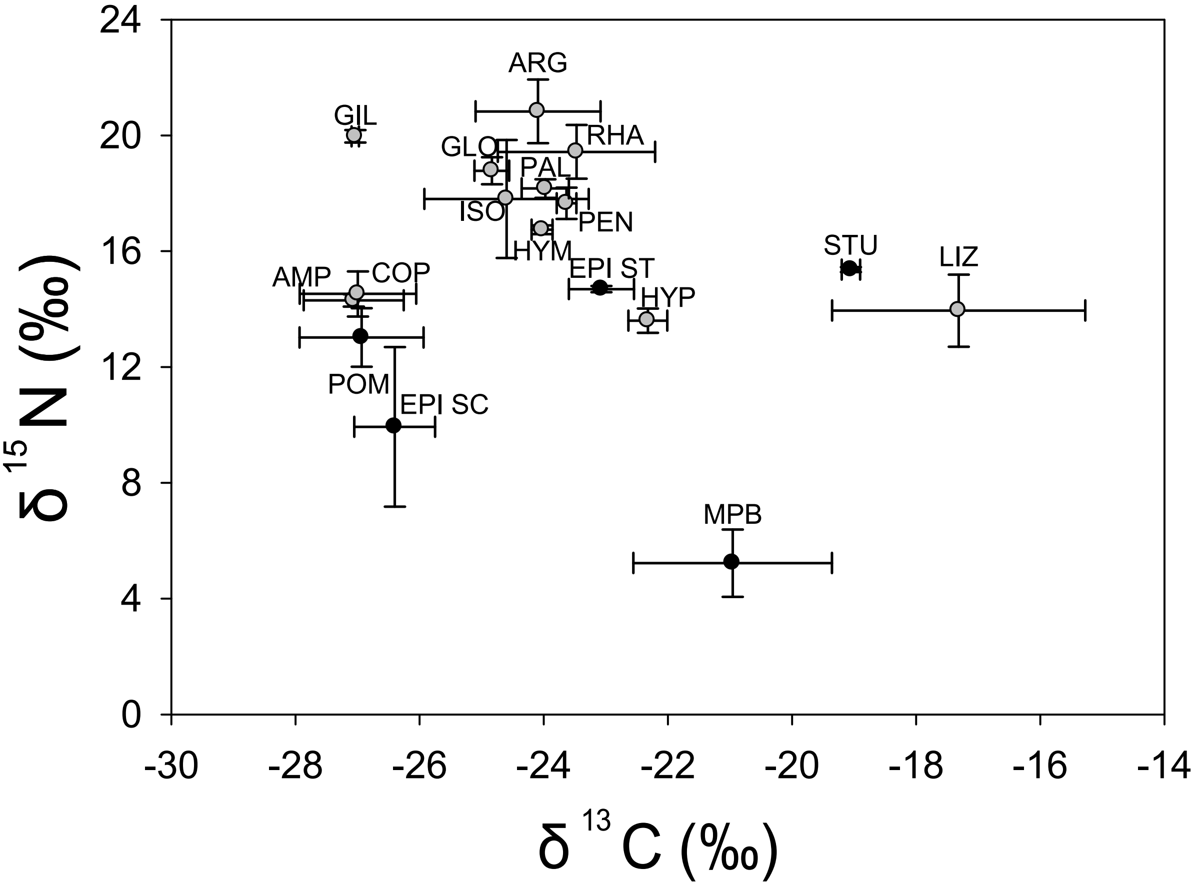


Figure 5.1. Mean (±SD) values of δ^{13}C and δ^{15}N for organic matter sources (black circles), and consumers (grey circles) in the Kowie Estuary in February 2013. POM: particulate organic matter; STU: *Stukenia pectinatus;* EPI SC: epibionts from *Schoenoplectus corymbosus*; EPI ST: epibionts from *Stukenia pectinatus;* MPB: microphytobenthos; COP: copepods; HYP: *Hyporhamphus capensis;* LIZ: *Liza dumerilii*; HYM: *Hymenosoma* sp.; ISO: *Exosphaeroma hylecoetes;* AMP: *Grandidierella lignorum;* PAL: *Palaemon peringueyi;* PEN: *Penaeus monodon;* GLO: *Glossogobius callidus*; GIL: *Gilchristella aestuaria;* RHA: *Rhabdosargus holubi;* ARG: *Argyrosomus japonicus*. Isotope values of particulate organic matter (POM), epibenthic material (EPI) and microphytobenthos (MPB) from the Kowie Estuary were collected by T. Dalu (unpublished data).

5.3.3 Trophic position (TP) and mixing model

The dusky kob *A. japonicus* was the top fish consumer in the Kowie Estuary (TP = 3.9), followed by the clupeid *G. aestuaria* (TP = 3.6; Fig. 5.2). The prawn *P. monodon*, isopod *E. hylecoetes*, shrimp *P. peringueyi* and fish *R. holubi* had similar intermediate trophic positions, ranging from 2.9 to 3.3. The copepods, amphipod *G. lignorum*, fish *H. capensis* and *L. dumerilii* occupied the lowest trophic levels and are regarded as primary consumers (Fig. 5.2). The crown crab *Hymenosoma* sp. had a $\delta^{15}N$ signal corresponding to a TP intermediate between the primary and secondary consumers (Fig. 5.2).

The mixing model using $\delta^{13}C$ and $\delta^{15}N$ suggested that three prey species made up almost 70% of the diet of *A. japonicus*. This predator utilized *G. aestuaria* as a principal food source (28%), with the goby *G. callidus* contributing 23% and the shrimp *P. peringueyi* 18% (Fig. 5.3).

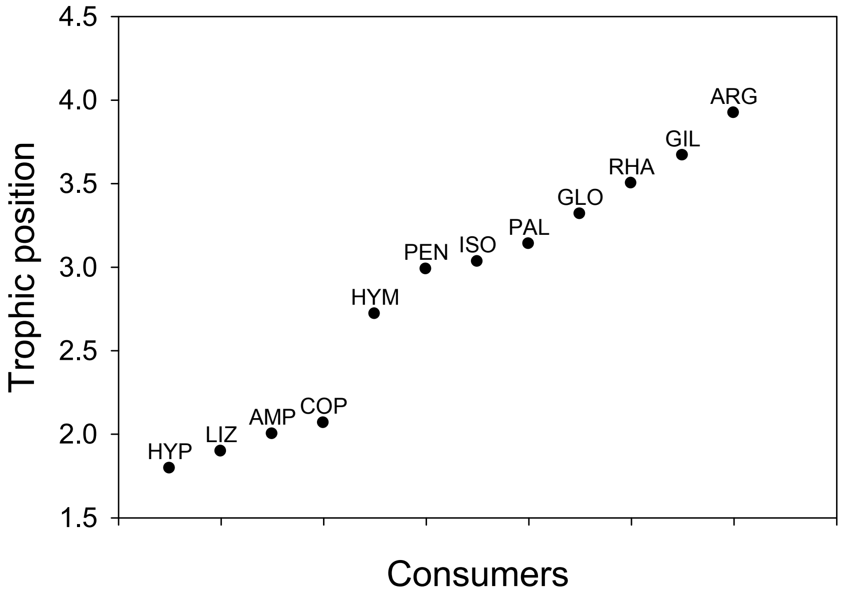


Figure 5.2. Trophic positions of consumers in the Kowie Estuary according to their δ^{15}N values. Abbreviations of species/groups are shown in Figure 5.1.

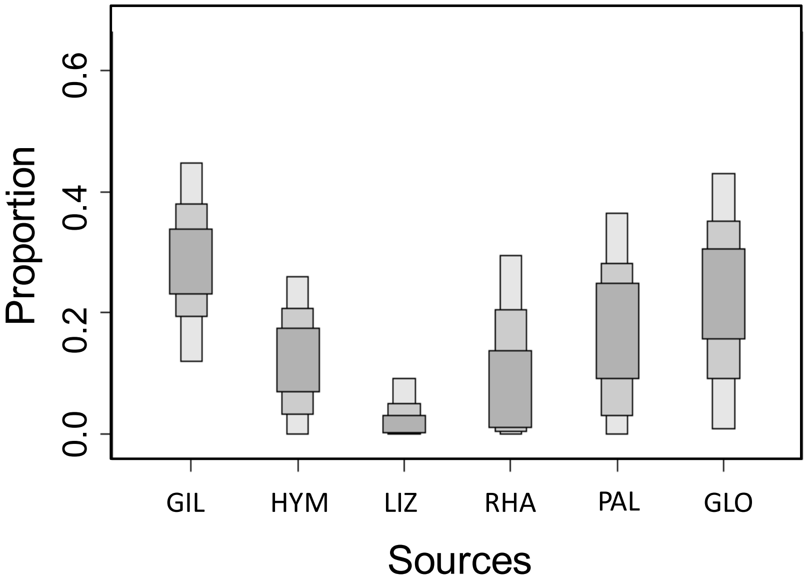


Figure 5.3. Results of the mixed model analysis SIAR (widths of bars showing the 95, 75 and 50% credibility intervals) to estimate source contributions to the predatory fish *Argyrosomus japonicus* in the Kowie Estuary. Abbreviations of sources are given in Figure 5.1.

5.4 Discussion

This study provides an indication of the diverse energy pathways used by *A. japonicus* in the Kowie Estuary. This top predator exploits herbivorous, detritivorous, omnivorous, zoobenthivorous and zooplanktivorous fishes, i.e. ichthyofaunal representatives from all major food chains within the system. Similar approaches to examine the trophic connectivity within estuaries that included top fish predators have been conducted elsewhere (Deegan and Garritt 1997, Kwak and Zedler 1997, Paterson and Whitfield 1997, Darnaude 2005, Abrantes and Sheaves 2009) and our data contribute significantly towards an improved understanding of the predatory role of a top fish predator in a temperate South African estuary. Moreover, the ecological role of *A. japonicus* in linking diverse food webs within an estuarine environment through its trophic behaviour and mobility in a food web context is highlighted for the first time.

Results of my study indicated that the littoral, pelagic and benthic habitats contain important energy sources for different consumers in the estuary. High $\delta^{13}C$ values in the littoral relative to pelagic food sources (e.g. epibionts versus suspended POM) were recorded, findings that were consistent with those from other estuarine systems (Moncreiff and Sullivan 2001) and confirmed the differentiation between the littoral and pelagic food chains (Paterson and Whitfield 1997; Froneman 2001b). The baseline differences among primary food sources in the estuary are of fundamental importance, as with some understanding of assimilation and fractionation processes, information about the ultimate sources of nutrition for higher consumers (i.e. whether they are littoral or pelagic-based) is contained within the isotope signatures of their tissues.

As expected in estuarine environments, consumers in the Kowie Estuary showed a wide range of carbon isotope values, suggesting the assimilation of food from a variety of sources. Assuming a ^{13}C-enrichment in consumers of ~1‰ compared to that of their food, in the Kowie Estuary the results for the clupeid *G. aestuaria* suggested a diet based primarily on planktonic sources such as copepods (Jerling and Wooldridge 1992) and amphipods (Read and Whitfield 1989). This fish species had a relatively high secondary consumer level (based on $\delta^{15}N$ signature) that is consistent with previous research that demonstrated a diet dominated by copepods, ostracods, mysids, macruran larvae, amphipods and chironomid larvae (Whitfield 1998).

In the Kowie Estuary, $\delta^{13}C$ values indicated that epibionts supported a littoral group of fishes including the omnivorous *R. holubi*. This result is in agreement with recent work that revealed the importance of epibionts in the diet of *R. holubi* (Sheppard et al. 2012). The high trophic position for *R. holubi* could be explained by its assimilation of ^{15}N-enriched epibionts derived from *S. pectinatus* and its consumption of a wide range of invertebrate prey (Whitfield 1998).

For the caridean shrimp *P. peringueyi,* epibionts living on the submerged macrophytes *S. pectinatus* and *S. brachyceras* were the most important food items (based on $\delta^{13}C$ signatures). This finding is consistent with previous Eastern Cape studies that showed a clear association of this species with submerged plants (Froneman 2006). In addition, the littoral community contains the goby *G. callidus,* which feeds predominantly on invertebrates such as copepods and amphipods (Wasserman 2012).

The carbon signatures in food sources and primary consumers allowed for discrimination between the channel and littoral food chains within the Kowie Estuary. The former is primarily supported by suspended POM, whereas the latter is based on a mixture of epibionts and MPB. This independent functioning of the littoral and channel habitats has been observed in other aquatic ecosystems (France 1995b; Paterson and Whitfield 1997; Froneman 2001b; Grimaldo et al. 2009), but little emphasis was placed on potential linkages between the habitats by top predators .

This study indicated that the dusky kob *A. japonicus* connects several energy flow pathways within the Kowie Estuary through its feeding activities on different fishes and invertebrates in the channel, benthic and littoral areas (Fig. 5.4). The $\delta^{13}C$ and $\delta^{15}N$ values have confirmed the role of *A. japonicus* as a top predator that feeds mainly upon smaller fishes such as *G. aestuaria* in the pelagic zone, *G. callidus* in the littoral and channel bottom areas, and *R. holubi* together with the shrimp *P. peringueyi* in the littoral zone. The isotope results were consistent with dietary information derived from stomach content analysis of this species in other South African estuaries, e.g. the most frequently occurring prey species (up to 94%) in the stomachs of juvenile *A. japonicus* were the small pelagic fish *G. aestuaria* and benthic dwelling *G. callidus* (Whitfield and Blaber 1978; Marais 1984). The new data from the Kowie Estuary indicated that epibenthic invertebrates such as *Hymenosoma* sp. may provide additional alternative food resources to this predatory fish.

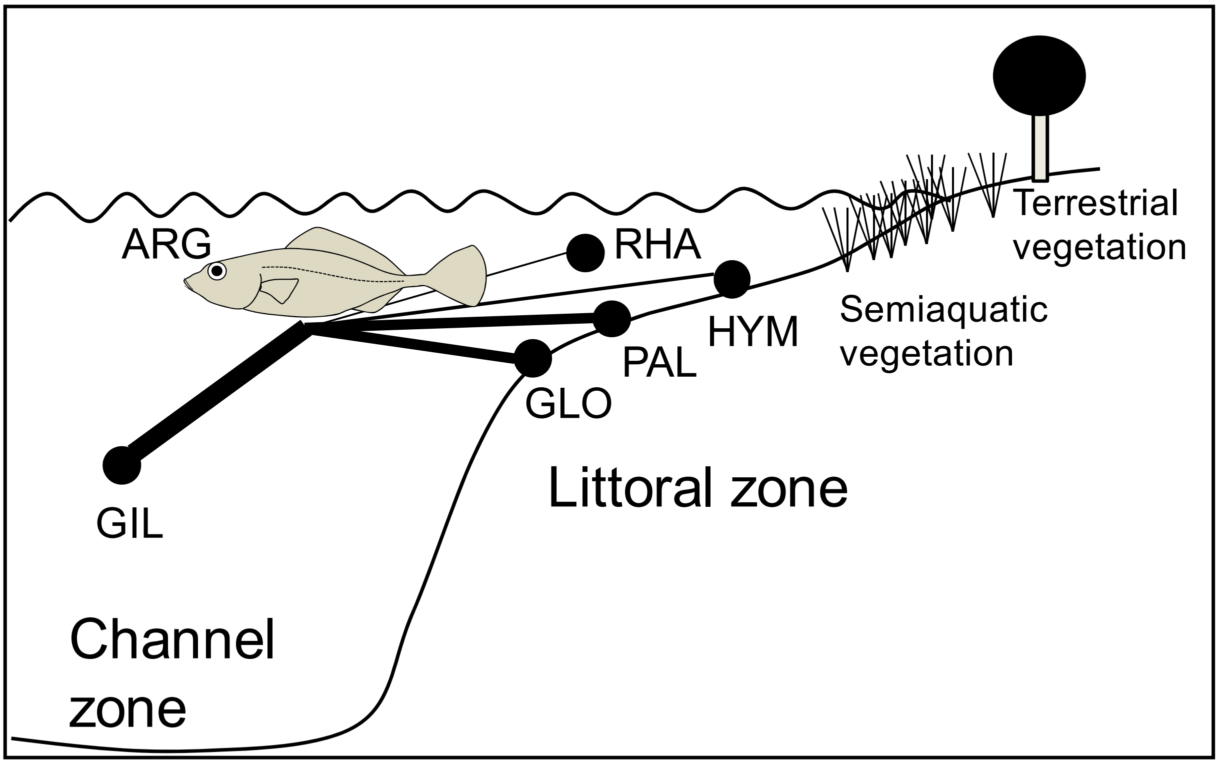


Figure 5.4. Diagrammatic representation of the location of major dietary components (black dots) of the predatory fish *Argyrosomus japonicus* within the Kowie Estuary. Abbreviations of prey sources are given in Figure 5.1. Size of the arrows are proportional to the contribution of it associated prey in the diet.

These results showed that the foraging behaviour of large mobile predatory fish in pelagic, littoral and demersal estuarine environments is an important way in which these habitats can be closely coupled. Researchers studying other estuarine food webs have highlighted the importance of top predators in the assimilation of a variety of food sources (Alfaro et al. 2006; Abrantes and Sheaves 2009). The link of food webs through diet diversification of consumers may impart stability to estuarine communities because it permits predators to have alternative food sources and thus contributes to the persistence of prey (Post et al. 2000; McCann et al. 2005; Vadeboncoeur et al. 2005). Such a coupling has the potential to promote the persistence of predators within a system once they have driven their preferred pelagic prey to low densities. However, this issue remains speculative and more work is needed to assess the influence of top predators in facilitating estuarine ecosystem stability.

In summary, this study demonstrated the existence of different pathways of organic matter in supporting a top consumer within an estuarine environment. The channel habitat supports both zooplanktivorous fishes such as *G. aestuaria* and benthic species such as *G. callidus* and *Hymenosoma* sp.. The littoral habitat supports *P. peringueyi, H. capensis* and *R. holubi* feeding predominantly on epibionts, together with *G. callidus* consuming mainly benthic invertebrates and *Hymenosoma* sp. on detritus. By preying upon all the above species in the littoral, channel, and benthic habitats, *A. japonicus* is integrating different energetic pathways within the Kowie Estuary. This food source diversity may help explain the dominance of this top fish predator in the Kowie Estuary (Whitfield et al. 1994), thus translating into a higher consumer biomass (Duffy et al. 2007). Further studies assessing the trophic role of other top predator species in South African estuaries may enhance our understanding of the relative importance of different habitats in the trophic ecology of these systems.

Chapter 6

General discussion

6.1 Food sources and trophic connections

In this dissertation I showed that a combination of processes alters the relative importance of different carbon sources to consumers within the estuary ecosystem, including the patterns of hydrology, feeding behavior and habitat characteristics. In particular, greater utilization of marsh grasses as a food source occurs in conditions of low freshwater discharge. Furthermore, future conservation and management plans in estuarine habitats need to maintain natural ecosystem processes such as the habitat connectivity through feeding activities of mobile organisms (which in turn promote healthy fish abundances) that may enhance the diversity of the estuarine biota.

Estuarine ecosystems represent spatially and temporally complex environments due to the fluctuations in abiotic factors such as salinity and temperature, and these fluctuations result in different responses in the biotic community structure along the estuary. Therefore, spatial and temporal approaches are important to consider to provide reliable information concerning ecosystem functioning. Although during the past several decades studies have been focused on describing the main trophic pathways in estuarine environments (e.g. Deegan and Garritt 1997; Connolly et al. 2005; Abrantes and Sheaves 2009), we still have much to learn regarding the relative importance of different food sources in estuaries given the dynamic nature of these ecosystems. This thesis incorporated SI signatures and FA profiles of consumers and organic matter sources to gain an understanding of spatial and temporal changes in estuarine food web structure. Furthermore, feeding experiments were used in this dissertation to validate some SI and FA patterns. Several key aims included: (1) identification of the main organic matter sources deposited in sediments and assimilated by consumers; (2) estimation of trophic position of consumers; and (3) quantification of the importance of food sources for consumers. Primary organic matter sources examined included particulate organic matter, sediment organic matter, macrophytes, terrestrial leaves, epibionts and MPB. The consumers were represented by zooplankton, macrobenthos and fishes. This holistic approach included sampling of all the trophic levels and major sources of organic matter. As a result of this dissertation, different spatial and temporal patterns of food webs were identified, and important trophic relationships have emerged that provide new insights into the structure of estuarine food webs. Furthermore, this thesis contributes in the understanding of the biological and physical process that affects the strcture of small temperate estuarine food webs.

SI data have been extensively used over several decades to describe food webs in a variety of ecosystems (Deegan and Garritt 1997; Kharlamenko et al. 2001; Bouillon et al. 2004; Alfaro et al. 2006; Richoux and Froneman 2007; Abrantes and Sheaves 2009; Pingram et al. 2012). However, the use of SI alone may lead to equivocal results for identifying sources of organic matter, particularly when there is an overlap among components in their isotopic signatures (Canuel et al. 1997; Phillips 2001). For example, in this dissertation I found similar isotopic signatures between detritus and fresh leaves of *Spartina maritima*, as has been documented by Currin et al. (1995). Furthermore, SI do not distinguish carbon sources from components such as bacteria. As a result, in dynamic and complex ecosystems such as estuaries, the use of SI in combination with FA analyses provides more comprehensive information regarding food web structure, as has been demonstrated in this dissertation and previous studies (Alfaro et al. 2006; Budge et al. 2008; Van den Meersche et al. 2009; Crawley et al. 2009; Dubois et al. 2014). However, a complication that I found during my study was the difficulty in deriving FA profiles from very small organisms such as the brachyuran crab *Hymenosoma,* isopods, and amphipods. In such cases, pooling of individuals is necessary, thus there is a loss in information about among-individual variation. Recent technological advances in molecular ecology have increased our ability to elucidate trophic relationships through analysis of mitochondrial DNA of gut contents (O'Rorke et al. 2012) and faeces (Deagle et al. 2009) compared to known DNA profiles of potential food sources (Carreon-Martinez and Heath 2010). The applicability of DNA shows great promise for resolving feeding behaviour in small organisms because very small amounts of material are necessary (e.g. Riemann et al. 2010; O'Rorke et al. 2012).

The first set of SI and FA data **(Chapter 2)** focused on spatial and seasonal variations in sediment organic matter composition along the Kowie Estuary. Both techniques indicated significant spatial and temporal changes in the organic matter deposited within the Kowie Estuary. Carbon isotopic values of the sediments were along the estuary and allowed me to link different regions with deposition of distinct organic matter sources. The FAs 16:1ω7 and 20:5ω3 were dominant in the MPB and estuarine POM, so it was possible to evaluate the influence of these carbon sources within the Kowie Estuary. Furthermore, *Spartina maritima* had large proportions of the long-chained FA 24:0, and this FA was useful for tracking the fate of marsh grass detritus in the estuary (Table 2.3). On average, in the upper and middle reaches sediment organic matter was derived mainly from MPB during all seasons, while in the lower reaches higher contributions of the marsh grass *S. maritima* were detected during periods of low freshwater discharge (autumn and winter). The deposition of marsh grass detritus in the lower estuarine reached was associated with the presence of the FA 24:0 together with higher carbon signatures in the sediments. These results provided useful information on the relative importance of different food sources available to consumers along the estuarine gradient and over time. My research supports previous hypotheses that the hydrology condition, vegetation type and primary productivity within an estuary are key features affecting the organic matter available in sediments (Zimmerman and Canuel 2001; Gogou and Stephanou 2004; Goñi et al. 2009; Palomo and Canuel 2010).

With the information on the different organic matter sources deposited and available within the estuary, I considered the benthic and pelagic food web structure in **Chapter 3**. My analysis provided new information on the spatial and temporal changes in food webs within the Kowie Estuary. Stable isotopes signatures and FA profiles were evaluated in food sources and consumers including invertebrates and fishes. Significant differences were detected in SI signatures of consumers inhabiting different estuarine reaches, with low $\delta^{13}C$ values occurring in consumers in the upper reaches during all seasons. Isotopic mixing models indicated that the diets of benthic consumers varied from the upper towards the lower reaches. This pattern was consistent with the general decrease in $\delta^{13}C$ from marine to terrestrial regions (Rubenstein and Hobson 2004). My results suggested a greater utilization of marsh grass by consumers such as brachyuran crabs inhabiting the lower reaches of the estuary during autumn and winter. Fatty acid data supported the isotopic results and indicated a significantly higher presence of plant-associated FAs (18:2ω6 and 18:3ω3) in the brachyuran crabs inhabiting the lower portion of the estuary. A possible explanation to this pattern is that the presence of salt marsh grass in the lower reaches reduced hydrodynamic forces, leading to calm physical conditions, and promoted the deposition of detritus (Bouma et al. 2005). I must note that intermediate carbon signatures of consumers from the lower reaches between the marsh grass *S. maritima* and other potential food sources including MPB and POM suggested that the utilization of the marsh grass as a dietary component may have been overestimated by the isotopic mixing model results.

To clarify SI and FA analyses I studied the trophic ecology of the sesarmid crabs *Sesarma catenata* through laboratory feeding experiments **(Chapter 4).** These experiments indicated that *S. catenata* fed preferentially on decomposed terrestrial leaves from riparian trees, followed by decomposed leaves of the marsh grass *S. maritima*. The low C/N ratios of *S. maritima* and the possible high bacterial activity in decomposed leaves may enhance the nutritional value by increasing the nitrogen content of these plant materials, hence explaining the feeding behavior of the crabs. Additional molecular analyses of the plants are needed to describe the mechanisms behind the food preferences of the crab *S. catenata*. Calculations based on consumption rates and mean densities of *S. catenata* within the Kowie Estuary suggested that although crabs play an important role in processing leaf material, large amounts of leaf litter can potentially be exported from the salt marsh area to subsidize adjacent aquatic areas.

Holistic approaches are necessary to provide reliable representations of energy flows within a system, and the inclusion of top predators in such studies is important due to their role in influencing community structure (McCann et al. 2005; Rooney et al. 2006; Duffy et al. 2007; Abrantes and Sheaves 2009). In **Chapter 5,** the trophic role of an abundant top predator (*Argyrosomus japonicus*: Sciaenidae: Teleostei) was examined within the Kowie Estuary. SI signatures of the dominant food sources and fauna from all trophic levels (herbivorous, detritivorous, omnivorous, zoobenthivorous, zooplanktivorous and piscivorous fishes) were evaluated. Mixing model applications suggested that the dusky kob *A. japonicus* utilized as main food sources pelagic fauna (i.e. the clupeid *Gilchristella aestuaria*), benthic fauna (the gobiid *Glossogobius callidus*) and littoral fauna (the shrimp *Palaemon peringueyi*). These results indicated that by preying upon the species in the littoral, channel, and benthic habitats, *A. japonicus* played a key role linking energetically these different habitats. In this way, each prey represented an alternative path of energy and allowed predators to switch among prey and promote their collective persistence (Rooney et al. 2006). The results from this work have implications for conservation and management practices, since the process of predator movements between patches of resources is important for determining system connectivity and the persistence of local taxa, thus preventing local competitive exclusion by prey species (Loreau et al. 2003). Future studies on food web structure should include the assessment of movements of top predators among distinct habitats, and the role of these movements in controlling ecosystem structure and functioning.

The results presented in this dissertation characterize the energy pathways in different estuarine reaches and identify the different abiotic and biotic factors that promote seasonal and spatial changes in the food web structure within the Kowie Estuary. This information may serve as a platform for conservation policies and management of resources showing the key issues associated with estuarine management and monitoring to permit ongoing exploitation of certain species such as the mudprawn *Upogebia africana*.

6.2 Future studies

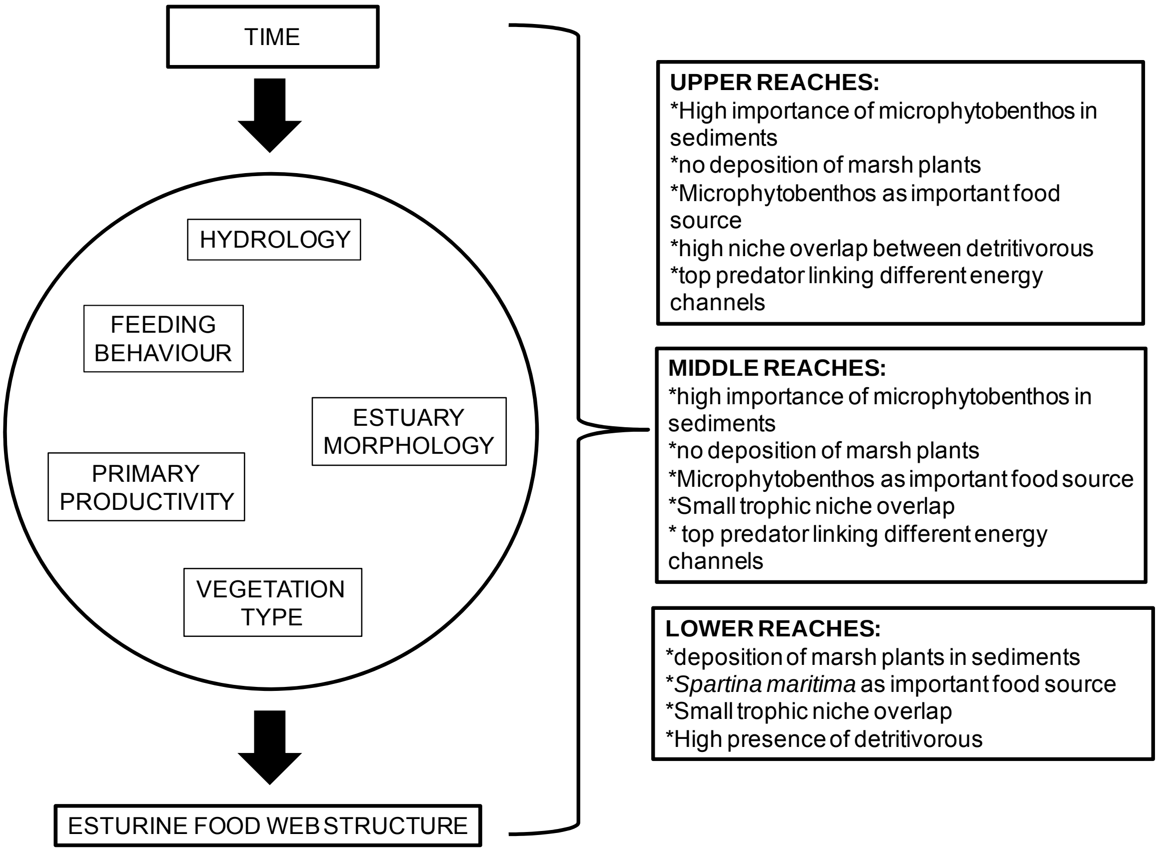
The different food sources available within the Kowie Estuary changed spatially along the estuary and seasonally corresponding with the fluctuation of different abiotic and biotic parameters. This dissertation stresses the need to consider both spatial and temporal aspects in estuarine environments to gain knowledge of the organisation of these ecosystems. Furthermore, lower in the food web different trophic chains tend to be separated within the estuary (benthic, pelagic and littoral), which are then coupled at higher trophic levels by predators (Rooney et al. 2006; McCann and Rooney 2009; McMeans et al. 2013). Continued research involving trophic tracers and more biotic components such as infaunal invertebrates (e.g. Galván et al. 2008) and birds may help us to fully understand the main trophic pathways within complex estuaries. In addition, this work showed that linking laboratory feeding experiments with SI and FA analyses help to improve food web models.

In a world with increasing anthropogenic impacts due to the rapidly increasing human population, questions regarding how food webs evolve and what factors provide resilience may become critical to assess (Levin and Lubchenco 2008; Valdovinos et al. 2010). For example, a particular food web pathway such as the contribution of organic matter from adjacent marsh areas could be important in restoration sites of coastal zones (Howe and Simenstad 2007). Furthermore, high mobility of animals could enhance the ecosystem resilience by connecting specific habitats and increasing the faunal abundance (Lundberg and Moberg 2003; Olds et al. 2012). In this context, understanding how the behaviour of animals, habitat and food web configuration influence the ecosystem resilience or robustness to perturbations can result in successful conservation practices of local habitats. Furthermore, under the current scenario of climate change, it is of great interest to predict impacts of climate change on the ecosystem structure (Grimm et al. 2008), and the assessments of factors that alter the integrity and resilience of ecosystems is therefore valuable.

The importance of *Spartina* as a food source in estuarine ecosytems has been discussed as showing high (e.g. Peterson et al. 1985; Kwak and Zedler 1997; Hoeinghaus and Davis 2007) and low assimilation by the estuarine fauna (e.g. Riera et al. 1999; Galván et al. 2008). These contrasting results lead to the critical question about under what conditions marsh grasses contribute to food webs. My results based on the isotopic signatures and fatty acid profiles of consumers suggested that the marsh grass *S. maritima* was a significant food source in a microtidal small estary during low periods of freshwater discharge, particularly in the shallow section of the system. Furthermore, decomposed material of *Spartina* with low C/N values and high bacterial activity may represent a high nutritional food item for other organisms inhabiting the estuarine environments (Haines and Montague 1979; Chapter 4). It is likely that the importance of salt marsh material in food web depends on a variety of conditions. Future works could explore the factors that affect the role *Spartina* in aquatic food webs, such as the tidal regime, degree of connectivity among adjacent habitats (Deegan and Garritt 1997) or anthropogenic activities.

Furthermore, the role of top predators linking different food chains needs further investigation in estuarine environments. In particular, future works could assess the functioning of trophic connectivity by top predators at different scales and their impact on diversity and ecosystem functioning. For example, previous studies demonstrated that such connectivity of distinct energy channels has the potential to promote the persistence of predators by offering multiple food choice (Schindler and Scheuerell 2002; Rooney et al. 2006). Furthermore, this food source diversity may enhance the growth and biomass of the predator through the "balanced diet hypothesis", which proposes that high prey diversity provides a more complete range of nutritional resources, thus translating into higher consumer biomass (Duffy et al. 2007). Studies that assess the effects of trophic connectivity by mobile organisms in a variety of estuarine ecosystems will help us to gain knowledge of the food web structures in estuaries.

Finally, this dissertation showed a comprehensive documentation of spatial and temporal changes in the food web structure within a small temperate estuarine ecosystem. The spatial and temporal dynamics of biotic and abiotic factors including the pattern of freshwater discharge, habitat configuration, vegetation type, feeding behaviour and primary productivity may alter the relative importance of different organic matter sources for the estuarine fauna (Fig. 6.1). These results reiterate the need for a spatial and temporal approach in food web studies (Chapter 2 and 3; Bergamino and Richoux 2014). This dissertation also emphasizes that the application of multiple trophic tracers including SI and FA analyses together with feeding experiments as complementary tools in an holistic food web approach will lead to significant improvements of our understanding of estuarine ecology (Gannes et al. 1997; Boecklen et al. 2011; Chapter 4). Furthermore, future works building ecosystem mass-balance models (e.g. Ecopath) that describe trophic interactions within ecosystems may help to give new insight in the functioning of estuarine environments. For example, Ecopath models may help to predict the impact of change in biomass of each biotic compartment on the other ecosystem compartments (Christensen and Walters 2004).



UPPER REACHES:
*High importance of microphytobenthos in sediments
*no deposition of marsh plants
*Microphytobenthos as important food source
*high niche overlap between detritivorous
*top predator linking different energy channels

MIDDLE REACHES:
*high importance of microphytobenthos in sediments
*no deposition of marsh plants
*Microphytobenthos as important food source
*Small trophic niche overlap
* top predator linking different energy channels

LOWER REACHES:
*deposition of marsh plants in sediments
**Spartina maritima* as important food source
*Small trophic niche overlap
*High presence of detritivorous

Figure 6.1. Conceptual model of the different factors affecting the estuarine food web structure. Circle represents abiotic and biotic factors within the ecosystem. Summary of the main effects within the Kowie Estuarie food web are also described.

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