FEEDING DYNAMICS OF SUSPENSION-FEEDERS IN THE NEARSHORE MARINE ENVIRONMENT ADJACENT TO TWO CONTRASTING ESTUARIES IN THE EASTERN CAPE, SOUTH AFRICA.

Manuscript submitted in the fulfilment of the requirements for the degree of
MASTER OF SCIENCE
of
RHODES UNIVERSITY

by
ILKE VERMEULEN

March 2011

ABSTRACT

Coastal transition zones form important interlinking regions where marine ecosystems, rivers and estuarine environments significantly influence each other. Coastal rocky shores are key habitats that sustain a variety of primary producers and invertebrates and due to the dynamic nature of coastal ecosystems, suspension-feeders on rocky shores can be influenced by an array of autochthonous and allochthonous food sources. Fatty acid and stable isotope trophic markers were employed to distinguish between regional and temporal changes in the potential food sources to rocky shore suspension-feeders in the Eastern Cape Province of South Africa. The primary aim was to assess the spatial and temporal influences of contrasting river flows on the available food sources to three indigenous coastal suspension-feeders, namely the volcano barnacle *Tetraclita serrata*, brown mussel *Perna perna* and tube-building polychaete *Gunnarea capensis*. This was done by examining the intra- and interspecific changes in the fatty acid and stable isotope signatures of the barnacles, mussels and polychaetes in the adjacent marine environment of a freshwater-restricted (Kariega) and freshwater-dominated (Great Fish) estuary during austral summer and winter.

Multivariate and Kruskal-Wallis analyses of variance on the fatty acid and isotopic signatures, respectively, identified significant regional changes in the barnacles and mussels, while only stable isotopes distinguished between the Kariega and Great Fish polychaetes ($P < 0.05$). In addition, significant temporal changes were observed in consumer fatty acids and isotope values in both regions ($P < 0.05$). Bacterial sources, detritus and phytoplankton assemblages, which are influenced by hydrology and vegetation, differed between regions and were mainly responsible for the regional and temporal separations. Principal component analyses on the consumer fatty acid signatures distinguished between animals situated upstream (i.e. north) and downstream (i.e. south) of the Kariega Estuary mouth in summer. The north/south separation was mainly due to greater contributions of diatoms to northern-located animals and dinoflagellates and detritus to southern-located consumers. In addition, the south-flowing Agulhas Current on the eastern shores of southern Africa appeared to influence the north/south separation in the Kariega region, as water leaving the estuary was probably entrained into the south-easterly flowing currents, thereby depositing estuarine-derived detritus to southern populations.

In general, diatoms and detritus were essential food sources to the filter-feeders in summer, and flagellates, diatoms, zooplankton and detritus were important in winter. Coastal macroalgae was a key food source in the Kariega and Great Fish regions during both seasons.

Consistently large levels of diatom markers (16:1n-7 and 20:5n-3) and dinoflagellate markers (22:6n-3) in consumer tissues in the Kariega and Great Fish regions identified that phytoplankton was their dominant food source. The barnacles, mussels and polychaetes had similar fatty acid markers and a fairly narrow δ^{13}C range (-16.5 to -14.4 ‰), suggesting that they probably consumed similar food. Carbon isotope analyses, however, separated the suspension-feeders into slightly depleted (barnacles; -16.5 to -16.1 ‰), intermediate (mussels; -15.8 to -15.0 ‰) and enriched (polychaetes; -15.0 to -14.4 ‰) consumers, but did not provide conclusive evidence of their preferences for specific phytoplankton. Conversely, fatty acid analyses highlighted that barnacles and mussels had greater proportions of dinoflagellate markers (22:6n-3; 7.0-15.3 % TFA), while polychaetes had larger diatom levels (20:5n-3; 15.1-22.2 % TFA). In addition, all three species had consistently large contributions from bacterial fatty acids (15:0, *i*-16:0, 17:0 and *i*-18:0; 4.2-13.6 % TFA) in summer and winter, and large proportions of saturated fatty acids (33.3-53.1 % TFA) including those with 14 to 18 carbons, indicating that bacterial and detritus food sources played an important role in their diets.

Barnacles had small levels of terrestrial markers (18:2n-6 and 18:3n-3; <2.5 % TFA) and demonstrated increased omnivorous feeding compared with the other suspension-feeders [increased levels of 20:1n-11 and 20:1n-9, higher 18:1n-9/18:1n-7 ratios at ~2.1, enriched δ^{15}N values at ~10.6 ‰; zooplankton (potentially including microzooplankton, larvae and protists) contribution of up to 61 % of the diet]. Mussels contained significant proportions of the terrestrial markers (18:2n-6 and 18:3n-3; >2.5 % TFA) and exhibited intermediate omnivory (intermediate levels of 20:1n-11 and 20:1n-9, intermediate 18:1n-9/18:1n-7 ratios at ~1.3, less enriched δ^{15}N values at ~7.9 ‰; zooplankton contribution of 10-15 % of the diet). The more depleted nitrogen signatures in the mussels relative to the barnacles and polychaetes possibly illustrated a stronger preference for autotrophic food. Polychaetes mainly consumed plant food sources (i.e. microalgae, macroalgae and detritus; high levels of *i*-18:0, 18:1n-9, 18:4n-3 and 20:5n-3) and displayed little omnivory (low levels of 20:1n-11 and 20:1n-9, low 18:1n-9/18:1n-7 ratios at ~0.4, intermediate δ^{15}N values at ~9.1 ‰; zooplankton contribution of <10 % of the diet). The barnacles, mussels and polychaetes are all suspension-feeders, originally presumed to consume the same food sources. The variations observed among the species, therefore, may result from differences in the proportional contributions of the various food sources to their diets as well as distinctions in metabolism. The distinct changes in the fatty acid and stable isotope signatures in all three filter-feeders in the Kariega and Great Fish regions are likely influenced by the diversity in

regional vegetation and hydrology in the different systems, combined with interspecific differences in resource partitioning among the species.

ACKNOWLEDGEMENTS

To my heavenly Father, I am incredibly thankful and fortunate to have been led by highly accomplished and brilliant scientists during my time as a graduate at Rhodes University. I would therefore like to extend a sincere and warm thank you to my supervisors, Dr Nicole Richoux and Professor William Froneman, without whom this thesis would not have been successfully completed. Your constructive feedback, continuous guidance and understanding of personal circumstances throughout the duration of our research, is genuinely appreciated.

Many thanks to Siyabonga Dyaloyi, Shan Ambrose and Candice Ryan for assisting me in the field with the collection of samples, and for enduring the clean air, beautiful beaches and occasional swim in the ocean with me. Tough life indeed. You guys were the best.

I would like to thank John Gillam and Liezel Strydom, Rhodes University and the Andrew Mellon Foundation, the Deutscher Akademischer Austausch Dienst (DAAD) and the Ernst and Ethel Eriksen Trust for providing funding which contributed towards me successfully completing this master’s degree. John and Liezel, many thanks for the hours (literally) I was able to spend in your office discussing everything from family life to the potential of innovative minds to become powerful leaders that should give back to the institutions that bestow knowledge to young graduates. John, I owe you some papers.

To my remarkable family, I extend my sincere gratitude for all your love and encouragement. It meant a great deal to me. Dad, you are the most generous and respectable man I know. I would not have been able to achieve half my goals had it not been for you. Thank you so much for your inspiration. Mam, my best friend, you always know how to lift my spirits and make me laugh. Your support and love throughout the past year has been unrelenting, so needed, and deeply appreciated. I thank you sincerely for that.

Throughout my studies at Rhodes University and the duration of writing up this manuscript there were undeniably some hard times, personal challenges, memorable moments and good laughs. I would like to thank those people that formed a part of my life and that have contributed genuine friendship, positive experiences and good memories. You know who you are, and you mean a lot to me. Thank you.

I dedicate this work to Kaelan Vermeulen...
One day you will realise what a profound impact and change you have made in my life since
14 June 2004. You have given me perspective and focus,
and I love you more than anything.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

LIST OF APPENDICES

CHAPTER 1

GENERAL INTRODUCTION AND OVERVIEW

1.1 General Introduction

1.1.1 Coastal transition zone

The coastal transition zone (CTZ) connects the terrestrial with the marine habitat (Ray, 2005) and encompasses that region where land and ocean influence one another (Schaefer, 1972). The CTZ is a highly dynamic region where species diversity and productivity are enhanced, and where human activities and ecosystem services are numerous (Costanza et al., 1997; Levin et al., 2001). Ecosystem services in the CTZ include the important role of biogeochemical cycles in sustaining water, dissolved and particulate nutrients via surface runoff or groundwater flow, and vast amounts of matter and energy that are exchanged with the open ocean and estuarine environments (Moberg & Rönnbäck, 2003). CTZs are important sites for microbial processes, nutrient cycling and primary production and they are characterized by rapid changes in water chemistry, nutrients and community composition (Levin et al., 2001). Suspension-feeding organisms are closely associated with critical ecosystem functions in the CTZ such as decomposition, nutrient cycling and sediment movement (Herman et al., 2000; Levin et al., 2001). As human populations continue to increase inland and in the coastal regions of the world, pressure on freshwater resources becomes more noticeable (Peschak, 2005). Threats to biodiversity include persistent organic pollutants (e.g. storm water, agricultural runoff and pesticides), nutrient loading and possible anoxic or eutrophic conditions, heavy metal contamination, and physical changes such as dredging, and habitat loss or fragmentation through development (Levin, 2001). The study of CTZs and the species responsible for maintaining their ecological functioning is therefore of fundamental importance to ecologists, coastal zone managers and the general public.

An important characteristic of CTZs where rivers link to the marine environment is the presence of an estuarine front (Largier, 1993). Estuarine fronts form zones generally associated with high productivity due to the supply of nutrients from freshwater, as well as increased food availability ascribed to the presence of particulate organic matter (POM) and estuarine detritus (Forrest et al., 2007). Chavez et al. (1991) noted that the zooplankton species composition of a nearshore marine ecosystem off the coast of northern California reflected the presence of two diverse phytoplankton communities. These communities formed as result of the temporal hydrodynamic processes occurring within the associated CTZ estuarine front, and highlighted that estuarine fronts can contribute to the spatial variations in the plankton assemblages within the nearshore environment. Seasonal and regional dynamics influencing the amount of freshwater flow from estuaries are therefore important, as even transitory fronts can influence food availability to organisms on the nearby coastal rocky shores. A survey conducted along the east coast of South Africa bordering the Thukela Banks assessed the impacts of river runoff to the nearby marine ecosystem (DWAF, 2004). Results of this study indicated that the recruitment of fish was strongly coupled to the magnitude of freshwater outflow, with elevated recruitment of the dominant line fish species, including slinger *Chrysoblephus puniceus* and squaretail cob *Argyrosomus thorpei*, recorded during periods of high outflow (DWAF, 2004). Vorwerk (2006) also found a positive relationship between food availability in the estuarine frontal zone and the biomass and abundance of zooplankton in the region opposite the river mouths of two contrasting estuaries. The importance of flow patterns to offshore productivity within the frontal region may be profound, and more studies underlining potential links between reduced river outflows and food webs in the nearshore marine ecosystems of southern Africa are needed.

Research on the effect of different freshwater inflow on the trophic dynamics of nearshore coastal communities within the CTZ of South Africa has been limited. CTZ studies have mainly focussed on assessing ecological impacts of hydrodynamics (Berry & Schleyer, 1983; McQuaid & Branch, 1985; Bustamante & Branch, 1996; Branch et al., 2010; McQuaid & Mostert, 2010), dependence of biological components on estuaries (Vorwerk et al., 2003; 2008), estuarine trophic relationships (Froneman, 2001; 2004; Richoux & Froneman, 2007) and physico-chemical and biological characteristics in the river-estuary interface zone (Grange & Allanson, 1995; Grange et al., 2000; Bate et al., 2002; Vorwerk, 2006). These studies have contributed to the knowledge of estuaries and their connectivity with adjacent marine environments. Further investigation into this dynamic zone is required to assess the influences of altered river flows on nearby coastal feeding dynamics and to broaden our knowledge of the potential responses of these ecosystems to human impacts.

1.1.2 South African coastal dynamics

South Africa is an arid country with average rainfall of around 450 mm per annum (Davies & Day, 1998; Van Rooyen, 2002). The arid nature of the sub-region has resulted in the construction of a large number of impoundments within the catchment areas of the main river systems which has contributed to reduce freshwater flow into estuaries. Since freshwater inflow represents the primary source of nutrients to estuaries, the decreased freshwater inflow into southern African estuaries has contributed to nutrient-limitation in these systems (Grange & Allanson, 1995; Grange et al., 2000). Strong coastal circulation processes such as eastern and western boundary currents are responsible for determining the physical, chemical and biological characters of CTZs around the world (Chavez et al., 1991; Kosro et al., 1991). South Africa is bordered by two of the major ocean current systems in the world's oceans, the Benguela Current on the west coast; and the Agulhas Current on the south-east coast (Figure 1.1).

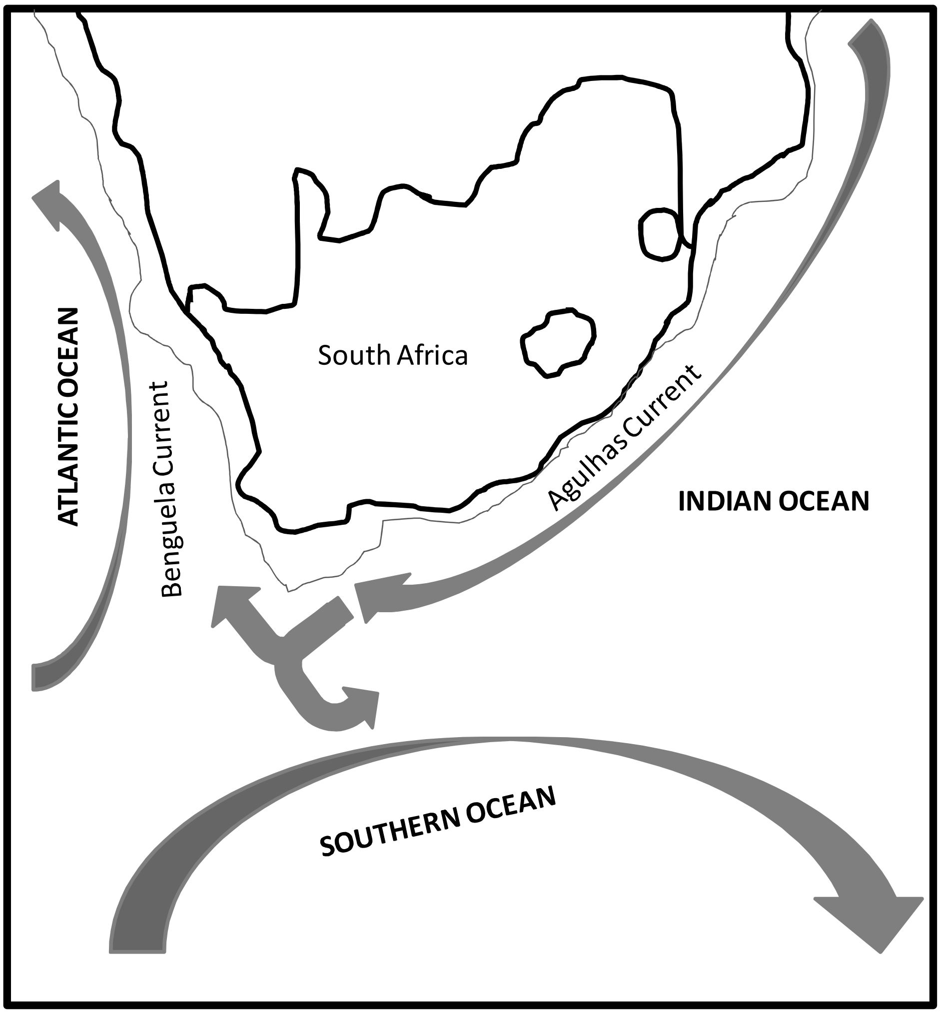

Figure 1.1 Major oceans (Atlantic, Indian and Southern) and oceanic currents (Agulhas and Benguela) bordering South Africa. Adapted from Van der Elst and King (2000).

The Benguela Current represents the eastern boundary current of the south Atlantic gyre and is characterised by strong seasonal coastal upwelling (Lutjeharms et al., 2000; Dijkstra & De Ruijter, 2001; Beal et al., 2006; Hill et al., 2006). The upwelling of the cold nutrient-rich waters contributes to strong seasonal patterns in physico-chemical and biological variables in the region (Machu et al., 2005). The Agulhas Current comprises water derived from the south Indian gyre and tropical surface water which flows down the Mozambique Channel as a series of eddies (Lutjeharms et al., 2000; Dijkstra & De Ruijter, 2001; Beal et al., 2006; Hill et al., 2006). Decreased nutrient concentrations in the eastern marine waters of southern Africa may have dramatic influences on the overall pelagic primary productivity, macroalgal species diversity and food availability in the area (Hill et al., 2006; Hill & McQuaid, 2008). Coastal and estuarine algae and macrophytes are also essential to CTZs and can contribute considerably to water column primary production (~100 g C m^2 yr^{-1}; Underwood & Kromkamp, 1999). Rocky shore intertidal filter-feeders may experience immense variation in the quantity and quality of nutrition available for utilisation, and localised estuaries may therefore, represent important sources of nutrients to the adjacent marine ecosystem.

Within the terrestrial environment, decaying plant matter may produce significant amounts of dissolved and particulate organic material (Mann 1988). Since water is a good vector for POM, carbon is highly mobile in aquatic systems and connects rivers to marine habitats, thereby readily influencing possible food sources for nearshore rocky intertidal organisms (Mann, 1988; Odum, 1980; Shi et al., 2001; Connolly et al., 2005). The magnitude of this connection is largely dependent on the river catchment hydrology that feeds the associated estuaries (Cravo et al., 2000). High freshwater flows that result in the resuspension of particulates could increase the coupling of the benthos to the vertical pelagic environment, and up- and downwelling of such materials may be important in connecting the estuary with the coastal marine environment and providing potential food sources to suspension-feeders (Grange & Allanson, 1995; Freites et al., 2002). Various South African and international studies have recorded substantial hydrodynamically-driven river influences on links between estuaries and the sea (Baird et al., 1987; Jennings, 2005; Forrest et al., 2007). Jennings (2005) assessed the temporal nutrient dynamics in two South African estuaries along the eastern shoreline. A direct link was made between the amount of freshwater input from the estuary and the nutrient content of the adjacent marine environment. Low nutrient concentrations were observed in the marine system adjacent to the estuary receiving little freshwater. Conversely, elevated nutrient concentrations in the nearshore marine environment were associated with high river discharge (Jennings, 2005). Seasonal variations in physico-chemical and nutrient concentrations for the low flow system were negligible, but highly variable in the system with high freshwater discharge, reflecting variable flow rates within the system (Jennings, 2005). Further away, spatial and temporal effects of river water have become evident in physico-chemical factors and the biota in an adjacent coastal environment. Stable carbon and nitrogen ratios, lipid biomarkers and trace elements provided evidence of terrestrial influences from a river system with large freshwater flows on a coastal embayment of New Zealand (Forrest et al., 2007). By analysing sediments, terrigenous signals were found up to six kilometres from the mouth; however, the river plume contributed only locally to the diets of suspension-feeding bivalves, and marine phytoplankton played a major role in sustaining communities further from the mouth (Forrest et al., 2007).

1.1.3 South African estuaries

The southern African coastline has approximately 260 estuaries (Whitfield, 1998), most of which have major ecological and socio-economic importance and are under threat of anthropogenic over-utilisation, freshwater abstraction and degradation (Harrison et al., 2001; Turpie & Clark, 2007). Estuaries are productive systems, and although they are geologically ephemeral (Levinton, 2001), they are biologically rich and especially important as breeding grounds or sanctuaries for many estuarine- or marine-breeding species (Hall et al., 2006). Estuaries represent suitable habitat for a variety of species (Branch & Branch, 1983), and due to their highly diverse nature, they present challenging areas for scientific research (Pasquaud et al., 2007).

South Africa is broadly divided into arid and mesic regions, although it generally displays an arid trend (Van Rooyen, 2002). Eastern Cape estuaries in particular receive reduced freshwater inputs, as they fall within the semi-arid region with low and/or unreliable rainfall and high evapotranspiration rates, and are strongly influenced by the presence of man-made impoundments along the rivers within the catchment areas (Hodgson, 1987; Maree & Casey, 1993; Whitfield, 1998). Consequently, these environmental constraints are expected to influence the associated coastal food webs (Hill & McQuaid, 2008).

Flux of materials from the sea into an estuary is known as inwelling and from an estuary to the ocean or nearshore marine environment as outwelling (Dame & Allen, 1996). Odum (1980) suggested the "outwelling hypothesis", or the movement of surplus materials of estuarine origin to the marine environment. The crucial role that the export of particulates and detritus plays in coastal food webs has been documented and the relative reliance of coastal communities on allochthonous estuarine and autochthonous marine sources identified (Dame & Allan, 1996; Vorwerk & Froneman, 2009). Autochthonous or locally-produced food sources in coastal habitats include marine phytoplankton and macroalgae (Marín Leal et al., 2008). Allochthonous carbon sources that originate elsewhere and are transported to coastal regions as micro- or macroalgal detritus may comprise riverine or pelagic phytoplankton, and microphytobenthos, sediment organic matter and detritus from terrestrial or estuarine origins (Marín Leal et al., 2008). The numerous hydrodynamic features in coastal environments (i.e. local geography, catchment terrestrial influences, magnitude and direction of tides and currents) and the seasonal characteristics in hydrology (i.e. temperature and river flow) therefore largely determine the importance of estuaries in the CTZ (Baird et al., 1987; Winter et al., 1996; Schumann & Pearce, 1997; Vizzini & Mazzola, 2006).

Vorwerk (2006) examined the relevance of the estuarine outwelling hypothesis in the CTZ adjacent to two permanently open South African estuaries. The results from his study showed that systems with high river runoff that formed plumes in the adjacent marine environment were associated with increased zooplankton biomass, POM and chlorophyll-a concentrations along the coastal environment. Conversely, in those systems with mostly attenuated freshwater inflow, the biological response was restricted to the region immediately adjacent to the estuary mouth. It was suggested that the high zooplankton abundances associated with both regions were due to increased food availability in the frontal zone rather than from the flux from the estuaries (Vorwerk, 2006). Vorwerk (2006) further demonstrated, using stable isotope analysis, that the hydrology of the estuary played a significant role in the dietary composition of suspension-feeders in the nearshore marine environment. Riverine phytoplankton arising from high river inflows was the main food source to zooplankton and suspension-feeders in the freshwater-dominant system, whereas macroalgal detritus and estuarine phytoplankton sustained similar organisms in the freshwater-restricted system (Froneman, 2002; Vorwerk, 2006). It is important to note, however, that estuarine-derived productivity contributed to the food webs in both systems through the outflow of POM and chlorophyll-a, although the magnitude of carbon-export was dependent on hydrology (Vorwerk, 2006). Considering the variety of human impacts on South African estuaries and the significant modification of freshwater inputs into most river systems, it is vital to assess systems with distinct hydrology patterns, as the influences of altered inflows into estuaries on the nearshore marine ecosystems of southern Africa are poorly understood.

1.1.4 Food sources for marine suspension-feeders

Two ultimate sources have been identified for supplying carbon to aquatic consumers in coastal lakes – authochthonous and allochthonous forms (Perga et al. 2006). Carbon sources contributing to coastal suspension-feeder diets inhabiting rocky shores near estuaries could arise from similar alternate sources (Dame & Allen, 1996). Identifying autochthonous and allochthonous carbon sources in estuarine and coastal regions are, however, more challenging than in less complex aquatic habitats such as lakes. Allochthonous sources can include input from terrestrial or estuarine plants (i.e. transferred through particulate organic material) and microbial heterotrophic production (i.e. terrestrially-derived dissolved carbon) and estuarine zooplankton. Autochthonous sources include anything produced locally at the coastal site, including oceanic phytoplankton, bacteria and zooplankton in addition to macroalgae. Rocky shore suspension-feeders probably rely largely on autochthonous plankton produced in the surf zone, and allochthonous food particles that have been transported from rivers (and potentially the open ocean) (Freites et al., 2002; Erlandsson et al., 2005; McQuaid & Mostert, 2010). Due to physical and biological processes carrying autotrophic resources over distance, substantial spatial segregation between animals and their food sources can occur (Able, 2005; Connolly et al., 2005). Heterotrophic bacteria and fungi and many benthic and epi-benthic invertebrates form key functional groups in aquatic ecosystems (Hickman et al., 2001). These organisms maintain ecosystem processes by shredding and recycling organic material, and collecting and transporting sediments and nutrients across the seabed and sediment-water interface (Herman et al., 2000). The detrital food chain thus plays a major role within estuaries, as bacterial decomposition renders the normally indigestible particulate fragments more palatable for associated organisms, and consequently rocky shore suspension-feeders bordering estuaries can benefit from their nutrient outwelling (Mann, 1988; Odum, 1980).

The productivity of the nearshore marine phytoplankton and extensive macroalgal communities, particularly those in rocky shores, form an important food source for filter-feeders via direct consumption to the detrital pathway (McQuaid & Branch, 1985; Ricciardi & Bourget, 1999; Kharlamenko et al., 2001; Hill et al., 2006). Rossi et al. (2004) showed with stable isotopic analysis that a suspension-feeding bivalve in the CTZ of a northern European estuary fed almost exclusively on microphytoplankton. The relatively depleted carbon signatures of the bivalve reflected marine phytoplankton as the dominant food source, and were too depleted to suggest that benthic algae were a major carbon source (France, 1995). A study conducted by Maksymowska et al. (2000) also revealed with isotopic ratios that coastal regions may be influenced by marine phytoplankton sources and heterogeneous riverine discharges. Stable carbon isotopes showed that the two sources contributing ~95 % to coastal organic matter were autochthonous marine primary production and riverine particulate material (Maksymowska et al. 2000). Along the South African coastline, Hill & McQuaid (2008) studied rocky intertidal consumers in four biogeographic regions and recorded that various coastal suspension-feeders showed dependence on nearshore carbon and nitrogen sources derived from phytoplankton and macroalgae. Carbon signatures were most depleted in the producers, while consumers were relatively enriched, and the suspension-feeders showed a strong reliance on nearshore organic carbon (>40 %). Distinction among the contributions of macroalgal detritus or terrestrial sources within the nearshore SPM was not done, but the importance of coastal production relative to offshore phytoplankton was highlighted (Hill & McQuaid, 2008). In addition, distinct east to west nitrogen enrichment was observed along the coast of South Africa (Hill & McQuaid, 2008). The enrichment in nitrogen signatures in the western region was related to oceanographic features, with the increased phytoplankton productivity along the west coast compared with decreased productivity in the east due to the influence of the nutrient-limited Agulhas Current (Peschak, 2005; Hill et al., 2006).

A study conducted in the estuary-marine interface of a CTZ in France assessed the temporal dynamics of organic food sources available to suspension-feeders (Lefebvre et al., 2009). Suspension-feeders of two regions showed isotopic signatures reflecting their environment and season. A macrotidal estuarine bay was influenced by two river channels that displayed low freshwater output in summer (annual mean flow 13.1 m^3 m^{-1}) and high output in winter (annual mean flow 20.1 m^3 m^{-1}), and animals from this region fed mainly on estuarine microphytobenthos and macroalgal detritus (Lefebvre et al., 2009). In contrast, an intertidal coastal region with direct marine influences received terrestrial inputs from a small catchment (759 m^2) with seasonal river flows (annual mean flow 10.7 m^3 m^{-1}), and the suspension-feeders mainly consumed marine phytoplankton. During seasonal blooms, all suspension-feeders relied on phytoplankton as their major food source, but during winter when stocks were limited, the suspension-feeders were supplemented with alternative sources. These alternative sources included outwelled organic particulates from the estuary and continental origin (Lefebvre et al., 2009). It is clear that coastal environments are influenced by a diversity of locally-produced and imported food sources. Further investigation into the potential food sources and their influence on suspension-feeder diets in the coastal rocky shores bordering estuaries is therefore essential.

1.1.5 Study organisms

Sessile suspension-feeders are generally the most abundant organisms on wave-exposed coastal rocky shores, while intertidal grazers may attain substantial abundance on sheltered shores (Little & Kitching, 1996). In South Africa, greater grazing and filter-feeding consumer abundances have been recorded on the west coast compared to the east coast

(Bustamante, 1994). However, suspension-feeders comprise the bulk of the invertebrate biomass on the rocky shores of both the western and eastern coastlines (McQuaid & Branch, 1985; Bustamante et al., 1995; Bustamante & Branch, 1996). Rocky shore invertebrates are influenced by an array of physical factors (i.e. wave action and tides) and biological interactions (i.e. competition for space and food; Hiscock & Marshall, 2006). Although grazing by benthic herbivores can be substantial on rocky shores, these organisms do not graze directly on macroalgae but rather consume the settling microalgae and algal sporelings (McQuaid & Branch, 1985). This implies that the contribution of macroalgae as a food source to consumers is mainly via the detrital food chain (Mann, 1988). The importance of feeding by rocky shore animals has been identified by the effects water filtration can have on energy transfer, phyto- and mesozooplankton biomass and the development of microalgal blooms (McQuaid & Branch, 1985; Davenport et al., 2000; Prins & Escaravage, 2005; Alfaro, 2006). In particular, filtration by filter-feeding consumers allows the transfer of energy from the water column to the rocky shore community, and therefore these organisms form important biological components in linking adjacent aquatic habitats (McQuaid & Branch, 1985). Dense beds of suspension-feeding invertebrates also have key ecological functions on rocky shores as they can offer direct nutrition to marine predators, and may create microhabitats for other intertidal organisms (Branch & Branch, 1983).

The three indigenous target organisms collected for this study, namely the barnacle *Tetraclita serrata*, the mussel *Perna perna* and the polychaete *Gunnarea capensis*, were selected on the basis of their feeding mode (i.e. all three are suspension-feeders), and their wide distribution along the South African coastline (Branch & Branch, 1983). These organisms form a representative depiction of the Eastern Cape rocky shore suspension-feeding trophic guild. By studying different species within a specific trophic level, we can more readily determine whether differences in fatty acid and stable isotopic signatures of the animals were caused by variations in diet rather than factors such as reproduction or growth. LaBarbera (1984) distinguished among the various types of filter-feeders. Bivalve molluscs and serpulid polychaetes were categorized as active suspension-feeders, as they employ metabolic energy to steer water currents. Barnacles were grouped as facultatively active suspension-feeders due to their ability to optimise favourable conditions by producing their own feeding currents or feed passively using their cirral fans (LaBarbera, 1984).

The grey volcano barnacle *T. serrata* (subphylum Crustacea, class Cirripedia) is a sessile intertidal organism enclosed in a shell of four calcareous plates, and feeds non-selectively by extending its flexible legs or cirri from its shell opening to collect food particles via a stroking action from the water column (Hickman et al., 2001). The presence of long and short cirri enhances feeding efficiency by capturing zooplankton organisms and large phytoplankton cells in size range 20-200 µm, as well as smaller phytoplankton (2-20 µm; Riisgard & Larsen, 2010). A study assessing possible food sources for different barnacle species distinguished this invertebrate as an omnivorous feeder (Zhukova, 2000). Marchinko (2007) recorded a significant relationship between wave exposure and barnacle feeding appendages that influenced their feeding efficiency. Barnacles exposed to less intense wave action developed long, thin cirri which could not withstand full cirral extension when water velocity increased. In contrast, barnacles exposed to intense wave action had short, thick feeding limbs that were able to overcome drag forces associated with high water velocity and were thus advantageous for capturing food particles (Marchinko, 2007). Barnacle populations from distinct environments, like those occurring within estuary mouths as compared to coastal environments, can potentially show different feeding abilities.

Most bivalves, including the brown mussel *P. perna* (phylum Mollusca, class Bivalvia), have enlarged plate-like gills positioned on the lateral sides of the body which serve important respiration and feeding functions (Hickman et al., 2001). Water is circulated through the gills and mantle cavity with the aid of fine cilia, and food particles are extracted and moved to the palps and mouth where they are sorted according to size. *P. perna* is able to filter particles in the size range of 2-100 µm (Schleyer, 1981). Particles in this size fraction contain substantial amounts of organic material comprised of detritus, free-living coccoid bacteria and microflagellates (Schleyer, 1981; Berry & Schleyer, 1983), although mussels are able to select and consume a wide variety of food sources (Espinosa et al., 2008; Lefebvre et al., 2009). Recent evidence suggests that mussels have the ability to consume larger particles including mesozooplankton (200 µm to 2 cm) and zooplankton (i.e. rotifers; Davenport et al., 2000; Alfaro, 2006; Wong & Levinton, 2006). Espinosa et al. (2008) conducted feeding experiments that showed that mussel diet could shift significantly depending on food availability. Summer carbon sources comprising marsh detritus and bacteria contributed as much as 40 % to metabolic requirements, whereas bacteria contributed 70 % of the nitrogen diet during winter (Espinosa et al., 2008).

The tube-forming or serpulid polychaetes *G. capensis* (phylum Annelida, class Polychaeta) build extensive banks of strong sandy tubes and feed on suspended particles by extending their tentacles from the tube opening (Noffke et al., 2009). Davies et al. (1989) conducted feeding trials with an analogous sessile marine annelid (*Ficopomatus enigmaticus*) to assess the food particles consumed. These tubeworms efficiently retained particles in the size range 2-12 µm, which highlighted detritus and small flagellates as accessible food sources. Another study identified a wider variety of particles consumed by two different marine polychaetes (Phillips & Pernet, 1996). These authors found that the tubeworms (*Serpula vermicularis*) fed on particles ≤12 µm in size, whereas the scaleworms (*Arctonoe vittata*) ingested larger particles in the range 2-60 µm, which mainly comprised diatoms and bivalve veligers. Sessile marine polychaetes thus exhibit diverse particle selection abilities, and their diets include phytoplankton and smaller particles such as micro- and macroalgal detritus, autotrophic flagellates and bacteria.

1.2 Trophic research

Three key techniques for understanding food web ecology have been described by Pasquaud et al. (2007) and comprise stomach content, stable isotope ratio and fatty acid analyses. Stomach contents can provide both quantitative and qualitative information on organism diets, but there are biases associated with this method arising from food regurgitation and digestion. Measuring assimilated diet is challenging and requires accurate baseline data for the study organisms to account for fractions of food consumed, digested and metabolised (Maree & Casey, 1993; Thompson et al., 2008). Furthermore, stomach content analysis is not practical for studying suspension-feeding invertebrates, as it will not give information on the origin of the detritic organic matter within the diet. Stable isotopes have been applied in ecological studies of marine food webs since they are useful tools in tracing the flow of organic matter (Grall et al., 2006; Bodin et al., 2007). In particular, $^{13}C/^{12}C$- and $^{15}N/^{14}N$-ratios are used to trace the primary sources of carbon and nitrogen and can indicate an organism's position in the food web (Richoux & Froneman, 2007).

The underlying principle of the stable isotope method is based on the enrichment of the heavier isotopes (^{13}C and ^{15}N) with each step of assimilation along the food chain, mainly ascribed to metabolic losses via respiration and excretion of the lighter isotopes (^{12}C and ^{14}N; Alfaro et al., 2006; Carabel et al., 2006; Bodin et al., 2007). Due to the somewhat predictable fractionation factors of carbon and nitrogen signatures in organisms from one trophic level to the next, stable isotopes can indicate the distance of the predator from the base of the food web (Shin et al., 2008). The standard fractionation factors of $\delta^{13}C$ and $\delta^{15}N$ per trophic level are 0 to 1 ‰ and 3 to 4 ‰, respectively (Michener & Schell, 1994). Fractionation values, however, vary within and among species, different tissues and geographic location (Kupfer et al., 2006; Dubois et al., 2007; Hill & McQuaid, 2008). To base trophic shift conclusions on isotopic results that have not considered species-specific variability may thus lead to flawed interpretations of organic matter pathways and contributions of consumer dietary sources (Dubois et al., 2007; Marín Lean et al., 2008). Trophic fractionation in rocky shore suspension-feeding invertebrates has not been studied extensively, and information on enrichment factors for these consumers remains limited. Consumer-diet discrimination values that do exist for aquatic invertebrates are continuously being questioned due to the specificity of trophic fractionation (McCutchan et al., 2003). Some research has identified fractionation values for suspension-feeders at ~1.3 ‰ for carbon and ~2.2 ‰ for nitrogen (McCuthcan et al., 2003; Grall et al., 2006; Riera et al., 2009), although variability in fractionation values within this trophic guild may be substantial. Dubois et al. (2007) examined the isotopic fractionation of the suspension-feeding pacific oyster (*Crassostrea gigas*) and blue mussel (*Mytilus edulis*) under laboratory conditions and identified diet-tissue fractionation values at ~2.0 ‰ and ~3.6 ‰ for carbon and nitrogen, respectively. During their study on assessing the variability of fractionation values in distinct groups such as taxon, habitat and food type, Vander Zanden and Rasmussen (2001) identified that nitrogen fractionation is more variable in invertebrates compared to vertebrates, herbivores relative to carnivores, and under laboratory conditions in contrast to field estimates. A whole array of factors should therefore be considered when studying food web ecology and energy-flow in aquatic systems. In addition, even though stable isotopic analyses can aid in revealing carbon origins, direct (POM) or indirect (bacteria or microzooplankton) assimilation pathways may be less discernable (Van den Meersche et al., 2009). Such distinctions of trophic pathways are important, as detritus may enter aquatic food chains as either dissolved or particulate organic content (Mann, 1988). Further studies into the specific isotopic fractionation values for various species (faunal and floral) are therefore very important to better understand the natural variation associated with these measurements (Kupfer et al., 2006; Dubois et al., 2007).

Tamelander et al. (2006) noted that animal tissue with lower turnover rates, such as muscle or body structures free of calcium carbonate ($CaCO_3$) (Marieb & Hoehn, 2010), are ideal for stable isotope analysis as they can provide a time-integrated picture of the community trophic dynamics. A disadvantage in using the stable isotope technique is that there are no generally accepted criteria for the pre-analysis preparation of samples, including collection and storage methods (Hobson et al., 1996). Several authors have identified that the sample treatment or processing method may influence isotopic ratios (McCutchan et al., 2003; Jacob et al., 2005; Carabel et al., 2006; Dannheim et al., 2007). Although opposing results have been obtained regarding the effects of sample acidification, removal of $CaCO_3$

through acidification with hydrochloric acid may significantly influence both $\delta^{13}C$ and $\delta^{15}N$ signatures (McCutchan et al., 2003; Jacob et al., 2005). Samples free from $CaCO_3$ may demonstrate a significant reduction in tissue nitrogen content when acidified prior to isotopic analysis, leading to elevated C/N ratios (Jacob et al., 2005). There also appears to be a common lack of data on species-specific fractionation values in ecosystem studies using stable isotopes (Dubois et al., 2007), and trophic shifts can thus be influenced by choice of sample tissue and biochemical composition of different organisms (McCutchan et al., 2003). There is a general trend to focus on single level trophodynamics rather than multiple trophic level research, the latter increasingly more useful in understanding the relationships within aquatic food webs (Alfaro et al., 2006; Hall et al., 2006) and in improving interpretations of potential food sources when information is limited (Fry & Sherr, 1984).

Fatty acids can aid in identifying the trophic status of an organism, and are suitable as biomarkers to trace the origin and paths of organic matter (Drazen et al., 2008). Fatty acid composition is thus a useful technique for studying aquatic trophic ecology through space and time, and fatty acids are generally reported in the technical notation as prescribed by the International Union of Pure and Applied Chemistry (IUPAC) by X:An-*X*, where X is the number of carbon bonds within the molecule, A the total number of double bonds and *X* the position of the first double bond from the terminal methyl carbon, n. Lipids classes such as triacylglycerols, phospholipids and sterols form an integral energy-supplying nutrient to living organisms, and their components are involved with maintaining cell or membrane functions (Garrett & Grisham, 1999). Fatty acids, which form the backbone of triacylglycerols and phospholipids, comprise long carbon atom chains surrounded by hydrogen (Thompson et al., 2008). Saturated fatty acids (SFAs) contain maximum hydrogen atoms adjoining every carbon and thus have no double bonds (e.g. 14:0). Unsaturated fatty acids have double bonds between carbon atoms; monounsaturated fatty acids (MUFAs) lack hydrogen atoms in a single region (i.e. single double bond, e.g. 18:1n-7), and polyunsaturated fatty acids (PUFAs) lack hydrogen atoms in multiple locations (i.e. contain numerous double bonds, e.g. 18:3n-3). The level of saturation determines the shape of triacylglycerols, and the hydrogen atoms at the unsaturated region can be arranged in different positions: in *cis*-configuration both hydrogen atoms are on the same side of the double bond, while for *trans*-configuration the hydrogen atoms are on diagonally opposite sides of the double bond (Thompson et al., 2008).

Specific fatty acids and fatty acid profiles may be used to trace diet origins in consumers. The components 16:1n-7 and 20:5n-3 have been used to identify diatoms, 18:4n-3 and 22:6n-3 for dinoflagellates (Graeve et al., 1994; Kharlamenko et al., 2001), 18:1n-9 and 18:1n-7 as markers for plankton (excluding diatoms) and bacterial consumption, respectively (Brett & Muller-Navarra, 1997), 18:2n-6 and 18:3n-3 for terrestrial sources (>2.5 % TFA as significant; Budge & Parrish, 1998), 20:1n-11 and 20:1n-9 for calanoid copepod markers (Graeve et al., 1997), and 20:4n-6 for heterotrophic protists or sediment micro-eukaryotes (Dalsgaard et al., 2003). Bacterial fatty acids (BFAs) tend to have odd-numbered carbon chains with *iso*- or *anteiso*- branches, and also include non-methylene interrupted dienes (Ackman & Hooper, 1973; Dalsgaard et al., 2003; Howell et al., 2003). It is clear that due to the wide array of fatty acids contributing to SPM proportions, that many possible sources of origin can be found within coastal rocky shores.

Essential fatty acids (EFAs) cannot efficiently be synthesised by most consumers and must be obtained from the diet (Thompson et al., 2008). The EFAs are particularly valuable, as they can provide useful information on organism dietary intake. For aquatic systems, the PUFAs 20:4n-6 (arachidonic acid), 20:5n-3 (eicosapentaenoic acid) and 22:6n-3 (docosahexaenoic acid) have been identified as particularly important (Olsen, 1999). In phytoplankton assemblages diatoms (16:1n-7 and 20:5n-3) are important marine nutrient sources as they can contain large quantities of PUFAs; however, 22:6n-3 is not found solely in dinoflagellates but in other phytoplankton species as well (Rousch et al., 2003). An inverse relationship has been noted for the levels of 20:5n-3 and 22:6n-3 in phytoplankton assemblages, and therefore when diatoms and dinoflagellates are the dominant producers in a particular ecosystem, ratio markers can be used to reflect the relevant dominance of dinoflagellates *versus* diatoms (22:6n-3/20:5n-3) in consumer diets (Budge & Parrish, 1998). High ratios of 16:1/16:0 and 20:5n-3/22:6n-3 in consumers are indicative of the utilisation of diatom food sources, while elevated ratios of 22:6n-3/20:5n-3 identifies the consumption of flagellates (ratio of ≥1 represents a significant contribution; Jeffries, 1970; St. John & Lund, 1996; Budge & Parrish, 1998; Kharlamenko et al., 2001).

Most dietary fatty acids are assimilated by consumers (Schmidt-Nielsen, 2007; Shin et al., 2008). Even though modification of assimilated fatty acids can be substantial, limited calibration data are available to aide interpretation of field collected data. Fatty acids have largely been used as qualitative markers in marine ecosystems since they can differentiate between some sources of organic matter and can provide information on primary carbon sources for consumers (Dalsgaard et al., 2003; Shin et al., 2008). Since coastal environments are influenced by a magnitude of estuarine-derived detritus and other particulates, using stable isotopes and fatty acids as biomarkers is particularly challenging in these ecosystems

(Pasquaud et al., 2007). However, many such studies have been conducted with these techniques and have revealed intricate trophic relationships within estuarine and marine habitats (Schlechtriem et al., 2003; Richoux et al., 2005; Alfaro et al., 2006; Hall et al., 2006; Richoux & Froneman, 2007, 2008; Allan et al., 2010).

Alfaro et al. (2006) suggested that fatty acid biomarkers should best be used in conjunction with supplementary techniques such as stable isotopic signatures since both methods have certain limitations. A limitation to the stable isotopes method include the considerable variation in trophic shift that can occur among analogous consumers, and may cause inaccuracy in determining the contribution of food sources to consumer diets (McCutchan et al., 2003). The limitations of the fatty acid technique are the non-specific nature of most fatty acids, as few organisms or groups of organisms display unique fatty acids, and the processes for lipid digestion and fatty acid metabolisation may differ (Garrett & Grisham, 1999). Stable isotopes and fatty acids, however, can be used concurrently to provide information on food sources and feeding dynamics in highly fluctuant aquatic systems (Pasquad *et al*., 2007). Fatty acid and stable isotope analyses have been successfully integrated to identify inter- and intraspecific trophic links in various aquatic habitats (Kharlamenko et al., 2001; 2008; Henninger et al., 2009; Allan et al., 2010), and have been employed in this study as tools for assessing the feeding dynamics among suspension-feeders in the nearshore coastal ecosystem of the Eastern Cape Province.

1.3 Thesis overview

The broad aim of this research was to assess the influence of different food sources in the diets of selected nearshore filter-feeders (the volcano barnacle *Tetraclita serrata*, brown mussel *Perna perna*, and tube-building polychaete *Gunnarea capensis*) in the immediate vicinity of two contrasting permanently open southern African estuaries. The study was conducted near a freshwater deprived (Kariega Estuary) and freshwater dominated (Great Fish Estuary) system. The importance of the terrestrially-derived carbon in the diets of the selected filter-feeders was assessed using both stable isotope and fatty acid analyses.

This study also aims to assess the temporal and spatial variations in the diets of the three indigenous suspension-feeders in the nearshore marine coastal habitats of the Eastern Cape coastline. The Kariega and Great Fish Estuaries were chosen as they represent contrasting systems with distinct hydrology and freshwater inputs along the South African coastline. Crucial baseline data for fatty acid and stable isotope tissue values for these common filter-feeders are provided, and possible energetic links on smaller spatial scales were assessed. It should be emphasised, however, that although this work is adequate to address small-scale questions on the contributions of potential food sources to the diets of three common rocky shore suspension-feeders, a more comprehensive study and sampling design is required to properly evaluate larger spatial and seasonal patterns in trophic food webs.

The main findings of the fatty acid analyses are described in Chapter 2, and those of the stable isotope analyses in Chapter 3. As part of the main objectives for this study, the following hypotheses were tested:

1) Intraspecific fatty acid and stable isotopic signatures for the barnacle, mussel and polychaete vary regionally due to the differences in freshwater inputs of the two systems influencing regional food sources.
2) Intraspecific fatty acid and stable isotopic signatures for the barnacle, mussel and polychaete differ temporally as result of seasonal variation in available food sources.
3) Since all three species fall within the same trophic guild (i.e. suspension-feeders) and are thus assumed to be consuming the same food sources, similar temporal and regional effects occur among the species.
4) Given that the coastal region of the Eastern Cape is dominated by the south flowing Agulhas Current, suspension-feeders found downstream (south) of the estuary mouth demonstrate a higher dependence on terrestrially-derived carbon than those recorded upstream (north) of the two estuaries.

CHAPTER 2

SPATIAL AND TEMPORAL CHANGES IN THE DIETS OF ROCKY SHORE INVERTEBRATES ALONG THE COAST OF SOUTH AFRICA: A FATTY ACID PERSPECTIVE

2.1 Introduction

Coastal suspension-feeders derive their food from a variety of sources including microalgae, macroalgae, bacteria, zooplankton and detritus (Dalsgaard et al., 2003). Terrestrially-derived organic matter can play an important role in the provision of supplementary food sources to nearshore coastal communities, particularly in the vicinity of estuaries (Grange & Allanson, 1995; Dalsgaard et al., 2003). However, the literature suggests that higher plants generally represent a low quality food source to suspension-feeders unless substantially decomposed by microbes (Deegan & Garritt, 1997; Forrest et al., 2007; Marin Leal et al., 2008; McLeod & Wing, 2009). As plankton communities in coastal waters are dynamic and many rocky shores are situated adjacent to estuaries that may contribute to the outflow of particulates, consumer diets may change through space and time (Peschak, 2005; Kang et al., 2006; Vorwerk, 2006). Fatty acid biomarkers are useful tools for identifying the importance of various food sources and their contribution to animal diets (Graeve et al., 1994; Peters et al., 2006) and can reveal seasonal and spatial inputs of food sources to suspension-feeders (Meziane et al., 1997; Falk-Peterson et al., 2000; Hudson et al., 2004; García-Alonso et al., 2008).

Fatty acids can be used to trace carbon in food webs, and have been successfully used in a variety of ecological studies involving microalgae (Hama, 1991; Budge & Parrish, 1998; Volkman et al., 1998; Budge & Parrish, 1999; Budge et al., 2001) macroalgae (Sanina et al., 2004; Shin et al., 2008), zooplankton (Copeman & Parrish, 2003; Richoux et al., 2005; Peters et al., 2006) and higher organisms such as freshwater and marine fish (Ackman et al., 1980; St. John & Lund, 1996) and seabirds (Richoux et al., 2010). Limited fatty acid research has been conducted along the South African coastline (Richoux & Froneman, 2008; Henninger et al., 2009; Allan et al, 2010), although some international studies have focussed on estuarine and/or marine filter-feeding invertebrates (Zhukova et al., 1992; Meziane et al., 1997; Zhukova et al., 1998; Kharlamenko et al., 2001; 2008; Freites et al., 2002; Alfaro et al., 2006). Studies identifying spatial and temporal patterns in coastal rocky shore food sources of invertebrates with the aid of fatty acid analysis are limited, although some research has been conducted in boreal and austral marine systems to determine seasonal patterns in fatty acid profiles in SPM (Mayzaud et al., 1989; Canuel, 2001) and marine invertebrates (Falk-Peterson et al., 2000; Hudson et al., 2004; Peters et al., 2006).

The essential fatty acids (EFAs; 20:4n-6, 20:5n-3 and 22:6n-3) can represent a measure of the quality of food sources obtained by suspension-feeders (Brett & Müller-Navarra, 1997). It is important to evaluate the overall quality of food sources for rocky shore suspension-feeders to determine how regional and temporal changes in local hydrology and vegetation can influence their food composition. A study on seston food-quality identified that seston was strongly influenced by EFAs, and related a marine invertebrate’s biomass to seston variables (i.e. carbon, nitrogen, phosphorus and fatty acids; Brett & Müller-Navarra, 1997). These authors recorded moderate to weak interactions for the elements with *Daphnia sp.*, but a strong relationship between *Daphnia sp.* and eicosapentaenoic acid (20:5n-3). The relevant contributions of EFAs in the diets of suspension-feeders can provide information on the phytoplankton food available to the consumers. Budge et al. (2001) studied fatty acid profiles of the blue mussel (*Mytilus edulis*), phytoplankton and sediments in a sheltered Newfoundland cove with negligible freshwater and terrestrial inputs, limited benthic vegetation and high sediment resuspension. Specifically, the total contribution of polyunsaturated fatty acids (PUFAs), the levels of arachidonic acid (20:4n-6), and the ratio of n-3/n-6 fatty acids (used to identifiy the degree of herbivory; Stevens et al., 2004) were assessed to establish if phytoplankton could meet mussel maintenance requirements in a bivalve aquaculture site. The SPM contained high levels of 20:5n-3 and docosahexaenoic acid (22:6n-3), and substantial bacterial and terrigenous signals due to sediment resuspension and terrestrial material deposited from the surrounding forest via small connecting streams (Budge et al., 2001). The higher plant fatty acids (HPFAs), or terrestrial markers 18:2n-6 and 18:3n-3, have significant inputs when their contribution is larger than 2.5 % of the total identified fatty acids (TFA; Budge & Parrish, 1998). The blue mussels contained substantial amounts of 20:5n-3 and 22:6n-3, indicating that phytoplankton was an important food source in their diets, and high levels 20:4n-6 that was suggested to be due to selective retention in response to shifting food availability on rocky shores (Budge et al., 2001; Freites et al., 2002). Phytoplankton food is important in the provision of the EFAs to coastal suspension-feeders, and by assessing temporal and regional changes in their contributions to consumers, information can be obtained regarding the dominant phytoplankton assemblages and the importance of this food source in the consumer diets.

Coastal rocky shore and pelagic marine food sources vary since pelagic systems are largely influenced by oceanic phytoplankton assemblages and rocky shores by an array of locally-produced and imported phytoplankton, detritus, and macroalgae (Frances & Guerrero, 2008). In addition, bacterial food sources (markers include *i*-15:0, 15:0, *i*-16:0, *i*-17:0, 17:0, *i*-18:0 and 18:1n-7) generally contribute substantially to suspension-feeder diets (Ackman & Hooper, 1973; Dalsgaard et al., 2003; Howell et al., 2003). Studies considering spatial and temporal trends in fatty acids of marine consumers can provide useful information on the contribution of dominant food sources to consumer diets, and the changes observed between regions and seasons. For example, fatty acid signatures in the copepod, *Pseudocalanus acuspes* from the Bornholm Basin in the Baltic Sea demonstrated strong seasonality (Peters et al., 2006). Copepod tissue fatty acids largely reflected seasonal food inputs, whereby during spring, high levels of diatoms and dinoflagellate markers were recorded, during summer substantial contributions of cyanobacteria, during autumn increasing 18:2n-6 and 18:3n-3 levels, and during winter high fractions of palmitic and stearic acids (Peters et al., 2006). However, large proportions of 18:1n-9 were recorded throughout all seasons, signifying the importance of ciliate food sources in copepod diets (Peters et al., 2006). Studying seasonal trends in fatty acid biomarkers can allow trophic ecologists to differentiate between the food sources that remain consistently important to rocky shore suspension-feeders and those that are seasonally utilised due to their availability.

Spatial and temporal variation in food sources, in addition to animal food preferences, can influence fatty acid signatures in consumers (Kharlamenko et al., 2001). Falk-Peterson et al. (2000) assessed the spatial and temporal patterns in fatty acid profiles of Arctic and Antarctic krill species and found that food preference, not seasonal trends in food sources, largely determined the fatty acid profiles. The carnivorous Arctic species *Thysanoessa longicaudata* and *Meganyctiphanes norvegica,* which largely fed on copepods, had high levels of the monounsaturated fatty acids (MUFAs) 20:1n-9 and 20:1n-11, while the Arctic *T. raschii* and Antarctic *Euphasia superba* showed significant ratios of 18:1n-9/18:1n-7, also identifying substantial animal carnivory (Falk-Peterson et al., 2000). The true polar herbivores *T. inermis* and *E. crystallorophias* contained large proportions of 18:4n-4 and 20:5n-3, indicating that phytoplankton was a major food source. In addition to phytoplankton, decaying algae from sea ice contributed to the diets of the herbivorous Arctic *T. raschii* and Antarctic *E. crystallorophias* in winter (Falk-Peterson et al., 2000). Similarly, spatial trends in the contribution of salt marsh detritus to consumers with different feeding modes in an intertidal sand flat in the Bay of Mont-Saint-Michel in France were assessed with the aid of fatty acid markers (Meziane et al., 1997). Salt marsh detritus was exported to the intertidal zone up to a distance of 2.4 km and was found in significant levels in the sediments and consumers (Meziane et al., 1997). However, the contributions of diatoms and bacterial fatty acids (BFAs) in the tissues of the consumers identified phytoplankton and bacterial detritus as important food sources for the filter- and deposit-feeders, respectively (Meziane et al., 1997).

Due to potential interspecific variability in fatty acid composition of organisms within the same trophic guild, and the variations in fatty acid signatures that can be obtained when assessing possible food sources, investigation into the feeding dynamics of rocky shore invertebrates is necessary. The aim of this chapter was to assess the spatial and temporal changes in fatty acid signatures of the barnacle *Tetraclita serrata*, mussel *Perna perna*, and polychaete *Gunnarea capensis* in the coastal marine environment adjacent to two contrasting estuaries in the Eastern Cape Province of South Africa. The study regions were adjacent to the freshwater-deprived Kariega Estuary (refer to Section 2.2.1.1) and freshwater-dominated Great Fish Estuary (refer to Section 2.2.1.2). As part of the main objectives for this chapter the following hypotheses were tested:

1) Since all three species fall within the same trophic guild (i.e. suspension-feeders) and are thus assumed to be subject to the same food quality and quantity, similar temporal and regional effects in the fatty acid profiles occur among the species.
2) Intraspecific fatty acid signatures for the barnacle, mussel and polychaete vary regionally due to the differences in freshwater inputs of the two systems (which influence the regional food sources).
3) Intraspecific fatty acid signatures for the barnacle, mussel and polychaete differ temporally as result of seasonal variation in the available food sources.
4) Given that the coastal region of the Eastern Cape is dominated by the south flowing Agulhas Current, suspension-feeders found downstream (south) of the estuary mouth demonstrate a greater dependence on terrestrially-derived food sources than those recorded upstream (north) of the two estuaries. As a result, suspension-feeders located south of an estuary mouth with greater terrestrial sources in their diets should have larger contributions of terrestrial plant markers (18:2n-6 and 18:3n-3) compared to those consumers situated north of the estuary mouth.

2.2 Materials and Methods

2.2.1 Study estuaries

The Kariega and Great Fish Estuaries are positioned on the south coast of South Africa within the Eastern Cape Province at 33°40’55’’S, 26°41’04’’E and 33°29’48’’S, 27°08’01’’E, respectively (Figure 2.1). Both river systems are permanently open to the ocean. The Kariega Estuary is a freshwater-deprived and well-mixed marine dominated system, while the Great Fish River Estuary system receives sustained freshwater inflow, demonstrates distinct longitudinal gradients in salinity, and has a stratified water column (Grange et al., 2000; Vorwerk, 2006). The main features differentiating these river systems are the size of their catchments, their tidal to river volume ratios influencing the amount of freshwater within each system, and the presence or absence of littoral macrophytes and salt marshes (Jennings, 2005; Vorwerk, 2006). Since the sampling sites were mainly situated in the nearshore coastal environments adjacent to the estuaries, reference to the geographic locations will be referred to as the Kariega and Great Fish regions.

2.2.1.1. Kariega Estuary

The warm temperate Kariega Estuary has a small catchment size of approximately 680 km^2, an average length of ~18 kilometres and width of ~100 metres in the lower reaches, narrowing down to a channel of about 40 to 60 metres towards the upper reaches (Hodgson, 1987) (Figure 2.1). The tidal to river volume ratio is estimated at 106:1, resulting in an almost uniform salinity of ~35 ‰. Hypersaline conditions have been recorded in the upper reaches of the system during the dry months when evaporative losses exceeded river inflows (Bate et al., 2002). Richoux and Froneman (2007; 2008) recorded the average maximum temperatures for summer and winter of ~29 °C and ~15 °C, respectively. The limited freshwater input into the estuary is related to sporadic rainfall, the presence of several impoundments along the length of the Kariega River, and the relatively small catchment area. The intertidal banks of the estuary remain undisturbed by encroachment and farming enterprises (Froneman & McQuaid, 1997; Paterson & Whitfield, 1997).

Floristically, the Kariega Estuary is dominated by the eelgrass *Zostera capensis* and cord grass *Spartina maritima*, which are found interspersed with bare mud and sand patches along its intertidal banks (Paterson & Whitfield, 1997; Richoux & Froneman, 2008). The middle and lower reaches of the estuary have benthic algal mats colonising the sediments with few macrophytes (Vorwerk, 2006). This region has salt marshes comprising the plants *Sarcocornia perennis* and *Chenolea diffusa*.

As result of the overall stability of the Kariega Estuary, there appears to be limited seasonal effects on the food web structure within the system, and producers generally show little isotopic variation within the lower reaches (Richoux & Froneman, 2007). The total chlorophyll concentrations in the Kariega Estuary are low (<0.5 mg chl-*a* l^{-1}), generally reflecting the slow phytoplankton growth rates conferred by limited macronutrient availability due to the reduced freshwater inflow (Paterson & Whitfield, 1997). The general water transparency is high within the system (surface turbidity ~6.9 NTU, Grange et al., 2000).

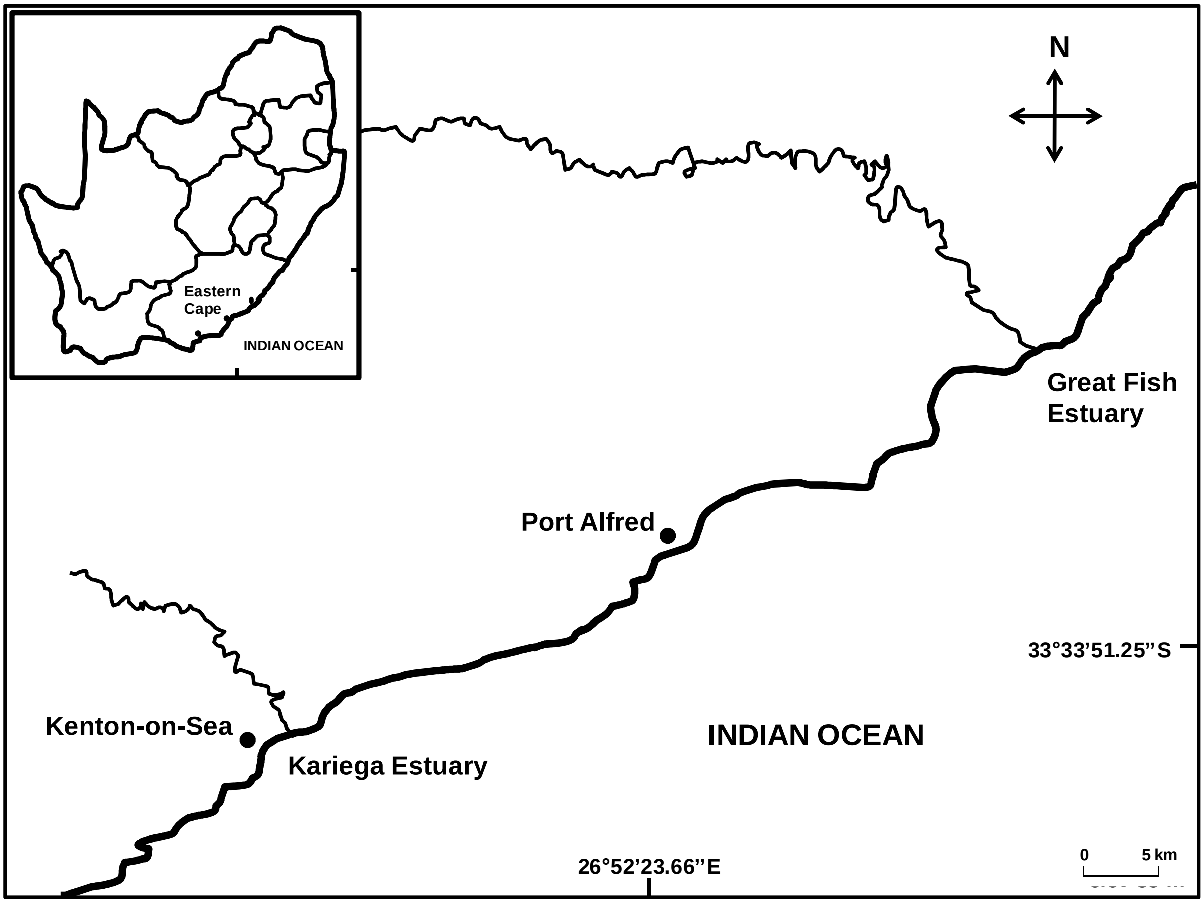

Figure 2.1 The location of the Eastern Cape Province of South Africa. The Kariega and Great Fish Estuaries and associated nearshore regions are situated within a 25 kilometre radius north and south of the coastal town, Port Alfred.

Seston, or suspended particulate material (SPM; average 39 to 76 mg l^{-1}), in the estuary is largely comprised of detritus, phytoplankton, inorganic particles, bacteria and other microorganisms (Grange & Allanson, 1995). The organic fraction of the SPM, or particulate organic matter (POM; average 11 to 23 mg l^{-1}), is remarkably reduced during winter but represents an important food source for estuarine suspension-feeders (Grange et al., 2000;

Richoux & Froneman, 2007). Due to the shallow nature and enhanced stability of the system, the SPM is uniformly distributed along the length of the estuary, with a pronounced increase in concentrations during summer (Allanson & Read, 1995). The predominant size class of the suspended matter (SPM and POM) and chlorophyll-*a* within the Kariega Estuary falls within the nanoplankton size class (2-20 µm; Allanson & Read, 1995).

2.2.1.2 Great Fish Estuary

The Great Fish Estuary has a large catchment system, ~30,400 km^2, which enters the Indian Ocean between the cities of East London and Port Elizabeth (Figure 2.1). Due to its substantial freshwater input, the estuary is classified as a river dominated meso-eutrophic system with significant terrestrial influences (Jennings, 2005; Vorwerk, 2006). The interbasin transfer of water from the Orange River to the Great Fish River system augments the freshwater input to the system. The hydrodynamic conditions are determined by channel morphology and catchment management procedures directing water release from the Gariep Dam (Bate et al., 2002). The upper reaches of the estuary are particularly freshwater dominant, entering a river-sea mixing zone along the middle reaches with strong marine interactions present in the lower region (Grange et al., 2000).

The Great Fish Estuary has an estimated length of ~12 kilometres and a narrow head water region that broadens to approximately ~180 metres in width in the lower reaches (Vorwerk et al., 2001). Maximum temperatures during summer and winter months are recorded as ~30 °C and ~11 °C, respectively (Grange et al., 2000). Grange and Allanson (1995) noted that geological leaching causes increased salt concentrations; however, these are masked by the large freshwater inputs to the estuary, which in accordance with the highly erodible catchment soils, causes elevated water turbidity levels (surface turbidity ~65.2 NTU, Grange et al., 2000). In addition, the shallow nature of the estuary in the middle to upper reaches is responsible for large amounts of silt deposition, thus forming vast intertidal mudbanks. Vorwerk et al. (2001) identified the Great Fish Estuary as having sparse aquatic vegetation with no submerged macrophytes. A few supratidal salt marshes are located within the extreme western lower reaches, and sporadic sedges and reeds are found along the banks of the estuary (Allanson & Read, 1987; Grange et al., 2000).

During the ebb tides, large concentrations of suspended sediment are associated with increased SPM levels and water turbidity, and as such, vast amounts of terrestrially-derived matter are transported to sea (Grange et al., 2000). Similarly, the net movement of terrestrial materials downstream has restricted the distribution of marine sediments locally to the estuary mouth (Grange et al., 2000). Seston concentrations are much higher compared to the Kariega Estuary, and the dominant organic fraction mostly comprises phytoplankton within the nanoplankton (2-20 µm) and larger micro-phytoplankton (>20 µm) size-ranges (Grange & Allanson, 1995; Froneman, 2000).

Due to the continuous supply of nutrients, elevated phytoplankton biomass has been recorded within the system. Jennings (2005), however, recorded increased turbidity within the system mainly caused by allochtonous inputs of detritic matter, and consequently phytoplankton production was generally limited within the Great Fish Estuary. Periodically though, high phytoplankton biomass following sudden large freshwater pulses is evident due to phytoplankton accumulation from the river inflows associated with the hydrodynamic trapping from the marine environment (Grange & Allanson, 2000; Vorwerk, 2006). Average values for SPM (126 to 509 mg l^{-1}), POM (29 to 76 mg l^{-1}) and chlorophyll-*a* concentrations (0.4 to 21.8 µg l^{-1}) are generally higher than those recorded in the Kariega Estuary (Grange et al., 2000; Vorwerk et al., 2001).

2.2.2 Sample collection

Sampling was conducted in 2009 during austral summer (March) and winter (July) to allow for temporal comparisons. Five sampling sites were selected at both the Kariega (Figure 2.2) and Great Fish regions (Figure 2.3). Two sites north (Site 1, most northern; Site 2, just north of mouth) and two sites south (Site 3, just south of mouth; Site 4, most southern) of each estuary mouth were sampled in each region during both seasons. Site 2 was located within ~0.5 kilometres from the estuary, while Sites 1, 3 and 4 were sampled at a distance of ~1-1.5 kilometres upstream and downstream of the estuary mouth. One site was situated inside the estuary mouth in each region (Site 5).

Due to the marine dominance of the Kariega Estuary, the water samples for Site 5 were obtained from the upper reaches of the estuary to represent an estuarine signal. For the Kariega region, Site 5 was therefore divided into two sub-sites: Site 5a (estuary mouth) and Site 5b (upper reaches). Physico-chemical data were collected at each site with a YSI-meter (temperature, °C; and dissolved oxygen, mg l^{-1}) and refractometer (salinity, psu). Triplicate water samples of 500 ml each were collected at each site for the determination of total chlorophyll-*a* (chl-*a*, µg l^{-1}) and SPM/POM concentrations (mg l^{-1}). All samples were stored in a cooler box with ice blocks during transport to the laboratory in Grahamstown. Additional water sample collections at each site comprised single five litre aliquots each for determination of SPM (mg l^{-1}) fatty acid signatures for the Kariega and Great Fish regions (*n*

= 5 per region). All filters were visually inspected after water filtration to remove any zooplankton or debris.

Data on the different hydrology regimes or daily average flow rates (m^2 s^{-1}) were obtained from the Department of Water Affairs and Forestry (http://www.dwaf.gov.za/hydrology). The Smithfield (P2H001; 33°33’08’’S and 26°36’07’’E) and Matomela (Q9H018; 33°14’16’’S and 26°29’42’’E) stations were used for proxies of the flow data from the Kariega and Great Fish Estuaries, respectively. Sampling of the three indigenous target organisms, the barnacle *Tetraclita serrata*, the mussel *Perna perna* and the polychaete *Gunnarea capensis*, was as follows. A preliminary study was done to determine the number of individuals needed per species to make up the required total dry mass (i.e. 20 to 100 mg) necessary for fatty acid determination (Parrish, 1999). These results indicated that a single mussel, ten barnacles and five polychaetes were each sufficient for one fatty acid sample. At each site, haphazardly selected patches were chosen where three replicates each of ten barnacles, one mussel, and five polychaetes were collected, totalling 12 to 15 samples per organism for each study region. No macroalgae were collected as biomarker data already existed for the dominant species in the regions (Richoux & Froneman, 2007; 2008; Allan et al., 2010). Due to a rapid tidal shift experienced during the summer (March) sampling event at the Great Fish Estuary, no mussels could be collected at the most northern site (Site 1).

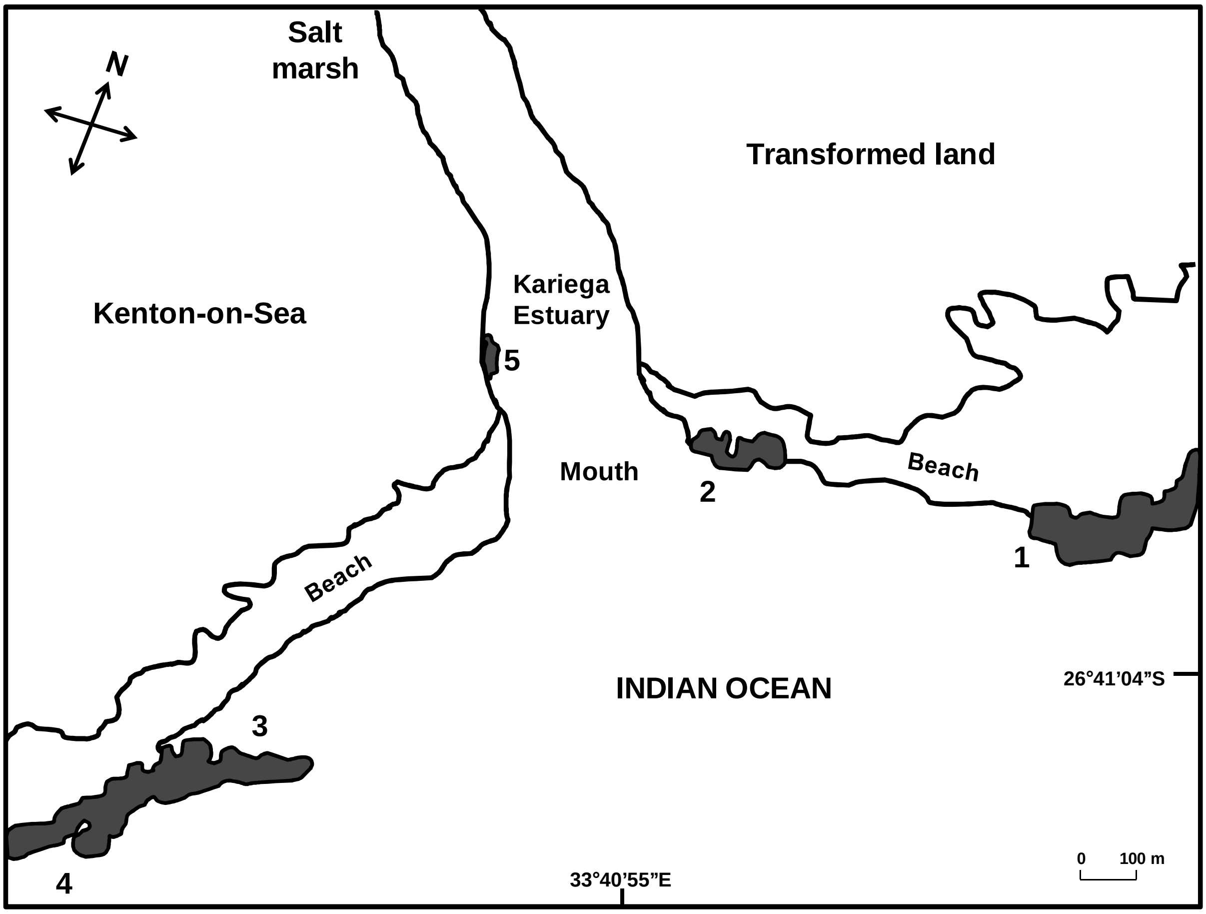

Figure 2.2 The Kariega Estuary mouth with positions of the five sample sites indicated along the coastal rocky shore. Shaded areas represent rocky outcrops where animals and water samples were collected. Sites 1 and 2 correspond to the northern sites and Sites 3 and 4 to the southern sites. Site 5 represents an estuarine location. Barnacles were the only suspension-feeders found at Site 5.

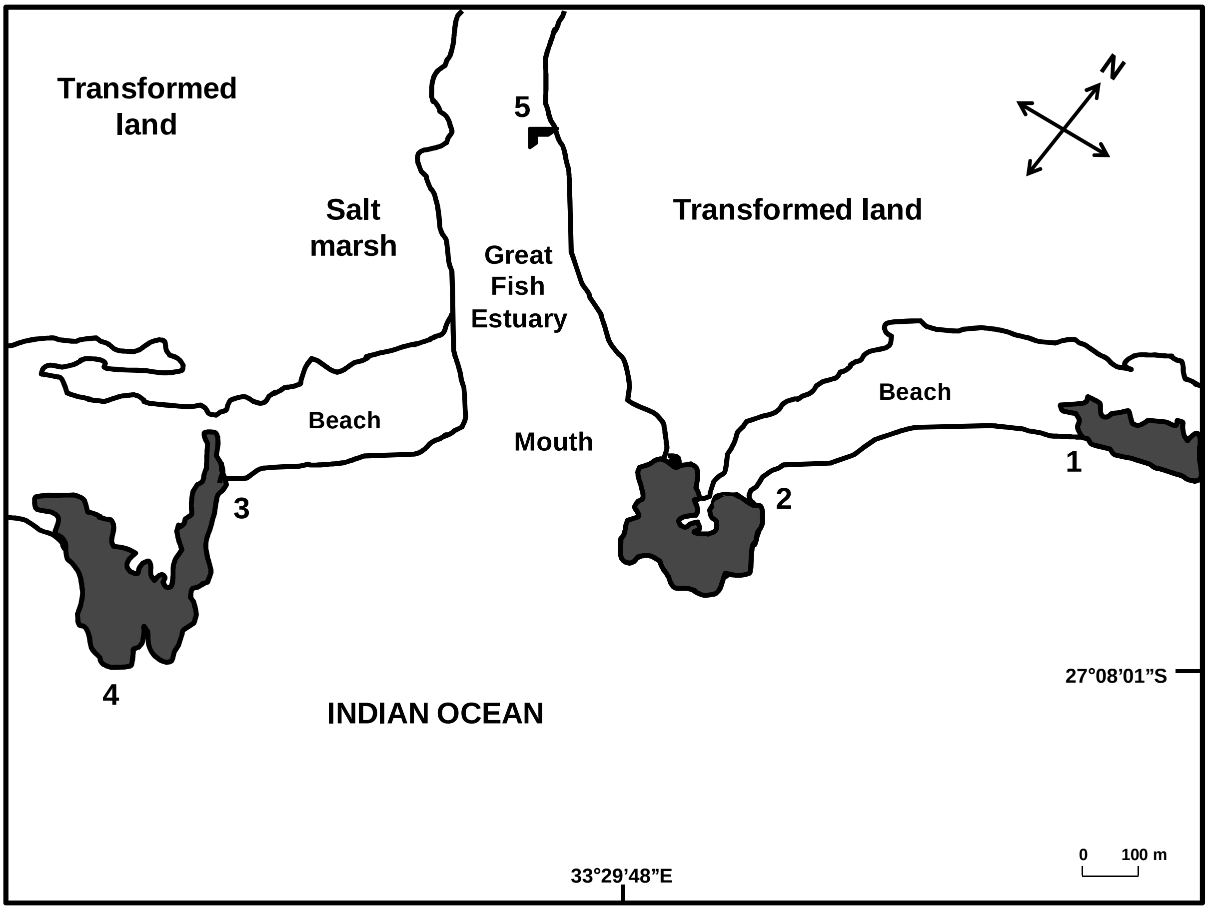

Figure 2.3 The Great Fish Estuary mouth with positions of the five sample sites indicated along the coastal rocky shore. Shaded areas represent rocky outcrops where animals and water samples were collected. Sites 1 and 2 correspond to the northern sites and Sites 3 and 4 to the southern sites. Site 5 represents an estuarine location. Due to the nature of the water salinity no animal samples were found at Site 5.

2.2.3 Sample preparation

2.2.3.1 Physico-chemical parameters

All water samples were filtered (<5 cm Hg vacuum filtration) onto pre-combusted (~450 °C, six hours) and pre-weighed glass fibre filters (GF/F Whatman, 47 mm), after which they were dried at 50 °C in an oven for 24 hours and weighed to determine SPM dry mass (DM, mg l^{-1}). Subtraction of initial filter weight from the ash mass following ignition at 450 °C for 12 hours allowed for estimation of the organic fraction dry mass of POM (mg l^{-1}).

An adjusted method from Parsons et al. (1984) was used for total chl-*a* concentration determination. Chl-*a* concentrations were estimated by submersion of the filtered samples in 8 ml of 90 % acetone and kept overnight in a refrigerator. Two drops of hydrochloric acid (HCl) were added to the extraction tubes and shaken. Before and after acidification readings were taken with a Turner Designs 10AU Fluorometer, and chl-*a* concentration was determined with the following formula (Lorenzen & Jeffrey, 1980):

$$\text{Chl-}a\ (\mu g\ l^{-1}) = 0.325 \times (\text{reading prior} - \text{reading post HCl}) \times [(\text{volume acetone}) / (\text{volume water filtered})]$$

2.2.3.2 Fatty acid signatures

Animals were kept alive and aerated for one day to allow gut clearing prior to processing. Dissection of all three organisms was done on ice with the aid of a dissecting microscope, and different tissues for each species were selected and excised. For barnacles, the whole body except the gut and seminal vesicles were kept for analysis; for mussels, the adductor muscle and mantle margin were kept (Hill & McQuaid, 2008; Shin et al., 2008); and for polychaetes the entire body (Jacob et al., 2005). The various tissues were selected based on studies that have indicated certain components, such as muscle, to closely reflect diet (Tieszen et al., 1983; Thompson et al., 2008).

All materials used in the preparation of tissue samples for fatty acid determination were lipid-cleaned prior to and during processing. Clean glass tubes were ashed at 450 °C for a minimum of four hours in a muffle furnace. Tube caps were cleaned with three consecutive rinses each of methanol (MeOH) followed by chloroform ($CHCl_3$). Grinding and separation utensils were also lipid cleaned following the same MeOH-$CHCl_3$ procedure between each sample.

Each tissue sample was placed inside a separate foil packet, and all samples lyophilized with a VirTis BenchTop K Freeze Dryer at -60 °C for a minimum of 24 hours to remove moisture while maintaining the integrity of the lipid components (Garrett & Grisham, 1999). All animal tissues were homogenised with mortar and pestle. After lyophilisation and homogenisation, tissues destined for fatty acid analysis were weighed on a microbalance prior to transferring into glass tubes, and covered with 2 ml of $CHCl_3$ containing 0.01 % butylated hydroxytoluene, topped with nitrogen gas and sealed with teflon tape. All samples were kept in a freezer at -20 °C until further processing, or at -80 °C for long-term storage.

A method modified from Folch et al. (1957) was used for the lipid extractions and from Budge et al. (2006) for the fatty acid methyl ester (FAME) synthesis procedure. For lipid extraction, 1 ml ice-cold MeOH and 1 ml 2:1 ($CHCl_3$:MeOH, *v:v*) solutions were added to each sample, after which samples were vortexed and sonicated for four minutes in an ice bath. Test tubes were topped with nitrogen, capped and stored for 24 hours at -20 °C. A small plug of cotton wool was inserted into the top of glass Pasteur pipettes. Sample contents were transferred to clean tubes through the cotton wool plugged pipettes, and rinsed twice with 1 ml 2:1 ($CHCl_3$:MeOH) solution (total solvent volume 6 ml). A 1.5 ml solution of 0.9 % potassium chloride (KCl) was added to each sample, and the top layer discarded after centrifugation. An additional 0.5 ml KCl and 0.5 ml MeOH were added to the tubes and the top aqueous phase discarded a second time. Anhydrous sodium sulphate (Na_2SO_4) was added to each extract and transferred to a third test tube, and the remaining organic phase concentrated down to dryness under a moderate stream of nitrogen. Samples were covered in 1.5 ml Na_2SO_4-dried dichloromethane, covered with nitrogen, sealed with teflon tape and stored at -20 °C until FAME synthesis.

Prior to FAME synthesis, an internal standard (tricosanoic acid; 23:0) in known quantities was added to all samples to allow for the quantification of FAMEs. All extracts received 3 ml of Hilditch reagent (1.5 ml concentrated sulphuric acid added to Na_2SO_4-dried MeOH) and were placed in an oven at 100 °C for 1 hour. After the tubes were cooled to room temperature, 3 ml of hexane and 1 ml of MilliQ water were added, and the top layer transferred to a second lipid clean tube after centrifugation for five minutes. The hexane extraction was repeated twice using 1 ml of hexane each time, following the addition of 2 ml of MilliQ to the pooled extract. After two minutes of centrifugation, the top layer was pipetted into a third tube and dried with Na_2SO_4, after which the extracts were transferred to 2 ml vials, concentrated to dryness under nitrogen and covered in 0.5 ml hexane. All vials were topped with nitrogen, capped and sealed with teflon tape until injection into a gas chromatogram (GC) fitted with a flame ionization detector (splitless injections). Analyses of the FAMEs were conducted using a Hewlett Packard 5890A Series II GC fitted with ZB-Waxplus 320 column (bonded and cross-linked 78 % cyanopropyl methylpolysiloxane fused silica capillary) and helium as the carrier gas. One microlitre (1 µl) samples were manually injected at 250 °C and the flame ionisation detector set at 260 °C. Oven temperature was 150 °C for five minutes, and raised at the rate of 2.5 °C min^{-1} to 225 °C and held for five minutes. All fatty acid peaks were processed with Clarity 2.6 and quantified with the aid of comparisons to the known internal standard (23:0) peaks and external standards (marine PUFA no. 1 and 37 component FAMEs mix, Supelco).

Fatty acids were reported in the technical notation as prescribed by the International Union of Pure and Applied Chemistry (IUPAC) as X:An-*X*, where X is the number of carbon bonds within the molecule, A the total number of double bonds and *X* the position of the first double bond from the terminal methyl carbon, n. Each fatty acid was reported quantitatively as µg fatty acid mg^{-1} dry mass (DM) and proportionally as a percentage of the total identified fatty acids (% TFA).

2.2.4 Data analyses

For the physico-chemical parameters, the mean (± standard deviation) values for temperature (°C), salinity (psu), dissolved oxygen (mg l^{-1}), SPM and POM (mg l^{-1}) and chl-*a* (µg l^{-1}) were measured at both geographic locations (Kariega and Great Fish regions) and seasons (summer and winter). Multivariate analysis of variance (MANOVA followed with a Tukey post hoc test, when applicable) was conducted separately for each region to test for significant differences among sites and seasons (independent variables) for SPM, POM and chl-a concentrations (dependent variables). MANOVA was also applied to the proportions of diatom (16:1n-7 and 20:5n-3) and dinoflagellate (22:6n-3) biomarkers (dependent variables) in SPM, and to test for seasonal and regional changes (independent variables) in the SPM and animal fatty acid groups (i.e. SFAs, MUFAs, PUFAs, BFAs, HPFAs and EFAs; n = 4 or 5 per region and season; dependent variables) in the two study regions in March and July.

All quantitative data (µg fatty acid mg^{-1} dry mass) were natural-logarithm transformed and checked for normality with a Shapiro-Wilk test. Proportional data were arc sine square-root transformed. Separate analyses were done to distinguish between the total identified fatty acids (TFA) as well as those that comprised more than one percent of the TFA. As similar temporal and spatial patterns emerged from both sets of data, fatty acids that comprised >1 % TFA were used in analyses. Principal Component Analysis (PCA; covariance matrices) of both the quantitative and qualitative fatty acid data was performed to determine intraspecific similarities at the separate regions and sites. Two separate PCAs of the combined SPM data, and consumer signature data, were also used to examine broad-scale differences between the regions and sample times. Non-parametric models were used (i.e. non-metric multidimensional scaling; n-MDS) to examine the SPM and consumer data prior to the PCAs; however, similar patterns were observed in both the n-MDS and the PCA results. Although the n-MDS was more appropriate to represent the regional and temporal variations in SPM and consumer data as result of small sample sizes (i.e. small number of samples relative to variables), the PCA plots are presented as they are visually superior (PCA loadings can be superimposed). As PCA of the quantitative (µg fatty acid mg^{-1} dry mass) and proportional data (% TFA) revealed similar patterns, only the percentage data are presented here (see Appendix 2.8 for information on quantitative data).

Multivariate analysis of variance (MANOVA) or Kruskal-Wallis analysis of variance (H) was applied to the PCA scores data, and principal components (PC) -1 and/or -2 were assigned as dependent variables, while site and/or date were used as independent variables (significance level at $P < 0.05$). Fatty acids with loadings >2 % were considered influential to the observed PCA groupings. Ellipses were placed around significantly different groups, as determined by Tukey multiple comparisons or Kruskal-Wallis ranked sum comparisons tests. A Levene's test for homogeneity was used in all cases. Where Tukey multiple comparisons tests of PCA scores for suspension-feeder tissue fatty acid composition revealed no distinctions among sites within each region or sample date, data from the northern and southern sites were pooled and assessed for potential north *versus* south distinctions with independent t-tests ($n = 6$ per region and season).

Several methods were used to conduct statistical testing for differences within and among the SPM and consumer groups of the combined data PCAs. These methods included SIMPER analysis of average similarity within- and dissimilarity among groups, non-parametric Bray-Curtis dissimilarity measures, and non-parametric Kruskal-Wallis analysis of variance. All of the statistical methods gave similar results for the SPM and consumer fatty acids in the Kariega and Great Fish regions in summer and winter. The fatty acids that were identified by the PCA loadings of the SPM and consumer plots to contribute >2 % (thus observed as influential to the groupings), were the same as those identified by SIMPER as contributing most to the dissimilarity detected among the different groups. Based on these results, the PCAs were used to visually represent the combined SPM data and consumer signatures, and the Kruskal-Wallis test results chosen to report the significant groupings. Analyses were conducted with the use of Primer (v5), SYSTAT (v12.0) and PAST 1.42 (Hammer et al., 2001).

2.3 Results

2.3.1 Environmental variables

The daily average flow rates for the Kariega and Great Fish Estuaries in summer were 0.008 and 14.475 $m^2 s^{-1}$, respectively (Table 2.1). During winter, the daily average flow rates were 0.005 and 5.109 $m^2 s^{-1}$ for the Kariega and Great Fish Estuaries, respectively (Table 2.1). Seawater temperatures ranged from 17.7 to 26.5 °C in March and from 13.9 to 17.9 °C in July in the Kariega region (Table 2.1). During July and March, hypersaline conditions were recorded in the upper reaches of the Kariega Estuary (Site 5b: up to 44 psu), whereas reduced salinities were recorded in the lower mouth area of the Great Fish region (Site 5: 10

and 28 psu for March and July, respectively). The particulate matter concentrations were higher in both regions in March (Kariega: SPM 34.8 ± 9.9 and POM 5.8 ± 0.9 mg l^{-1}; Great Fish: SPM 96.6 ± 74.3 and POM 9.5 ± 4.9 mg l^{-1}) compared to July (Kariega: SPM 26.9 ± 5.6 and POM 4.9 ± 1.0 mg l^{-1}; Great Fish: SPM 32.8 ± 7.3 and POM 7.2 ± 5.0 mg l^{-1}). The total chl-*a* concentrations were low during both sample dates in both regions (Kariega: 0.2-0.4 µg l^{-1}; Great Fish: 0.1-0.7 µg l^{-1} Table 2.1).

Multivariate ANOVA indicated that the biological variables were not significantly different among sites or between July and March in the Kariega region (Levene's $P > 0.001$; SPM: $F_{1,9} = 1.714$, $P = 0.151$; POM: $F_{1,9} = 1.973$, $P = 0.099$; Chl-a: $F_{1,9} = 1.303$, $P = 0.296$). Although in general chl-*a* values were low in the Great Fish region, significant seasonal differences were observed for the SPM and chl-*a* concentrations (Levene's $P > 0.001$; SPM: $F_{1,9} = 2.760$, $P < 0.05$; POM: $F_{1,9} = 1.152$, $P = 0.375$; Chl-a: $F_{1,9} = 3.513$, $P < 0.05$; Tukey: $P < 0.001$ between March and July surveys).

Table 2.1 Water characteristics in the Kariega and Great Fish regions at Sites 1 to 5 (Site 1, most northern; Site 2, just north of mouth; Site 3, just south of mouth; Site 4, most southern; Site 5, mouth). SPM, suspended particulate matter; POM, particulate organic matter; DM, dry mass; AFDM, ash free dry mass; Chl-*a*, chlorophyll-*a*; SD, standard deviation. Daily average flow rate data were obtained from the Department of Water Affairs and Forestry, measured at the Smithfield and Matomela stations for the Kariega and Great Fish Estuaries, respectively.

	Kariega Region					Great Fish Region				
March 2009	Site 1	Site 2	Site 3	Site 4	Site 5b	Site 1	Site 2	Site 3	Site 4	Site 5
Water temperature (°C)	18.2	19.6	17.7	18.6	26.5	15.6	14.3	16.6	13.6	21.5
Salinity (psu)	35	35	35	36	41	38	35	27	35	10
Dissolved oxygen (mg l^{-1})	8.3	7.5	8.2	8.6	3.7	8.7	8.3	7.9	9.8	6
SPM (DM ± SD, mg l^{-1})	40.9 ± 7.0	29.4 ± 1.7	29.8 ± 5.3	29.6 ± 1.1	48.7 ± 19.3	216.9 ± 4.4	29.9 ± 1.1	110.9 ± 24.1	27.5 ± 1.8	98.0 ± 46.3
POM (AFDM ± SD, mg l^{-1})	6.0 ± 0.6	5.5 ± 0.5	5.4 ± 1.6	5.5 ± 0.3	6.6 ± 1.6	14.2 ± 0.6	5.2 ± 0.2	8.1 ± 0.9	5.0 ± 0.5	15.1 ± 4.7
Chl-*a* (µg l^{-1})	0.3 ± 0.3	0.2 ± 0.1	0.2 ± 0.1	0.2 ± 0.2	0.2 ± 0.1	0.3 ± 0.2	0.1 ± 0.1	0.3 ± 0.2	0.4 ± 0.3	0.1 ± 0.2
Daily avg flow rate (m^2 s^{-1})			0.008					14.475		
July 2009										
Water temperature (°C)	17.9	17.5	17.6	17.6	13.9	17.4	17.1	17.4	17.4	15.1
Salinity (psu)	38	38	38	38	44	38	38	38	38	28
Dissolved oxygen (mg l^{-1})	6.2	6.6	6.1	6.5	4.5	6.5	6.5	6.2	6.7	4.7
SPM (DM ± SD, mg l^{-1})	23.3 ± 3.0	31.4 ± 5.0	24.7 ± 1.3	18.5 ± 0.0	30.7 ± 5.1	27.4 ± 2.9	26.0 ± 0.0	30.3 ± 3.5	44.5 ± 7.0	36.2 ± 0.8
POM (AFDM ± SD, mg l^{-1})	4.2 ± 0.6	6.4 ± 1.2	4.6 ± 0.2	3.8 ± 0.0	5.1 ± 0.5	4.8 ± 0.3	4.5 ± 0.0	7.6 ± 4.3	14.2 ± 8.3	5.0 ± 0.2
Chl-*a* (µg l^{-1})	0.2 ± 0.0	0.2 ± 0.1	0.3 ± 0.1	0.4 ± 0.1	0.2 ± 0.1	0.7 ± 0.3	0.3 ± 0.1	0.4 ± 0.1	0.1 ± 0.0	0.0 ± 0.0
Daily avg flow rate (m^2 s^{-1})			0.005					5.109		

2.3.2 Fatty acid signatures

Thirty-five fatty acids were measured at concentrations >1 % TFA in March, whereas twenty-nine were detected in July. These data represent 1 % TFA in at least one sample of SPM (Appendix 2.1), barnacle *T. Serrata* (Appendices 2.2 and 2.3), mussel *P. perna* (Appendices 2.4 and 2.5) or polychaete *G. capensis* tissues (Appendices 2.6 and 2.7).

2.3.2.1 Suspended particulate matter

The SPM samples contained large proportions of saturated fatty acids (SFAs; 35.7-59.4 % TFA) and monounsaturated fatty acids (MUFAs; 31.3-53.8 % TFA) in both regions during March and July (Appendix 2.1). No changes were observed in the SFA levels ($F = 0.367$, $P > 0.05$, $df = 3,16$), although the MUFAs were significantly greater during both sample times in the Great Fish region (32.1-53.8 % TFA; $F = 3.970$, $P < 0.05$, $df = 3,16$) compared to the Kariega region (31.4-46.2 % TFA; Appendix 2.1). The polyunsaturated fatty acids (PUFAs; 3.1-23.4 % TFA) showed no distinct patterns during both seasons in the Kariega and Great Fish regions ($F = 2.545$, $P > 0.05$, $df = 3,16$; Appendix 2.1).

Bacterial fatty acids (BFAs) occurred in substantial fractions in the Kariega (March: 5.1-11.6 % TFA; July: 0.1-8.0 % TFA) and Great Fish regions (March: 0.1-7.4% TFA; July: 5.4-10.5 % TFA), and varied significantly between March and July in both regions ($F = 3.385$, $P < 0.05$, $df = 3,16$). The essential fatty acids (EFAs) 20:4n-6, 20:5n-3 and 22:6n-3 were present in larger proportions during July in both regions (Kariega: 3.8-9.1 % TFA; Great Fish: 2.6-8.3 % TFA; $F = 6.516$, $P < 0.01$, $df = 3,16$; Appendix 2.1). Proportions of higher plant fatty acids (HPFAs) were significantly different between the Kariega and Great Fish regions, and between March and July in the Great Fish region ($F = 8.867$, $P < 0.01$, $df = 3,16$; Appendix 2.1). The Kariega region experienced negligible HPFA inputs during July and March (0.0-2.2 % TFA), except for the site in the direct vicinity of the estuary mouth (Site 2; 8.9 % TFA) in March (Appendix 2.1). The Great Fish region showed significant HPFA influences during March (3.6-6.7 % TFA) and July (4.9-14.3 % TFA; Appendix 2.1).

The diatom marker 16:1n-7 occurred in substantial proportions in SPM during both sample periods in the Kariega (2.9-7.7 % TFA) and Great Fish regions (3.6-8.3 % TFA), and showed the greatest contributions of all biomarkers in March (Table 2.2; Figure 2.4). The dinoflagellate marker 22:6n-3, however, was present in small proportions during March (0.9 ± 1.6 % TFA) and July (0.5 ± 1.2 % TFA), and was negligible during July in the Great Fish region (0.0 % TFA, Figure 2.4). A significant difference between sample dates in the proportions of 20:5n-3 ($F = 16.905$, $P < 0.001$, $df = 3$) was recorded in both regions (Figure

2.4), and increased significantly during July in the Kariega (1.4 ± 0.5 to 4.6 ± 3.3 % TFA) and Great Fish regions (0.1 ± 0.0 to 4.6 ± 2.4 % TFA; Figure 2.4).

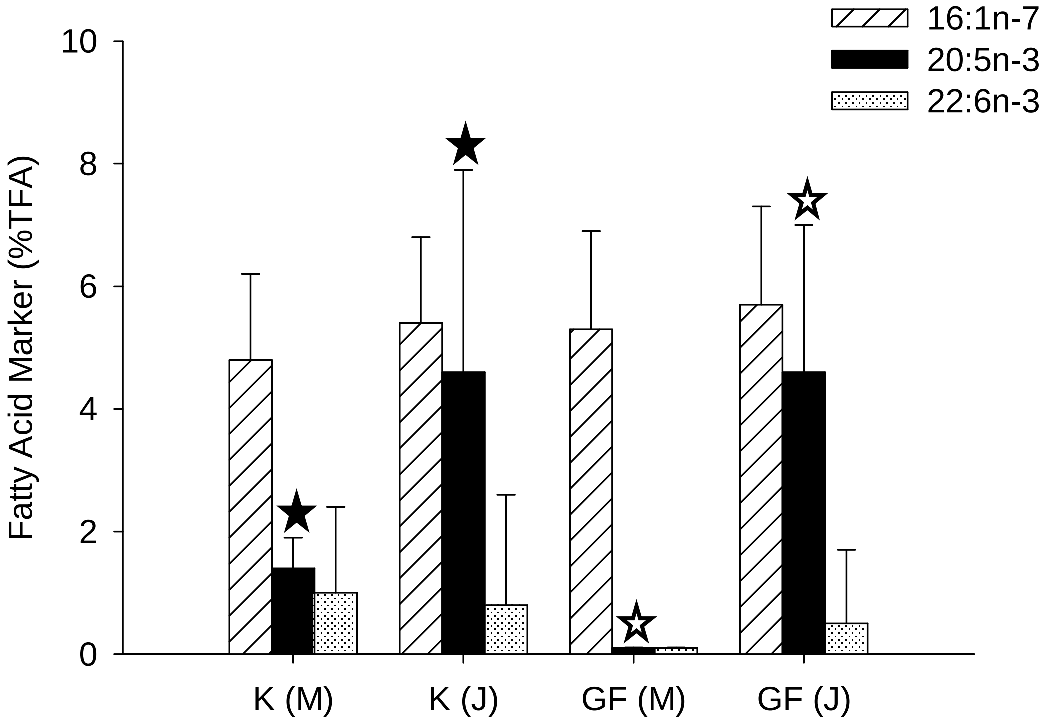

Figure 2.4 Proportions of diatom (16:1n-7 and 20:5n-3) and dinoflagellate (22:6n-3) biomarkers in SPM in the two study regions (Kariega and Great Fish) in March and July. Values are presented as means ± standard deviation for arcsine-transformed proportional data. Data were pooled for all five sites per region ($n = 5$). Multivariate analysis of variance on SPM data revealed a significant temporal distinction in the diatom marker 20:5n-3 proportions, between March and July in both regions (indicated by stars). K, Kariega; GF, Great Fish; M, March; J, July.

The PCA scores of the proportional fatty acids in SPM showed significant changes between sample times in the Kariega and Great Fish regions ($H = 14.223$; $P < 0.01$; $df = 3$; Figure 2.5). A significant spatial difference in SPM fatty acids was evident between the Kariega and Great Fish regions in March only ($H = 14.223$; $P < 0.01$; $df = 3$; Figure 2.5). Three distinct SPM groupings in fatty acid signatures were identified, including the Kariega signatures in March, the Great Fish signatures in March and the signatures of both regions in July (Figure 2.5). The principal components (PC) -1 and -2 explained much of the regional and seasonal variance in the data (Figure 2.5). The Kariega and Great Fish SPM signatures (79 % variance; PC-1: 60 % and PC-2: 19 %) were characterised by the influential fatty acids 16:0, 18:0, 18:1n-7, 20:1n-7, 20:1n-9 and 20:2n-6 on PC-1 and by 18:1n-7, 18:n1-9, 20:1n-7 and 20:2n-6 on PC-2 (Figure 2.5).

Table 2.2 Temporal changes in diatom (16:1n-7 and 20:5n-3) and dinoflagellate (22:6n-3) fatty acid markers (% TFA) in the suspended particulate matter (SPM) and tissues of the barnacle (*Tetraclita serrata*), mussel (*Perna perna*) and polychaete (*Gunnarea capensis*) in the Kariega and Great Fish regions. Values are presented as means ± standard deviation for replicated data; - no data.

		March 2009						July 2009					
Kariega	Site	16:1n-7	20:5n-3	22:6n-3	16:1n-7 /16:0	22:6n-3 /20:5n-3	20:5n-3 /22:6n-3	16:1n-7	20:5n-3	22:6n-3	16:1n-7 /16:0	22:6n-3 /20:5n-3	20:5n-3 /22:6n-3
SPM	1	4.3	1.4	2.9	0.2	2.0	0.5	4.0	4.0	4.0	0.0	1.0	1.0
	2	4.5	2.2	0.0	0.2	0.0	-	7.7	3.8	0.0	0.3	0.0	-
	3	6.3	1.0	0.0	0.2	0.0	-	5.9	5.9	0.0	0.3	0.0	-
	4	6.3	1.1	0.0	0.3	0.0	-	4.5	9.1	0.0	0.2	0.0	-
	5	2.9	1.5	2.2	0.1	1.5	0.7	5.0	0.0	0.0	0.2	0.0	0.0
T. serrata	1	3.2 ± 0.9	12.5 ± 0.4	13.0 ± 1.4	0.2 ± 0.0	1.0 ± 0.0	1.0 ± 0.0	2.4 ± 0.3	19.5 ± 2.9	21.4 ± 3.4	0.1 ± 0.0	1.1 ± 0.0	0.9 ± 0.0
	2	4.7 ± 0.5	12.5 ± 0.3	15.6± 1.2	0.2 ± 0.0	1.3 ± 0.0	0.8 ± 0.0	2.4 ± 0.2	19.3 ± 1.1	23.7 ± 2.3	0.1 ± 0.0	1.2 ± 0.1	0.8 ± 0.0
	3	3.4 ± 0.8	16.2 ± 2.2	19.7 ± 5.7	0.2 ± 0.1	1.2 ± 0.1	0.8 ± 0.1	2.4 ± 0.2	19.4 ± 0.4	20.8 ± 1.3	0.1 ± 0.0	1.1 ± 0.1	0.9 ± 0.0
	4	4.6 ± 0.3	13.4 ± 1.4	13.7 ± 0.6	0.2 ± 0.0	1.0 ± 0.0	1.0 ± 0.0	1.8 ± 0.2	18.4 ± 4.1	22.1 ± 4.7	0.1 ± 0.0	1.2 ± 0.0	0.8 ± 0.0
	5	4.8 ± 0.6	12.7 ± 1.1	12.7 ± 1.4	0.2 ± 0.0	1.0 ± 0.1	1.0 ± 0.0	2.2 ± 0.4	15.4 ± 0.4	20.8 ± 0.8	0.1 ± 0.0	1.3 ± 0.0	0.7 ± 0.0
P.perna	1	9.3 ± 2.0	14.6 ± 1.7	11.3 ± 2.1	0.5 ± 0.0	0.8 ± 0.1	1.3 ± 0.0	4.6 ± 1.2	10.8 ± 2.1	13.1 ± 1.0	0.3 ± 0.1	1.2 ± 0.1	0.8 ± 0.1
	2	9.2 ± 1.6	11.7 ± 0.9	8.9 ± 2.0	0.4 ± 0.0	0.8 ± 0.1	1.3 ± 0.0	1.9 ± 0.4	9.3 ± 1.0	13.9 ± 1.4	0.1 ± 0.0	1.5 ± 0.0	0.7 ± 0.0
	3	4.9 ± 2.6	9.1 ± 2.2	11.2 ± 2.0	0.3 ± 0.1	1.2 ± 0.3	0.8 ± 0.1	5.4 ± 2.4	10.2 ± 3.9	15.0 ± 1.8	0.3 ± 0.2	1.5 ± 0.3	0.7 ± 0.2
	4	5.1 ± 1.3	9.6 ± 1.6	11.8 ± 2.4	0.3 ± 0.1	1.2 ± 0.3	0.8 ± 0.1	2.2 ± 0.6	6.7 ± 1.1	14.4 ± 2.2	0.1 ± 0.0	2.2 ± 0.2	0.5 ± 0.0
G. capensis	1	2.9 ± 0.7	17.2 ± 1.5	2.3 ± 0.2	0.2 ± 0.0	0.1 ± 0.0	7.4 ± 0.0	3.1 ± 0.4	20.7 ± 2.2	3.8 ± 1.0	0.2 ± 0.0	0.2 ± 0.0	5.4 ± 0.0
	2	2.3 ± 0.3	18.3 ± 2.6	3.3 ± 0.6	0.2 ± 0.0	0.1 ± 0.0	8.0 ± 0.0	2.9 ± 0.3	19.1 ± 0.9	3.3 ± 1.0	0.2 ± 0.0	0.2 ± 0.1	5.7 ± 0.0
	3	3.4 ± 0.3	15.1 ± 0.4	3.8 ± 0.4	0.2 ± 0.0	0.1 ± 0.0	7.2 ± 0.0	2.7 ± 0.6	21.7 ± 1.6	3.8 ± 0.2	0.2 ± 0.0	0.2 ± 0.0	5.7 ± 0.0
	4	3.3 ± 1.2	17.4 ± 0.3	3.5 ± 0.3	0.2 ± 0.1	0.1 ± 0.0	6.8 ± 0.1	2.9 ± 0.3	21.6 ± 1.7	3.5 ± 0.6	0.2 ± 0.0	0.2 ± 0.0	6.2 ± 0.0
Great Fish													
SPM	1	6.7	0.0	0.0	0.3	0.0	0.0	5.4	2.7	2.7	0.2	1.0	1.0
	2	5.9	0.0	0.0	0.3	0.0	0.0	5.3	5.3	0.0	0.5	0.0	-
	3	6.7	0.0	0.0	0.3	0.0	0.0	4.2	4.2	0.0	0.1	0.0	-
	4	3.7	0.0	0.0	0.1	0.0	0.0	5.1	2.6	0.0	0.3	0.0	-
	5	3.6	0.0	0.0	0.1	0.0	0.0	8.3	8.3	0.0	0.5	0.0	-
T. serrata	1	4.0 ± 0.2	8.9 ± 3.0	11.2 ± 4.0	0.2 ± 0.0	1.3 ± 0.1	0.8 ± 0.0	2.3 ± 0.6	20.1 ± 2.4	24.6 ± 2.6	0.1 ± 0.0	1.2 ± 0.0	0.8 ± 0.0
	2	4.2 ± 1.4	13.6 ± 1.7	14.2 ± 0.8	0.2 ± 0.0	1.0 ± 0.1	1.0 ± 0.0	1.6 ± 0.5	18.1 ± 3.0	24.6 ± 3.3	0.1 ± 0.0	1.3 ± 0.0	0.8 ± 0.0
	3	5.9 ± 1.1	8.1 ± 0.8	8.4 ± 0.8	0.2 ± 0.0	1.0 ± 0.0	1.0 ± 0.0	1.8 ± 0.5	19.8 ± 1.6	22.5 ± 2.5	0.1 ± 0.0	1.1 ± 0.1	0.9 ± 0.0
	4	6.0 ± 0.9	11.1 ± 2.3	11.2 ± 1.7	0.2 ± 0.0	1.0 ± 0.1	1.0 ± 0.0	2.1 ± 0.5	19.0 ± 1.9	23.6 ± 4.0	0.1 ± 0.0	1.2 ± 0.1	0.8 ± 0.0
P.perna	1	-	-	-	-	-	-	5.0 ± 1.8	10.0 ± 2.7	12.5 ± 1.0	0.3 ± 0.1	1.3 ± 0.2	0.8 ± 0.1
	2	3.0 ± 0.8	7.5 ± 1.4	9.8 ± 1.3	0.2 ± 0.0	1.2 ± 0.1	0.8 ± 0.1	3.2 ± 1.8	8.8 ± 1.8	13.6 ± 0.7	0.2 ± 0.1	1.5 ± 0.1	0.6 ± 0.1
	3	4.2 ± 2.6	7.3 ± 1.7	8.7 ± 4.2	0.2 ± 0.1	1.2 ± 0.3	1.1 ± 0.1	8.2 ± 3.6	8.2 ± 1.5	9.8 ± 4.7	0.4 ± 0.1	1.2 ± 0.3	0.8 ± 0.1
	4	4.3 ± 1.0	8.3 ± 1.4	7.8 ± 1.2	0.2 ± 0.1	0.9 ± 0.1	0.8 ± 0.1	4.5 ± 1.4	9.0 ± 1.9	15.3 ± 3.1	0.3 ± 0.0	1.7 ± 0.1	0.6 ± 0.0
G. capensis	1	2.7 ± 0.1	18.1 ± 2.0	2.2 ± 0.6	0.2 ± 0.0	0.1 ± 0.0	8.2 ± 0.0	3.5 ± 0.9	20.9 ± 0.4	3.5 ± 0.2	0.2 ± 0.0	0.2 ± 0.0	5.9 ± 0.0
	2	2.7 ± 0.3	17.2 ± 1.0	1.8 ± 0.1	0.2 ± 0.0	0.1 ± 0.0	9.6 ± 0.0	2.6 ± 0.5	21.1 ± 0.5	4.1 ± 0.3	0.2 ± 0.0	0.2 ± 0.0	5.1 ± 0.0
	3	2.1 ± 0.5	19.3 ± 1.4	2.7 ± 0.5	0.1 ± 0.0	0.1 ± 0.0	7.3 ± 0.0	3.9 ± 0.2	22.2 ± 0.8	4.8 ± 1.0	0.3 ± 0.0	0.2 ± 0.1	4.6 ± 0.0
	4	2.6 ± 0.4	18.3 ± 1.6	2.4 ± 0.6	0.2 ± 0.0	0.1 ± 0.0	7.7 ± 0.0	3.5 ± 0.4	20.9 ± 1.6	4.3 ± 0.1	0.2 ± 0.0	0.2. ± 0.0	4.9 ± 0.0

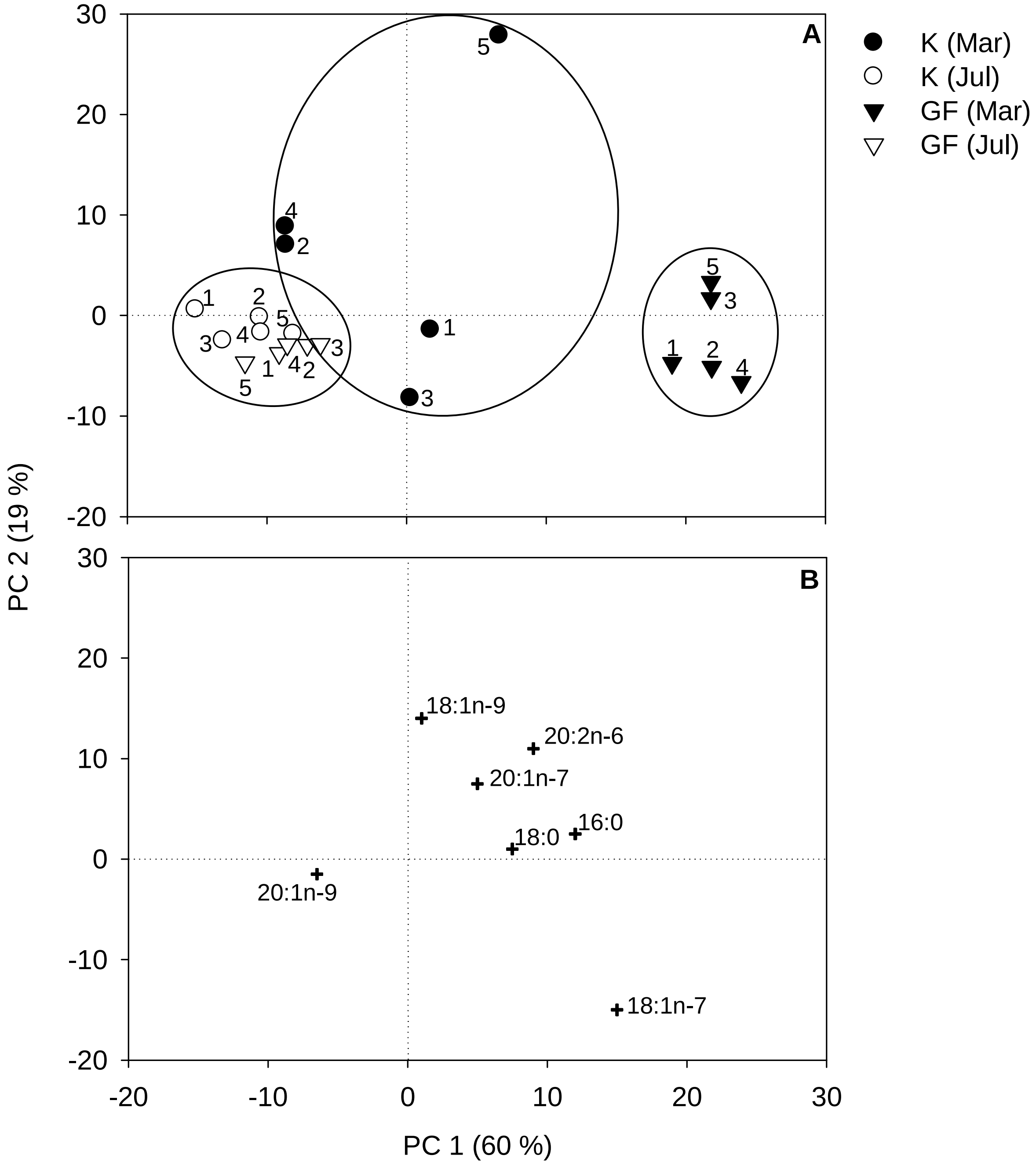

Figure 2.5 Principal Component Analysis (PCA) of the suspended particulate matter fatty acid signatures (arcsine-transformed proportional values; % TFA) in the Kariega and Great Fish regions during March and July 2009. Proportional variance of each principal component is explained by percentage values. (A) PCA scores demonstrating regional and temporal separation. (B) PCA loadings of the influential fatty acids (>2 %). Ellipses indicate significantly different groups as determined by Kruskal-Wallis ranked sum comparisons on PC scores (n = 20). Dashed lines denote the origin. TFA, total fatty acids; K, Kariega; GF, Great Fish; M, March; J, July; Site 1, most northern; Site 2, just north of mouth; Site 3, just south of mouth; Site 4, most southern; Site 5, mouth/estuary.

2.3.2.2 Fatty acid groups and biomarkers in suspension-feeders

The SFAs (March: 34.4-53.1 % TFA; July: 33.3-44.3 % TFA) and PUFAs (March: 25.6-49.3 % TFA; July: 35.2-50.9 % TFA) showed the greatest contributions to the diets of all three suspension-feeders, namely the barnacle *Tetraclita serrata*, mussel *Perna perna*, and polychaete *Gunnarea capensis* ($P < 0.05$ for both fatty acid groups; Figures 2.6 to 2.8). The dominant SFAs in the tissues of all three suspension-feeders during both sampling periods were 14:0, 16:0 and 18:0 (Figures 2.9 to 2.12; Appendices 2.2 to 2.7). The MUFAs were comparable among species and between regions and dates (March: 13.2-21.3 % TFA; July: 12.9-20.5 % TFA; Figures 2.6 to 2.8). The dominant MUFAs in March and July in all three suspension-feeders were 16:1n-7 (1.6-9.3 % TFA), 18:1n-9 (1.1-7.5 % TFA) and 18:1n-7 (1.1-7.6 % TFA; Figures 2.12 to 2.15). Levels of HPFAs were consistently low (<5 % TFA) in all species from both regions during March and July (Figures 2.6 to 2.8).

The SFAs decreased from March (37.5-53.1 % TFA) to July (33.3-40.1 % TFA) in *T. serrata* tissues ($F = 11.016$, $P < 0.01$, $df = 3,14$; Figure 2.6). The PUFAs (March: 25.6-49.3 % TFA; July: 45.6-50.9 % TFA) and EFAs (March: 17.7-43.6 % TFA; July: 38.7-46.4 % TFA), however, increased in proportions from March to July. Large fractions of EFAs were observed during July (38.7-46.4 % TFA) in both regions ($F = 34.315$, $P < 0.001$, $df = 3,14$). Consistent proportions of MUFAs (13.2-20.3 % TFA), BFAs (4.2-9.1 % TFA) and HPFAs (1.2-2.7 % TFA) were found in the barnacles at the Kariega and Great Fish regions during both sample times (Figure 2.6). The BFA *i*-16:0 was more abundant in *T. serrata* and occurred at fairly constant levels through time in the Kariega (March: 1.3-2.0 % TFA; July: 1.6-2.4 % TFA) and Great Fish regions (March: 1.4-2.3 % TFA; July: 2.1-2.4 % TFA). Two MUFAs appeared particularly important in barnacle tissues (Appendices 2.2 and 2.3). The component 18:1n-9 was abundant in March (4.7-6.5 % TFA) and July (4.8-7.5 % TFA) in both regions, and 18:1n-7 in the Great Fish region during both sample times (5.5-7.6 % TFA; Figures 2.9 to 2.12). The MUFA 18:1n-7 was present in the barnacles in the Kariega region in March only (4.1-6.4 % TFA; Figure 2.9; Appendix 2.2). High incidences of omnivory (18:1n-9/18:1n-7) and herbivory (n-3/n-6) were recorded for *T. serrata*. Omnivory was more pronounced in barnacles from the Kariega region (March: ratios 0.9 to 1.4; July: ratios 1.8 to 4.8) relative to the Great Fish region (March: ratios 0.8 to 1.1; July: ratios 0.7 to 0.9), while herbivory was prevalent through time in both regions (Kariega: ratios 5.3 to 13.9; Great Fish: ratios 3.5 to 15.3).

The SFAs (March: 34.4-47.0 % TFA; July: 37.0-44.3 % TFA), PUFAs (March: 37.0-45.0 % TFA; July: 35.2-46.7 % TFA) and EFAs (March: 20.5-29.8 % TFA; July: 21.3-29.9

% TFA) showed consistent proportions in *P. perna* from the Kariega and Great Fish regions during both sample times (Figure 2.7). Mussel tissues showed large BFA components in the Kariega (6.0-13.6 % TFA) and Great Fish regions (9.0-17.7 % TFA; $F = 9.375$, $P < 0.01$, $df = 3,11$; Figure 2.7). Approximately equal proportions of MUFAs (13.9-20.5 % TFA) and HPFAs (2.5-4.9 % TFA) were found in mussel tissues in the Kariega and Great Fish regions during both sample times (Figure 2.7). The 16:1n-7 component occurred in mussel tissues in greater proportions at the northern sites (Sites 1 and 2; 9.2-9.3 % TFA) during March in the Kariega region (Appendix 2.4). The MUFA 20:1n-7, and PUFAs 22:2 non-methylene interrupted (NMI) 1 and 22:2 NMI 2 were important in both regions during March (1.3-4.6 % TFA) and July (2.5-5.6 % TFA; Figures 2.9 to 2.12). Although omnivory was moderate in March and July in both regions (18:1n-9/18:1n-7 ratios 0.6 to 1.0), greater animal inputs to mussel diet were noticed in the Kariega region in July (18:1n-9/ 18:1n-7 ratios 1.0 to 3.0). Herbivory remained prevalent through space and time in the mussels (n-3/n-6 ratios 2.0 to 4.0).

The SFA proportions showed a small decrease in *G. capensis* from March (34.9-47.2 % TFA) to July (35.7-43.4 % TFA) in both regions ($F = 3.966$, $P < 0.05$, $df = 3,12$; Figure 2.8). Consistent proportions of MUFAs (16.0-19.0 % TFA) and EFAs (19.9-30.9 % TFA) were recorded in polychaetes between sample dates in both regions (Figure 2.8). The MUFA 20:1n-11 was abundant in *G. capensis* from both regions during March (4.8-6.1 % TFA) and July (4.3-5.4 % TFA; Figures 2.9 to 2.12). PUFA proportions in the polychaetes showed a minor increase from March to July in both regions (Kariega: 44.3 to 47.9 % TFA; Great Fish: 43.2 to 47.9 % TFA), and 22:2 NMI 2 remained important through time (2.7-3.8 % TFA; Figures 2.9 to 2.12). BFAs were substantial in polychaetes from both regions during March (10.8-13.6 % TFA) and July (8.7-12.2 % TFA), with consistently low proportions of HPFAs (1.3-2.1 % TFA). Polychaetes displayed limited omnivory in both regions (18:1n-9/18:1n-7 ratios 0.2 to 0.6), while herbivory was prominent (n-3/n-6 ratios 2.2 to 3.8).

For *G. capensis* and *P. perna*, the proportions of BFA *i*-18:0 were consistent during both sample times in the Kariega (mussel: 3.3-10.1 % TFA; polychaete: 5.4-7.6 % TFA) and Great Fish regions (mussel: 6.3-12.8 % TFA; polychaete: 4.2-7.2 % TFA; Figures 2.9 to 2.12), respectively. The diatom marker 16:1n-7 occurred in smaller levels during March and July in *G. capensis* (Kariega: 2.3-3.4 % TFA; Great Fish: 2.1-3.9 % TFA; Appendices 2.6 to 2.7) compared to *P. perna* (Kariega: 1.9-9.2 % TFA; Great Fish: 3.0-8.2 % TFA; Appendices 2.4 to 2.5) and *T. serrata* (Kariega: 1.8-4.8 % TFA; Great Fish: 1.6-6.0 % TFA; Appendices 2.2 to 2.3).

Three major PUFAs were identified in all three species in both sample times and regions, namely 20:4n-6, 20:5n-3 and 22:6n-3 (Figures 2.9 to 2.12). These fatty acids comprise the EFAs and occurred in relatively equal proportions across species and time. For *T. serrata*, however, 20:4n-6 was present in smaller proportions (Kariega: 1.5-2.4 % TFA; Great Fish: 1.1-2.6 % TFA; Appendices 2.2 to 2.3) than in *P. perna* (Kariega: 3.5-5.6 % TFA; Great Fish: 3.3-5.6 % TFA; Appendices 2.4 to 2.5) and *G. capensis* (Kariega: 3.0-3.8 % TFA; Great Fish: 3.1-4.1 % TFA; Appendices 2.6 to 2.7). A similar pattern was observed with the dinoflagellate marker 22:6n-3, where considerably reduced levels were observed in *G. capensis* (Kariega: 2.1-3.8 % TFA; Great Fish: 1.8-4.8 % TFA) compared to *T. serrata* (Kariega: 12.7-23.7 % TFA; Great Fish: 8.4-24.6 % TFA) and *P. perna* (Kariega: 8.9-15.0 % TFA; Great Fish: 7.8-15.3 % TFA). An increase from March to July in 22:6n-3 (March: 8.4-14.2 % TFA; July: 22.5-24.6 % TFA), 20:4n-6 (March: 1.1-2.1 % TFA; July: 1.7-2.6 % TFA) and 20:5n-3 (March: 8.9-13.6 % TFA; July: 18.1-20.0 % TFA) proportions were also observed in *T. serrata* in the Great Fish region (Figures 2.11 and 2.12; Appendices 2.2 to 2.3).

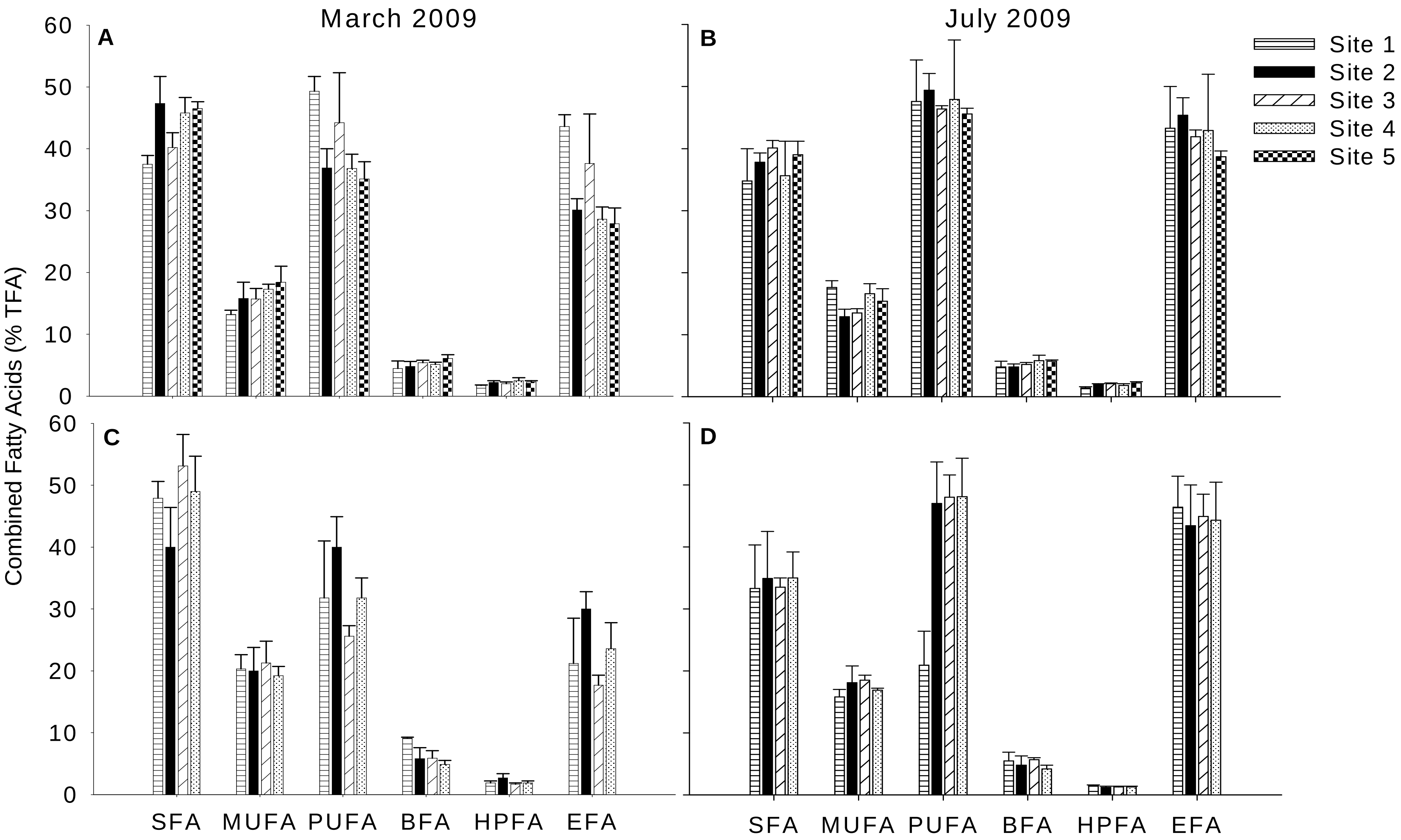

Figure 2.6 Fatty acid composition (% TFA) of fatty acid groups in the barnacle (*Tetraclita serrata*) during March and July 2009. Panels A and B represent the Kariega region, whereas C and D the Great Fish region. Values are presented as means ± standard deviation (*n* = 3 per site). SFA, saturated fatty acid; MUFA, monounsaturated fatty acid; PUFA, polyunsaturated fatty acid; BFA, bacterial fatty acid; HPFA, higher plant fatty acid; EFA, essential fatty acid. Site 1, most northern; Site 2, just north of mouth; Site 3, just south of mouth; Site 4, most southern; Site 5, mouth.

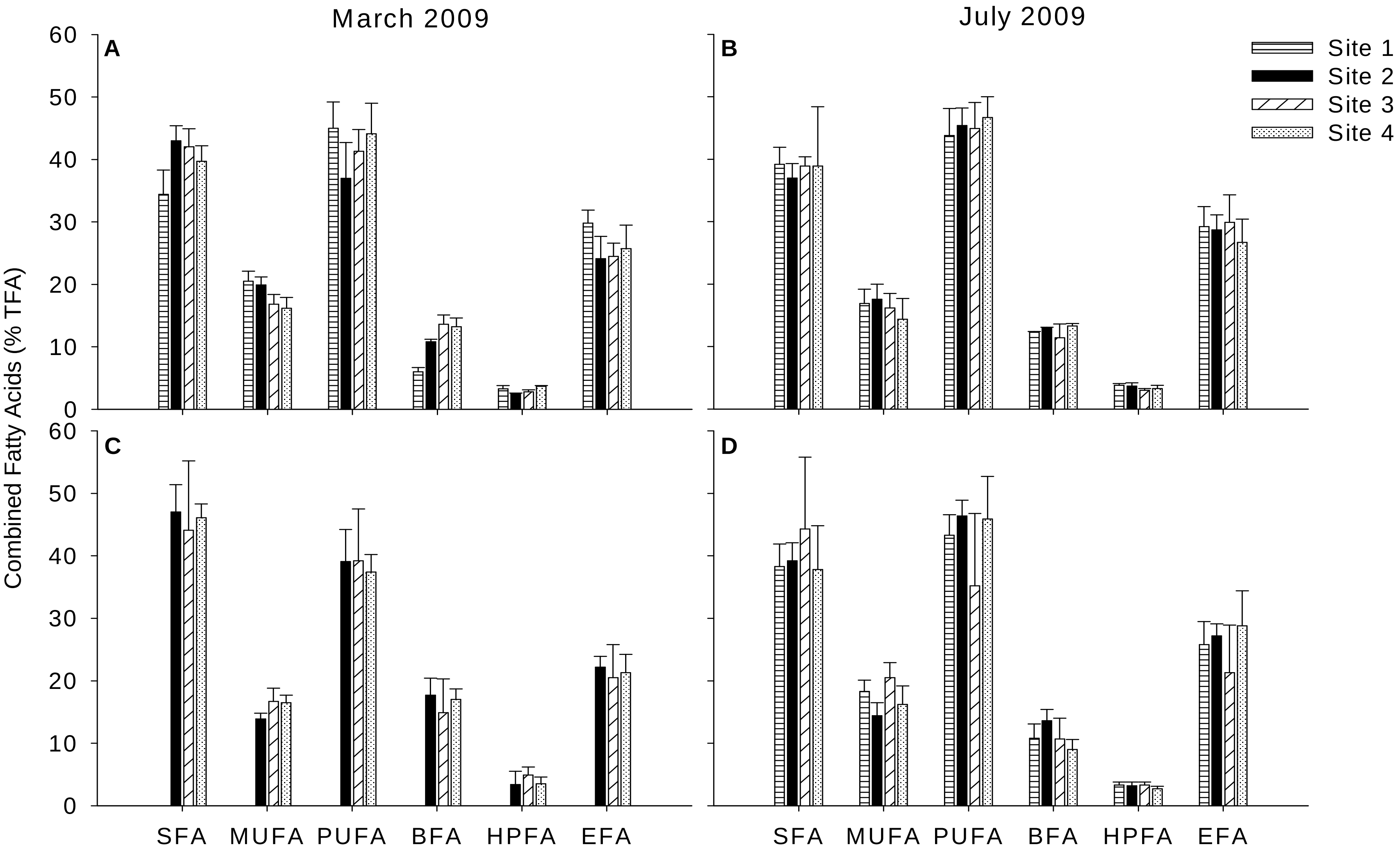

Figure 2.7 Fatty acid composition of fatty acid groups (% TFA) in the mussel (*Perna perna*) during March and July 2009. Panels A and B represent the Kariega region, whereas C and D the Great Fish region. Values are presented as means ± standard deviation ($n = 3$ per site). Rapid tidal shift resulted in no mussel collection at Site 1 during March in the Great Fish region. SFA, saturated fatty acid; MUFA, monounsaturated fatty acid; PUFA, polyunsaturated fatty acid; BFA, bacterial fatty acid; HPFA, higher plant fatty acid; EFA, essential fatty acid. Site 1, most northern; Site 2, just north of mouth; Site 3, just south of mouth; Site 4, most southern; Site 5, mouth.

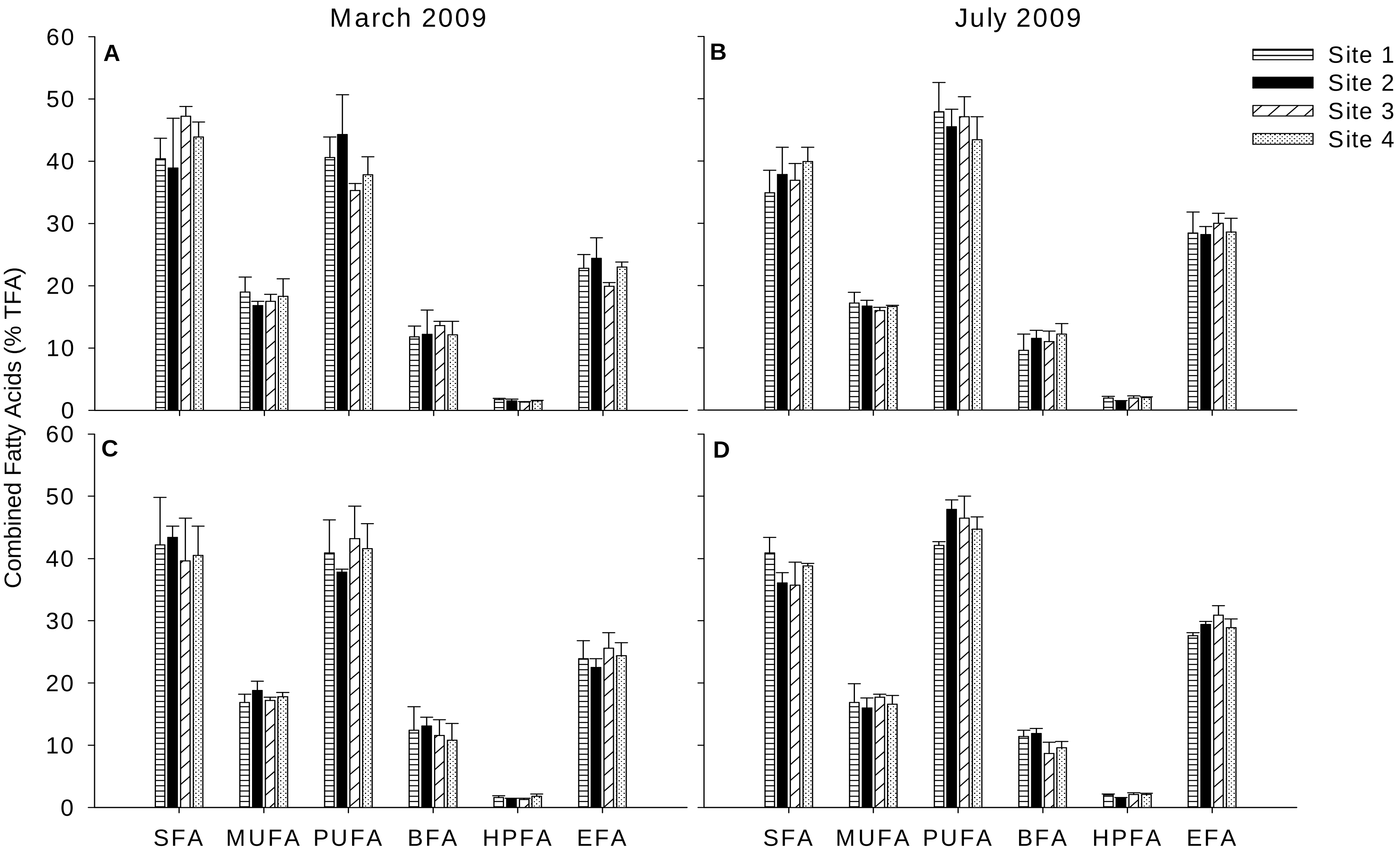

Figure 2.8 Fatty acid composition of fatty acid groups (% TFA) in the polychaete (*Gunnarea capensis*) during March and July 2009. Panels A and B represent the Kariega region, whereas C and D the Great Fish region. Values are presented as means ± standard deviation (*n* = 3 per site). SFA, saturated fatty acid; MUFA, monounsaturated fatty acid; PUFA, polyunsaturated fatty acid; BFA, bacterial fatty acid; HPFA, higher plant fatty acid; EFA, essential fatty acid. Site 1, most northern; Site 2, just north of mouth; Site 3, just south of mouth; Site 4, most southern; Site 5, mouth.

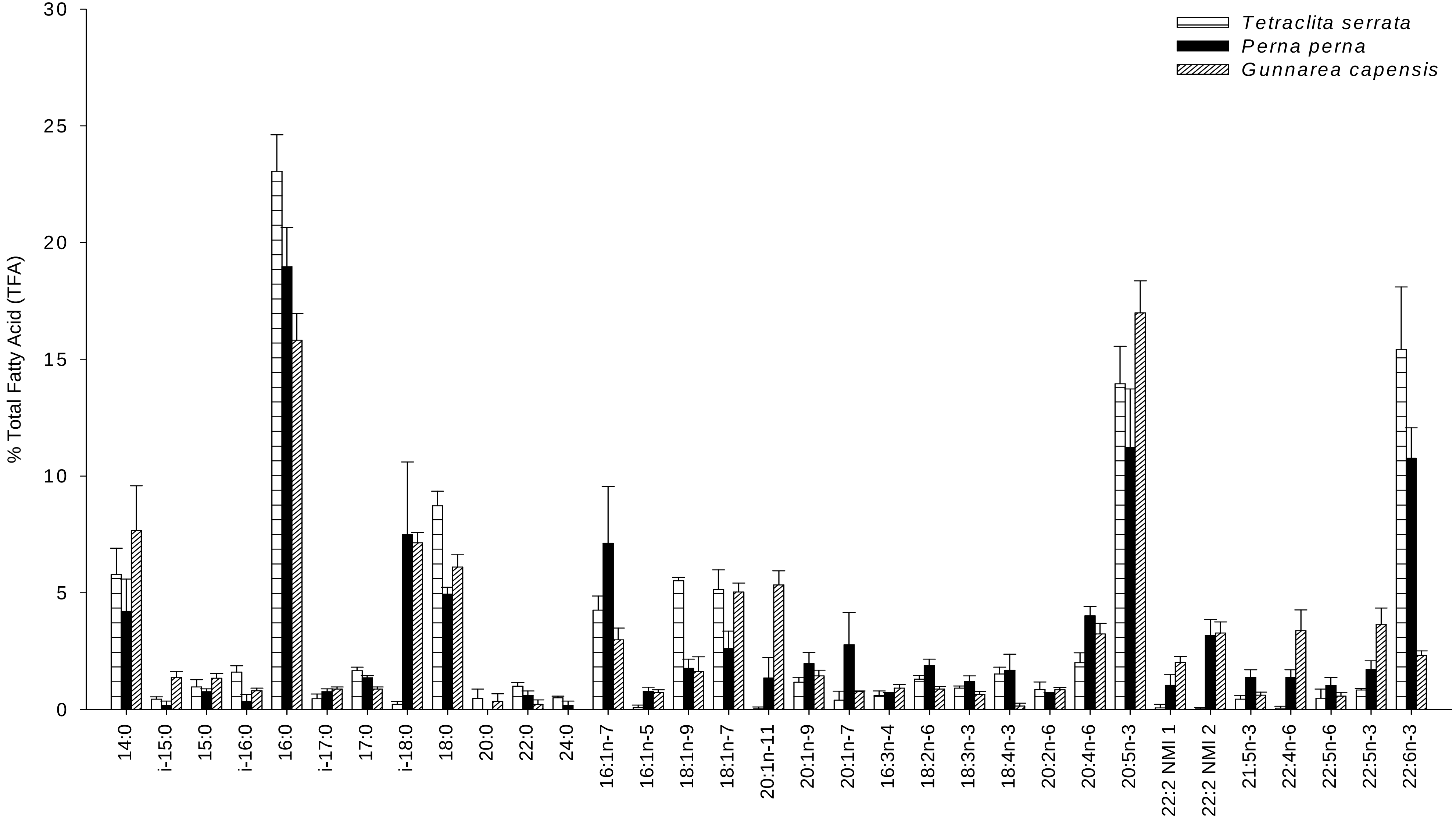

Figure 2.9 Fatty acid profiles (% TFA) of the barnacle *Tetraclita serrata*, mussel *Perna perna*, and polychaete *Gunnarea capensis* tissues in the Kariega region during March 2009. Values are presented as means ± standard deviation (*n* = 4 to 5 per species).

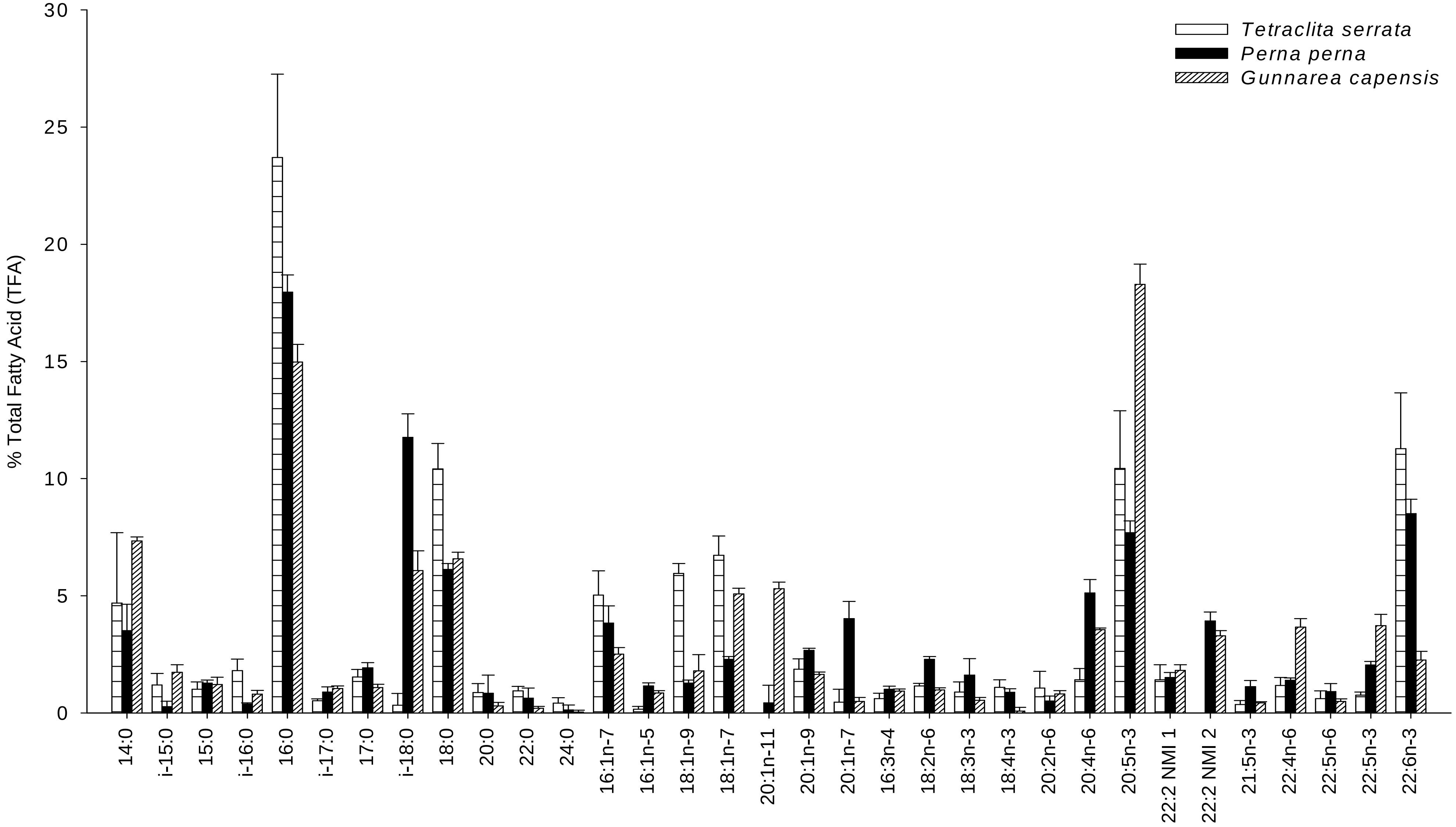

Figure 2.10 Fatty acid profiles (% TFA) of the barnacle *Tetraclita serrata*, mussel *Perna perna*, and polychaete *Gunnarea capensis* tissues in the Kariega region during July 2009. Values are presented as means ± standard deviation (*n* = 4 to 5 per species).

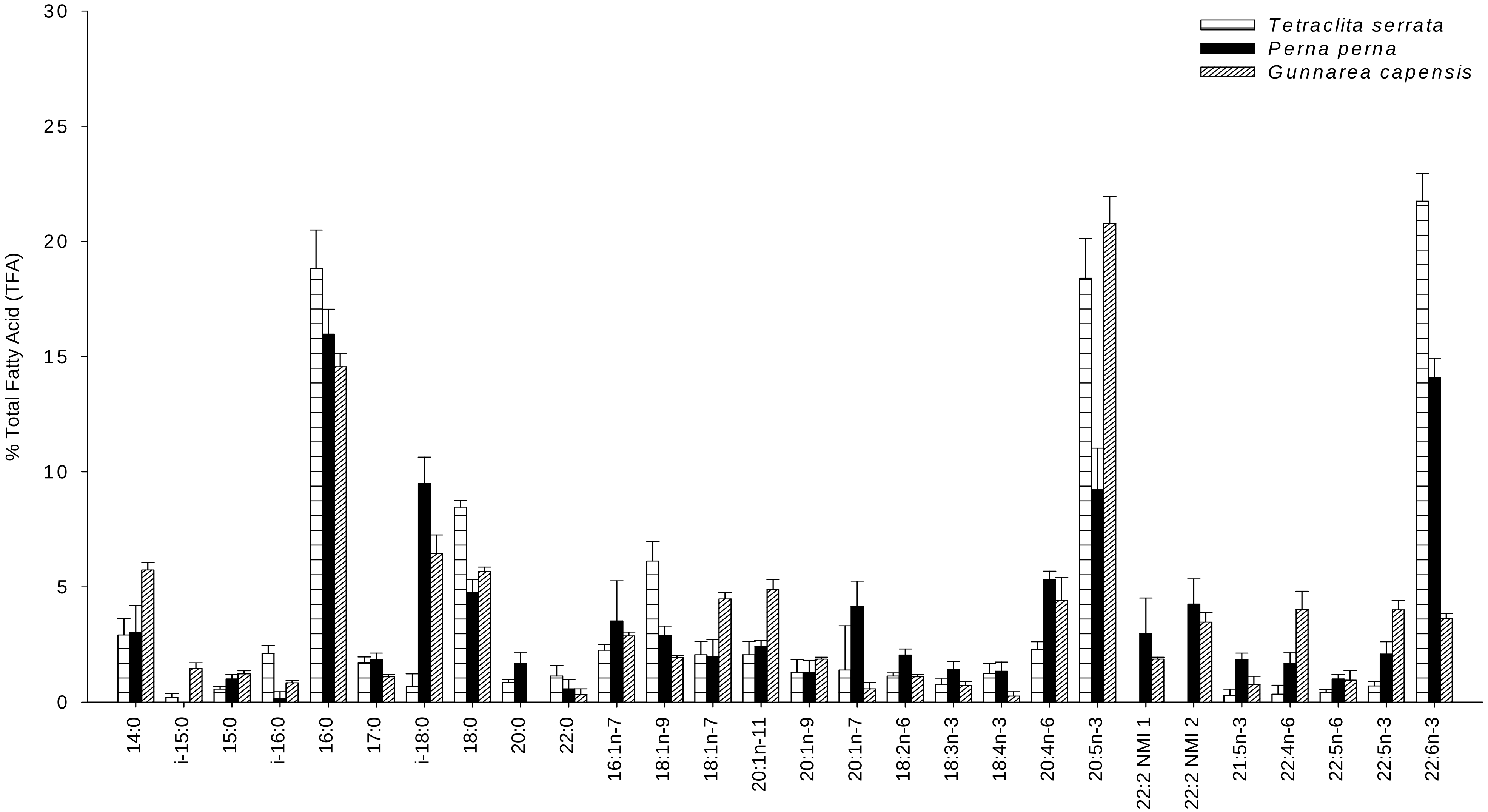

Figure 2.11 Fatty acid profiles (% TFA) of the barnacle *Tetraclita serrata*, mussel *Perna perna*, and polychaete *Gunnarea capensis* tissues in the Great Fish region during March 2009. Values are presented as means ± standard deviation (*n* = 4 to 5 per species).

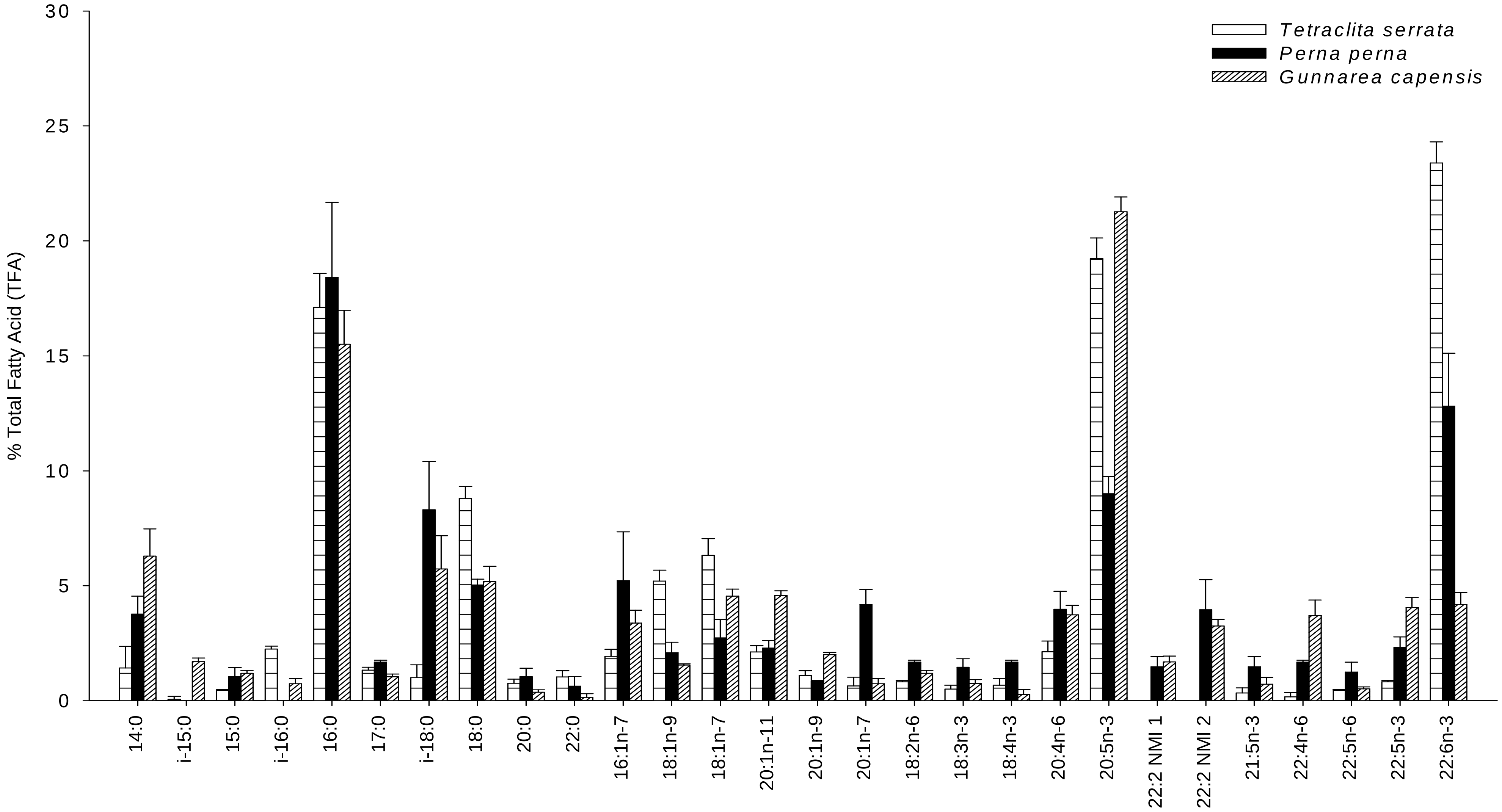

Figure 2.12 Fatty acid profiles (% TFA) of the barnacle *Tetraclita serrata*, mussel *Perna perna*, and polychaete *Gunnarea capensis* tissues in the Great Fish region during July 2009. Values are presented as means ± standard deviation (*n* = 4 to 5 per species).

2.3.2.3 Diatom and dinoflagellate markers in suspension-feeders

The diatom (16:1n-7 and 20:5n-3) and dinoflagellate (22:6n-3) biomarkers showed distinct patterns in the suspension-feeders (Table 2.2; Figures 2.13 to 2.15). Multivariate ANOVA (with the three biomarkers as dependent, and region or date as independent variables) revealed the following results. Significant seasonal differences in the proportions of all three biomarkers were recorded in *T. serrata* tissues in the Kariega and Great Fish regions ($P < 0.05$ in all cases; Figure 2.13). The diatom marker 16:1n-7 had a larger contribution in March (3.2-6.0 % TFA) than in July (1.8-2.4 % TFA; Figure 2.13) in both regions. However, levels of the diatom markers 16:1n-7 and 16:1/16:0 (ratios 0.1 to 0.2), were reduced compared to the other phytoplankton biomarkers (Table 2.2). Proportions of 20:5n-3 and 22:6n-3 in barnacle tissues were higher in July (15.4-24.6 % TFA) than in March (8.1-19.7 % TFA) in both regions (Figure 2.13). Consistently large contributions of the dinoflagellate markers 22:6n-3 and 22:6n-3/20:5n-3 (ratios 1.0 to 1.3) were recorded in barnacle tissues in both regions during both sampling times (Table 2.2).

A significant temporal difference in *P. perna* tissues was observed in all three biomarkers in the Kariega region ($P < 0.05$ in all cases; Figure 2.14). Diatom proportions were significantly greater in March, and at Sites 1 and 2 (16:1n-7: 9.2-9.3 % TFA, 16:1/16:0 ratios 0.4 to 0.5; 20:5n-3: 11.7-14.6 % TFA, 22:6n-3/20:5n-3 ratios ~1.3), compared to Sites 3 and 4 (16:1n-7: 4.9-5.1 % TFA, 16:1/16:0 ratios ~0.3; 20:5n-3: 9.1-9.6 % TFA, 22:6n-3/20:5n-3 ratios ~0.8) in the Kariega region ($P < 0.05$; Figure 2.14). The diatom marker 16:1/16:0 (ratios 0.1 to 0.5) was small, while the dinoflagellate markers 22:6n-3 (7.8-15.3 % TFA) and 22:6n-3/20:5n-3 (ratios 0.8 to 2.2) were enhanced in mussels at the Kariega and Great Fish regions during both sample times ($P < 0.05$; Figure 2.14).

Significant regional changes were recorded in the proportions of all three biomarkers in *G. capensis* ($P < 0.05$ in all cases; Figure 2.15). The component 20:5n-3 (15.1-22.2 % TFA) represented the most dominant fatty acid in polychaete tissues, with consistently small proportions of 16:1n-7 (2.1-3.5 % TFA) and 22:6n-3 (1.8-4.8 % TFA) recorded in the Kariega and Great Fish polychaetes during both sample times (Figure 2.15). These results compare with the ratio markers for diatoms 16:1/16:0 (ratios ~0.2), 20:5n-3/ 22:6n-3 (ratios 5.1 to 9.6) and dinoflagellates 22:6n-3/20:5n-3 (ratios 0.1 to 0.2) signifying the importance of the component 20:5n-3 in polychaete diets (Table 2.2).

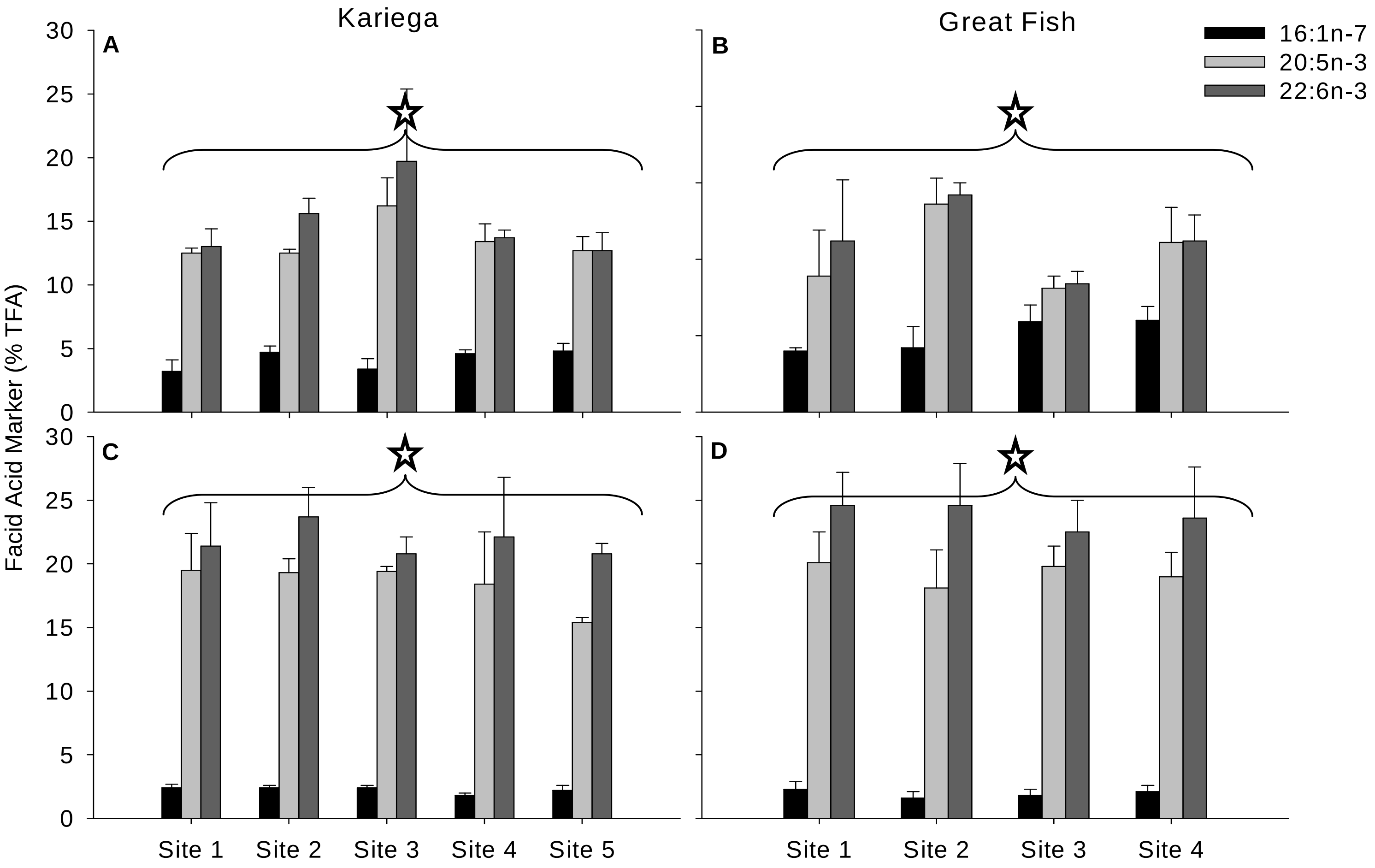

Figure 2.13 Changes in the diatom (16:1n-7 and 20:5n-3) and dinoflagellate (22:6n-3) fatty acid biomarkers in the barnacle (*Tetraclita serrata*) between dates in the Kariega and Great Fish regions. Panels A and B represent March whereas C and D July. Values are presented in means ± standard deviation for arcsine-transformed proportional data (n = 3 per site). Stars indicate significant temporal and regional differences in the proportions of all three biomarkers in the barnacle ($P < 0.05$ in all cases; MANOVA). Site 1, most northern; Site 2, just north of mouth; Site 3, just south of mouth; Site 4, most southern; Site 5, mouth.

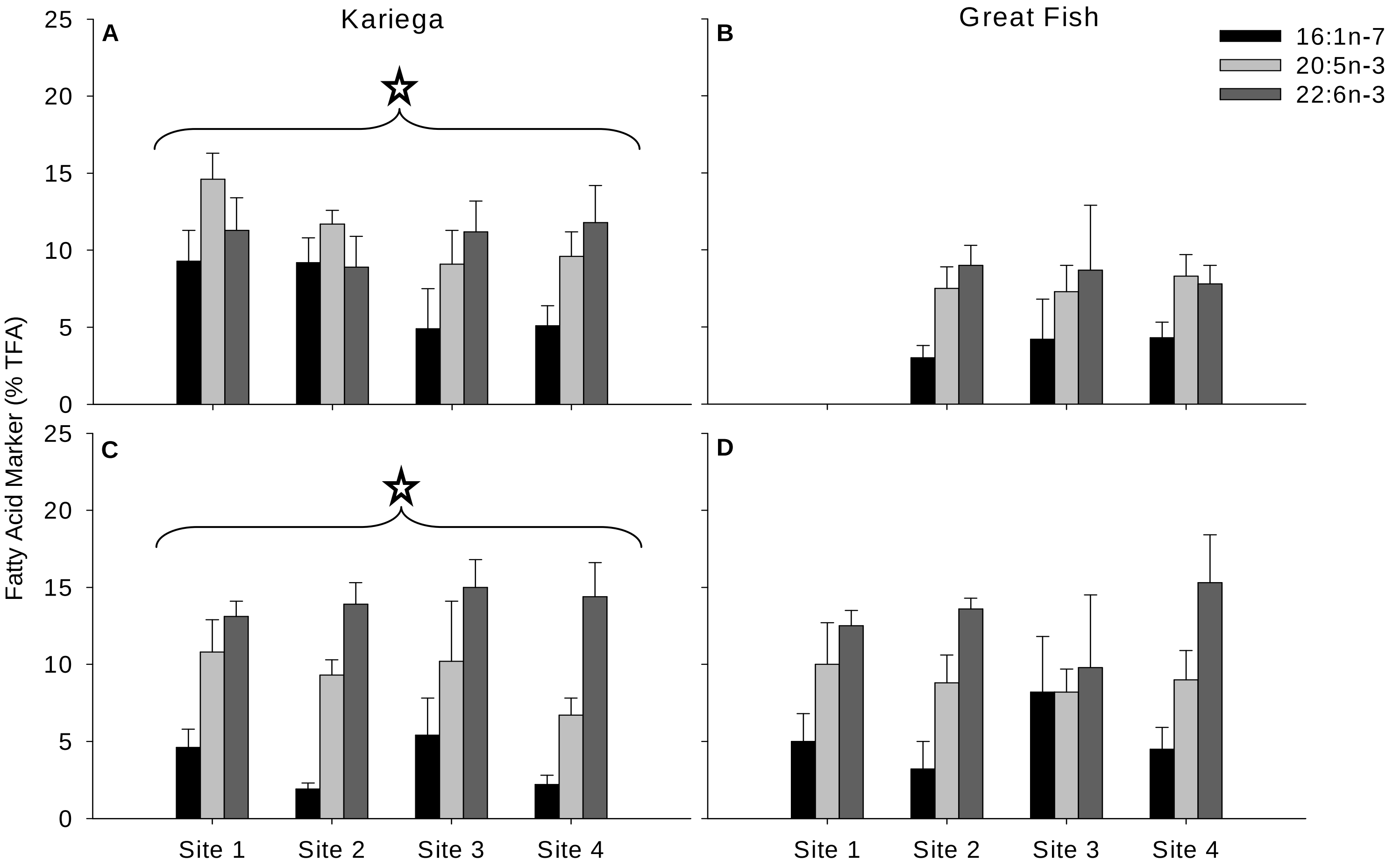

Figure 2.14 Changes in the diatom (16:1n-7 and 20:5n-3) and dinoflagellate (22:6n-3) fatty acid biomarkers in the mussel (*Perna perna*) between dates in the Kariega and Great Fish regions. Panels A and B represent March whereas C and D July. Values are presented in means ± standard deviation for arcsine-transformed proportional data ($n = 3$ per site). Stars indicate significant temporal differences in the proportions of all three biomarkers in the mussel ($P < 0.05$ in all cases; MANOVA). Rapid tidal shift resulted in no mussel collection at Site 1 during March in the Great Fish region. Site 1, most northern; Site 2, just north of mouth; Site 3, just south of mouth; Site 4, most southern; Site 5, mouth.

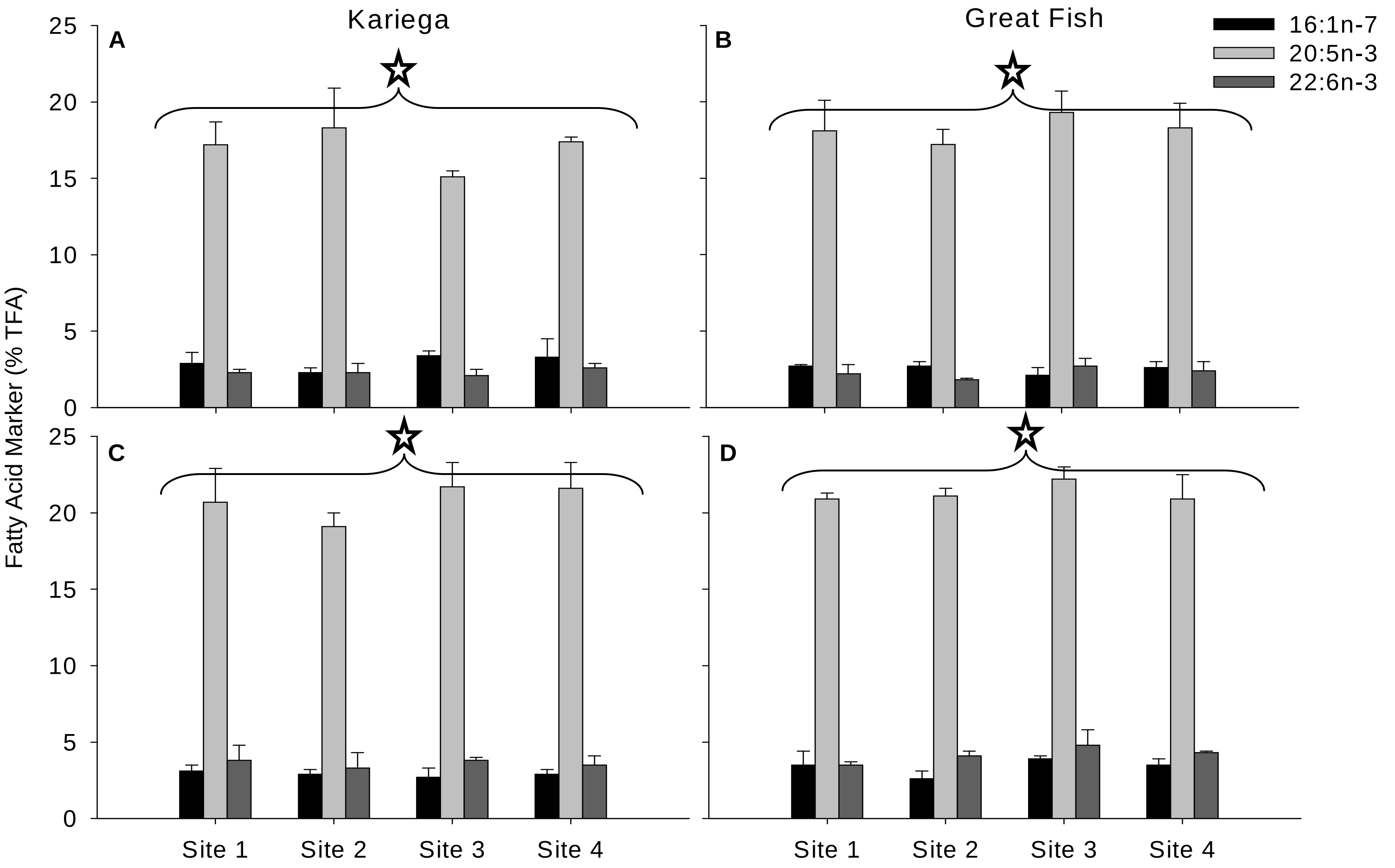

Figure 2.15 Changes in the diatom (16:1n-7 and 20:5n-3) and dinoflagellate (22:6n-3) fatty acid biomarkers in the polychaete (*Gunnarea capensis*) between dates in the Kariega and Great Fish regions. Panels A and B represent March whereas C and D July. Values are presented in means ± standard deviation for arcsine-transformed proportional data (n = 3 per site). Stars indicate significant temporal and regional differences in the proportions of all three biomarkers in the polychaete ($P < 0.05$ in all cases; MANOVA). Site 1, most northern; Site 2, just north of mouth; Site 3, just south of mouth; Site 4, most southern; Site 5, mouth.

2.3.2.4 Principal component analyses of fatty acids in suspension-feeders

Significant regional and temporal differences were observed in *T. serrata* fatty acid signatures ($P < 0.01$ for all models; Table 2.3). No significant changes were noted among sites in the Kariega and Great Fish regions during both sample times ($P > 0.05$ for all models). The influential fatty acids (loadings >2 %) in the Kariega region were 16:1n-7 during March, and during July *i*-18:0, 20:1n-11, 20:5n-3 and 22:6n-3. In the Great Fish region the influential fatty acids for March were *i*-15:0, 16:1n-7, 22:2 NMI 1 and for July were 20:1n-11, 20:5n-3 and 22:6n-3. Since Tukey multiple-comparisons tests failed to differentiate among sites within each region or sample date, data for the northern (Sites 1 and 2) and southern sites (Sites 3 and 4) were pooled. Independent *t*-tests were done for each region to test for significant differences between sites situated north and south of the estuary mouth. Significant north *versus* south spatial variation was observed in *T. serrata* fatty acid signatures during March in the Kariega ($t = -4.86$, $P < 0.001$, $df = 10$) and Great Fish regions ($t = 5.88$, $P < 0.001$, $df = 10$), respectively (Figure 2.16). Principal components described 55 % of the observed variance (PC-1: 32 % and PC-2: 23 %) in *T. serrata* fatty acid signatures during March in both regions, and the influential fatty acids contributing to the north/south separation were 20:5n-3 and 22:6n-3 (Figure 2.16).

The qualitative PCA results in *P. perna* fatty acid signatures indicated a significant spatial difference between the Kariega and Great Fish regions in March ($P < 0.01$), and a distinct temporal difference was observed in both regions ($P < 0.05$; Table 2.3). No significant changes were noted among sites in the Kariega and Great Fish regions during both month ($P > 0.05$ for all models). In the Kariega region, influential fatty acids comprised 16:1n-7, 18:1n-7, 18:4n-3, 20:5n-3 in March, and *i*-18:0, 20:1n-11, 22:6n-3 in July. The influential fatty acids in the Great Fish region were *i*-18:0, 16:1n-7, 20:1n-9, 20:4n-6, 22:2 NMI 2 in March and 16:1n-7, 20:1n-11, 22:6n-3 in July. PCA on the *P. perna* fatty acid signatures explained 69 % of the proportional variance in the data (PC-1: 50 % and PC-2: 19 %), and distinct northern and southern groupings were recorded in the Kariega region in March ($t = -4.61$, $P < 0.001$, $df = 10$; Figure 2.17). The fatty acids 16:1n-7 and 22:6n-3 were regarded as influential to the north/south separation (Figure 2.17).

Significant temporal variations in *G. capensis* tissue fatty acid signatures were recorded in the Kariega and Great Fish regions ($P < 0.01$ for both models; Table 2.3). No significant changes were noted among sites in the Kariega and Great Fish regions during both sample times ($P > 0.05$ for all models). The influential fatty acids in the Kariega region in March were 16:1n-7, 18:1n-9, 20:1n-11 and in July were 18:4n-3, 22:6n-3. In the Great Fish region the fatty acids *i*-18:0, 18:1n-7, 20:1n-11, 20:5n-3, 22:2 NMI 2 were influential during March, and 18:4n-3, 20:5n-3, 22:6n-3 in July. In March, principal components explained 58 % of the variance (PC-1: 45 % and PC-2: 13 %) in *G. capensis* fatty acid compositions (Figure 2.18). Distinctive north *versus* south groups were recorded in *G. capensis* during March in the Kariega region (t = -3.73, P < 0.01, df = 10; Figure 2.18).

The PCA scores of the proportional fatty acids of the combined consumer data showed significant differences among the suspension-feeders from the Kariega and Great Fish regions (H = 136.087; P < 0.001; df = 11; Figure 2.19). Kruskal-Wallis ranked sum comparisons further identified a significant temporal distinction in signatures of barnacles, mussels and polychaetes in both regions (H = 109.550; P < 0.001; df = 11; Figure 2.19). The principal components (PC) -1 and -2 explained much of the regional and temporal variance in the data (Figure 2.19). The Kariega and Great Fish consumer signatures (73 % variance; PC-1: 52 % and PC-2: 21 %) were characterised by the influential fatty acids 14:0, 16:0, 16:1n-7, 18:0, 18:1n-9, 18:1n-7 and 20:5n-3 on PC-1 and by *i*-18:0, 18:n1-9, 22:2 NMI 1, 22:5n-3 and 22:6n-3 on PC-2 (Figure 2.19).

Table 2.3 Multivariate ANOVA results (with PC-1 and -2 scores data as dependent, and region or date as independent variables) indicating temporal and regional differences in fatty acid signatures of three indigenous suspension-feeding organisms (barnacle *Tetraclita serrata*; mussel *Perna perna*; and polychaete *Gunnarea capensis*). Univariate *F*-test results for PC-1 and -2 scores and percentage contribution of PC-1 and -2 axes (%) to the variance observed within the data were given. Data represent arcsine-transformed proportional values (% TFA). Levene's test for homogeneity was conducted to verify the validity of each model. K, Kariega; GF, Great Fish; Mar, March; Jul, July; ns, not significant.

Species	Season	Region	ANOVA			Levene's Test		PCA
			F-ratio	*P*	*df*	Statistic	*P*	%
PC-1 results								
Tetraclita serrata	Mar	K x GF	17.409	<0.001	8	3.042	ns	32
	Jul	K x GF	16.361	<0.001	8	2.929	ns	28
	Mar x Jul	K	45.296	<0.001	9	1.759	ns	40
	Mar x Jul	GF	38.592	<0.001	7	4.504	ns	59
Perna perna	Mar	K x GF	8.312	<0.01	6	5.051	ns	50
	Jul	K x GF	1.238	ns	7	4.152	ns	42
	Mar x Jul	K	5.038	<0.01	7	2.899	ns	53
	Mar x Jul	GF	68.885	<0.001	6	5.625	ns	42
Gunnarea capensis	Mar	K x GF	1.454	ns	7	2.808	ns	42
	Jul	K x GF	4.614	ns	7	2.919	ns	29
	Mar x Jul	K	15.284	<0.001	7	2.846	ns	45
	Mar x Jul	GF	4.766	<0.01	7	5.705	ns	37
PC-2 results								
Tetraclita serrata	Mar	K x GF	9.403	<0.001	8	3.042	ns	23
	Jul	K x GF	5.735	<0.01	8	2.929	ns	22
	Mar x Jul	K	4.649	<0.01	9	1.759	ns	23
	Mar x Jul	GF	4.495	<0.01	7	4.504	ns	14
Perna perna	Mar	K x GF	1.843	ns	6	5.051	ns	19
	Jul	K x GF	0.350	ns	7	4.152	ns	17
	Mar x Jul	K	3.135	<0.05	7	2.899	ns	12
	Mar x Jul	GF	0.541	ns	6	5.625	ns	23
Gunnarea capensis	Mar	K x GF	0.926	ns	7	2.808	ns	19
	Jul	K x GF	1.228	ns	7	2.919	ns	21
	Mar x Jul	K	2.495	ns	7	2.846	ns	13
	Mar x Jul	GF	2.611	ns	7	5.705	ns	21

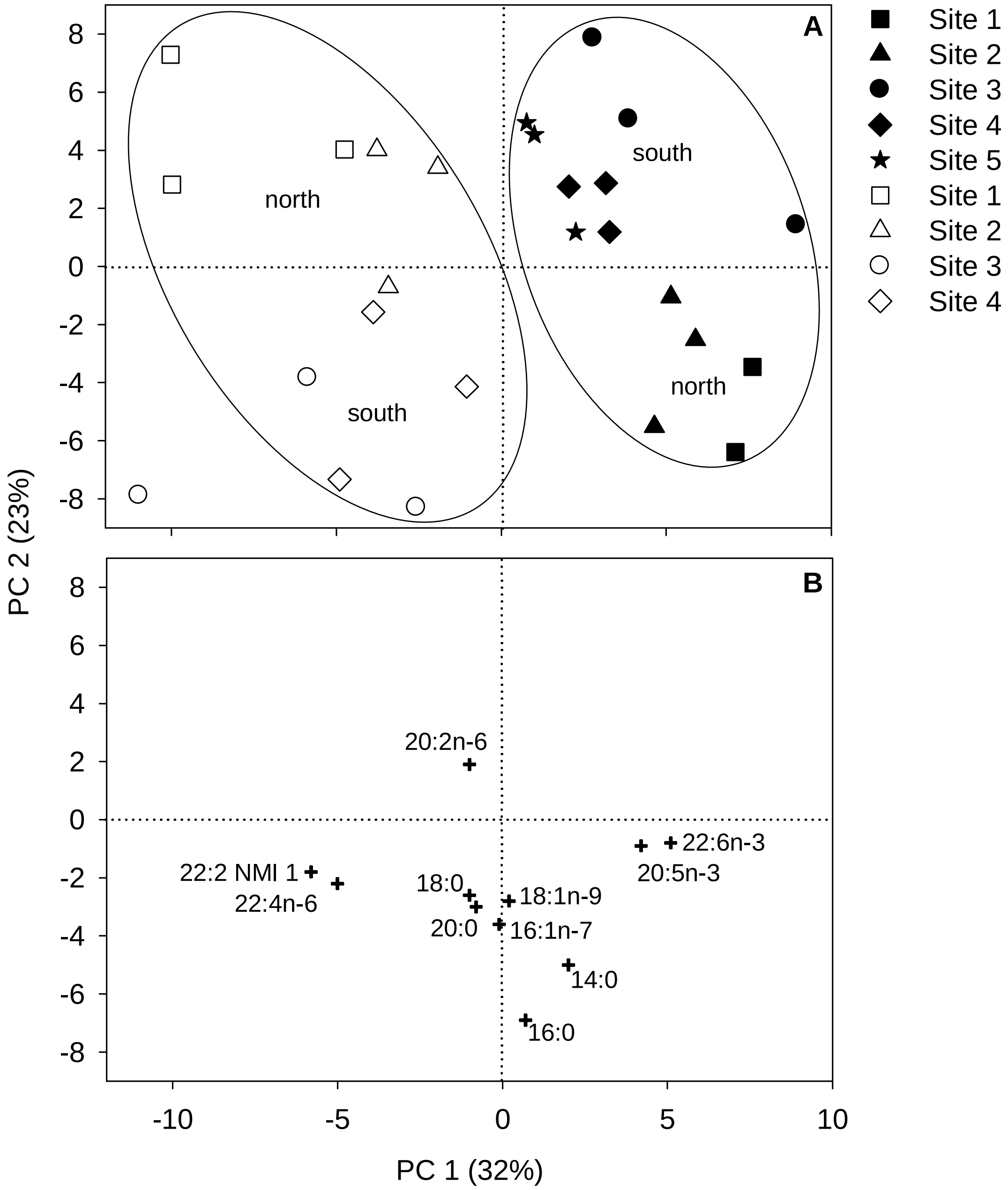

Figure 2.16 Principal Component Analysis (PCA) of the fatty acid signatures in the barnacle (*Tetraclita serrata*) during March 2009 in the Kariega and Great Fish regions. Data represent arcsine-transformed proportional values (% TFA). Proportional variance of each principal component is explained by percentage values. (A) PCA scores demonstrating clear separation between geographic localities, with significantly different regions indicated by ellipses, as determined by MANOVA on PC scores (n = 27). An independent t-test on PC-1 or -2 scores revealed distinct northern *versus* southern site separation in both regions. Datum point in left lower quadrant should be included within Great Fish ellipsis. (B) PCA loadings illustrating the influential fatty acids (>2 %). Dashed lines denote the origin. The small population of barnacles that were located within the estuary mouth at the Kariega region is represented by Site 5. NMI, nonmethylene interrupted; K, Kariega; GF, Great Fish; Site 1, most northern; Site 2, just north of mouth; Site 3, just south of mouth; Site 4, most southern; Site 5, mouth.

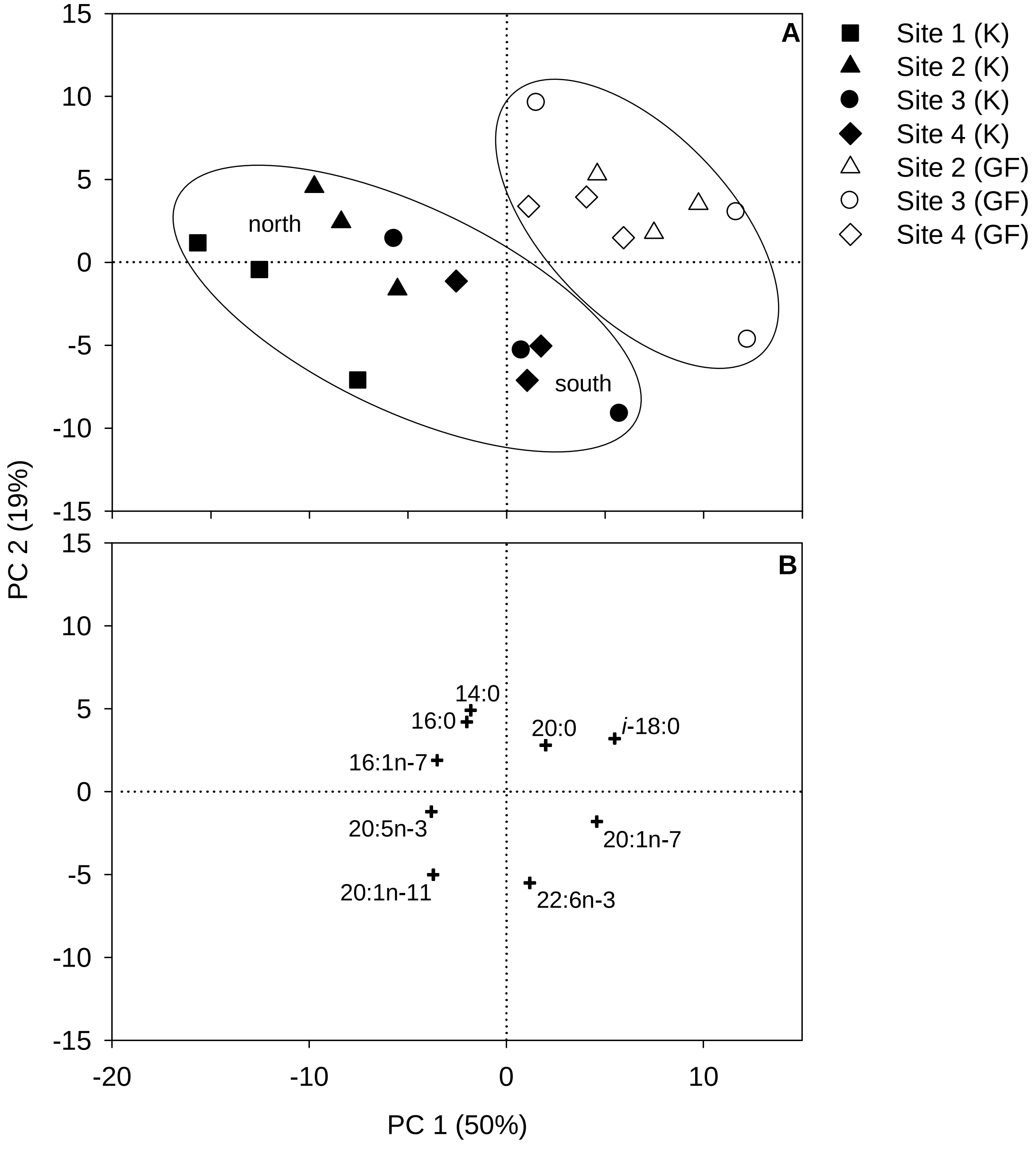

Figure 2.17 Principal Component Analysis (PCA) of the fatty acid signatures in the mussel (*Perna perna*) during March 2009 in the Kariega and Great Fish regions. Data represent arcsine-transformed proportional values (% TFA). Proportional variance of each principal component is explained by percentage values. (A) PCA scores demonstrating clear separation between geographic localities, with significantly different regions indicated by ellipses, as determined by MANOVA on PC scores (*n* = 21). An independent *t*-test on PC-1 or -2 scores revealed distinct northern *versus* southern site separation in the Kariega region. (B) PCA loadings illustrating the influential fatty acids (>2 %). Dashed lines denote the origin. Due to rapid tidal shift no mussels were collected at Site 1 in the Great Fish region. NMI, nonmethylene interrupted; K, Kariega; GF, Great Fish; Site 1, most northern; Site 2, just north of mouth; Site 3, just south of mouth; Site 4, most southern.

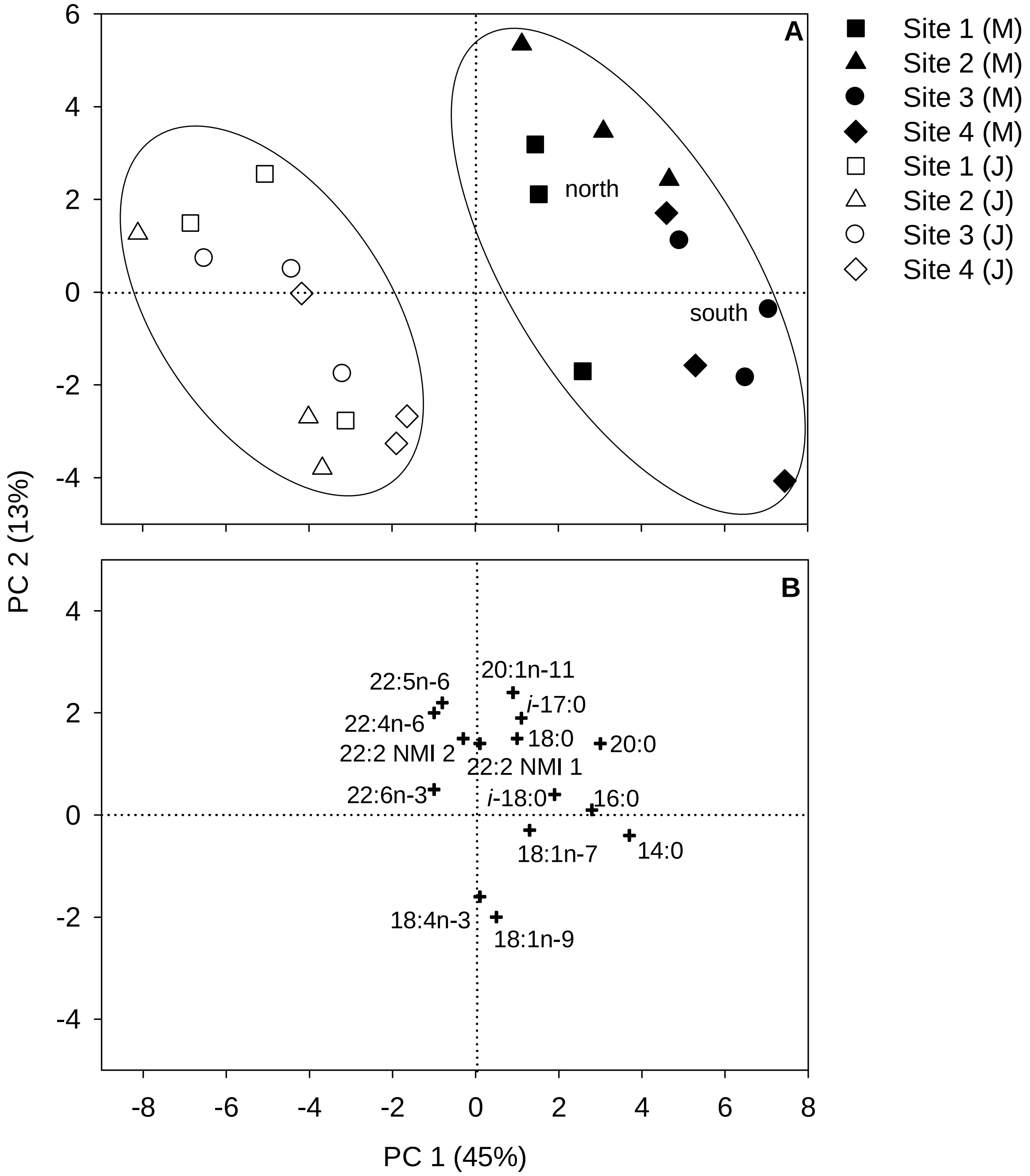

Figure 2.18 Principal Component Analysis (PCA) of the fatty acid signatures in the polychaete (*Gunnarea capensis*) during March and July 2009 in the Kariega region. Data represent arcsine-transformed proportional values (% TFA). Proportional variance of each principal component is explained by percentage values. (A) PCA scores demonstrating clear separation between March and July, with significantly different times indicated by ellipses, as determined by MANOVA on PC scores (n = 24). An independent t-test on PC-1 or -2 scores revealed distinct northern *versus* southern site separation in March. (B) PCA loadings illustrating the influential fatty acids (>2 %). Dashed lines denote the origin. NMI, nonmethylene interrupted; M, March; J, July; Site 1, most northern; Site 2, just north of mouth; Site 3, just south of mouth; Site 4, most southern

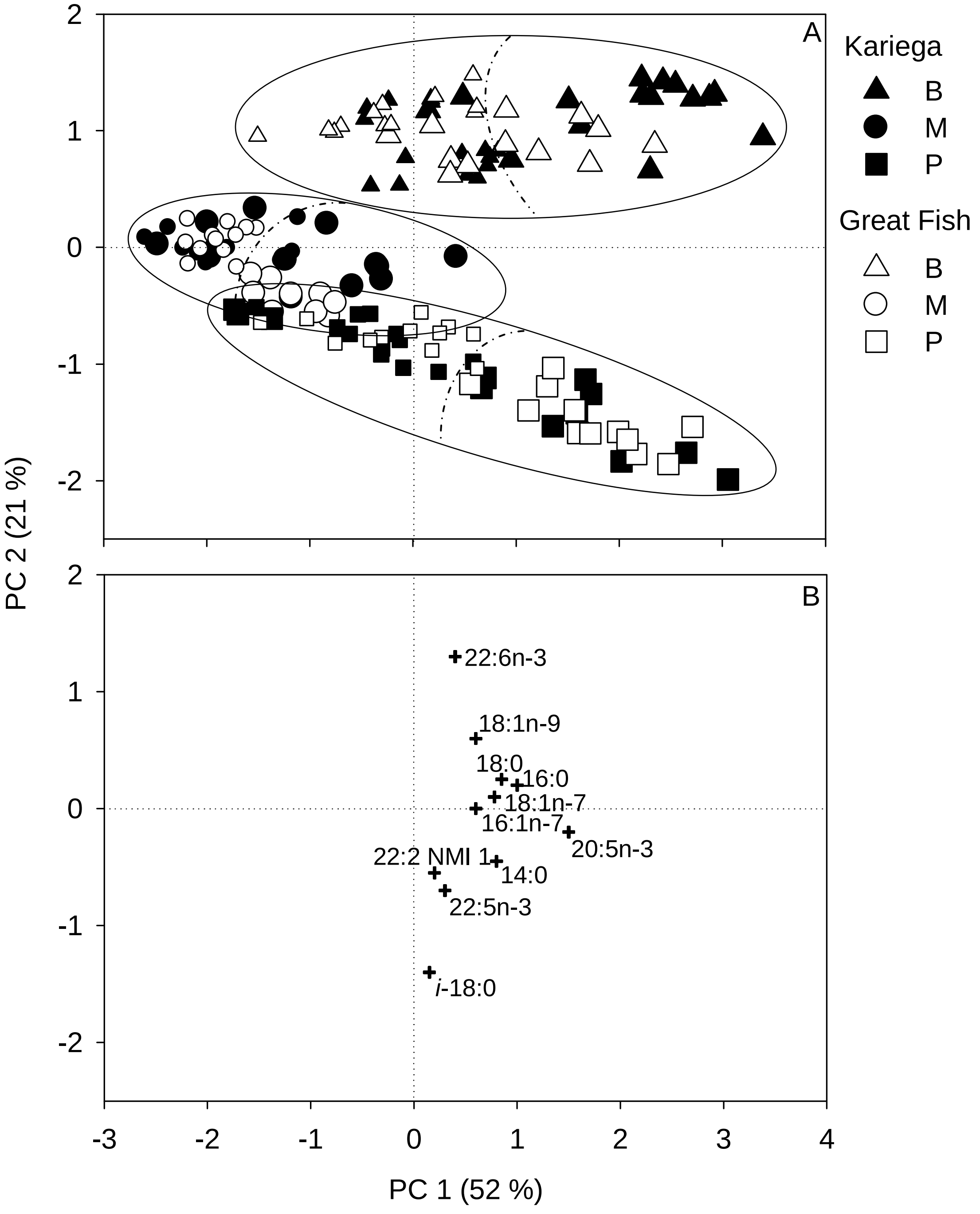

Figure 2.19 Principal Component Analysis (PCA) of the fatty acid signatures in the barnacle (*Tetraclita serrata*), mussel (*Perna perna*) and polychaete (*Gunnarea capensis*) during March and July 2009 in the Kariega and Great Fish regions. Data represent arcsine-transformed proportional values (% TFA). Proportional variance of each principal component is explained by percentage values. (A) PCA scores demonstrating separation among the consumers. Significantly different groups indicated by ellipses, as determined by Kruskal-Wallis ranked sum comparisons on PC scores (*n* = 147). (B) PCA loadings illustrating the influential fatty acids (>2 %). Dashed lines denote the origin. The size of the symbol corresponds to the seasons, i.e. March (large) and July (small) and dotted curves divide these dates. TFA, total fatty acids; NMI, nonmethylene interrupted; B, barnacle; M, mussel; P, polychaete.

2.4 Discussion

2.4.1 Environmental variables

The Kariega Estuary was characterised by a reverse salinity gradient during both sample times, reflecting the low freshwater inflow into the system and high evaporation rates (Table 2.1; Vorwerk et al., 2008). As a consequence, there were no clear distinctions in environmental variables such as salinity and chl-*a* concentration in the marine environment upstream and downstream of the estuary mouth. In contrast, the influence of the freshwater-dominated Great Fish Estuary on the adjacent marine environment was clearly evident. In March, when daily average flow rates were high, reduced salinities were recorded in the regions situated inside and directly south of the Great Fish Estuary mouth (Table 2.1).

Due to the low freshwater input, total chlorophyll concentrations in the Kariega Estuary and its associated rocky shores are generally low (Grange et al., 2000; Allan et al., 2010). The total chlorophyll-*a* (0.2-0.4 µg l^{-1}) and SPM (18.5-48.7 mg l^{-1}) values recorded in the Kariega region in 2009 coincided with literature values (<0.5 µg l^{-1} and 39-76 mg l^{-1}, respectively), although POM concentrations were slightly lower (5.4-6.6 mg l^{-1} compared to 11-23 mg l^{-1}; Table 2.1; Grange & Allanson, 1995; Froneman, 2001; Vorwerk et al., 2008). The general lack of clear temporal trends in SPM, POM and total chl-a biomass in the Kariega region are associated with the prevailing homogeneity of the estuary and the small influence of freshwater inflows (Grange & Allanson, 1995; Froneman, 2001). Grange & Allanson (1995) also found no seasonal trends in POM values in the Kariega Estuary, nor any distinct correlations between freshwater inflows and SPM, POM and chl-*a* biomass. Attenuated freshwater inflows are likely responsible for the lack of distinct temporal and among-site differences in particulate and chl-*a* values in the Kariega region.

For the freshwater-dominated Great Fish region, total chl-a concentrations fell within the lower-extreme range of that recorded in previous studies in the estuary (0.1-0.7 µg l^{-1} compared to 0.4-21.8 µg l^{-1}; Table 2.1; Grange et al., 2000; Bate et al., 2002). High river flows are generally associated with elevated chlorophyll concentrations ascribed to increased production by added nutrients or imported riverine chlorophyll (Vorwerk et al., 2008). However, standing stocks of phytoplankton are generally greater in the middle to upper reaches of the estuary, as opposed to closer to the marine environment (Grange & Allanson, 1995; Jennings, 2005). Allan et al. (2010) also recorded low chl-*a* values (0.14 ± 0.01 µg l^{-1}) in the nearshore coastal area adjoining the Great Fish region, thus highlighting the dynamic nature of biological variables in the coastal transition zone (Bate et al., 2002). Although the SPM concentrations in the Great Fish region during this investigation (26.0-216.9 mg l^{-1})

were in the range reported in other studies (126-509 mg l^{-1}), POM values were substantially lower than that previously recorded in the estuary (ranged from 4.5-15.1 mg l^{-1} in current study; ranged from 29-76 mg l^{-1} in literature; Grange & Allanson, 1995; Allan et al., 2010). River flows notably influence seston in the Great Fish Estuary, as evident by the significant temporal variation in SPM and chl-*a* concentrations recorded in 2009.

The enhanced concentrations of SPM and POM in both regions during March corresponded to elevated water temperatures in summer, and were greater in the Great Fish region as result of high river flows (although possibly not from added nutrients; Grange & Allanson, 1995; Bate et al., 2002; Frances & Guerrero, 2008; Vorwerk et al., 2008). The increase in SPM but decrease in chl-*a* concentration during March in the Great Fish region might therefore reflect an elevation in detritus and not necessarily chlorophyll from phytoplankton production. The total chl-*a* values recorded in the nearshore marine environment of both regions were broadly similar. The water flowing from the estuary is mixing with the marine waters in the nearshore marine environment (Frances & Guerrero, 2008). As a consequence, with increasing distance from the estuary mouth, a greater dilution of the estuarine water with the marine waters would occur. Additionally, the total chl-*a* values recorded in both regions reflected the reduced productivity of the coastal waters on the eastern shores of South Africa due to the nutrient-limited south-flowing Agulhas Current (Lutjeharms et al., 2000; Lutjeharms, 2007).

2.4.2 Fatty acid signatures of suspended particulate matter

The patterns in the proportional SPM fatty acid groups (SFAs, MUFAs, PUFAs, BFAs and HPFAs) differed between the Kariega and Great Fish regions (Appendix 2.1). The proportional PCA results for the SPM fatty acid signatures agree with the results of the qualitative summaries of the fatty acid groups in the Great Fish region. Distinct variation between March and July fatty acid proportions, and the SPM and chl-*a* content, was recorded in the Great Fish region (Figure 2.5). There was a lack of temporal variation in the environmental variables and the MUFAs, PUFAs and HPFAs in the SPM in the Kariega region, although a significant temporal difference was observed in the SPM fatty acid signatures (Figure 2.5). It is apparent that certain seasonal hydrodynamic factors influence the SPM fatty acid signatures in the Kariega and Great Fish regions as distinct differences were recorded between March and July (Figure 2.5). No significant spatial difference was recorded for the July SPM signatures, although 20:1n-9 appeared to significantly influence SPM signatures in both regions (Figure 2.5). During March, significant regional differences between the Kariega and Great Fish SPM signatures were recorded (Figure 2.5). It would appear that similar factors influence the regional and temporal patterns in the SPM fatty acid groups. The insignificant and substantial contributions of HPFAs to the Kariega and Great Fish SPM, respectively, are possibly associated with the regional flora and hydrodynamics in each system (Paterson & Whitfield, 1997; Quiñones & Montes, 2001; Vorwerk et al., 2001). Floristically, the littoral zone of the Kariega Estuary comprises eelgrasses and salt marsh vegetation with a lack of terrestrial inputs due to low freshwater flow. In comparison, the Great Fish Estuary has reeds and sedges along its banks and increased terrestrial plant influences due to the freshwater washing terrestrial matter into the river and estuary (Grange et al., 2000). The small levels of SPM HPFAs in the Kariega region in March and July are likely related to the small annual freshwater flow through the system. However, the site in the immediate vicinity of the mouth (<500 m) that showed a significant HPFA signal comes into direct contact with the river outflow and can possibly explain why an HPFA signal was detected at this site. In contrast, the Great Fish Estuary has substantial river flows that maintain consistent and high inputs of terrestrial detritus that reach the ocean during summer and winter (Grange et al., 2000; Vorwerk, 2006).

In both regions, bacterial components of the SPM appeared to increase during July when odd-numbered and *iso*-branched SFAs were detected (Appendix 2.1). This is possibly due to the increased colonisation of heterotrophic bacteria in sediments and SPM, especially when freshwater inputs are low (i.e. during austral winter) and hydrodynamic mixing forces are reduced, so allowing suspended particulates to settle (Morris, 1984). However, the BFAs (15:0 and 17:0) were elevated relative to HPFAs in the SPM during March in the Kariega region. This can possibly be explained by the residence times of water within the system (Largier & Taljaard, 1991; Jennings, 2005). Detritus, bacteria and other heterotrophic microorganisms form a substantial part of the seston in the Kariega Estuary (Grange & Allanson, 1995). Increased degradation of plant materials within the freshwater-restricted Kariega Estuary with long water retention times (~2 weeks; Jennings, 2005) could increase bacterial colonisation of detritus in summer when water temperatures are high. In contrast, the seston in the Great Fish Estuary largely comprises nano- and micro-phytoplankton and terrigenous detritus (Grange et al., 2000; Vorwerk, 2006). The high freshwater inflows and short retention time (<1 day; Jennings, 2005) possibly limits bacterial inputs. Small proportions of HPFAs (0.4-3.0 % TFA) compared to elevated proportions of BFAs (20.4-22.2 % TFA) in SPM were previously recorded in the Kariega and Great Fish regions (Allan et al., 2010).

The EFAs demonstrated a temporal change in the Kariega and Great Fish regions, reaching higher levels in July and potentially indicating that the quality of the SPM increased in winter. Brett & Müller-Navarra (1997) in their review on 20:5n-3 and 22:6n-3 in aquatic food webs noted that these two fatty acids increased phytoplankton quality, as a significant increase in fatty acid ratios was recorded in the animals that consumed the higher quality food. The diatom biomarker 16:1n-7 had the highest contribution relative to the other phytoplankton markers to SPM in the Kariega and Great Fish regions during both sample times (Figure 2.4). Distinct temporal differences were also noted in diatom marker 20:5n-3 levels in the Kariega and Great Fish regions, and were significantly increased in July (Figure 2.4). The 20:5n-3 component was also responsible for the significant contribution of EFAs noted in July in both regions. The dinoflagellate marker 22:6n-3 occurred in consistently low proportions relative to the diatoms, and was negligible in the Great Fish region in March. The regional consistency in 16:1n-7 proportions are indicative of the coastal waters in these parts of the Eastern Cape Province and are associated with the predominance of diatoms (St. John & Lund, 1996; Machu et al., 2005).

Diatoms and dinoflagellates form an integral part of the phytoplankton assemblages along the Eastern Cape shoreline. A study examining spatial changes on a larger scale than the current study in the diet of mussels along the eastern coast of South Africa, recorded substantial contributions of the diatom markers 16:1n-7 and 20:5n-3 to mussels from the coastal waters upstream and in the vicinity of the Kariega and Great Fish regions, with an increase in dinoflagellate marker 22:6n-3 levels downstream of the two regions all the way to the south-eastern Cape (Allan et al., 2010). In the Great Fish region in 2009, the marked temporal difference in diatom proportions may coincide with river flows during this period as result of increased nutrient inputs to the system (flow rates three times those noted during July; Table 2.1). However, since the marine-dominated Kariega system has negligible freshwater outflows, the changes observed in 20:5n-3 proportions must be ascribed to alternative factors, likely including changes in the phytoplankton communities, influencing the variability of this biomarker between sample times. It should also be noted that although diatoms were abundant in the Great Fish region in both months (Figure 2.4), terrestrial inputs (18:2n-6 and 18:3n-3) equalled and surpassed phytoplankton levels in March and July, respectively. Due to the high variability in SPM in a dynamic region like the coastal transition zone, making conclusions based on a few samples is challenging as each individual sample could have given quite different results, and further investigation is therefore necessary.

2.4.3 Fatty acid signatures of consumers

The interspecific patterns in the qualitative fatty acids groups of the barnacle, mussel and polychaete provided information on temporal changes in dietary compositions in the Kariega and Great Fish regions. The fatty acid profiles of all three suspension-feeders intermittently reflected the patterns in the SPM in the Kariega and Great Fish regions (Figures 2.6 to 2.8; Appendix 2.1). As SPM provides information on the current composition of particulate sources and animal tissues a longer and averaged view of assimilated nutrients, discrepancies between the SPM and consumers can be expected (Hill et al., 2006). Large proportions of the SFAs 14:0, 16:0 and 18:0 were expected since these components represent the most common fatty acids in nature and form integral parts of cellular membrane components (Garrett & Grisham, 1999). Detritus is recognised as a source of SFAs with 14 to 18 carbons (Freites et al., 2002), and high levels of these fatty acids could indicate significant inputs of detritic and bacterial material in the diets of the suspension-feeders. All three species consistently had substantial levels of BFAs in their tissues (4.2-17.7 % TFA), again reflecting detrital contributions to the diets of the suspension-feeders in the Kariega and Great Fish regions. The mussels and polychaetes, however, had greater fractions of BFAs (i.e. 15:0, 17:0, *i*-18:0; 9.0-17.7 % TFA) than the barnacles. Similar high BFA levels in mussels have been previously recorded along the Eastern Cape coastline (12.3-21.0 % TFA; Allan et al., 2010). During March, however, proportions of BFAs were one to five percent greater at the southern sites in the Kariega region (Figures 2.6 to 2.8). As the Kariega Estuary is characterised by salt marshes in the middle to lower reaches (Froneman, 2000), marsh detritus may be more prevalent at southern locations in summer due to the flow of water from the estuary mouth captured in a southward direction.

The large levels of PUFAs relative to MUFAs, with slight increases in PUFAs during winter, have been observed in marine invertebrates (Budge et al., 2001; Kharlamenko et al., 2001; Freites et al., 2002; Saito et al., 2002). Budge et al. (2001) noted that bivalves utilise PUFAs for growth and development, especially long-chain n-3 and n-6 PUFAs. High levels of 16:1n-7 and 18:1n-7 MUFAs reflected the dominance of phytoplankton as food source for the intertidal consumers (Freites et al., 2002; Narváez et al., 2008). Specifically, the diatom marker 16:1n-7 in the suspension-feeders mirrored the fairly equal proportions found in the SPM in both regions and seasons, and indicated consistent contributions of phytoplankton to their diets. The fatty acid composition data generally identified phytoplankton, detritus and bacterial sources as important food to the rocky shore suspension-feeders in the Kariega and

Great Fish regions (Figure 2.19). Although the invertebrates consumed these food sources in different proportions, the results for this study supported the hypothesis that all three species consume similar food sources since they fall within the same trophic guild.

Certain changes in the diatom and dinoflagellate markers indicated intraspecific temporal and spatial patterns in the contributions of phytoplankton food to rocky shore barnacles, mussels and polychaetes (Figures 2.9 to 2.11). The significant increase in 20:5n-3 in the SPM during July was reflected in the high levels of EFAs and PUFAs observed in the barnacles and polychaetes (Table 2.2; Figures 2.4, 2.8 and 2.19; Appendix 2.3). The importance of the diatom markers 16:1n-7 and 20:5n-3 in barnacle tissues varied temporally, and 16:1n-7 relative to the other markers was greater in March and 20:5n-3 and 22:6n-3 were more important in July (Table 2.2; Figure 2.13). The presence of the dinoflagellate marker 22:6n-3 in substantial proportions, along with the ratio marker (22:6n-3/20:5n-3) which was significant in both months in the Kariega and Great Fish regions, indicated dinoflagellates as potentially important in the diets of the barnacles (Figure 2.19). The importance of dinoflagellates has been observed in the genus *Tetraclita* (i.e. *T. squamosa*), where the substantial contribution of phytoplankton (~53 % of gut contents) in their diet showed slightly greater levels of dinoflagellates (~30 %) relative to diatoms (~23 %; Hunt & Alexander, 1991). Mussels also had greater levels of the dinoflagellate marker in the Kariega and Great Fish regions during both sample times (7.8-15.3 % TFA), and reflected values similar to those recorded previously for mussels along these coastal regions (9.5-9.7 % TFA; Table 2.2; Figure 2.14; Allan et al., 2010). However, the higher levels of diatoms in mussel diets in March compared to July in the Kariega region may indicate selection of these microalgae when they are available (Raby et al., 1997), but more information on food availability is needed to conclude this with confidence. Alternatively, a strong seasonal pattern in the phytoplankton community composition may be present. It is well known that under conditions of high turbulence, dinoflagellates outcompete diatoms, and such a scenario would likely exist during winter (Frances & Guerrero, 2008). Polychaetes had small proportions of the microalgal components 16:1n-7 and 22:6n-3, and had significantly greater concentrations of 20:5n-3 (4.0-13.4 µg fatty acid mg^{-1} dry mass) in both regions (Figures 2.15 and 2.19). Similar levels (5.3-6.2 µg fatty acid mg^{-1} dry mass) of 20:5n-3 have been recorded in an analogous nereidid marine polychaete from an intertidal sand flat in Cardiff Bay (García-Alonso et al., 2008).

Seston food quantity and quality varied notably over space and time (Berg & Newell, 1986). A study evaluating the value of different fatty acids (18:2n-6, 18:3n-3, 20:4n-6 and

22:6n-3) in the quality of the diets for a marine invertebrate (*Penaeus chinensis*) identified 22:6n-3 as the EFA with the highest value for ensuring growth and survival (Xu et al., 1993). The diets containing 22:6n-3 (~16.3 % TFA) were responsible for the highest survival and growth rates of all the PUFA-supplemented diets, with greater moulting frequency and weight gains per moult observed in the animals consuming this diet (Xu et al., 1993). As the EFAs can mirror the quality of the food sources obtained by the suspension-feeders (Stutzman, 1995; Brett & Müller-Navarra, 1997), the contribution of these fatty acids to the diets of the mussels and polychaetes (17.0-29.0 % TFA) signify that food sources for these two species were of consistent high quality in the Kariega and Great Fish regions during both sample times (Figures 2.6 to 2.8). The increase in quality of available food sources to barnacles during July (27-44 % TFA) can likely be ascribed to the elevated contributions of the 20:5n-3 and 22:6n-3 markers to their diet (Figures 2.13 and 2.19). The literature suggests that barnacles generally feed unselectively, and that the size of particles consumed is largely a function of body size (Sanford & Menge, 2001; Dubois et al., 2007; Riisgard & Larssen, 2010). As such, the significant increase in 20:5n-3 EFA levels in the water during July possibly has a greater influence on the lipid content of the barnacles, while mussels and polychaetes, due to their wide particle selection abilities, are thus able to maintain a more consistent high quality diet through time (Schleyer, 1981; Phillips & Pernet, 1996; Espinosa et al., 2008). The greater levels of the 22:6n-3 marker in *P. perna* and *T. serrata* tissues may reflect the successional change in phytoplankton assemblages when diatoms become silicate-depleted and dinoflagellates become more abundant (Peschak, 2005). However, as 20:5n-3 and 22:6n-3 are essential to marine invertebrates, the variation in fatty acid composition between species could be due to differences in metabolism and not necessarily diet (Iverson et al., 2004; Schmidt-Nielsen, 2007; Thompson et al., 2008). Although intraspecific temporal and spatial trends were detected through changes in the fatty acid composition of consumers, interspecific variation was also apparent. These variations in fatty acid composition among species disputed the hypothesis that no variation occurs among the suspension-feeders.

The degree of omnivory among the suspension-feeders showed differences in the rocky shore invertebrates' nutrition. Apart from two sites in March, terrestrial food sources appeared insignificant in the diet of the barnacles in both regions (Figure 2.6). The presence of the copepod marker 20:1n-11, and high ratios of the omnivory ratio 18:1n-9/18:1n-7 (~2.1) observed in *T. serrata* in both regions during July indicates that barnacles consume zooplankton (Achituv et al., 1997; Teegarden, 1999; Teegarden et al., 2001). Zooplankton density peaked during winter in the Kariega (~3788 ind.m^{-3}) and Great Fish regions (~4860 ind.m^{-3}) in 2006 mainly as result of the copepods *Calanus agulhensis* and *C. simillimus* and their nauplii (Vorwerk, 2006). In addition, the SPM fatty acid signatures in July were also observed to be influenced by the copepod marker 20:1n-9 (Figure 2.5). Large contributions of small crustaceans (i.e. copepods) have been recorded in the gut contents of barnacles (~18 %) and have highlighted the importance of zooplankton in the diet of this suspension-feeder (Hunt & Alexander, 1991). Mussels are known to ingest zooplankton (Davenport et al., 2000) and appeared to have an overall moderate contribution to their diet (18:1n-9/18:1n-7 ratio ~1.3) in the Kariega and Great Fish regions. Similar to barnacles, omnivory appeared to increase in mussels in the Kariega region in July (18:1n-9/18:1n-7 ratio ~1.5) when copepods reach maximum densities (Vorwerk, 2006). As phytoplankton growth may be limited by the decrease in water temperature in July, supplementation of phytoplankton food with additional animal nutrients may become important during winter (Jeffries, 1970). The polychaetes did not contain large levels of heterotrophic (18:1n-9/18:1n-7 ratio ~0.4) or terrestrial markers, which was consistent with the literature regarding food preferences of serpulid marine polychaetes (Figure 2.8; Phillips & Pernet, 1996; Yokoyama et al., 2005). Fatty acids indicated the contribution of coastal benthic macroalgae to the diets of the polychaetes in both regions, as 18:1n-7 (10.8 ± 0.8 % TFA) and 18:4n-3 (5.3 ± 2.7 % TFA) have been recorded in *Ulva sp*., and 20:4n-6 in *Gelidium pristoides* and *G. enterobium* (20-22 % TFA; Allan et al., 2010).

In contrast to the low contributions of 18:2n-6 and 18:3n-3 to the diets of the barnacles and polychaetes, mussels clearly assimilated terrestrial sources during March and July in both regions (Table 2.2). Preferential selection has been identified as a mechanism by which bivalves (i.e. mussels) sort and ingest particles that would contribute to a higher quality of food in their diet (Jørgensen, 1996). However, terrestrial food sources as direct nutrition without microbial decomposition contribute very little to rocky shore suspension-feeder diets (Kharlamenko et al., 2001; 2008; McLeod & Wing, 2009; Lebreton et al., 2011). The BFAs (i.e. *i*-16:0 and *i*-18:0) that were abundant in mussel tissues in both regions probably reflected bacteria from the SPM detritus (Figures 2.9 to 2.12; Allan et al., 2010). Detritus was an important food source for *P. perna* on a rocky shore in Durban (South Africa), where the organic fraction of the available food comprised 94 % detritus with attached bacteria (Berry & Schleyer, 1983). In addition, macroalgae appeared to be important to the diets of mussels in the Kariega and Great Fish regions (Figure 2.17; Appendix 2.4). Moderate proportions of 18:1n-7 (10.8 ± 0.8 % TFA) and 18:4n-3 (5.3 ± 2.7 % TFA) have been noted in the fatty acid profiles of *Ulva sp*. in the Kariega region (Allan et al., 2010). The contribution of 20:4n-6 in mussel tissues in the Great Fish region in March and July may indicate the macroalgae *G. pristoides* (21.6 ± 2.6 % TFA) and *G. enterobium* (20.0 ± 1.9 % TFA) as important food sources to the mussels (Allan et al., 2010).

The distinct intraspecific changes in food sources for the barnacles recorded during the current study correspond to the literature regarding omnivory on phytoplankton, macroalgae, bacteria and zooplankton (Achituv et al., 1997; Zhukova, 2000; Hill & McQuaid, 2008). The significant regional and temporal changes in the fatty acid profiles of the barnacles concurred with the regional and temporal hypotheses, as the diatoms (16:1n-7) and detritus (*i*-16:0 and 17:0) were important in March, and the diatoms (20:5n-3), flagellates (18:4n-3 and 22:6n-3) and zooplankton sources (18:1n-9 and 20:1n-11) essential during July in both regions (Figures 2.16 and 2.19). Although mussels showed intermediate levels of omnivory, their preference for autotrophic food was reflected by their increased proportions of macroalgae (18:1n-7 and 18:4n-4), and terrigenous (18:2n-6 and 18:3n-3) and detrital food sources (17:0 and *i*-18:0). Furthermore, the diatoms were more abundant in mussel tissues in March in the Kariega and Great Fish regions, and the dinoflagellates, copepod marker and detritus during July in both regions. The temporal differences in the mussel fatty acid signatures did not closely reflect the SPM, although large dependence on detritus supports the abundant BFAs that were detected in mussels from both regions (Appendix 2.1). However, the regional hypothesis of distinct differences between the two regions was applicable in March when elevated diatom markers were detected in the Kariega mussels (Figure 2.14). Similarly, the difference between sample times in polychaete fatty acids agreed with the temporal hypothesis predicting distinct temporal variation, as the diatoms (16:1n-7 and 20:5n-3) were consumed in March, and to a lesser extent flagellates (18:4n-3 and 22:6n-3) and detritus (*i*-15:0, 15:0, 17:0 and *i*-18:0) were important in July in both regions (Figure 2.18; Appendices 2.6 and 2.7). For the degree of omnivory, the results for this study thus opposed the hypothesis predicting no variation among the species, as barnacles appeared to show increased omnivory compared to mussels and polychaetes. Mussels appeared to consume food from animal and plant sources, while polychaetes had greater contributions of fatty acid markers from plant origins.

Some potential north *versus* south intraspecific differences in fatty acid composition among the suspension-feeders were observed, and were probably related to flow dynamics down the coast creating a difference in food for consumers at a relatively small spatial scale (Table 2.2; Figures 2.4, 2.14, 2.16, 2.17 and 2.18). To determine what might be driving any differences more specifically, follow up studies will need to be conducted. The distinct north/south separation observed for all three invertebrates in the Kariega region in March, while not in the Great Fish region, can probably be linked to the hydrology of the different systems. The Great Fish Estuary is always freshwater dominated due to the inter-basin transfer system from the Orange River (Bate et al., 2002), while the Kariega Estuary can switch from net exporter to net importer of nutrients under conditions of upwelling (Grange & Allanson, 1995). Mouth morphology and plume formation could be important contributing factors influencing the outflow of river water and food sources to consumers (Vorwerk, 2006). The morphology of the estuary mouth could play a vital role in determining the direction of water outflow, and seasonal changes in coastal topography could occur and influence the direction and magnitude of estuarine outwelling. Additionally, the flow of river water into the coastal water on the eastern shores of South Africa is influenced by the Agulhas Current (Lutjeharms et al., 2000; Peschak, 2005).

The outflow of river water from the Kariega Estuary, with reduced freshwater inputs, does not form a large plume in the adjoining coastal waters (Grange et al., 2000). This was clearly shown along the marine shore near the Kariega Estuary, where estuarine-derived carbon sources were only detected in the waters immediately adjacent to the estuary mouth (Vorwerk, 2006). Water flowing out of the Kariega Estuary is likely entrained by the water flowing in a south-easterly direction, thereby influencing nutrient availability that could otherwise have contributed to the food sources of northern populations. This pattern was evident in the Kariega region during March in 2009, as dinoflagellates and detritus were recorded in higher proportions at the sites situated south of the estuary mouth. In contrast, the Great Fish Estuary, with high freshwater inputs, causes local turbulent mixing and the formation of a large plume upstream and downstream of the estuary mouth (Grange & Allanson, 1995; Grange et al., 2000). The absence of any spatial patterns in the fatty acid profiles is therefore not unexpected. It is worth noting that Vorwerk (2006) demonstrated that POM and chl-*a* derived from the Great Fish Estuary could be detected up to 12 km downstream (southwards) of the estuary mouth. The transport of the estuarine-derived carbon was probably largely due to the estuarine plume being entrained in the southward-flowing Agulhas Current. The results for my study therefore opposed the hypothesis predicting a higher dependence by southern populations on terrestrially-derived plant material, since although there was a distinct difference in fatty acid signatures between northern- and southern-located populations in the Kariega region in March, higher plants did not appear to influence this separation.

2.5 Summary

The fatty acid compositions of the suspension-feeders reflected that similar food sources generally contributed to their diets in the Kariega and Great Fish regions during both sampling times. However, both intra- and inter-specific changes were noted in the fatty acid profiles of the suspension-feeders. Small differences relating to proportional contributions of various fatty acid groups, like the seasonal increase in PUFAs relative to SFAs in the barnacles and mussels, and the higher levels of BFAs compared to EFAs in the mussels and polychaetes, indicated that as a trophic guild the barnacles, mussels and polychaetes still consume similar food sources in the Kariega and Great Fish regions. All three suspension-feeders clearly demonstrated herbivorous feeding, although omnivory was more prevalent in the barnacles, followed by the mussels based on their moderate to high omnivory ratios (18:1n-9/18:1n-7). In general, diatoms, macroalgae and detritus were food sources in March; and flagellates, zooplankton, detritus, and diatoms became important in July. The interspecific similarities in fatty acid signatures in the suspension-feeders between March and July largely mirrored the SPM. The most important food source in the diets of all three suspension-feeders was phytoplankton. The diatom (16:1n-7 and 20:5n-3) and dinoflagellate (22:6n-3) markers consistently influenced intraspecific spatial patterns through time, signifying the importance of phytoplankton assemblages as food sources to suspension-feeders. It appeared that levels of the diatom marker 16:1n-7 did not change in the consumer tissues between regions or dates, but contributions of the diatom marker 20:5n-3 increased in the consumers at both regions in July, possibly indicating a species-shift in the phytoplankton communities through time. Barnacles and mussels had moderate and high contributions of the diatom and dinoflagellate markers, respectively, whereas the polychaetes showed greater levels of diatoms. The high levels of BFAs relative to HPFAs in the tissues of the suspension-feeders illustrated that SPM and macroalgal detritus contributed to consumer diets. However, the Great Fish region is generally characterised by large contributions of terrestrial sources and the significant levels of HPFAs in the mussel tissues in both regions suggested a reliance of this suspension-feeder on higher plants.

Distinct spatial and temporal patterns were recorded for the SPM and three indigenous suspension-feeders, indicating that regional flora and hydrology play a role in influencing diets of rocky shore organisms. The results for this study supported the regional hypothesis for the barnacles in March and July and for the mussels in March, signifying that the differences in freshwater inputs in the two systems influence regional food sources for these suspension-feeders. A change in the contributions of phytoplankton assemblages and bacterial inputs between the two regions is likely the main factor influencing barnacle and mussel food source variation. No regional changes in fatty acid profiles were observed in the polychaetes in March or July, which is reflected by the consistent assimilation of diatoms as major phytoplankton food. The hypothesis predicting distinct temporal changes in fatty acid composition as result of seasonal variation in food sources, however, was supported by data on all three suspension-feeders. This possibly relates to distinctions in metabolism and the seasonal differences in river flows, phytoplankton communities and the subsequent variation in available food. Specifically, detritus appeared to play a major role in structuring north *versus* south communities in the Kariega region in March.

No evidence was found for increased utilisation of terrestrial sources (i.e. higher plants) by southern-located populations in the Kariega and Great Fish regions during March or July, although mussels incorporated significant levels of terrigenous material in their diets. Bacterial-derived sources, however, occurred in higher levels in the tissues of all three suspension-feeders at the southern sites of the Kariega region in March. In addition, the barnacles and mussels fatty acid profiles reflected potential differences in phytoplankton consumption, whereby higher levels of diatoms were recorded at the northern sites, and predominance to dinoflagellates at the southern sites. Macroalgal detritus also played a role in influencing southern-located populations in the Kariega region during March. Although the key hypothesis regarding increased utilisation of riverine carbon (i.e. higher plant) by southern populations was not supported, geographic location and date strongly influenced fatty acid composition of the invertebrates.

Appendix 2.1 Fatty acid compositions (% TFA) for the suspended particulate matter (SPM) in the Kariega and Great Fish regions during austral March and July 2009. Due to logistical constraints no replicate samples were collected per site (1 to 5). Values are represented by fatty acids at concentrations >1 % TFA (present in at least one sample; *n* = 5 per region or date). TFA, total fatty acids; SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; BAFA, bacterial fatty acids; HPF, higher plant fatty acids; EFA, essential fatty acids (20:4n-6; 20:5n-3 and 22:6n-3).

	March 2009										July 2009									
	Kariega					Great Fish					Kariega					Great Fish				
Fatty Acid	**1**	**2**	**3**	**4**	**5**	**1**	**2**	**3**	**4**	**5**	**1**	**2**	**3**	**4**	**5**	**1**	**2**	**3**	**4**	**5**
14:0	2.9	2.2	4.2	3.2	0.7	0.0	5.6	0.0	7.4	7.1	4.0	3.8	5.9	4.5	5.0	2.7	4.5	3.8	2.4	0.0
i-15:0	1.4	0.0	0.0	1.1	0.7	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
15:0	4.3	2.2	4.2	4.2	1.5	0.0	0.0	0.0	3.7	3.6	4.0	3.8	0.0	0.0	5.0	2.7	4.5	3.8	4.9	7.1
i-16:0	1.4	0.0	2.1	0.5	0.7	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
16:0	27.1	22.2	26.0	22.1	20.4	25.0	22.2	26.7	29.6	32.1	28.0	26.9	23.5	27.3	30.0	27.0	22.7	26.9	17.1	14.3
i-17:0	0.0	0.0	1.0	0.0	0.7	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
17:0	2.9	4.4	2.1	4.2	1.5	0.0	0.0	6.7	3.7	0.0	4.0	3.8	5.9	0.0	0.0	2.7	4.5	3.8	2.4	0.0
i-18:0	0.0	0.0	0.0	1.6	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
18:0	7.1	8.9	10.4	5.8	6.6	12.5	11.1	13.3	11.1	10.7	8.0	7.7	11.8	9.1	10.0	8.1	9.1	11.5	7.3	7.1
20:0	0.0	2.2	0.0	0.0	0.7	6.3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
22:0	5.7	4.4	2.1	4.2	1.5	6.3	0.0	0.0	3.7	3.6	4.0	3.8	5.9	4.5	5.0	2.7	4.5	3.8	4.9	7.1
24:0	2.9	2.2	7.3	1.1	1.5	0.0	5.6	0.0	0.0	0.0	4.0	0.0	0.0	4.5	0.0	0.0	0.0	0.0	2.4	0.0
ΣSFAs	**55.7**	**48.9**	**59.4**	**47.9**	**36.5**	**50.0**	**44.4**	**46.7**	**59.3**	**57.1**	**56.0**	**50.0**	**52.9**	**50.0**	**55.0**	**45.9**	**50.0**	**53.8**	**41.5**	**35.7**
16:1n-7	4.3	4.4	6.3	6.3	2.9	6.3	5.6	6.7	3.7	3.6	4.0	7.7	5.9	4.5	5.0	5.4	4.5	3.8	4.9	7.1
16:1n-5	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
18:1n-9	7.1	20.0	2.1	26.3	27.0	0.0	5.6	6.7	3.7	7.1	20.0	19.2	17.6	18.2	15.0	18.9	18.2	11.5	17.1	14.3
18:1n-7	14.3	0.0	20.8	0.0	0.0	18.8	22.2	20.0	25.9	14.3	0.0	3.8	5.9	4.5	5.0	8.1	9.1	7.7	9.8	7.1
20:1n-11	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
20:1n-9	0.0	8.9	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	8.0	11.5	11.8	9.1	10.0	10.8	4.5	7.7	12.2	7.1
20:1n-7	5.7	6.7	8.3	5.8	10.2	6.3	5.6	6.7	0.0	7.1	4.0	3.8	0.0	4.5	5.0	0.0	0.0	3.8	7.3	7.1
ΣMUFAs	**31.4**	**40.0**	**37.5**	**38.4**	**40.1**	**31.3**	**38.9**	**40.0**	**33.3**	**32.1**	**36.0**	**46.2**	**41.2**	**40.9**	**40.0**	**43.2**	**36.4**	**34.6**	**51.2**	**42.9**
16:3n-4	0.0	0.0	0.0	1.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
18:2n-6	1.4	8.9	1.0	1.1	0.7	6.3	5.6	6.7	3.7	3.6	0.0	0.0	0.0	0.0	0.0	2.7	4.5	3.8	2.4	7.1
18:3n-3	0.0	0.0	0.0	0.0	1.5	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	2.7	4.5	3.8	2.4	7.1
18:4n-3	2.9	0.0	0.0	3.7	2.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	5.0	0.0	0.0	0.0	0.0	0.0
20:2n-6	4.3	0.0	1.0	6.8	14.6	6.3	5.6	6.7	7.4	7.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
20:4n-6	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
20:5n-3	1.4	2.2	1.0	1.1	1.5	0.0	0.0	0.0	0.0	0.0	4.0	3.8	5.9	9.1	0.0	2.7	4.5	3.8	2.4	7.1
22:2 NMI 1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
22:2 NMI 2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
21:5n-3	0.0	0.0	0.0	0.0	0.7	6.3	5.6	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
22:4n-6	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
22:5n-6	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
22:5n-3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
22:6n-3	2.9	0.0	0.0	0.0	2.2	0.0	0.0	0.0	0.0	0.0	4.0	0.0	0.0	0.0	0.0	2.7	0.0	0.0	0.0	0.0
ΣPUFAs	**12.9**	**11.1**	**3.1**	**13.7**	**23.4**	**18.8**	**16.7**	**13.3**	**11.1**	**10.7**	**8.0**	**3.8**	**5.9**	**9.1**	**5.0**	**10.8**	**13.6**	**11.5**	**7.3**	**21.4**
ΣBFAs	**10.0**	**6.7**	**9.4**	**11.6**	**5.1**	**0.0**	**0.0**	**6.7**	**7.4**	**3.6**	**8.0**	**7.7**	**5.9**	**0.0**	**5.0**	**5.4**	**9.1**	**7.7**	**7.3**	**7.1**
ΣHPFAs	**1.4**	**8.9**	**1.0**	**1.1**	**2.2**	**6.3**	**5.6**	**6.7**	**3.7**	**3.6**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**5.4**	**9.1**	**7.7**	**4.9**	**14.3**
ΣEFAs	**4.3**	**2.2**	**1.0**	**1.1**	**3.6**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**8.0**	**3.8**	**5.9**	**9.1**	**0.0**	**5.4**	**4.5**	**3.8**	**2.4**	**7.1**

Appendix 2.2 Fatty acid composition (% TFA) in barnacle (*Tetraclita serrata*) tissues at the Kariega and Great Fish regions during March 2009. Values are represented by means ± standard deviation for all fatty acids at concentrations >1 % TFA (present in at least one sample; *n* = 3). TFA, total fatty acids; SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; BAFA, bacterial fatty acids; HPF, higher plant fatty acids; EFA, essential fatty acids (20:4n-6; 20:5n-3 and 22:6n-3).

Fatty Acid	Kariega					Great Fish			
	Site 1	Site 2	Site 3	Site 4	Site 5	Site 1	Site 2	Site 3	Site 4
14:0	4.9 ± 2.0	5.5 ± 0.7	3.1 ± 1.0	4.7 ± 0.6	3.9 ± 0.1	1.6 ± 0.3	1.2 ± 0.8	5.8 ± 2.8	5.7 ± 1.8
i-15:0	0.4 ± 0.0	0.3 ± 0.2	0.2 ± 0.1	0.3 ± 0.0	0.3 ± 0.0	1.2 ± 0.4	0.7 ± 0.2	0.9 ± 0.3	0.6 ± 0.2
15:0	0.6 ± 0.1	0.7 ± 0.1	0.7 ± 0.2	0.6 ± 0.0	0.9 ± 0.1	0.8 ± 0.4	0.6 ± 0.2	0.7 ± 0.2	0.6 ± 0.1
i-16:0	1.6 ± 0.6	1.2 ± 0.4	1.2 ± 0.5	1.1 ± 0.4	0.9 ± 0.3	1.4 ± 0.0	1.7 ± 0.3	1.1 ± 0.4	1.1 ± 0.2
16:0	21.7 ± 1.4	20.8 ± 1.8	14.2 ± 1.1	16.5 ± 1.5	15.0 ± 0.9	12.6 ± 1.6	15.1 ± 3.8	22.7 ± 1.9	20.3 ± 3.2
i-17:0	0.4 ± 0.1	0.3 ± 0.1	0.2 ± 0.1	0.3 ± 0.1	0.5 ± 0.2	0.3 ± 0.1	0.5 ± 0.2	0.5 ± 0.1	0.4 ± 0.0
17:0	1.6 ± 0.2	1.4 ± 0.2	0.9 ± 0.1	1.2 ± 0.2	1.2 ± 0.2	1.1 ± 0.4	0.9 ± 0.3	1.5 ± 0.4	1.1 ± 0.3
i-18:0	0.3 ± 0.3	0.1 ± 0.1	0.2 ± 0.2	0.1 ± 0.1	0.1 ± 0.0	0.7 ± 0.5	0.1 ± 0.1	0.3 ± 0.2	0.1 ± 0.0
18:0	8.9 ± 0.9	7.4 ± 2.0	5.5 ± 0.4	5.9 ± 0.6	5.8 ± 0.0	7.0 ± 0.8	7.9 ± 1.9	8.8 ± 2.7	7.0 ± 0.7
20:0	0.5 ± 0.5	0.9 ± 0.7	0.3 ± 0.5	0.1 ± 0.1	0.3 ± 0.5	0.8 ± 0.4	0.4 ± 0.3	1.0 ± 0.7	0.5 ± 0.2
22:0	0.7 ± 0.2	0.8 ± 0.4	0.8 ± 0.2	0.6 ± 0.1	0.8 ± 0.1	0.7 ± 0.3	0.7 ± 0.2	0.8 ± 0.6	0.5 ± 0.1
24:0	0.4 ± 0.4	0.5 ± 0.2	0.3 ± 0.1	0.3 ± 0.3	0.3 ± 0.1	0.4 ± 0.1	0.4 ± 0.1	0.2 ± 0.1	0.2 ± 0.2
ΣSFAs	**37.5 ± 1.4**	**47.3 ± 4.4**	**40.2 ± 2.4**	**45.8 ± 2.5**	**46.5 ± 1.1**	**47.9 ± 2.7**	**40.0 ± 6.4**	**53.1 ± 5.1**	**49.0 ± 5.7**
16:1n-7	3.6 ± 0.9	4.0 ± 0.5	2.4 ± 0.8	3.1 ± 0.3	3.1 ± 0.6	2.5 ± 0.2	3.1 ± 1.4	4.9 ± 1.1	4.6 ± 0.9
16:1n-5	0.1 ± 0.1	0.0 ± 0.1	0.0 ± 0.0	0.2 ± 0.1	0.1 ± 0.1	0.2 ± 0.3	0.0 ± 0.0	0.3 ± 0.0	0.1 ± 0.2
18:1n-9	5.3 ± 0.3	4.8 ± 1.0	3.7 ± 0.3	3.6 ± 0.2	3.6 ± 0.5	3.4 ± 0.5	4.4 ± 1.2	5.5 ± 0.7	4.8 ± 0.5
18:1n-7	4.5 ± 0.7	3.6 ± 0.9	3.4 ± 0.3	3.6 ± 0.7	4.0 ± 1.1	4.1 ± 0.3	5.5 ± 1.4	5.7 ± 2.9	4.4 ± 0.7
20:1n-11	0.0 ± 0.0	0.2 ± 0.2	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.1 ± 0.1
20:1n-9	1.3 ± 0.6	1.0 ± 0.2	0.8 ± 0.1	0.8 ± 0.2	0.6 ± 0.1	1.5 ± 0.4	1.7 ± 0.4	1.4 ± 0.1	1.1 ± 0.1
20:1n-7	0.0 ± 0.0	0.0 ± 0.0	0.5 ± 0.5	0.4 ± 0.5	0.3 ± 0.3	1.0 ± 0.6	0.3 ± 0.4	0.2 ± 0.3	0.0 ± 0.0
ΣMUFAs	**13.2 ± 0.7**	**15.8 ± 2.6**	**15.7 ± 1.7**	**17.3 ± 0.8**	**18.4 ± 2.6**	**20.3 ± 2.3**	**20.0 ± 3.8**	**21.3 ± 3.5**	**19.2 ± 1.5**
16:3n-4	0.7 ± 0.5	0.3 ± 0.1	0.3 ± 0.1	0.4 ± 0.1	0.5 ± 0.1	0.5 ± 0.4	0.6 ± 0.4	0.5 ± 0.2	0.3 ± 0.1
18:2n-6	1.1 ± 0.0	1.0 ± 0.0	0.9 ± 0.2	1.0 ± 0.4	1.0 ± 0.1	0.8 ± 0.1	0.8 ± 0.1	0.9 ± 0.1	0.8 ± 0.1
18:3n-3	0.8 ± 0.2	0.8 ± 0.3	0.5 ± 0.2	0.7 ± 0.1	0.6 ± 0.1	0.4 ± 0.2	1.0 ± 0.6	0.6 ± 0.2	0.6 ± 0.3
18:4n-3	1.4 ± 0.5	1.2 ± 0.1	1.0 ± 0.4	1.4 ± 0.2	0.9 ± 0.2	0.7 ± 0.9	1.2 ± 0.4	0.7 ± 0.6	0.9 ± 0.1
20:2n-6	0.4 ± 0.2	0.5 ± 0.2	0.7 ± 0.1	0.6 ± 0.1	0.8 ± 0.2	1.4 ± 0.7	0.8 ± 0.3	0.5 ± 0.2	0.5 ± 0.2
20:4n-6	2.0 ± 0.2	1.6 ± 0.3	1.2 ± 0.3	1.1 ± 0.0	1.7 ± 0.3	0.7 ± 0.3	1.6 ± 0.5	1.0 ± 0.0	1.0 ± 0.2
20:5n-3	14.0 ± 0.4	10.3 ± 0.3	11.5 ± 2.2	9.3 ± 1.4	8.1 ± 1.1	6.2 ± 3.0	10.3 ± 1.7	6.7 ± 0.8	8.8 ± 2.3
22:2 NMI 1	0.0 ± 0.0	0.3 ± 0.5	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	1.4 ± 0.5	0.7 ± 0.3	1.3 ± 0.7	0.8 ± 0.1
22:2 NMI 2	0.1 ± 0.2	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
21:5n-3	0.7 ± 0.4	0.4 ± 0.1	0.1 ± 0.1	0.2 ± 0.2	0.2 ± 0.2	0.2 ± 0.1	0.5 ± 0.2	0.3 ± 0.1	0.3 ± 0.1
22:4n-6	0.0 ± 0.0	0.2 ± 0.3	0.1 ± 0.1	0.0 ± 0.0	0.0 ± 0.1	0.6 ± 0.5	0.7 ± 0.2	1.2 ± 0.8	1.2 ± 0.4
22:5n-6	0.5 ± 0.6	0.4 ± 0.5	0.1 ± 0.1	0.8 ± 0.5	0.1 ± 0.1	0.6 ± 0.2	0.5 ± 0.2	0.3 ± 0.3	0.3 ± 0.3
22:5n-3	0.8 ± 0.3	0.7 ± 0.1	0.6 ± 0.3	0.5 ± 0.2	0.6 ± 0.2	0.5 ± 0.4	0.7 ± 0.1	0.7 ± 0.4	0.5 ± 0.0
22:6n-3	14.4 ± 1.4	13.1± 1.2	14.3 ± 5.7	9.4 ± 0.6	8.0 ± 1.4	7.7 ± 4.0	10.8 ± 0.8	7.0 ± 0.8	8.8 ± 1.7
ΣPUFAs	**49.3 ± 2.4**	**36.9 ± 3.1**	**44.2 ± 8.1**	**36.8 ± 2.3**	**35.1 ± 2.8**	**31.8 ± 9.2**	**40.0 ± 4.9**	**25.6 ± 1.7**	**31.8 ± 3.2**
ΣBFAs	**4.5 ± 1.2**	**4.8 ± 0.8**	**5.4 ± 0.4**	**5.1 ± 0.4**	**6.1 ± 0.6**	**9.1 ± 0.2**	**5.8 ± 1.8**	**5.9 ± 1.2**	**4.9 ± 0.6**
ΣHPFAs	**1.7 ± 0.1**	**2.2 ± 0.3**	**2.0 ± 0.3**	**2.5 ± 0.5**	**2.3 ± 0.2**	**1.9 ± 0.3**	**2.7 ± 0.7**	**1.7 ± 0.2**	**1.9 ± 0.3**
ΣEFAs	**43.6 ± 1.9**	**30.1 ± 1.8**	**37.6 ± 8.0**	**28.6 ± 2.0**	**27.9 ± 2.5**	**21.2 ± 7.3**	**30.0 ± 2.8**	**17.7 ± 1.6**	**23.6 ± 4.2**

Appendix 2.3 Fatty acid composition (% TFA) in barnacle (*Tetraclita serrata*) tissues in the Kariega and Great Fish regions during July 2009. Values are represented by means ± standard deviation for all fatty acids at concentrations >1 % TFA (present in at least one sample; *n* = 3). TFA, total fatty acids; SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; BAFA, bacterial fatty acids; HPF, higher plant fatty acids; EFA, essential fatty acids (20:4n-6; 20:5n-3 and 22:6n-3).

Fatty Acid	Kariega					Great Fish			
	Site 1	Site 2	Site 3	Site 4	Site 5	Site 1	Site 2	Site 3	Site 4
14:0	1.7 ± 0.5	1.9 ± 0.0	2.6 ± 0.2	1.5 ± 0.4	2.1 ± 1.0	2.1 ± 1.2	1.3 ± 0.7	1.0 ± 0.1	1.7 ± 1.0
i-15:0	0.2 ± 0.0	0.1 ± 0.1	0.2 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.2 ± 0.1	0.0 ± 0.0	0.0 ± 0.1	0.1 ± 0.0
15:0	0.4 ± 0.1	0.4 ± 0.1	0.4 ± 0.0	0.3 ± 0.0	0.3 ± 0.2	0.4 ± 0.2	0.3 ± 0.2	0.4 ± 0.2	0.3 ± 0.1
i-16:0	1.8 ± 0.3	1.4 ± 0.1	1.7 ± 0.0	1.4 ± 0.5	0.9 ± 0.2	1.6 ± 0.3	1.7 ± 0.5	1.6 ± 0.2	1.4 ± 0.5
16:0	13.6 ± 2.5	12.7 ± 1.2	15.2 ± 1.1	12.0 ± 0.3	10.8 ± 0.6	13.4 ± 3.9	11.9 ± 4.3	10.7 ± 0.8	12.7 ± 3.0
17:0	1.3 ± 0.4	1.2 ± 0.1	1.1 ± 0.1	1.0 ± 0.2	1.0 ± 0.2	1.0 ± 0.2	0.9 ± 0.2	1.0 ± 0.1	0.9 ± 0.1
i-18:0	0.2 ± 0.2	0.3 ± 0.3	0.0 ± 0.0	0.9 ± 0.1	0.7 ± 0.2	0.7 ± 0.5	0.7 ± 0.6	1.1 ± 0.1	0.5 ± 0.5
18:0	6.4 ± 1.4	5.9 ± 0.5	6.0 ± 0.2	5.9 ± 1.5	4.9 ± 0.7	6.0 ± 1.0	6.3 ± 1.3	6.8 ± 0.1	6.4 ± 0.1
20:0	0.6 ± 0.0	0.6 ± 0.0	0.5 ± 0.0	0.6 ± 0.1	0.6 ± 0.1	0.3 ± 0.2	0.5 ± 0.0	0.6 ± 0.2	0.5 ± 0.0
22:0	0.7 ± 0.1	0.7 ± 0.2	0.5 ± 0.2	0.6 ± 0.0	1.2 ± 0.9	0.6 ± 0.1	0.8 ± 0.1	0.8 ± 0.2	0.7 ± 0.2
ΣSFAs	**34.8 ± 5.2**	**37.8 ± 1.5**	**40.1 ± 1.2**	**35.6 ± 5.6**	**39.0 ± 2.2**	**33.3 ± 7.0**	**34.9 ± 7.6**	**33.5 ± 1.5**	**35.0 ± 4.2**
16:1n-7	1.8 ± 0.3	1.6 ± 0.2	1.8 ± 0.2	1.2 ± 0.2	1.3 ± 0.4	1.8 ± 0.6	1.1 ± 0.5	1.3 ± 0.5	1.6 ± 0.5
18:1n-9	4.4 ± 0.5	3.7 ± 0.8	4.5 ± 0.4	4.0 ± 1.2	4.4 ± 1.0	3.4 ± 0.5	3.4 ± 0.8	4.2 ± 0.6	3.9 ± 0.2
18:1n-7	4.3 ± 0.6	4.2 ± 0.4	4.0 ± 0.3	4.3 ± 1.2	3.4 ± 0.9	3.9 ± 0.2	4.6 ± 1.1	5.1 ± 0.1	4.5 ± 0.3
20:1n-11	1.6 ± 0.6	1.4 ± 0.3	1.3 ± 0.2	1.9 ± 0.7	1.0 ± 0.4	1.2 ± 0.1	1.5 ± 0.1	1.8 ± 0.3	1.6 ± 0.3
20:1n-9	0.6 ± 0.4	0.6 ± 0.3	0.8 ± 0.2	1.3 ± 0.3	1.0 ± 0.5	0.6 ± 0.4	0.7 ± 0.5	1.1 ± 0.3	0.7 ± 0.3
20:1n-7	0.5 ± 0.2	0.0 ± 0.0	0.1 ± 0.2	0.6 ± 0.5	0.6 ± 0.3	0.4 ± 0.0	0.7 ± 0.3	0.4 ± 0.2	0.3 ± 0.2
ΣMUFAs	**17.6 ± 1.1**	**12.9 ± 1.2**	**13.5 ± 0.7**	**16.6 ± 1.6**	**15.4 ± 2.0**	**15.8 ± 1.2**	**18.1 ± 2.7**	**18.5 ± 0.8**	**16.9 ± 0.3**
18:2n-6	0.7 ± 0.1	0.9 ± 0.1	0.8 ± 0.1	0.8 ± 0.1	0.8 ± 0.2	0.6 ± 0.1	0.5 ± 0.1	0.6 ± 0.0	0.6 ± 0.1
18:3n-3	0.5 ± 0.1	0.6 ± 0.0	0.6 ± 0.0	0.4 ± 0.2	0.5 ± 0.1	0.5 ± 0.1	0.4 ± 0.1	0.4 ± 0.1	0.4 ± 0.0
18:4n-3	0.6 ± 0.2	0.6 ± 0.1	0.9 ± 0.1	0.8 ± 0.2	1.0 ± 0.6	0.7 ± 0.3	0.4 ± 0.3	0.4 ± 0.1	0.6 ± 0.3
20:4n-6	1.9 ± 0.3	1.7 ± 0.1	1.2 ± 0.0	1.7 ± 0.3	1.4 ± 0.2	1.3 ± 0.2	1.6 ± 0.3	2.0 ± 0.1	1.4 ± 0.2
20:5n-3	15.0 ± 2.9	12.6 ± 1.1	13.9 ± 0.4	13.0 ± 4.1	9.0 ± 0.4	14.5 ± 2.4	12.6 ± 3.0	14.4 ± 1.6	14.4 ± 1.9
22:2 NMI 1	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
22:2 NMI 2	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
21:5n-3	0.4 ± 0.1	0.1 ± 0.1	0.1 ± 0.2	0.3 ± 0.2	0.3 ± 0.1	0.3 ± 0.1	0.2 ± 0.0	0.1 ± 0.1	0.3 ± 0.1
22:4n-6	0.2 ± 0.1	0.0 ± 0.0	0.1 ± 0.2	0.2 ± 0.1	0.5 ± 0.5	0.2 ± 0.1	0.1 ± 0.1	0.0 ± 0.0	0.1 ± 0.0
22:5n-6	0.3 ± 0.1	0.2 ± 0.1	0.2 ± 0.1	0.4 ± 0.1	0.2 ± 0.1	0.3 ± 0.2	0.3 ± 0.1	0.3 ± 0.1	0.2 ± 0.1
22:5n-3	0.4 ± 0.1	0.6 ± 0.1	0.4 ± 0.0	0.5 ± 0.1	0.5 ± 0.1	0.6 ± 0.1	0.5 ± 0.1	0.7 ± 0.2	0.6 ± 0.1
22:6n-3	16.2 ± 3.4	15.7 ± 2.3	14.8 ± 1.3	15.6 ± 4.7	12.1 ± 0.8	17.2 ± 2.6	15.6 ± 3.3	16.6 ± 2.5	18.6 ± 4.0
ΣPUFAs	**47.6 ± 6.7**	**49.4 ± 2.7**	**46.4 ± 0.5**	**47.9 ± 9.6**	**45.6 ± 0.9**	**50.9 ± 5.5**	**47.0 ± 6.7**	**48.0 ± 3.6**	**48.1 ± 6.2**
ΣBFAs	**4.8 ± 0.9**	**4.8 ± 0.5**	**5.2 ± 0.3**	**5.8 ± 0.9**	**5.7 ± 0.2**	**5.5 ± 1.4**	**4.8 ± 1.5**	**5.7 ± 0.3**	**4.2 ± 0.6**
ΣHPFAs	**1.4 ± 0.2**	**2.0 ± 0.1**	**2.1 ± 0.1**	**1.8 ± 0.3**	**2.2 ± 0.2**	**1.5 ± 0.1**	**1.2 ± 0.2**	**1.3 ± 0.1**	**1.3 ± 0.1**
ΣEFAs	**43.3 ± 6.7**	**45.4 ± 2.8**	**41.9 ± 1.1**	**42.9 ± 9.1**	**38.7 ± 0.9**	**46.4 ± 5.0**	**43.3 ± 6.6**	**44.9 ± 3.6**	**44.3 ± 6.1**

Appendix 2.4 Fatty acid composition (% TFA) in mussel (*Perna perna*) tissues in the Kariega and Great Fish regions during March 2009. Values are represented by means ± standard deviation for all fatty acids at concentrations >1 % TFA (present in at least one sample; *n* = 3). Due to rapid tidal shift no mussels were collected at Site 1 for the Great Fish Estuary. TFA, total fatty acids; SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; BAFA, bacterial fatty acids; HPF, higher plant fatty acids; EFA, essential fatty acids (20:4n-6; 20:5n-3 and 22:6n-3).

Fatty Acid	Kariega				Great Fish			
	Site 1	Site 2	Site 3	Site 4	Site 1	Site 2	Site 3	Site 4
14:0	3.3 ± 2.6	6.0 ± 1.3	4.5 ± 2.0	2.9 ± 1.8	-	2.6 ± 0.7	3.1 ± 1.9	4.8 ± 0.8
i-15:0	0.0 ± 0.1	0.3 ± 0.1	0.3 ± 0.2	0.0 ± 0.1	-	0.4 ± 0.2	1.0 ± 0.1	0.4 ± 0.2
15:0	0.7 ± 0.3	0.9 ± 0.1	0.7 ± 0.1	0.7 ± 0.1	-	1.1 ± 0.1	1.4 ± 0.5	1.3 ± 0.3
i-16:0	0.0 ± 0.0	0.3 ± 0.1	0.3 ± 0.1	0.7 ± 0.1	-	0.4 ± 0.1	0.3 ± 0.2	0.4 ± 0.1
16:0	19.9 ± 4.7	20.9 ± 1.7	17.5 ± 2.8	17.6 ± 1.3	-	17.3 ± 2.0	18.8 ± 4.5	17.8 ± 0.8
i-17:0	0.7 ± 0.2	0.9 ± 0.1	0.7 ± 0.2	0.7 ± 0.1	-	1.1 ± 0.3	0.7 ± 0.2	0.9 ± 0.2
17:0	1.3 ± 0.1	1.3 ± 0.1	1.4 ± 0.0	1.5 ± 0.3	-	1.9 ± 0.3	1.7 ± 0.2	2.2 ± 0.4
i-18:0	3.3 ± 0.8	7.0 ± 0.6	10.1 ± 1.7	9.6 ± 1.2	-	12.8 ± 1.9	10.8 ± 4.2	11.7 ± 0.9
18:0	4.6 ± 0.4	4.7 ± 0.2	5.2 ± 0.0	5.1 ± 0.4	-	6.4 ± 1.4	5.9 ± 0.8	6.1 ± 0.5
20:0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.1	0.0 ± 0.0	-	1.5 ± 0.2	1.0 ± 0.7	0.0 ± 0.1
22:0	0.7 ± 0.1	0.3 ± 0.1	0.7 ± 0.3	0.7 ± 0.2	-	1.1 ± 0.5	0.3 ± 0.2	0.4 ± 0.1
24:0	0.0 ± 0.1	0.3 ± 0.1	0.3 ± 0.3	0.0 ± 0.1	-	0.4 ± 0.1	0.0 ± 0.1	0.0 ± 0.0
ΣSFAs	**34.4 ± 3.9**	**43.0 ± 2.4**	**42.0 ± 2.9**	**39.7 ± 2.5**	-	**47.0 ± 4.4**	**44.1 ± 11.1**	**46.1 ± 2.2**
16:1n-7	9.3 ± 2.0	9.2 ± 1.6	4.9 ± 2.6	5.1 ± 1.3	-	3.0 ± 0.8	4.2 ± 2.6	4.3 ± 1.0
16:1n-5	0.7 ± 0.0	0.2 ± 0.2	1.0 ± 0.2	0.7 ± 0.2	-	1.1 ± 0.1	1.0 ± 0.2	1.3 ± 0.3
18:1n-9	2.0 ± 0.2	2.2 ± 0.3	1.4 ± 0.3	1.5 ± 0.2	-	1.1 ± 0.2	1.4 ± 0.4	1.3 ± 0.2
18:1n-7	3.3 ± 0.4	3.2 ± 0.8	1.7 ± 0.4	2.2 ± 0.7	-	2.3 ± 0.4	2.4 ± 0.9	2.2 ± 0.4
20:1n-11	2.6 ± 1.0	0.9 ± 0.2	1.0 ± 0.2	0.7 ± 0.1	-	0.0 ± 0.0	0.0 ± 0.0	1.3 ± 0.2
20:1n-9	1.3 ± 0.4	1.9 ± 0.3	2.4 ± 0.3	2.2 ± 0.6	-	2.6 ± 0.2	2.8 ± 1.3	2.6 ± 0.3
20:1n-7	1.3 ± 1.1	1.9 ± 0.4	4.2 ± 1.2	3.7 ± 1.0	-	3.8 ± 0.9	4.9 ± 2.8	3.5 ± 0.4
ΣMUFAs	**20.5 ± 1.6**	**19.9 ± 1.3**	**16.8 ± 1.6**	**16.2 ± 1.7**	**-**	**13.9 ± 0.9**	**16.7 ± 2.1**	**16.5 ± 1.2**
16:3n-4	0.7 ± 0.1	0.6 ± 0.1	0.7 ± 0.2	0.7 ± 0.1	-	1.1 ± 0.2	1.0 ± 0.3	0.9 ± 0.2
18:2n-6	2.0 ± 0.3	1.6 ± 0.1	1.7 ± 0.0	2.2 ± 0.1	-	2.3 ± 0.6	2.4 ± 0.5	2.2 ± 0.6
18:3n-3	1.3 ± 0.2	0.9 ± 0.0	1.0 ± 0.2	1.5 ± 0.1	-	1.1 ± 1.5	2.4 ± 0.8	1.3 ± 0.5
18:4n-3	2.6 ± 0.9	1.6 ± 0.1	1.0 ± 0.7	1.5 ± 0.1	-	0.8 ± 0.3	1.0 ± 0.3	0.9 ± 0.6
20:2n-6	0.7 ± 0.1	0.6 ± 0.2	0.7 ± 0.4	0.7 ± 0.1	-	0.8 ± 0.3	0.3 ± 0.4	0.4 ± 0.4
20:4n-6	4.0 ± 0.8	3.5 ± 0.7	4.2 ± 0.7	4.4 ± 0.5	-	5.6 ± 0.4	4.5 ± 0.9	5.2 ± 0.1
20:5n-3	14.6 ± 1.7	11.7 ± 0.9	9.1 ± 2.2	9.6 ± 1.6	-	7.5 ± 1.4	7.3 ± 1.7	8.3 ± 1.4
22:2 NMI 1	0.7 ± 0.5	0.6 ± 0.2	1.4 ± 0.5	1.5 ± 0.5	-	1.5 ± 0.2	1.7 ± 0.9	1.3 ± 0.2
22:2 NMI 2	2.6 ± 1.5	2.5 ± 0.8	3.8 ± 1.1	3.7 ± 0.9	-	4.1 ± 0.7	4.2 ± 2.2	3.5 ± 0.4
21:5n-3	1.3 ± 0.2	0.9 ± 0.3	1.7 ± 0.6	1.5 ± 0.2	-	1.1 ± 0.4	1.4 ± 0.4	0.9 ± 0.2
22:4n-6	1.3 ± 0.4	0.9 ± 0.3	1.7 ± 0.5	1.5 ± 0.2	-	1.5 ± 0.4	1.4 ± 0.6	1.3 ± 0.1
22:5n-6	0.7 ± 0.2	0.9 ± 0.2	1.0 ± 0.2	1.5 ± 0.2	-	0.8 ± 0.0	0.7 ± 0.3	1.3 ± 0.5
22:5n-3	1.3 ± 0.6	1.6 ± 0.3	1.7 ± 0.2	2.2 ± 0.2	-	1.9 ± 0.9	2.7 ± 0.9	2.2 ± 0.3
22:6n-3	11.3 ± 2.1	8.9 ± 2.0	11.2 ± 2.0	11.8 ± 2.4	-	9.0 ± 1.3	8.7 ± 4.2	7.8 ± 1.2
ΣPUFAs	**45.0 ± 4.2**	**37.0 ± 5.7**	**41.3 ± 3.5**	**44.1 ± 4.9**	**-**	**39.1 ± 5.1**	**39.2 ± 8.3**	**37.4 ± 2.8**
ΣBFAs	**6.0 ± 0.7**	**10.8 ± 0.4**	**13.6 ± 1.5**	**13.2 ± 1.4**	**-**	**17.7 ± 2.7**	**14.9 ± 5.4**	**17.0 ± 1.7**
ΣHPFAs	**3.3 ± 0.5**	**2.5 ± 0.1**	**2.8 ± 0.3**	**3.7 ± 0.1**	**-**	**3.4 ± 2.1**	**4.9 ± 1.3**	**3.5 ± 1.1**
ΣEFAs	**29.8 ± 2.1**	**24.1 ± 3.6**	**24.5 ± 2.1**	**25.7 ± 3.8**	**-**	**22.2 ± 1.7**	**20.5 ± 5.3**	**21.3 ± 2.9**

Appendix 2.5 Fatty acid composition (% TFA) in mussel (*Perna perna*) in the Kariega and Great Fish regions during July 2009. Values are represented by means ± standard deviation for all fatty acids at concentrations >1 % TFA (present in at least one sample; *n* = 3). TFA, total fatty acids; SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; BAFA, bacterial fatty acids; HPF, higher plant fatty acids; EFA, essential fatty acids (20:4n-6; 20:5n-3 and 22:6n-3).

Fatty Acid	Kariega				Great Fish			
	Site 1	Site 2	Site 3	Site 4	Site 1	Site 2	Site 3	Site 4
14:0	3.7 ± 0.8	1.8 ± 0.3	4.2 ± 1.6	2.2 ± 1.0	2.4 ± 1.0	3.2 ± 1.3	4.9 ± 0.9	3.6 ± 1.7
i-15:0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.1 ± 0.1	0.0 ± 0.1	0.0 ± 0.1	0.0 ± 0.1
15:0	0.7 ± 0.3	0.9 ± 0.1	1.2 ± 0.1	1.1 ± 0.3	0.5 ± 0.1	0.8 ± 0.2	1.6 ± 0.3	0.9 ± 0.2
i-16:0	0.0 ± 0.0	0.0 ± 0.1	0.6 ± 0.1	0.0 ± 0.1	0.2 ± 0.1	0.0 ± 0.0	0.0 ± 0.1	0.0 ± 0.1
16:0	15.6 ± 2.0	14.2 ± 2.0	17.4 ± 1.6	15.6 ± 3.0	11.7 ± 2.0	15.2 ± 3.1	23.0 ± 7.2	18.0 ± 3.5
17:0	1.5 ± 0.1	1.8 ± 0.2	1.8 ± 0.4	2.2 ± 0.4	0.9 ± 0.1	1.6 ± 0.1	1.6 ± 0.5	1.8 ± 0.4
i-18:0	9.6 ± 0.3	9.7 ± 0.3	7.8 ± 2.0	10.0 ± 3.4	5.9 ± 2.2	11.2 ± 2.0	7.4 ± 2.2	6.3 ± 1.1
18:0	4.4 ± 0.5	4.4 ± 0.2	4.2 ± 0.3	5.6 ± 1.0	3.3 ± 0.3	4.8 ± 0.1	4.9 ± 0.3	5.4 ± 0.4
20:0	1.5 ± 0.1	1.8 ± 0.2	1.2 ± 0.5	2.2 ± 0.5	0.7 ± 0.1	1.6 ± 0.4	0.8 ± 0.3	0.9 ± 0.3
22:0	0.7 ± 0.3	0.9 ± 0.2	0.6 ± 0.1	0.0 ± 0.1	0.3 ± 0.0	0.8 ± 0.1	0.0 ± 0.2	0.9 ± 0.4
ΣSFAs	**37.8 ± 2.7**	**35.4 ± 2.3**	**38.9 ± 1.5**	**38.9 ± 9.5**	**38.3 ± 3.6**	**39.2 ± 2.9**	**44.3 ± 11.5**	**37.8 ± 7.0**
16:1n-7	4.4 ± 1.2	1.8 ± 0.4	5.4 ± 2.4	2.2 ± 0.6	3.5 ± 1.8	3.2 ± 1.8	8.2 ± 3.6	4.5 ± 1.4
18:1n-9	3.0 ± 0.2	2.7 ± 0.9	2.4 ± 0.6	3.3 ± 1.4	1.5 ± 0.7	1.6 ± 0.6	8.5 ± 0.9	1.8 ± 1.0
18:1n-7	2.2 ± 1.0	2.7 ± 0.6	1.8 ± 1.0	1.1 ± 0.4	2.0 ± 0.3	1.6 ± 0.3	3.3 ± 0.3	2.7 ± 0.6
20:1n-11	2.2 ± 0.4	2.7 ± 0.4	2.4 ± 0.0	2.2 ± 0.9	1.5 ± 0.2	2.4 ± 0.2	2.5 ± 1.0	1.8 ± 0.0
20:1n-9	1.5 ± 0.2	1.8 ± 0.7	0.6 ± 0.2	1.1 ± 0.7	0.8 ± 0.3	0.8 ± 0.2	0.8 ± 0.2	0.9 ± 0.8
20:1n-7	3.0 ± 0.5	5.3 ± 1.0	2.6 ± 1.4	4.4 ± 1.1	2.9 ± 0.9	4.8 ± 0.4	3.3 ± 1.1	4.5 ± 1.3
ΣMUFAs	**16.3 ± 2.3**	**16.8 ± 2.4**	**16.2 ± 2.3**	**14.4 ± 3.3**	**18.3 ± 1.8**	**14.4 ± 2.1**	**20.5 ± 2.4**	**16.2 ± 3.0**
18:2n-6	2.2 ± 0.2	1.8 ± 0.2	1.8 ± 0.2	2.2 ± 0.2	1.1 ± 0.3	1.6 ± 0.3	1.6 ± 0.3	1.8 ± 0.2
18:3n-3	1.5 ± 0.2	1.8 ± 0.4	1.2 ± 0.2	1.1 ± 0.1	0.9 ± 0.2	1.6 ± 0.4	1.6 ± 0.2	0.9 ± 0.1
18:4n-3	1.5 ± 0.4	0.9 ± 0.2	1.8 ± 1.0	1.1 ± 0.2	1.1 ± 0.5	1.6 ± 0.7	1.6 ± 0.4	1.8 ± 0.5
20:4n-6	5.2 ± 0.2	5.3 ± 0.3	4.8 ± 1.0	5.6 ± 0.7	2.5 ± 0.8	4.8 ± 0.5	3.3 ± 1.4	4.5 ± 0.9
20:5n-3	10.4 ± 2.1	8.8 ± 1.0	10.2 ± 3.9	6.7 ± 1.1	6.4 ± 2.7	8.8 ± 1.8	8.2 ± 1.5	9.0 ± 1.9
22:2 NMI 1	3.7 ± 0.2	4.4 ± 0.0	1.2 ± 0.3	2.2 ± 0.3	0.9 ± 0.2	1.6 ± 0.3	0.8 ± 0.5	1.8 ± 0.2
22:2 NMI 2	3.7 ± 0.3	4.4 ± 0.2	3.0 ± 0.6	5.6 ± 0.3	3.0 ± 0.3	5.6 ± 0.4	2.5 ± 1.2	3.6 ± 0.8
21:5n-3	1.5 ± 0.1	1.8 ± 0.1	1.8 ± 0.0	2.2 ± 0.1	0.9 ± 0.1	1.6 ± 0.2	0.8 ± 0.6	1.8 ± 0.2
22:4n-6	1.5 ± 0.2	1.8 ± 0.1	1.2 ± 0.3	2.2 ± 0.1	1.0 ± 0.2	1.6 ± 0.4	1.6 ± 0.7	1.8 ± 0.2
22:5n-6	0.7 ± 0.0	0.9 ± 0.1	1.2 ± 0.1	1.1 ± 0.3	0.7 ± 0.3	1.6 ± 0.2	1.6 ± 1.2	0.9 ± 0.1
22:5n-3	1.5 ± 0.1	2.7 ± 0.2	1.8 ± 0.1	2.2 ± 0.2	1.6 ± 0.3	2.4 ± 0.3	1.6 ± 0.9	2.7 ± 0.5
22:6n-3	12.6 ± 1.0	13.3 ± 1.4	15.0 ± 1.8	14.4 ± 2.2	8.5 ± 1.0	13.6 ± 0.7	9.8 ± 4.7	15.3 ± 3.1
ΣPUFAs	**45.9 ± 4.3**	**47.8 ± 2.8**	**44.9 ± 4.2**	**46.7 ± 3.3**	**43.3 ± 3.3**	**46.4 ± 2.5**	**35.2 ± 11.6**	**45.9 ± 6.8**
ΣBFAs	**11.9 ± 0.1**	**12.4 ± 0.1**	**11.4 ± 2.2**	**13.3 ± 0.4**	**10.8 ± 2.3**	**13.6 ± 1.8**	**10.7 ± 3.3**	**9.0 ± 1.6**
ΣHPFAs	**3.7 ± 0.3**	**3.5 ± 0.5**	**3.0 ± 0.3**	**3.3 ± 0.5**	**3.3 ± 0.5**	**3.2 ± 0.6**	**3.3 ± 0.5**	**2.7 ± 0.4**
ΣEFAs	**28.1 ± 3.2**	**27.4 ± 2.4**	**29.9 ± 4.4**	**26.7 ± 3.7**	**25.8 ± 3.7**	**27.2 ± 1.9**	**21.3 ± 7.6**	**28.8 ± 5.6**

Appendix 2.6 Fatty acid composition (% TFA) in polychaete (*Gunnarea capensis*) in the Kariega and Great Fish regions during March 2009. Values are represented by means ± standard deviation for all fatty acids at concentrations >1 % TFA (present in at least one sample; *n* = 3). TFA, total fatty acids; SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; BAFA, bacterial fatty acids; HPF, higher plant fatty acids; EFA, essential fatty acids (20:4n-6; 20:5n-3 and 22:6n-3).

Fatty Acid	Kariega				Great Fish			
	Site 1	Site 2	Site 3	Site 4	Site 1	Site 2	Site 3	Site 4
14:0	7.0 ± 1.7	5.3 ± 1.8	9.7 ± 0.5	8.6 ± 3.0	7.4 ± 1.7	7.2 ± 0.9	7.2 ± 2.6	7.6 ± 1.2
i-15:0	1.3 ± 0.3	1.5 ± 0.5	1.6 ± 0.2	1.1 ± 0.7	1.4 ± 0.3	1.9 ± 0.1	1.5 ± 0.4	2.1 ± 0.4
15:0	1.3 ± 0.2	1.5 ± 0.5	1.5 ± 0.2	1.1 ± 0.2	1.4 ± 0.3	1.5 ± 0.3	0.9 ± 0.6	1.0 ± 0.7
i-16:0	0.7 ± 0.1	0.8 ± 0.5	1.0 ± 0.2	0.8 ± 0.3	0.8 ± 0.3	1.0 ± 0.2	0.8 ± 0.2	0.6 ± 0.3
16:0	15.3 ± 0.7	14.5 ± 2.1	17.0 ± 1.6	16.5 ± 2.0	14.6 ± 1.5	16.0 ± 0.5	14.2 ± 2.6	15.1 ± 2.0
i-17:0	0.9 ± 0.1	0.8 ± 0.2	1.0 ± 0.1	0.9 ± 0.1	0.9 ± 0.1	1.2 ± 0.1	0.9 ± 0.1	1.1 ± 0.1
17:0	0.9 ± 0.2	0.8 ± 0.1	1.0 ± 0.1	0.9 ± 0.3	1.3 ± 0.4	1.0 ± 0.1	0.9 ± 0.1	1.1 ± 0.2
i-18:0	6.7 ± 1.0	6.9 ± 2.0	7.6 ± 0.5	7.4 ± 1.3	6.6 ± 2.4	6.4 ± 1.6	6.4 ± 1.5	4.8 ± 1.9
18:0	5.8 ± 0.4	6.9 ± 0.3	6.0 ± 0.4	5.7 ± 1.0	6.9 ± 0.8	6.6 ± 0.5	6.3 ± 0.4	6.6 ± 1.0
20:0	0.1 ± 0.0	0.0 ± 0.2	0.6 ± 0.0	0.6 ± 0.0	0.5 ± 0.4	0.4 ± 0.3	0.2 ± 0.0	0.2 ± 0.1
22:0	0.4 ± 0.1	0.0 ± 0.0	0.2 ± 0.4	0.3 ± 0.4	0.3 ± 0.4	0.1 ± 0.2	0.2 ± 0.3	0.2 ± 0.2
24:0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.2 ± 0.2
ΣSFAs	**40.4 ± 3.3**	**38.9 ± 8.0**	**47.2 ± 1.6**	**43.9 ± 2.4**	**42.2 ± 7.6**	**43.4 ± 1.8**	**39.6 ± 6.9**	**40.5 ± 4.7**
16:1n-7	2.9 ± 0.7	2.3 ± 0.3	3.4 ± 0.3	3.3 ± 1.2	2.7 ± 0.1	2.7 ± 0.3	2.1 ± 0.5	2.6 ± 0.4
16:1n-5	0.9 ± 0.3	0.8 ± 0.1	0.6 ± 0.2	0.6 ± 0.3	0.8 ± 0.0	0.9 ± 0.2	0.8 ± 0.4	1.0 ± 0.2
18:1n-9	2.0 ± 0.7	0.8 ± 0.1	1.6 ± 0.5	2.1 ± 1.0	1.6 ± 0.6	2.8 ± 0.8	1.3 ± 0.1	1.4 ± 0.1
18:1n-7	5.2 ± 0.6	4.6 ± 0.1	4.9 ± 0.7	5.4 ± 1.3	4.7 ± 0.3	5.1 ± 0.2	5.3 ± 0.8	5.1 ± 0.4
20:1n-11	5.5 ± 0.7	6.1 ± 1.0	4.9 ± 0.4	4.8 ± 0.2	5.2 ± 0.5	5.0 ± 0.4	5.7 ± 1.1	5.3 ± 0.3
20:1n-9	1.7 ± 0.4	1.5 ± 0.3	1.3 ± 0.1	1.2 ± 0.2	1.6 ± 0.2	1.5 ± 0.1	1.7 ± 0.3	1.8 ± 0.1
20:1n-7	0.7 ± 0.0	0.8 ± 0.2	0.8 ± 0.2	0.8 ± 0.5	0.3 ± 0.2	0.6 ± 0.1	0.4 ± 0.0	0.6 ± 0.3
ΣMUFAs	**19.0 ± 2.4**	**16.8 ± 0.7**	**17.5 ± 1.1**	**18.3 ± 2.8**	**16.9 ± 1.3**	**18.8 ± 1.5**	**17.2 ± 0.5**	**17.8 ± 0.7**
16:3n-4	0.9 ± 0.2	0.8 ± 0.4	1.1 ± 0.1	0.9 ± 0.3	0.9 ± 0.2	1.0 ± 0.2	0.9 ± 0.2	0.8 ± 0.2
18:2n-6	1.0 ± 0.1	0.8 ± 0.3	0.8 ± 0.1	0.9 ± 0.0	0.9 ± 0.0	0.9 ± 0.0	0.9 ± 0.1	1.1 ± 0.3
18:3n-3	0.7 ± 0.1	0.8 ± 0.2	0.5 ± 0.1	0.6 ± 0.1	0.6 ± 0.3	0.5 ± 0.1	0.4 ± 0.1	0.6 ± 0.2
18:4n-3	0.3 ± 0.4	0.0 ± 0.0	0.2 ± 0.0	0.2 ± 0.1	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.3 ± 0.3
20:2n-6	0.9 ± 0.2	0.8 ± 0.1	1.0 ± 0.5	0.8 ± 0.2	0.9 ± 0.2	0.9 ± 0.3	0.8 ± 0.1	0.6 ± 0.1
20:4n-6	3.3 ± 0.5	3.8 ± 0.3	2.8 ± 0.0	3.0 ± 0.7	3.6 ± 0.6	3.5 ± 0.3	3.6 ± 0.6	3.5 ± 0.3
20:5n-3	17.2 ± 1.5	18.3 ± 2.6	15.1 ± 0.4	17.4 ± 0.3	18.1 ± 2.0	17.2 ± 1.0	19.3 ± 1.4	18.5 ± 1.6
22:2 NMI 1	2.2 ± 0.4	2.3 ± 0.8	1.8 ± 0.1	1.8 ± 0.3	1.7 ± 0.6	1.5 ± 0.1	2.1 ± 0.6	1.9 ± 0.5
22:2 NMI 2	3.5 ± 0.2	3.8 ± 1.5	3.1 ± 0.2	2.7 ± 0.5	3.3 ± 1.2	3.1 ± 0.0	3.6 ± 0.9	3.2 ± 0.9
21:5n-3	0.6 ± 0.0	0.8 ± 0.1	0.6 ± 0.2	0.5 ± 0.1	0.5 ± 0.1	0.4 ± 0.0	0.4 ± 0.1	0.5 ± 0.1
22:4n-6	3.5 ± 0.7	4.6 ± 1.3	2.6 ± 0.2	2.9 ± 0.7	3.9 ± 1.1	3.2 ± 0.1	4.0 ± 1.1	3.2 ± 0.9
22:5n-6	0.4 ± 0.1	0.8 ± 0.1	0.6 ± 0.7	0.5 ± 0.3	0.5 ± 0.2	0.6 ± 0.3	0.4 ± 0.2	0.5 ± 0.1
22:5n-3	3.8 ± 0.4	4.6 ± 1.5	3.1 ± 0.1	3.2 ± 0.5	3.6 ± 1.1	3.1 ± 0.2	4.2 ± 1.0	4.0 ± 1.0
22:6n-3	2.3 ± 0.2	2.3 ± 0.6	2.1 ± 0.4	2.6 ± 0.3	2.2 ± 0.6	1.8 ± 0.1	2.7 ± 0.5	2.4 ± 0.6
ΣPUFAs	**40.6 ± 3.3**	**44.3 ± 6.4**	**35.3 ± 1.1**	**37.8 ± 2.9**	**40.9 ± 5.3**	**37.8 ± 0.5**	**43.2 ± 5.2**	**41.6 ± 4.0**
ΣBFAs	**11.8 ± 1.7**	**12.2 ± 3.9**	**13.6 ± 0.7**	**12.1 ± 2.2**	**12.4 ± 3.8**	**13.1 ± 1.4**	**11.6 ± 2.5**	**10.8 ± 2.7**
ΣHPFAs	**1.7 ± 0.2**	**1.5 ± 0.3**	**1.3 ± 0.1**	**1.5 ± 0.1**	**1.6 ± 0.3**	**1.4 ± 0.1**	**1.3 ± 0.2**	**1.8 ± 0.4**
ΣEFAs	**22.8 ± 2.2**	**24.4 ± 3.3**	**19.9 ± 0.6**	**23.0 ± 0.8**	**23.9 ± 2.9**	**22.5 ± 1.4**	**25.6 ± 2.5**	**24.4 ± 2.1**

Appendix 2.7 Fatty acid composition (% TFA) in polychaete (*Gunnarea capensis*) in the Kariega and Great Fish regions during July 2009. Values are represented by means ± standard deviation for all fatty acids at concentrations >1 % TFA (present in at least one sample; *n* = 3). TFA, total fatty acids; SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; BAFA, bacterial fatty acids; HPF, higher plant fatty acids; EFA, essential fatty acids (20:4n-6; 20:5n-3 and 22:6n-3).

Fatty Acid	Kariega				Great Fish			
	Site 1	Site 2	Site 3	Site 4	Site 1	Site 2	Site 3	Site 4
14:0	5.7 ± 0.8	5.7 ± 0.8	5.3 ± 1.3	6.1 ± 0.4	7.5 ± 0.9	4.6 ± 0.5	6.6 ± 1.3	6.4 ± 0.5
i-15:0	1.1 ± 0.7	1.4 ± 0.1	1.5 ± 0.4	1.7 ± 0.2	1.6 ± 0.2	1.5 ± 0.3	1.8 ± 0.2	1.9 ± 0.1
15:0	1.1 ± 0.1	1.4 ± 0.1	1.1 ± 0.2	1.2 ± 0.5	1.2 ± 0.1	1.0 ± 0.2	1.2 ± 0.2	1.3 ± 0.1
i-16:0	0.8 ± 0.2	1.0 ± 0.3	0.8 ± 0.1	0.9 ± 0.2	0.8 ± 0.1	1.0 ± 0.0	0.6 ± 0.2	0.5 ± 0.1
16:0	13.8 ± 0.7	14.8 ± 1.8	14.4 ± 1.8	15.2 ± 1.1	16.1 ± 2.5	13.4 ± 1.5	15.6 ± 1.0	16.8 ± 0.9
17:0	1.1 ± 0.0	1.0 ± 0.1	1.1 ± 0.1	1.2 ± 0.1	1.2 ± 0.1	1.0 ± 0.0	0.9 ± 0.0	1.1 ± 0.0
i-18:0	5.4 ± 1.8	6.7 ± 0.5	6.5 ± 1.4	7.3 ± 1.9	6.7 ± 1.6	7.2 ± 1.2	4.2 ± 1.5	4.8 ± 1.0
18:0	5.4 ± 0.3	5.7 ± 0.9	5.7 ± 0.8	5.8 ± 0.4	5.5 ± 0.1	5.7 ± 0.3	4.2 ± 0.0	5.3 ± 0.5
20:0	0.0 ± 0.0	0.0 ± 0.1	0.0 ± 0.1	0.0 ± 0.0	0.4 ± 0.1	0.5 ± 0.2	0.3 ± 0.2	0.3 ± 0.1
22:0	0.4 ± 0.2	0.0 ± 0.2	0.4 ± 0.0	0.6 ± 0.1	0.0 ± 0.2	0.0 ± 0.3	0.3 ± 0.3	0.3 ± 0.3
ΣSFAs	**34.9 ± 3.6**	**37.8 ± 4.4**	**36.9 ± 2.7**	**39.9 ± 2.3**	**40.9 ± 2.5**	**36.1 ± 1.6**	**35.7 ± 3.7**	**38.8 ± 0.4**
16:1n-7	3.1 ± 0.4	2.9 ± 0.3	2.7 ± 0.6	2.9 ± 0.3	3.5 ± 0.9	2.6 ± 0.5	3.9 ± 0.2	3.5 ± 0.4
18:1n-9	1.9 ± 0.1	1.9 ± 0.4	1.9 ± 0.2	2.0 ± 0.2	1.6 ± 0.1	1.5 ± 0.2	1.5 ± 0.0	1.6 ± 0.3
18:1n-7	4.6 ± 0.3	4.8 ± 0.5	4.2 ± 0.4	4.4 ± 0.3	4.7 ± 0.3	4.1 ± 0.7	4.8 ± 0.3	4.5 ± 0.7
20:1n-11	5.4 ± 0.6	4.3 ± 1.1	4.9 ± 0.7	5.0 ± 0.3	4.3 ± 0.0	4.6 ± 0.2	4.8 ± 0.4	4.5 ± 0.5
20:1n-9	1.9 ± 0.2	1.9 ± 0.3	1.9 ± 0.4	1.7 ± 0.4	2.0 ± 0.1	2.1 ± 0.3	2.1 ± 0.2	1.9 ± 0.2
20:1n-7	0.4 ± 0.1	1.0 ± 0.7	0.4 ± 0.1	0.6 ± 0.0	0.8 ± 0.1	1.0 ± 0.1	0.6 ± 0.1	0.5 ± 0.1
ΣMUFAs	**17.2 ± 1.7**	**16.7 ± 0.9**	**16.0 ± 0.5**	**16.6 ± 0.2**	**16.9 ± 3.0**	**16.0 ± 1.6**	**17.7 ± 0.5**	**16.6 ± 1.4**
18:2n-6	1.1 ± 0.2	1.0 ± 0.0	1.1 ± 0.2	1.2 ± 0.1	1.2 ± 0.1	1.0 ± 0.2	1.2 ± 0.2	1.3 ± 0.3
18:3n-3	0.8 ± 0.1	0.5 ± 0.1	0.8 ± 0.1	0.9 ± 0.0	0.8 ± 0.1	0.5 ± 0.0	0.9 ± 0.1	0.8 ± 0.1
18:4n-3	0.4 ± 0.1	0.0 ± 0.0	0.4 ± 0.1	0.3 ± 0.1	0.0 ± 0.1	0.5 ± 0.3	0.3 ± 0.2	0.3 ± 0.1
20:4n-6	3.8 ± 0.3	5.7 ± 0.8	4.6 ± 0.9	3.5 ± 0.2	3.1 ± 0.2	4.1 ± 0.2	3.9 ± 0.2	3.7 ± 0.2
20:5n-3	20.7 ± 2.2	19.1 ± 0.9	21.7 ± 1.6	21.6 ± 1.7	20.9 ± 0.4	21.1 ± 0.5	22.2 ± 0.8	20.9 ± 1.6
22:2 NMI 1	1.9 ± 0.0	1.9 ± 0.3	1.9 ± 0.3	1.7 ± 0.2	1.6 ± 0.1	2.1 ± 0.2	1.5 ± 0.2	1.6 ± 0.1
22:2 NMI 2	3.8 ± 0.3	3.3 ± 0.8	3.8 ± 0.4	2.9 ± 0.1	3.1 ± 0.1	3.6 ± 0.1	3.3 ± 0.6	2.9 ± 0.3
21:5n-3	1.1 ± 0.4	1.0 ± 0.3	0.4 ± 0.1	0.6 ± 0.0	0.4 ± 0.1	1.0 ± 0.3	0.9 ± 0.5	0.5 ± 0.1
22:4n-6	4.2 ± 0.9	4.8 ± 1.5	4.2 ± 0.8	2.9 ± 0.3	3.1 ± 0.2	4.6 ± 0.2	3.3 ± 0.5	3.7 ± 0.6
22:5n-6	1.5 ± 1.2	1.0 ± 0.4	0.8 ± 0.3	0.6 ± 0.1	0.4 ± 0.1	0.5 ± 0.1	0.6 ± 0.2	0.5 ± 0.1
22:5n-3	4.6 ± 0.8	3.8 ± 0.3	3.8 ± 0.5	3.8 ± 1.1	3.9 ± 0.2	4.6 ± 0.1	3.6 ± 0.4	4.0 ± 0.1
22:6n-3	3.8 ± 1.0	3.3 ± 1.0	3.8 ± 0.2	3.5 ± 0.6	3.5 ± 0.2	4.1 ± 0.3	4.88 ± 1.0	4.3 ± 0.1
ΣPUFAs	**47.9 ± 4.7**	**45.5 ± 2.8**	**47.1 ± 3.2**	**43.4 ± 3.7**	**42.1 ± 0.6**	**47.9 ± 1.5**	**46.5 ± 3.5**	**44.7 ± 2.0**
ΣBFAs	**9.6 ± 2.6**	**11.5 ± 1.3**	**11.0 ± 1.7**	**12.2 ± 1.7**	**11.4 ± 1.0**	**11.9 ± 0.8**	**8.7 ± 1.8**	**9.6 ± 1.0**
ΣHPFAs	**1.9 ± 0.3**	**1.4 ± 0.1**	**1.9 ± 0.4**	**2.0 ± 0.1**	**2.0 ± 0.2**	**1.5 ± 0.1**	**2.1 ± 0.3**	**2.1 ± 0.2**
ΣEFAs	**28.4 ± 3.4**	**28.2 ± 1.3**	**30.0 ± 16**	**28.6 ± 2.2**	**27.6 ± 0.5**	**29.4 ± 0.5**	**30.9 ± 1.5**	**28.9 ± 1.4**

Appendix 2.8 Multivariate ANOVA results (with PC-1 and PC-2 scores data as dependent, and region or date as independent variables) indicating temporal and regional differences in fatty acid signatures of three indigenous suspension-feeding organisms (barnacle *Tetraclita serrata*; mussel *Perna perna*; and polychaete *Gunnarea capensis*). Univariate *F*-test results for PC-1 and -2 scores and percentage contribution of PC-1 and -2 axes (%) to the variance observed within the data were given. Data represent quantitative data (μg fatty acid mg^{-1} dry mass). Levene's test for homogeneity was conducted to verify the validity of each model. K, Kariega; GF, Great Fish; Mar, March; Jul, July; ns, not significant.

Species	Season	Region	ANOVA			Levene's Test		PCA
			df	*F*-ratio	*P*	Statistic	*P*	PC-2 (%)
Quantitative results								
Tetraclita serrata	Mar	K x GF	8	14.590	<0.001	0.841	ns	58
	Jul	K x GF	8	4.678	<0.01	3.765	ns	61
	Mar x Jul	K	9	7.735	<0.001	3.772	ns	81
	Mar x Jul	GF	7	12.983	<0.001	3.743	ns	65
Perna perna	Mar	K x GF	6	1.885	ns	2.541	ns	65
	Jul	K x GF	7	5.697	<0.01	3.056	ns	50
	Mar x Jul	K	7	8.682	<0.001	2.813	ns	70
	Mar x Jul	GF	6	20.903	<0.001	6.032	ns	66
Gunnarea capensis	Mar	K x GF	7	10.014	<0.001	6.617	ns	80
	Jul	K x GF	7	5.694	<0.01	3.220	ns	70
	Mar x Jul	K	7	12.597	<0.001	5.387	ns	85
	Mar x Jul	GF	7	33.834	<0.001	2.084	ns	80
Quantitative results								
Tetraclita serrata	Mar	K x GF	7	6.363	<0.01	0.841	ns	13
	Jul	K x GF	8	3.644	<0.05	3.765	ns	11
	Mar x Jul	K	8	7.735	<0.001	3.772	ns	4
	Mar x Jul	GF	7	8.766	<0.001	3.743	ns	12
Perna perna	Mar	K x GF	6	4.531	<0.01	2.541	ns	22
	Jul	K x GF	7	9.040	<0.001	3.056	ns	14
	Mar x Jul	K	7	5.822	<0.01	2.813	ns	14
	Mar x Jul	GF	6	3.168	<0.05	6.032	ns	10
Gunnarea capensis	Mar	K x GF	7	1.271	ns	6.617	ns	6
	Jul	K x GF	7	0.436	ns	3.220	ns	11
	Mar x Jul	K	7	8.719	<0.001	5.387	ns	5
	Mar x Jul	GF	7	6.487	<0.01	2.084	ns	5

CHAPTER 3

REGIONAL AND TEMPORAL CHANGES IN δ^{13}C AND δ^{15}N SIGNATURES IN THREE INDIGENOUS ROCKY SHORE SUSPENSION-FEEDERS

3.1 Introduction

Stable carbon and nitrogen isotopes have successfully been employed to study estuarine and marine trophic ecology (Jennings et al., 1997; Riera & Richard, 1996; Hill et al., 2006; Dubois et al., 2007). Stable isotopes are an important tool to assess the contributions of food in the diets of consumers by identifying the degree of dependency on certain food sources by consumers (Riera et al., 2009). Stable isotope analysis of estuarine and marine fauna have been widely applied in South Africa (Bustamante & Branch, 1996; Paterson & Whitfield, 1997; Froneman, 2000; 2001; 2002; Richoux & Froneman, 2007; Henninger et al., 2009), however, less attention has been given to trophic studies of rocky shore invertebrates (Hill et al., 2006; Hill & McQuaid, 2008; Allan et al., 2010).

Some studies have used stable isotope analysis to examine the influence of freshwater inputs on the biology and food webs in southern African estuaries (i.e. ichthyofaunal and plankton assemblages; Jerling & Wooldridge, 1995; Schlacher & Wooldridge, 1996; Paterson & Whitfield, 1997; Froneman, 2001; Richoux & Froneman, 2007). Little is known about the role of estuaries as suppliers of food for nearshore marine suspension-feeders. In South Africa, Vorwerk (2006) examined the effects of hydrological variation in two contrasting estuaries (i.e. Kariega and Great Fish Estuaries) on the contributions of estuarine-derived carbon to the nearshore marine organisms. Stable isotope data revealed that estuarine productivity contributed to the food of zooplankton (i.e. the copepods *Calanus agulhensis* and *Clausocalanus sp*., and the mysid *Rhopalophthalmus terranatalis*) and two suspension-feeders (i.e. unidentified sponges and the tunicate *Pyura stolonifera*) in the adjacent marine environment in both regions (Vorwerk, 2006). The suspension-feeders from the adjoining marine environment adjacent to the freshwater-limited Kariega Estuary reflected δ^{13}C values (-17.9 to -16.7 ‰) similar to the particulate organic detritus from the salt marshes and macrophytes (i.e. *Spartina maritima* and *Zostera capensis*; -16.9 ‰) from the lower reaches of the estuary (Vorwerk, 2006). In contrast, the particulate organic matter (POM) in the Great Fish Estuary adjoining marine environment was indistinguishable between sources from estuarine or oceanic origin (-20.2 to -18.25 ‰). The large riverine outflow to the marine environment was therefore responsible for maintaining a mixture of POM carbon sources between the estuary and coastal habitats up to ~12 km southwards from the Great Fish Estuary mouth (Vorwerk, 2006).

Hydrodynamic factors are important in structuring the spatial patterns in $\delta^{13}C$ and $\delta^{15}N$ signatures in suspension-feeders on rocky shores, especially as the distance between food and consumer may be influenced by river hydrology, coastal water currents and wave exposure (Machas et al., 2003; Vizzini & Mazzola, 2006; Schaal et al., 2009). A study conducted in Northern Brittany assessed the contribution of kelp, *Laminaria digitata*, as a potential food source in two rocky shore areas with contrasting wave exposure (Schaal et al., 2009). As the northeast Atlantic Ocean is characterised by distinct phytoplankton-dominated POM ($\delta^{13}C$: -23 ‰; Grall et al., 2006), the contribution of kelp-detritus as food source to filter-feeders was hypothesised to be limited. These authors recorded that the suspension-feeders in the region with high wave exposure had more depleted isotopic signatures and lower contributions of kelp organic matter (22-71 %) than those in the inner bay area with low wave exposure (31-90 %; Schaal et al., 2009). In addition, the suspension-feeders from the inner bay had enriched $\delta^{13}C$ and $\delta^{15}N$ signatures compared to the wave-exposed region. The higher accessibility of kelp detritus to inner bay organisms was explained by the decreased wave action and increased water retention in the bay which allowed significant kelp degradation. In the exposed bay, increased export of kelp material from the wave-exposed region contributed to reduced retention times, so lowering its degradation time and availability and thus contributing to the reduced input of organic matter derived from kelp to the diets of suspension-feeders (Schaal et al., 2009). Vizzini & Mazzola (2006) also identified the importance of hydrodynamics in influencing the spatial variability in organic matter food sources to the diets of invertebrates in a shallow coastal basin, Stagnone di Marsala, along the southern coast of Italy. The consumers from the central basin with low hydrodynamic dispersion had enriched $\delta^{13}C$ and depleted $\delta^{15}N$ values compared to the southern region which experienced vast water exchange. The distinct spatial and temporal changes in the isotopic signatures in the consumers were thus a result of the hydrodynamic regimes in the two regions which affected the distribution and transfer of organic matter (Vizzini & Mazzola, 2006).

In addition to spatial questions involving estuarine contributions to coastal systems, a second aspect of ecological interest is the potential temporal variation of these contributions. For example, a study examining the flux of particulates within the CTZ of the Swartkops

Estuary (a well-mixed permanently open system) in the Eastern Cape Province of South Africa identified a small-scale tidal influence on suspended particulate matter (SPM) concentrations (Baird et al., 1987). Baird et al. (1987) reported the amount of total suspended particulates outwelled annually from the estuary was equivalent to 5306 tonnes, of which particulate organic carbon comprised ~31 % of the total estuarine production, and consisted mainly of salt marsh detritus and intertidal invertebrate faecal material. The majority of the total suspended particulate export was recorded during summer and autumn when the frequency and quantity of net export surpassed that of net import (Baird et al., 1987). The extensive export of particulates from the estuary was directly related to increased freshwater outflow during monthly ebb-spring tides (Baird et al., 1987). Similarly, the seasonal changes in carbon and nitrogen signatures in intertidal mussels (*Perna perna* and *Mytilus galloprovincialis*) and their potential food sources (offshore and nearshore SPM; macroalgae) were examined along the coast of South Africa from Namibia to Mozambique (Hill et al., 2006). Although the isotopic signatures for the macroalgae and mussels showed large seasonal variations with no consistent trends, four biogeographic regions were identified (Hill et al., 2006). The nearshore SPM $\delta^{13}C$ values became enriched from the east coast to the south coast and were ascribed to the large-scale changes in regional nutrient status of the coastal waters of South Africa, shifting from eastern low-nutrient to western eutrophic conditions (Lutjeharms et al., 2000; Peschak, 2005). The potential of estuaries to supply nutrients to the coastal marine environment involves numerous and complex components, and further study is warranted.

As an example of component complexity, the large diversity of primary producers and other potential food sources for suspension-feeders along rocky shores results in a rich variety of food web structures through space and time. Terrestrial vascular material represents low quality organic matter until it has entered the detrital food chain and been exposed to microbial decomposition (Deegan & Garritt, 1997; Marín Leal et al., 2008). As a consequence, terrestrial vascular plants are rarely a direct source of nutrition for aquatic consumers (McLeod & Wing, 2009). Indeed, isotopic analysis in Fiordland, New Zealand, revealed that terrestrial organic matter (TOM) did not make a substantial contribution to the diets of benthic suspension-feeders (i.e. *Mytilus edulis* and various sponges; McLeod & Wing, 2009). In contrast, coastal macroalgae can represent a substantial direct source of nutrition for rocky shore invertebrates (Hill et al., 2006; Hill & McQuaid, 2008; Riera et al., 2009). A study by Hill et al. (2006) revealed that offshore SPM along the entire coastline of South Africa mainly comprised oceanic phytoplankton, while SPM from the nearshore reflected a mixture of macroalgal detritus, phytoplankton and inorganic debris which contributed more than 50 % to the organic carbon and nitrogen sources of intertidal mussels (Hill et al., 2006). Although the dynamic nature of particulates in coastal ecosystems can provide an array of local and imported food sources to suspension-feeders, species-specific food assimilation may also influence consumer diets.

Selective feeding has been well documented for co-occurring consumers including mussels and tube-building polychaetes (Davies et al., 1989; Rossi et al., 2004; Espinosa et al., 2008; Noffke et al., 2009). Carbon and nitrogen signatures in various suspension-feeders in an estuarine and marine habitat in Normandy (France) indicated that bivalves had the most depleted values, and polychaetes the most enriched (Lefebvre et al., 2009). These results suggested that bivalves mainly relied on phytoplankton food sources and polychaetes on organic matter from microphytobenthos and macroalgae (Lefebvre et al., 2009). Physical habitat and feeding behaviour were factors ascribed to explaining the food partitioning between these two suspension-feeders (Lefebvre et al. 2009). A further study by Schaal et al. (2010) proposed that selective feeding played an important role in the seasonal resource partitioning amongst the filter-feeders of an intertidal rocky shore in the Bay of Brest (France). The Bay of Brest has substantial anthropogenic influences (i.e. harbour and marina developments) and receives nitrogen inputs from two tributaries draining stock farming effluents (Schaal et al., 2010). A wide range of $\delta^{13}C$ signals in the food sources was recorded, and the POM varied seasonally as result of substantial sediment resuspension, with maximum contributions in November, January and May as 53, 66 and 95 % to the POM-pool, respectively (Schaal et al., 2010). Despite the large variation in food source isotopic signatures, suspension-feeders had fairly consistent seasonal $\delta^{13}C$ values, but showed significant variability in $\delta^{15}N$ signals (Schaal et al., 2010). The significant variation in nitrogen signatures between the barnacles (~11.6 ‰) and mussels (~8.0 ‰), however, clearly identified a higher degree of omnivory in the barnacles, and the presence of resource partitioning, as barnacles have been observed to assimilate zooplankton in their diet (Zhukova, 2000; Riisgard & Larsen, 2010; Schaal et al., 2010).

Spatial and temporal changes in potential food sources available to rocky shore suspension-feeders are influenced by regional vegetation and seasonal changes in the physical and biological parameters of aquatic environments. Further investigation into the factors contributing to the species-specific changes in consumer diets through space and time is therefore required. The aim of this chapter was to assess the spatial and temporal changes in $\delta^{13}C$ and $\delta^{15}N$ signatures of the barnacle *Tetraclita serrata*, mussel *Perna perna*, and polychaete *Gunnarea capensis* in the coastal marine environment adjacent to two contrasting estuaries in the Eastern Cape Province of South Africa. The study regions were located near the freshwater-deprived Kariega Estuary (refer to Section 2.2.1.1) and freshwater-dominated Great Fish Estuary (refer to Section 2.2.1.2). The following hypotheses were tested:

1) Intraspecific stable isotope signatures in the barnacles, mussels and polychaetes vary regionally due to the differences in freshwater inputs of the two systems influencing regional food sources.
2) Intraspecific stable isotope signatures in the barnacles, mussels and polychaetes differ temporally as result of seasonal variation in available food sources.
3) Since all three species fall within the same trophic guild (i.e. suspension-feeders), each species is assumed to experience similar temporal and regional changes in isotopic signatures.
4) Given that the coastal region of the Eastern Cape is dominated by the south flowing Agulhas Current, suspension-feeders found downstream (south) of an estuary mouth demonstrate greater dependence on terrestrially-derived carbon than those located upstream (north). As a result, suspension-feeders located south of an estuary mouth with greater terrestrial sources in their diets have significantly depleted carbon signatures compared to those consumers situated north of the estuary mouth.

3.2 Materials and Methods

3.2.1 Sample collection

A detailed description of the study regions and procedures followed for sample collection, preparation and analyses have been provided in Chapter 2. A brief summary is presented here, with particular focus on the methods relevant to the stable isotope procedure. Sampling was conducted during March 2009 and July 2009 at five sites along the coastal nearshore marine environment in the Kariega and Great Fish regions. Two sites north (Sites 1 and 2) and south (Sites 3 and 4) of the estuary mouth and one site situated within the mouth (Site 5) were sampled to determine physico-chemical and biological parameters (See Figures 2.2 and 2.3 for study sites in the Kariega and Great Fish regions, respectively).

Water samples comprised two replicates each of 2.5 litre aliquots for POM and SPM (mg l^{-1}) stable isotope signature determination per site in each region (*n* = 10 per region). All filters were visually inspected after water filtration to remove any zooplankton or debris. At each site, three replicate animal samples were taken from haphazardly selected patches of the barnacle *Tetraclita serrata*, the mussel *Perna perna* and the polychaete *Gunnarea capensis*.

The absence of mussels and polychaetes at the estuarine site (Site 5a) in the Kariega and Great Fish regions resulted in missing δ^{13}C and δ^{15}N values during both seasons. The rapid tidal shift in the Great Fish region during March resulted in no mussel collection at the most northern site (Site 1).

Isotopic data for potential food sources in the Kariega and Great Fish regions were derived from the literature (Richoux & Froneman, 2007; Vorwerk & Froneman, 2009; Allan et al., 2010). In the Kariega region, the following food sources were included in the δ^{13}C and δ^{15}N signature plots (Figures 3.1 and 3.2): macroalgae (*Ulva* sp., *Gelidium pristoides*), SPM (Allan et al., 2010); seagrass (*Zostera capensis* and *Spartina maritima*), seston, salt marsh plants (*Sarcocornia perennis* and *Chenolea diffusa*), macroalgae (*Codium* sp.) and benthic algae (Richoux & Froneman, 2007). Potential food sources were selected based on the sites and sampling time most representative of the data collected during the current study. Data from February 2006 were selected to coincide with the consumer plots for March (Figure 3.1) and from October 2005 for July (Figure 3.2). In the Great Fish region, macroalgal and SPM data from Vorwerk and Froneman (2009) and Allan et al. (2010) were incorporated into the plots for both March (Figure 3.3) and July (Figure 3.4). The δ^{13}C values for the zooplankton, dominant riparian plants, salt marsh plants, reeds and sedges located within the Kariega and Great Fish regions were obtained from Vorwerk and Froneman (2009). The isotopic data of the oceanic and riverine/estuarine phytoplankton originated from Fry and Sherr (1984) and Cloern et al. (2002).

3.2.2 Sample preparation

After collection, water samples were filtered onto pre-combusted and pre-weighed GF/F Whatman glass fibre filters (47 mm). The filters were lyophilized for 24 hours and then stored at -20 °C until further processing. All animal tissues were lyophilized and homogenised as in Section 2.2.3, and a small amount of each sample was separated into individual eppendorf vials.

To remove the effect of variable lipid content on carbon isotope ratios (Tamelander et al., 2006), the lipids were extracted from all the samples using a method from Folch et al. (1957). Acidification of the samples was not done, as the tissues contained few carbonates (Jacob et al., 2005; Hill & McQuaid, 2008; Shin et al., 2008). Each of the homogenised samples was transferred to a glass tube and covered with 2 ml of 2:1 chloroform-methanol ($CHCl_3$:MeOH *v:v*) and a single drop of MilliQ water. Samples were vortexed, capped and left for 24 hours before the solution was removed. The procedure was repeated and after the second removal of the 2:1 ($CHCl_3$:MeOH) solution, the samples were transferred to clean eppendorf vials and oven dried prior to final homogenisation.

3.2.3 Stable isotope analysis

All samples destined for isotopic evaluation were analysed by the Stable Light Isotope Laboratory at the University of Cape Town (Archaeology Department). All homogenised tissue samples were weighed in tin cups (Sartorius microbalance; accuracy of 1 µg). Combustion of samples occurred in a Flash EA 1112 series elemental analyser (Thermo Finnigan, Italy) and the gases were passed to a Delta Plus XP isotope ratio mass spectrometer (IRMS) (Thermo electron, Germany), via a Conflo III gas control unit (Thermo Finnigan, Germany). The in-house standards used for the analysis of the animal tissues were proteinaceous gel (Merck), crushed seal bone (demineralised, dissolved in acid and then reconstituted in gel form) and DL Valine (Sigma). For the filtered water, Merck Gel, dried lentils and Australian National University (ANU) sucrose were used. All in-house standards were calibrated against the International Atomic Energy Agency (IAEA) standards. Nitrogen was expressed in terms of its value relative to atmospheric nitrogen, and carbon in terms of its value relative to Vienna Pee-Dee Belemnite. Isotope ratios were expressed in standard delta notation (δ).

3.2.4 Data analyses

All data were checked for normality with the Shapiro-Wilks test. Independent *t*-tests were used to determine any temporal and regional differences in the $\delta^{13}C$ and $\delta^{15}N$ signatures of the SPM in the Kariega and Great Fish regions. Analyses of variance (Kruskal-Wallis ANOVA for $\delta^{13}C$; one-way ANOVA for $\delta^{15}N$) were used to examine regional and temporal differences in the $\delta^{13}C$ and $\delta^{15}N$ signatures of each suspension-feeder (intraspecific trends with isotope signatures as dependent, and date or region as independent variables). Kruskal-Wallis ANOVA was used to test for regional and temporal differences in the $\delta^{13}C$ and $\delta^{15}N$ signatures among the suspension-feeders (interspecific trends with isotope signatures as dependent, and date or region as independent variables), and ranked sum comparisons were used to identify significant groups (significance level at $P < 0.05$). A Levene’s test for homogeneity was used where appropriate. Where no differences among sites within each region or sample time were found in the isotope signatures of the SPM and suspension-feeder tissues, data from the northern and southern sites were pooled. The pooled data were then assessed for potential north *versus* south distinctions with independent *t*-tests (n = 10 and 6 per region and date for SPM and suspension-feeders, respectively). Coefficients of variation (CV; standard deviation expressed as percentage of the mean) were used to compare the variability in δ^{13}C and δ^{15}N signatures in the coastal SPM and among species in the Kariega and Great Fish regions in both months. Analyses were conducted with the use of SYSTAT (v12.0) and PAST 1.42 (Hammer et al., 2001).

The Bayesian mixing model SIAR (Stable Isotope Analysis in R; Parnell et al., 2010) was used to quantify the percentage contribution of the potential estuarine and oceanic suspended particulate matter (SPM) and zooplankton food sources in the diets of the three suspension-feeders. This model requires the inclusion of several dietary sources, consumer tissue isotopic values and trophic enrichment factors, and their associated variability, to generate robust probability distributions of potential food sources in consumer diets. The contributions of potential SPM or zooplankton food in the diet of the suspension-feeders are reported as means and the 95 % confidence intervals (CI). The SIAR mixing model was computed with the use of the SIAR software package (Parnell et al., 2010).

3.3 Results

3.3.1 δ^{13}C and δ^{15}N signatures of suspended particulate matter

The δ^{13}C signatures of the coastal SPM were significantly different between March and July in the Great Fish region, and between the Kariega and Great Fish regions in both months ($P < 0.05$ in all cases; Table 3.1). SPM δ^{15}N signatures were significantly different between March and July in both regions, and between the Kariega and Great Fish regions in July ($P < 0.05$ in all cases; Table 3.1). Variation in coastal SPM δ^{13}C signatures in the Kariega (CV: ~0.55 %) and Great Fish regions was low (CV: ~0.7 %) during both sample times (Tables 3.2 and 3.3). Variation in δ^{15}N signatures in March (CV: ~1.6 %) was greater compared to July (CV: ~0.65 %) in both regions (Tables 3.2 and 3.3).

Table 3.1 Independent *t*-test results for the suspended particulate matter (SPM) indicating differences in the $\delta^{13}C$ and $\delta^{15}N$ signatures (‰) in the Kariega and Great Fish regions during March and July. Variance equality was assessed with a Levene's test for homogeneity ($P > 0.05$ in all cases). K, Kariega; GF, Great Fish; Mar, March; Jul, July; ns, not significant.

Stable Isotope	Region	Date	Independent *t*-test		
			t-value	*P*	*df*
$\delta^{13}C$	K & GF	March	5.386	<0.001	14
	K & GF	July	2.658	<0.05	14
	Kariega	Mar & Jul	-0.409	ns	14
	Great Fish	Mar & Jul	-3.587	<0.01	14
$\delta^{15}N$	K & GF	March	-0.042	ns	13
	K & GF	July	3.042	<0.01	14
	Kariega	Mar & Jul	-4.111	<0.01	14
	Great Fish	Mar & Jul	-2.780	<0.05	13

Table 3.2 δ^{13}C and δ^{15}N signatures (‰) of the primary producers, suspended and particulate organic matter (SPM and POM) and zooplankton in the Kariega region. Values are presented as ranges or means ± standard deviation. Coefficients of variation (CV) document the variance in coastal SPM in March and July (CV δ^{13}C: 0.6 and 0.5 %, respectively; CV δ^{15}N: 1.7 and 0.8 %, respectively). Data derived from Fry & Sherr (1984)*, Cloern et al. (2002)*, Richoux & Froneman (2007)**; Vorwerk & Froneman (2009)***; and Allan et al., (2010)****; - no data.

Producers	Kariega Region	
March	δ^{13}C	δ^{15}N
Oceanic SPM (current study)	-15.2 ± 0.9	5.2 ± 0.9
Estuarine SPM (current study)	-25.7 ± 0.0	5.5 ± 0.4
SPM****	-14.8 ± 0.6	6.0 ± 0.5
Seston**	-23.0 ± 0.5	3.6 ± 0.9
Seagrass**,***	-16.9 to -8.0	7.1 to 8.3
Macroalgae**	-17.2 ± 0.9	9.3 ± 0.1
Salt marsh plants**,***	-29.9 to -14.3	9.3 to 11.1
Riparian plants***	-28.1 to -21.1	3.5 to 4.1
POM - lower estuary***	-20.9 ± 1.6	-
POM - opposite mouth***	-17.3 ± 1.9	-
POM - 0.5 km upstream***	-15.6 ± 0.5	-
POM - 0.5 km downstream***	-15.7 ± 0.2	-
Oceanic phytoplankton*	-20.0 ± 1.5	9.0 ± 1.7
Riverine/estuarine phytoplankton*	-27.0 ± 2.9	8.0 ± 2.6
Oceanic zooplankton**,***	-16.5 ± 0.3	8.5 to 10.5
Estuarine zooplankton**,***	-21.5 ± 1.4	8.5 to 10.5
July		
Oceanic SPM (present study)	-15.0 ± 0.8	6.7 ± 0.6
Estuarine SPM (present study)	-23.5 ± 0.2	5.7 ± 0.2
SPM****	-14.8 ± 0.6	6.0 ± 0.5
Seston**	-21.2 ± 0.2	8.4 ± 2.0
Seagrass**	-11.5 to -10.1	6.3 to 8.3
Benthic algae**	-15.5 ± 1.0	10.3 ± 2.7
Macroalgae****	-17.9 to -15.4	6.1 to 7.0
Salt marsh plants**	-26.5 to -13.3	6.6 to 8.4
Oceanic phytoplankton*	-20.0 ± 1.5	9.0 ± 1.7
Riverine/estuarine phytoplankton*	-27.0 ± 2.9	8.0 ± 2.6
Oceanic zooplankton**,***	-16.5 ± 0.3	8.5 to 10.5
Estuarine zooplankton**,***	-21.5 ± 1.4	8.5 to 10.5

Table 3.3 $\delta^{13}C$ and $\delta^{15}N$ signatures (‰) of the primary producers, suspended and particulate organic matter (SPM and POM) and zooplankton in the Great Fish region. Values are presented as ranges or means ± standard deviation. Coefficients of variation (CV) document the variance in coastal SPM in March and July (CV $\delta^{13}C$: 0.8 and 0.6 %, respectively; CV $\delta^{15}N$: 1.5 and 0.5 %, respectively). Data derived from Fry & Sherr (1984)*, Cloern et al. (2002)*, Richoux & Froneman (2007)**; Vorwerk & Froneman (2009)***; and Allan et al., (2010)****; - no data.

Producers	Great Fish Region	
March	$\delta^{13}C$	$\delta^{15}N$
Oceanic SPM (present study)	-18.4 ± 1.5	5.2 ± 0.8
Estuarine SPM (present study)	-20.4 ± 0.1	6.3 ± 0.1
SPM****	-16.5 ± 0.2	5.6 ± 1.4
Riparian plants***	-29.9 to -26.9	-
Reed/sedge***	-29.6 to -26.9	-
Salt marsh plants***	-27.7 to -14.4	-
POM - lower estuary***	-19.2 ± 0.3	-
POM - opposite mouth***	-20.2 ± 1.1	-
POM - 0.5 km upstream***	-19.7 ± 0.6	-
POM - 0.5 km downstream***	-19.4 ± 0.7	-
Oceanic phytoplankton*	-20.0 ± 1.5	9.0 ± 1.7
Riverine/estuarine phytoplankton*	-27.0 ± 2.9	8.0 ± 2.6
Oceanic zooplankton**,***	-18.8 ± 1.4	8.5 to 10.5
Estuarine zooplankton**,***	-16.8 ± 0.4	8.5 to 10.5
July		
Oceanic SPM (present study)	-16.2 ± 1.0	6.0 ± 0.3
Estuarine SPM (present study)	-24.4 ± 0.0	6.6 ± 0.1
SPM****	-16.5 ± 0.2	5.6 ± 1.4
Macroalgae****	-17.3 to -14.6	6.2 to 7.3
Oceanic phytoplankton*	-20.0 ± 1.5	9.0 ± 1.7
Riverine/estuarine phytoplankton*	-27.0 ± 2.9	8.0 ± 2.6
Oceanic zooplankton**,***	-18.8 ± 1.4	8.5 to 10.5
Estuarine zooplankton**,***	-16.8 ± 0.4	8.5 to 10.5

Table 3.4 δ^{13}C and δ^{15}N signatures (mean ± SD, ‰) of suspended particulate matter (SPM) and three indigenous filter-feeding organisms collected in the Kariega and Great Fish regions during March and July 2009. Site 1, most northern; Site 2, just north of mouth; Site 3, just south of mouth; Site 4, most southern; Site 5, mouth; - no data.

		Kariega Region				Great Fish Region			
		March 2009		July 2009		March 2009		July 2009	
		δ^{13}C	δ^{15}N	δ^{13}C	δ^{15}N	δ^{13}C	δ^{15}N	δ^{13}C	δ^{15}N
SPM	1	-15.7 ± 0.2	4.7 ± 0.6	-14.0 ± 0.3	6.0 ± 0.2	-16.7 ± 0.2	4.5 ± 1.2	-16.3 ± 0.1	5.9 ± 0.1
	2	-15.9 ± 0.1	4.2 ± 0.2	-14.7 ± 0.0	6.6 ± 0.2	-17.5 ± 0.1	5.6 ± 0.1	-17.4 ± 0.1	6.3 ± 0.1
	3	-13.9 ± 0.7	6.2 ± 0.1	-15.1 ± 0.1	6.9 ± 0.2	-20.1 ± 0.1	5.7 ± 0.5	-16.4 ± 0.1	5.7 ± 0.3
	4	-15.2 ± 0.6	5.7 ± 0.2	-16.1 ± 0.2	7.4 ± 0.2	-19.3 ± 0.0	3.5 ± 1.8	-14.7 ± 0.1	6.2 ± 0.1
	5	-25.7 ± 0.0	5.5 ± 0.4	-25.3 ± 0.2	5.7 ± 0.2	-20.4 ± 0.1	6.3 ± 0.1	-24.4 ± 0.0	6.6 ± 0.1
Tetraclita serrata (barnacle)	1	-15.7 ± 0.0	10.7 ± 0.1	-16.0 ± 0.0	11.0 ± 0.0	-16.1 ± 0.2	10.0 ± 0.2	-16.6 ± 0.1	10.2 ± 0.1
	2	-15.1 ± 0.4	10.3 ± 0.2	-16.1 ± 0.1	10.7 ± 0.2	-15.7 ± 0.0	10.8 ± 0.2	-16.2 ± 0.1	10.7 ± 0.2
	3	-15.9 ± 0.0	10.5 ± 0.0	-16.2 ± 0.1	10.7 ± 0.2	-16.6 ± 0.7	11.4 ± 0.2	-16.6 ± 0.0	11.4 ± 0.2
	4	-15.9 ± 0.0	10.2 ± 0.5	-16.0 ± 0.2	10.8 ± 0.1	-15.6 ± 0.3	10.8 ± 0.1	-16.4 ± 0.1	10.6 ± 0.1
	5	-14.9 ± 0.0	11.1 ± 1.4	-16.4 ± 0.1	10.3 ± 0.3	-	-	-	-
Perna perna (mussel)	1	-15.1 ± 0.2	7.9 ± 0.1	-15.0 ± 0.1	8.0 ± 0.4	-	-	-16.2 ± 0.1	7.7 ± 0.2
	2	-14.9 ± 0.2	8.0 ± 0.1	-15.0 ± 0.2	8.0 ± 0.2	-15.9 ± 0.4	8.0 ± 0.2	-16.4 ± 0.3	8.4 ± 0.3
	3	-15.1 ± 0.1	7.6 ± 0.1	-15.3 ± 0.4	7.6 ± 0.0	-16.2 ± 0.2	8.4 ± 0.3	-16.6 ± 0.3	8.0 ± 0.3
	4	-15.2 ± 0.0	7.4 ± 0.4	-14.9 ± 0.1	8.3 ± 0.2	-15.6 ± 0.1	7.8 ± 0.2	-15.8 ± 0.3	7.7 ± 0.1
	5	-	-	-	-	-	-	-	-
Gunnarea capensis (polychaete)	1	-14.7 ± 0.1	8.9 ± 0.0	-14.0 ± 0.2	9.4 ± 0.2	-14.7 ± 0.1	9.1 ± 0.2	-15.3 ± 0.3	9.0 ± 0.2
	2	-14.4 ± 0.2	9.1 ± 0.1	-14.2 ± 0.1	9.5 ± 0.3	-14.5 ± 0.1	9.5 ± 0.2	-14.4 ± 0.1	9.6 ± 0.2
	3	-14.5 ± 0.3	9.1 ± 0.2	-14.6 ± 0.0	9.0 ± 0.4	-15.5 ± 0.1	9.3 ± 0.2	-16.0 ± 0.3	9.5 ± 0.2
	4	-14.9 ± 0.2	8.7 ± 0.2	-14.6 ± 0.1	8.9 ± 0.2	-15.3 ± 0.3	8.9 ± 0.1	-15.3 ± 0.1	8.9 ± 0.2
	5	-	-	-	-	-	-	-	-

3.3.1.1 Kariega region

The δ^{13}C signatures of the SPM showed intermediate values at the coastal sites during March (Sites 1 to 4: -15.9 to -13.9 ‰) and July (-16.1 to -14.0 ‰), whereas the estuarine site was highly ^{13}C-depleted during both sample times (Site 5: -25.7 to -25.3 ‰; Table 3.4; Figures 3.1 and 3.2). The δ^{13}C and δ^{15}N signatures at the northern (Sites 1 and 2) and southern sites (Sites 3 and 4) were significantly different during March and July in the Kariega region ($P < 0.05$; Table 3.5). The SPM δ^{15}N signatures at the northern sites (Sites 1 and 2) in March were more depleted (4.2 to 4.7 ‰; $P < 0.05$) than the southern sites (Sites 3 and 4: 5.7 to 6.2 ‰; Table 3.4; Figure 3.1). In July, the most northern site (Site 1) had significantly ^{13}C-enriched and ^{15}N-depleted signatures compared to the other sites (-14.0 ± 0.3 ‰ and 6.0 ± 0.2 ‰; Table 3.4; Figure 3.2). A Tukey multiple-comparisons test did not reveal a significant temporal distinction in SPM δ^{13}C and δ^{15}N signatures at the estuarine site (Site 5) in the Kariega region ($P > 0.05$).

Table 3.5 Independent *t*-test results for the suspended particulate matter (SPM) δ^{13}C and δ^{15}N signatures (‰) indicating differences between the northern sites (Sites 1 and 2) and southern sites (Sites 3 and 4) in the Kariega and Great Fish regions during March and July; $n = 5$ per region or date; ns, not significant. Variance equality was assessed with a Levene's test for homogeneity ($P > 0.05$ in all cases).

Stable Isotope	Region	Date	Independent t-*t*est		
			t-value	*P*	*df*
δ^{13}C	Kariega	March	-2.539	<0.05	6
	Kariega	July	3.137	<0.05	6
	Great Fish	March	8.109	<0.05	6
	Great Fish	July	-2.275	ns	6
δ^{15}N	Kariega	March	-5.285	<0.05	6
	Kariega	July	-3.693	<0.05	6
	Great Fish	March	0.583	ns	5
	Great Fish	July	0.552	ns	6

3.3.1.2 Great Fish region

The δ^{13}C signatures of the SPM in March at the northern sites were significantly enriched (Sites 1 and 2: -17.5 to -16.7 ‰; $P < 0.05$) compared to the southern and estuarine sites (Sites 3 to 5: -20.4 to -19.3 ‰; Tables 3.4 and 3.5; Figure 3.3). The δ^{13}C values in July were intermediate at the coastal sites (Sites 1 to 4: -17.4 to -14.7 ‰) compared to the estuarine site, which was relatively depleted (Site 5: -24.4 ± 0.0 ‰; Table 3.4; Figure 3.4).

During March, the sites furthest from the mouth had the most depleted δ^{15}N signatures (Sites 1 and 4: 3.5 to 4.5 ‰), the sites closest to the mouth intermediate values (Site 2 and 3: 5.6 to 5.7 ‰), and the estuarine site the most enriched signatures (Site 5: 6.3 ± 0.1 ‰; Table 3.4; Figure 3.3). Tukey multiple-comparisons tests revealed significant temporal distinctions in SPM δ^{13}C and δ^{15}N signatures at the estuarine site (Site 5) in the Great Fish region ($P < 0.05$ in both cases). However, the SPM δ^{15}N signatures from the Great Fish region did not differ from north to south in March or July ($P > 0.05$; Table 3.5).

3.3.2 δ^{13}C and δ^{15}N signatures of consumers

The δ^{13}C and δ^{15}N signatures in the tissues of the three indigenous suspension-feeding invertebrates revealed several intraspecific trends through time and space. The δ^{13}C signatures in the barnacle *Tetraclita serrata* were significantly different between March and July in both regions, and were slightly enriched in March (Kariega: -15.1 ± 1.4 ‰; Great Fish: -16.0 ± 0.4 ‰; $P < 0.001$; Tables 3.6 and 3.7). The barnacles that were located within the estuary mouth (Site 5: -14.9 ± 0.0 ‰; 11.1 ± 1.4 ‰) were significantly more enriched in δ^{13}C and δ^{15}N during March in the Kariega region than those of the coastal sites (Sites 1 to 4: -15.9 to -15.1 ‰; 10.2 to 10.7 ‰; $P < 0.05$; Table 3.4; Figure 3.1). The δ^{13}C signatures in the barnacles were notably more depleted than those recorded for the other two invertebrates in the Kariega region in July (Kariega: -16.1 ± 0.2 ‰; $P < 0.001$; Table 3.9; Figure 3.2). In contrast, barnacle δ^{15}N signatures were significantly enriched relative to the mussel and polychaete in both regions in March and July (Kariega: 10.4 to 10.7 ‰; Great Fish: 10.7 to 10.8 ‰; $P < 0.001$ in all cases; Table 3.9; Figures 3.1 to 3.4). The barnacles in the Kariega region had δ^{13}C signatures similar to those of the coastal SPM, POM, benthic algae, macroalgae *G. pristoides* and salt marsh plant *Sporobolus virginicus* in both months. The signatures of the barnacles were, however, ^{13}C-enriched relative to the riparian vegetation and salt marsh plants *C. diffusa* and *S. perennis*, and ^{13}C-depleted compared to the seagrass *Z. capensis* (Table 3.2; Figures 3.1 and 3.2). In the Great Fish region, the δ^{13}C signatures in the barnacles closely reflected those of the macroalgae and were more ^{13}C-enriched than the SPM (not including the northern sites), riparian vegetation and POM (Table 3.3; Figures 3.3 and 3.4). The δ^{15}N values in the barnacles (10.0 to 11.4 ‰) showed no temporal or regional distinctions ($P > 0.05$; Table 3.8) but were substantially enriched compared to the SPM (3.5 to 7.4 ‰) in the Kariega and Great Fish regions (Table 3.4; Figures 3.1 to 3.4).

Table 3.6 δ^{13}C and δ^{15}N signatures (‰) in three indigenous suspension-feeders (barnacle *Tetraclita serrata*; mussel *Perna perna*; and polychaete *Gunnarea capensis*) in the Kariega and Great Fish regions during March and July. Values are presented as means ± standard deviation; and coefficient of variation (CV, %).

	Barnacle *(Tetraclita serrata)*		Mussel *(Perna perna)*		Polychaete *(Gunnarea capensis)*	
Kariega	δ^{13}C	δ^{15}N	δ^{13}C	δ^{15}N	δ^{13}C	δ^{15}N
March	-15.1 ± 1.4	10.4 ± 0.5	-15.1 ± 0.2	7.7 ± 0.2	-14.6 ± 0.3	8.9 ± 0.2
CV	0.9	0.4	0.1	0.4	0.2	0.3
July	-16.1 ± 0.2	10.7 ± 0.2	-15.0 ± 0.3	8.0 ± 0.3	-14.4 ± 0.3	9.2 ± 0.4
CV	0.1	0.2	0.1	0.4	0.2	0.4
Great Fish						
March	-16.0 ± 0.4	10.8 ± 0.5	-15.8 ± 0.3	8.1 ± 0.3	-15.0 ± 0.5	9.2 ± 0.3
CV	0.3	0.5	0.2	0.4	0.3	0.3
July	-16.5 ± 0.2	10.7 ± 0.5	-16.3 ± 0.4	7.9 ± 0.4	-15.3 ± 0.6	9.3 ± 0.4
CV	0.1	0.4	0.2	0.5	0.4	0.4

The δ^{13}C signatures in the mussel *Perna perna* were significantly different between the Kariega and Great Fish regions in March (Kariega: -15.1 ± 0.2 ‰; Great Fish: -15.8 ± 0.3 ‰) and July (Kariega: -15.0 ± 0.3 ‰; Great Fish: -16.3 ± 0.4 ‰; $P < 0.001$; Tables 3.6 and 3.7). The mussels had intermediate and distinctly different δ^{13}C values in comparison to the other filter-feeders in the Kariega region in July (-15.0 ± 0.3 ‰; $P < 0.001$; Figure 3.2), and significantly depleted δ^{15}N signatures in the Kariega and Great Fish regions during both sample times (Kariega: 7.7 to 8.0 ‰; Great Fish: 7.9 to 8.1 ‰; $P < 0.001$ in all cases; Table 3.9; Figures 3.1 to 3.4). Carbon signatures of the mussels were similar to those of the barnacles, and closely reflected the coastal SPM, benthic algae, and macroalgae *G. pristoides* and salt marsh plant *S. virginicus*. In addition, the mussels were ^{13}C-enriched relative to the riparian vegetation, salt marsh plants and macroalgae *Codium sp*. and *Ulva sp*., and ^{13}C-depleted compared to the seagrass *Z. capensis* (Table 3.2; Figures 3.1 and 3.2). The δ^{13}C signatures of the mussels closely resembled the SPM (northern sites only during March) and the dominant macroalgal species in the Great Fish region in both months (Table 3.3; Figures 3.3 and 3.4). Nitrogen signatures in the mussels (7.7 to 8.1 ‰) showed no temporal or regional distinctions ($P > 0.05$; Table 3.8) but were enriched compared to the SPM (3.5 to 7.4 ‰) in the Kariega and Great Fish regions, and resembled those of the seagrass *Z. capensis* in the Kariega region (8.0 to 8.3 ‰; Tables 3.2 and 3.4; Figures 3.1 to 3.4).

Table 3.7 Kruskal-Wallis ANOVA results (with isotope signature data as dependent, and date and region as independent variables) indicating geographic location and temporal differences in the $\delta^{13}C$ signatures (‰) of three indigenous suspension-feeding invertebrates (barnacle *Tetraclita serrata*; mussel *Perna perna*; and polychaete *Gunnarea capensis*). The between-groups comparisons among the species as indicated by their ranked sums were also given; K, Kariega; GF, Great Fish; Mar, March; Jul, July; *vs*., represents a significant difference between the stated variables.

Consumer	Variables	Kruskal-Wallis			Rank Sum
		df	statistic	*P*	Comparisons
Tetraclita serrata (Barnacle)	K, GF, Mar, Jul	3	31.730	<0.001	Mar *vs*. Jul (both regions)
Perna perna (Mussel)	K, GF, Mar, Jul	3	33.328	<0.001	K *vs*. GF (both seasons)
Gunnarea capensis (Polychaete)	K, GF, Mar, Jul	3	17.447	<0.01	K *vs*. GF (both seasons)

Table 3.8 One-way ANOVA results (with isotope signature data as dependent, and date and region as independent variables) indicating temporal and regional differences among the $\delta^{15}N$ signatures (‰) of three indigenous suspension-feeding invertebrates (barnacle *Tetraclita serrata*; mussel *Perna perna*; and polychaete *Gunnarea capensis*). Levene's test for homogeneity was conducted to verify the validity of each test ($P > 0.05$ in all cases). K, Kariega; GF, Great Fish; Mar, March; Jul, July; ns, not significant.

Consumer	Variables	$\delta^{15}N$		
		df	*F*-ratio	*P*
Tetraclita serrata (Barnacle)	K, GF, Mar, Jul	3,44	1.781	ns
Perna perna (Mussel)	K, GF, Mar, Jul	3,41	1.951	ns
Gunnarea capensis (Polychaete)	K, GF, Mar, Jul	3,44	2.417	ns

Table 3.9 Kruskal-Wallis ANOVA results (with isotope signature data as dependent, and estuary or date as independent variables) indicating geographic location and temporal differences between the $\delta^{13}C$ and $\delta^{15}N$ signatures (‰) of three indigenous suspension-feeding invertebrates (barnacle *Tetraclita serrata*; mussel *Perna perna*; and polychaete *Gunnarea capensis*). The between-groups comparisons among the species as indicated by their ranked sums were also given; *vs*., represents a significant difference between the stated variables.

Stable Isotope	Estuary	Date	Kruskal-Wallis			Rank Sum
			df	statistic	*P*	Comparisons
$\delta^{13}C$	Kariega	March	2	15.163	<0.01	polychaete *vs*. barnacle & mussel
	Kariega	July	2	33.656	<0.001	all species significantly different
	Great Fish	March	2	17.538	<0.001	polychaete *vs*. barnacle & mussel
	Great Fish	July	2	20.262	<0.001	polychaete *vs*. barnacle & mussel
$\delta^{15}N$	Kariega	March	2	33.646	<0.001	all species significantly different
	Kariega	July	2	33.653	<0.001	all species significantly different
	Great Fish	March	2	28.299	<0.001	all species significantly different
	Great Fish	July	2	31.135	<0.001	all species significantly different

The $\delta^{13}C$ and $\delta^{15}N$ signatures in the polychaete *Gunnarea capensis* were significantly distinct between the Kariega and Great Fish regions during both sample times ($P < 0.01$; Table 3.7). The polychaetes showed ^{13}C-enriched values compared to the other two suspension-feeders, and were significantly more enriched during both months in the Kariega (-14.6 to -14.4 ‰) and Great Fish regions (-15.3 to -15.0 ‰; $P < 0.01$ in all cases; Tables 3.6 and 3.9; Figures 3.2 to 3.4). The $\delta^{15}N$ values in the polychaete tissues remained consistently intermediate (8.9 to 9.3 ‰) between those recorded in the barnacles and mussels in both regions through time (Table 3.6; Figures 3.1 to 3.4). Carbon signatures in the polychaetes from the Kariega region reflected the coastal SPM, benthic algae, and macroalgae *G. pristoides* and salt marsh plant *S. virginicus*, but were more enriched relative to the POM, salt marsh plants and riparian vegetation, and more depleted than the seagrass *Z. capensis* (Table 3.2; Figures 3.1 and 3.2). In the Great Fish region, polychaete $\delta^{13}C$ values mirrored the coastal SPM (northern sites only in March), the macroalgae *Gelidium* spp. and *Codium* sp., and salt marsh plant *S. virginicus*, but were more enriched compared to the riparian vegetation, POM and macroalgae *C. filiformis* and *H. specifera* (Table 3.3; Figures 3.3 and 3.4). The polychaete $\delta^{15}N$ signal (8.7 to 9.6 ‰) showed no temporal or regional distinctions ($P > 0.05$; Table 3.8) but was enriched compared to the SPM (3.5 to 7.4 ‰) in the Kariega and Great Fish regions, and resembled those of *Codium* sp. and *S. perennis* in the Kariega region (9.3 to 9.5 ‰; Tables 3.2 and 3.4; Figures 3.1 to 3.4).

Intraspecific variation in the $\delta^{13}C$ and $\delta^{15}N$ signatures was small in all three suspension-feeders within both regions (CV: 0.1-0.9 %; Table 3.6). However, the interspecific differences in the $\delta^{13}C$ signatures among all three invertebrates closely reflected those of the coastal SPM, with the most ^{13}C-enriched values occurring in the Kariega region in March and the most ^{13}C-depleted values in the Great Fish region in July (Table 3.6). Differences in carbon signatures among the suspension-feeders within one region was significant between the barnacles and polychaetes (1.0 to 1.7 ‰), except in the Kariega region in March (~0.5 ‰), while among-species differences in nitrogen signatures were significant in the Kariega and Great Fish regions in both sample times (2.7 to 2.8 ‰; Table 3.6; Figures 3.1 to 3.4). No significant north *versus* south trends were observed in the $\delta^{13}C$ and $\delta^{15}N$ signatures in the suspension-feeders in the Kariega and Great Fish regions ($P > 0.05$ in all cases).

The results of SIAR indicated that oceanic SPM was the most important food source in the diets of the barnacles (mean = 30-60 %; 95 % CI = 25-65 %), mussels (mean = 60-85 %; 95 % CI = 50-90 %) and polychaetes (mean = 68-85 %; 95 % CI = 62-90 %; Figures 3.5 to 3.8). The barnacles, however, had a larger contribution of oceanic SPM in the Kariega region (mean = 54-60 %; 95 % CI = 50-65 %; Figures 3.5a and 3.6a), whereas those in the Great Fish region showed significant contributions of oceanic zooplankton to their diet during both sample times (mean = 31-68 %; 95 % CI = 32-68 %; Figures 3.7a and 3.8a). Oceanic SPM contributed more to mussel diet during March in the Kariega and Great Fish regions (mean = 75-85 %; 95 % CI = 69-90 %; Figures 3.5b and 3.7b), whereas oceanic zooplankton supplied important nutrients in July in both regions (mean = 10-15 %; 95 % CI = 1-24 %; Figures 3.6b and 3.8b). A consistently large contribution of oceanic SPM (mean = 68-85 %; 95 % CI = 62-90 %; Figures 3.5c to 3.8c) and small contribution of zooplankton (mean = ≤10 %; 95 % CI = 0-18 %; Figures 3.5c to 3.8c) to polychaete diet was recorded in both regions. The estuarine SPM contributed <20 % to the diets of the barnacles (95 % CI = 5-22 %) and <10 % to the diets of the mussels and polychaetes (95 % CI = 2-15 %; Figures 3.5 to 3.8). Contributions of estuarine zooplankton to consumer diets were <20 % (95 % CI = 5-22 %) for the barnacles, <10 % (95 % CI = 0-10 %) for the mussels and <5 % for the polychaetes (95 % CI = <5 %; Figures 3.5 to 3.8).

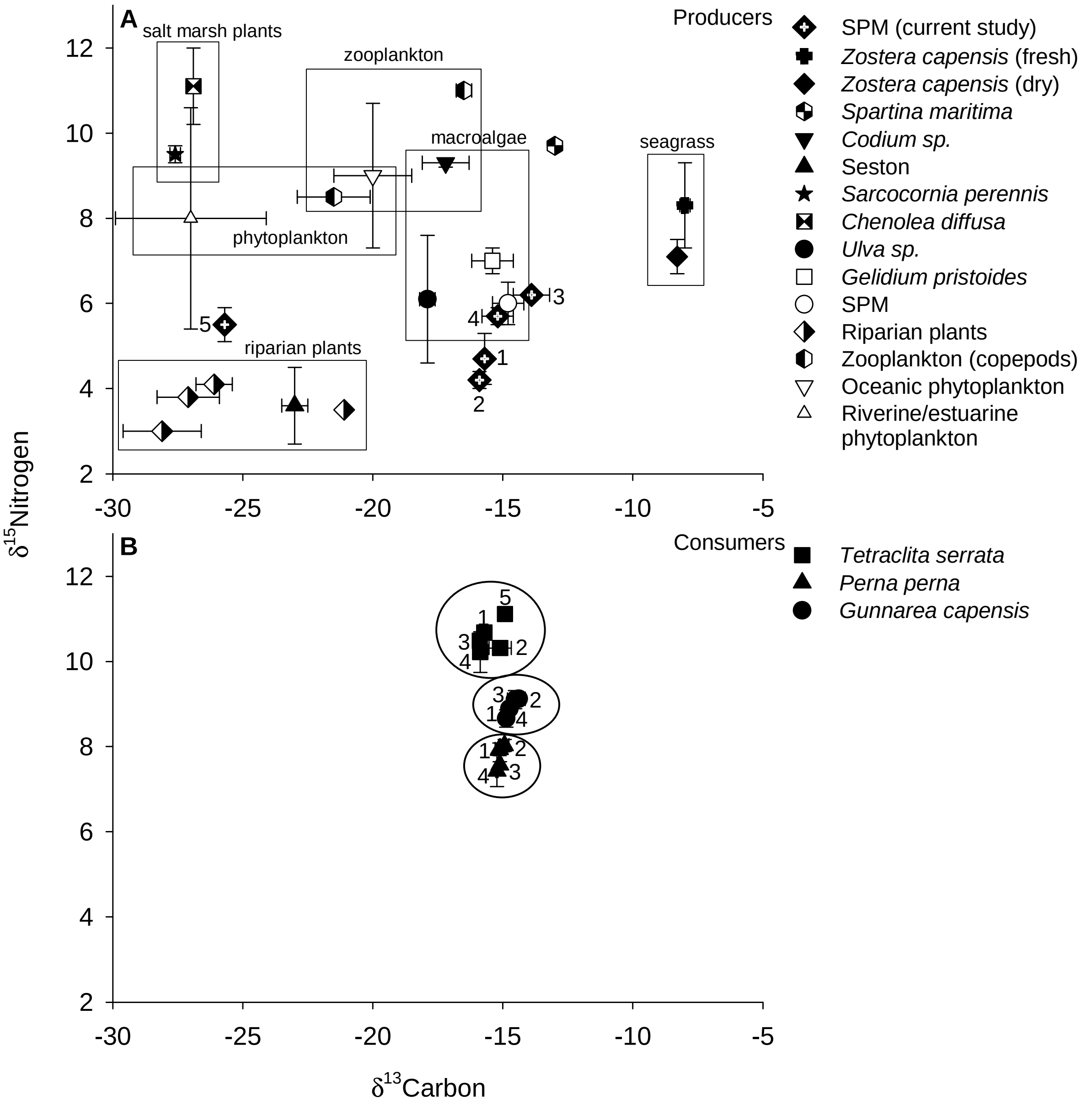

Figure 3.1 δ^{13}C and δ^{15}N signatures (‰) of (A) potential carbon sources and SPM, and (B) the barnacle *Tetraclita serrata*, mussel *Perna perna*, and polychaete *Gunnarea capensis* along the rocky shore environment in the Kariega region during March 2009 (*n* = 2 for barnacles at Sites 2 and 5 for carbon, and Site 5 for nitrogen; *n* = 3 for all other sites). Significant variations among the groups are indicated by ellipses (Kruskal-Wallis ANOVA ranked sum comparisons). Values are presented as means ± standard deviation. Data on producers, zooplankton and SPM in the Kariega region originate from Richoux & Froneman (2007), Vorwerk & Froneman (2009) and Allan et al. (2010). Phytoplankton data originate from Fry & Sherr (1984) and Cloern et al. (2002). Site 1, most northern; Site 2, just north of mouth; Site 3, just south of mouth; Site 4, most southern; Site 5, mouth.

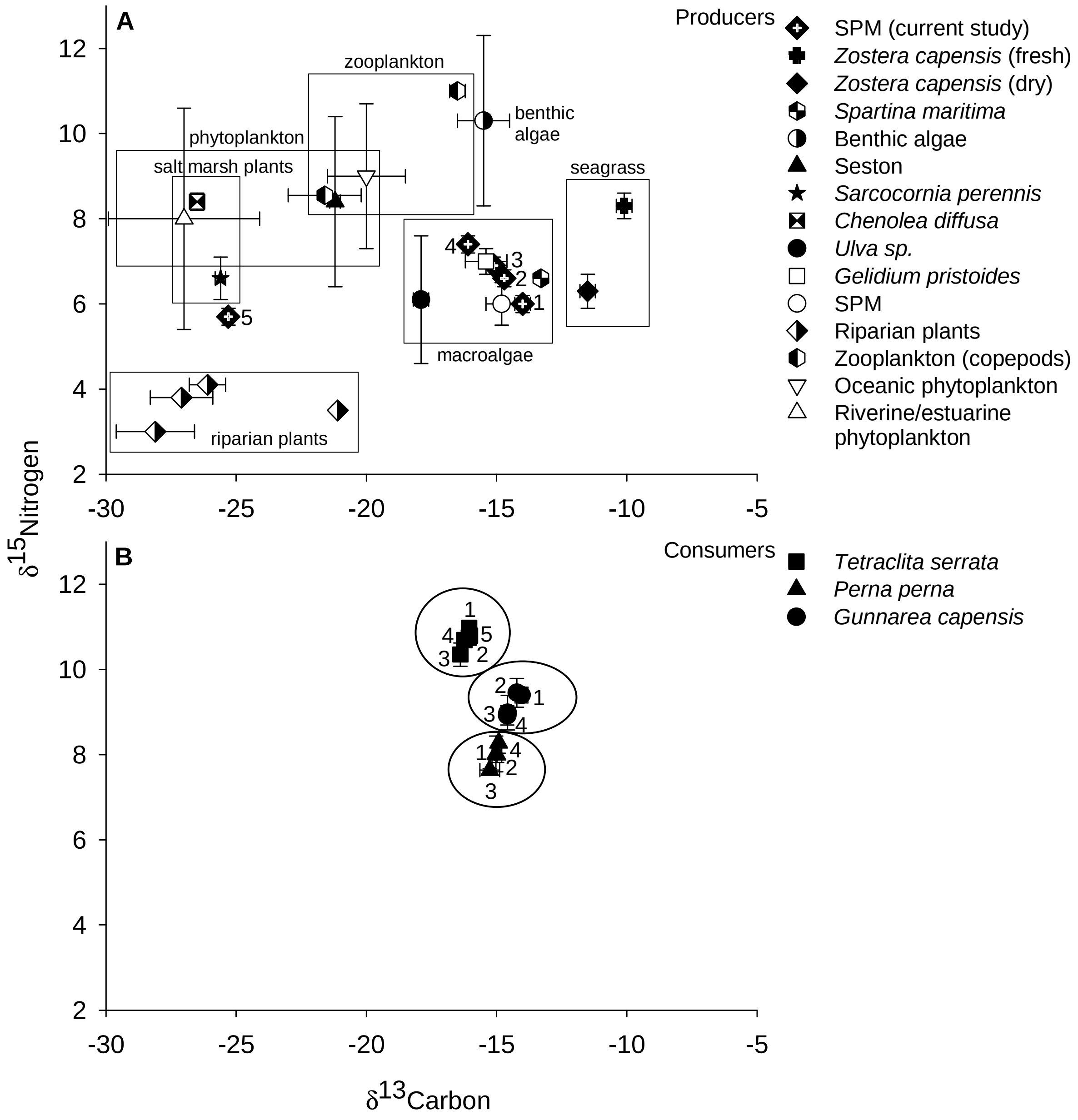

Figure 3.2 δ^{13}C and δ^{15}N signatures (‰) of (A) potential carbon sources and SPM, and (B) the barnacle *Tetraclita serrata*, mussel *Perna perna*, and polychaete *Gunnarea capensis* along the rocky shore environment in the Kariega region during July 2009 (*n* = 3 for all sites). Significant variations among the groups are indicated by ellipses (Kruskal-Wallis ANOVA ranked sum comparisons). Values are presented as means ± standard deviation. Data on producers, zooplankton and SPM in the Kariega region originate from Richoux & Froneman (2007), Vorwerk & Froneman (2009) and Allan et al. (2010). Phytoplankton data originate from Fry & Sherr (1984) and Cloern et al. (2002). Site 1, most northern; Site 2, just north of mouth; Site 3, just south of mouth; Site 4, most southern; Site 5, mouth.

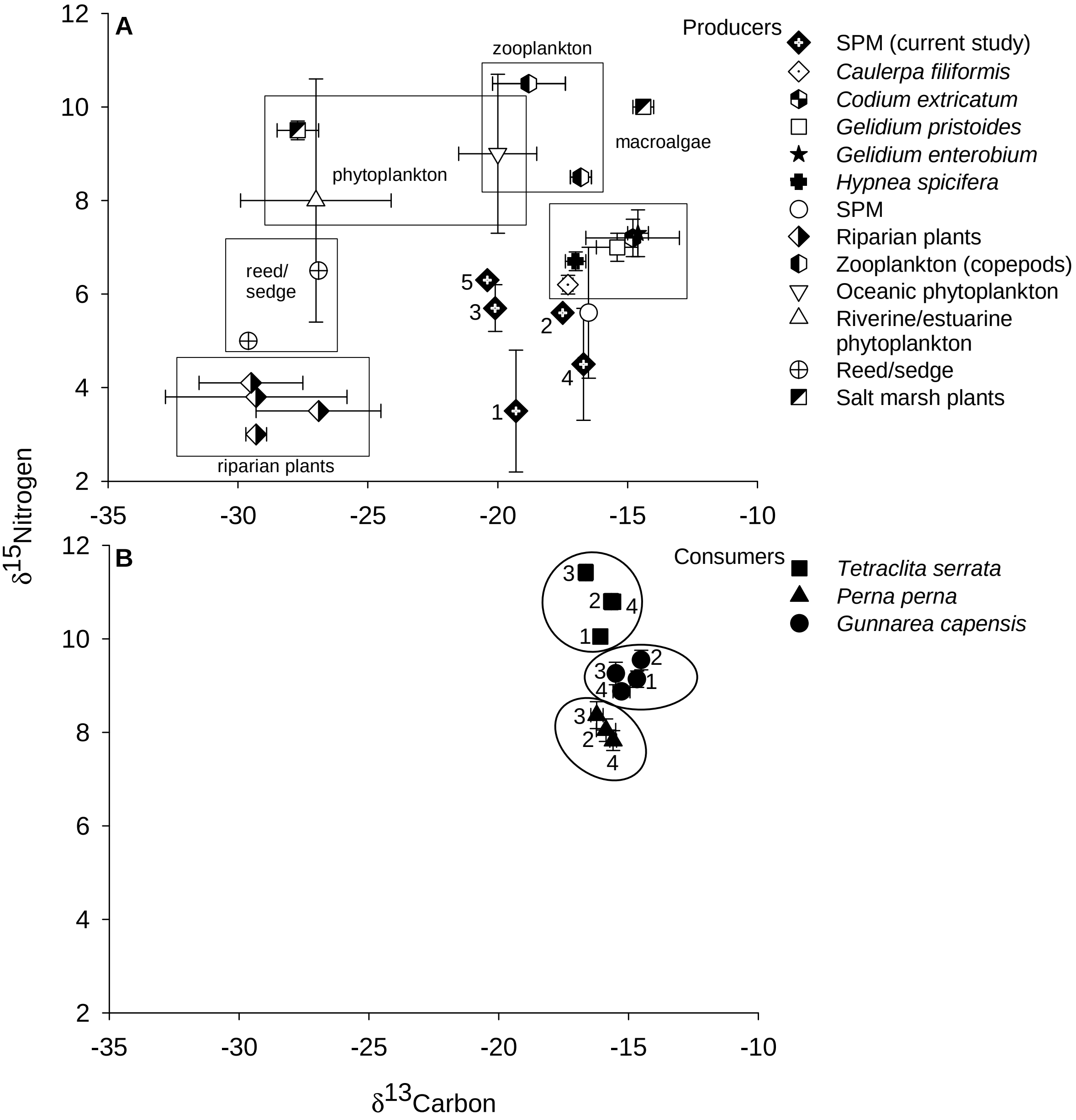

Figure 3.3 δ^{13}C and δ^{15}N signatures (‰) of (A) potential carbon sources and SPM, and (B) the barnacle *Tetraclita serrata*, mussel *Perna perna*, and polychaete *Gunnarea capensis* along the rocky shore environment in the Great Fish region during March 2009) (n = 2 for barnacles at Sites 3 and 4 for carbon, and mussels at Site 3 for carbon; n = 3 for all other sites). Significant variations among the groups are indicated by ellipses (Kruskal-Wallis ANOVA ranked sum comparisons). Values are presented by means ± standard deviation. Data on producers, zooplankton and SPM in the Great Fish region originate from Vorwerk & Froneman (2009) and Allan et al. (2010). Phytoplankton data originate from Fry & Sherr (1984) and Cloern et al. (2002). Site 1, most northern; Site 2, just north of mouth; Site 3, just south of mouth; Site 4, most southern; Site 5, mouth. Rapid tidal shift resulted in no mussel collection at Site 1 for March.

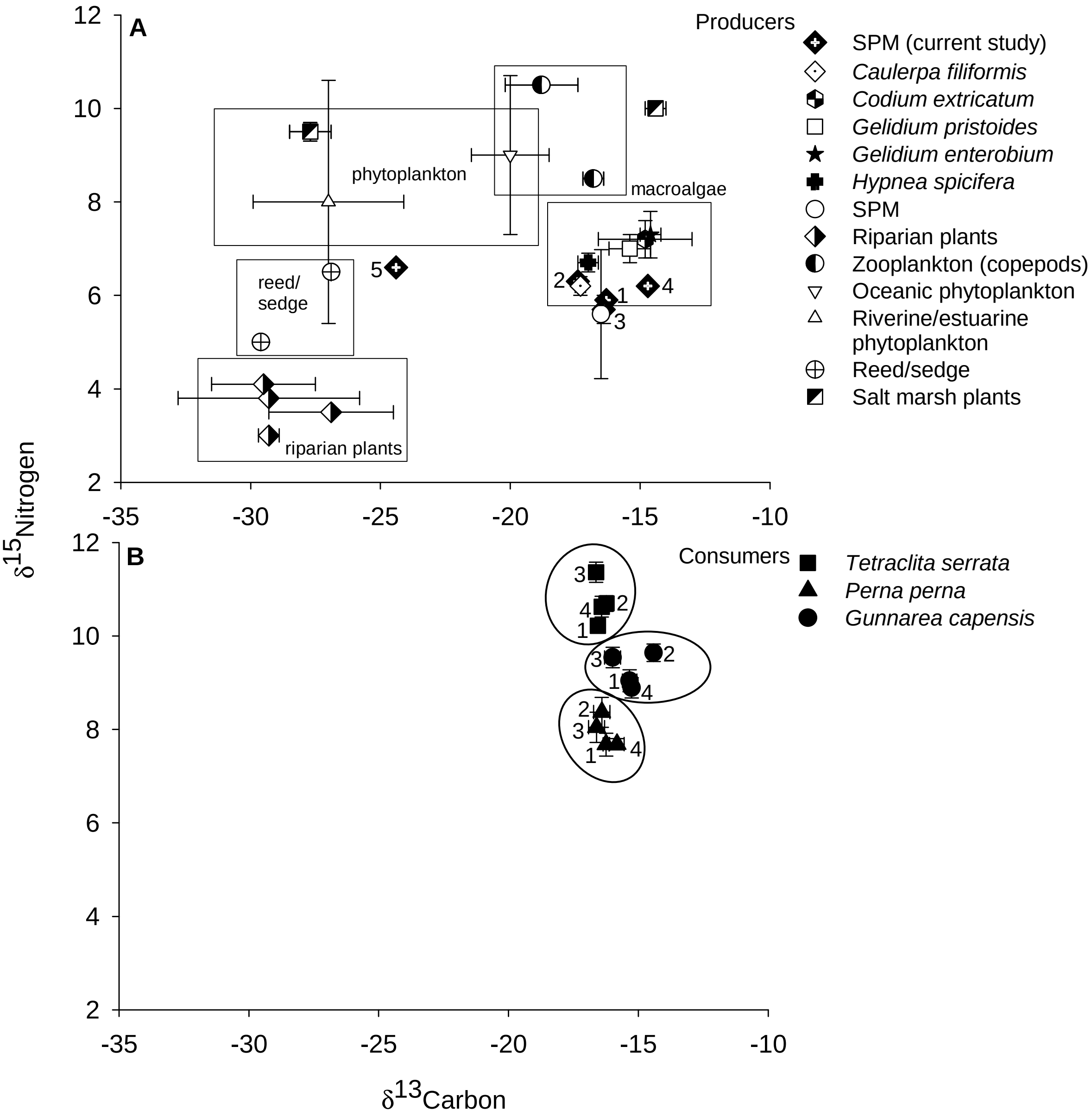

Figure 3.4 δ^{13}C and δ^{15}N signatures (‰) of (A) potential carbon sources and SPM, and (B) the barnacle *Tetraclita serrata*, mussel *Perna perna*, and polychaete *Gunnarea capensis* along the rocky shore environment in the Great Fish region during July 2009) (*n* = 3 for all sites). Significant variations between the groups are indicated by ellipses (Kruskal-Wallis ANOVA ranked sum comparisons). Values are presented as means ± standard deviation. Data on producers, zooplankton and SPM in the Great Fish region originate from Vorwerk & Froneman (2009) and Allan et al. (2010). Phytoplankton data originate from Fry & Sherr (1984) and Cloern et al. (2002). Site 1, most northern; Site 2, just north of mouth; Site 3, just south of mouth; Site 4, most southern; Site 5, mouth.

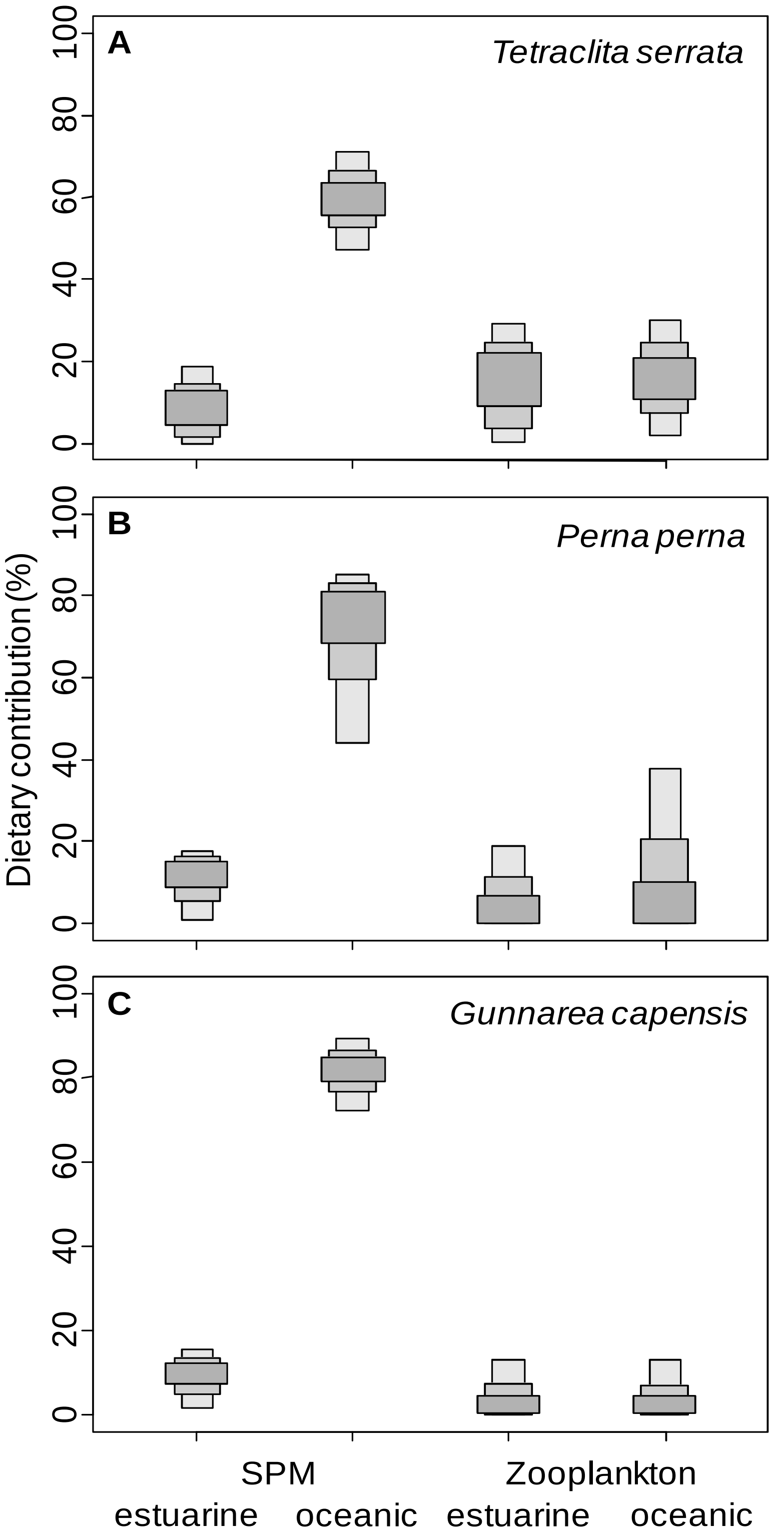

Figure 3.5 Dietary contribution (%) of potential suspended particulate matter (SPM) and zooplankton food sources for the barnacle *Tetraclita serrata*, mussel *Perna perna*, and polychaete *Gunnarea capensis* along the rocky shore environment in the Kariega region during March 2009. Results determined by a SIAR mixing model using carbon and nitrogen isotopic signatures of consumer tissues. Contribution of each food source to consumer diet is indicated by 95, 75, and 50 % credibility intervals.

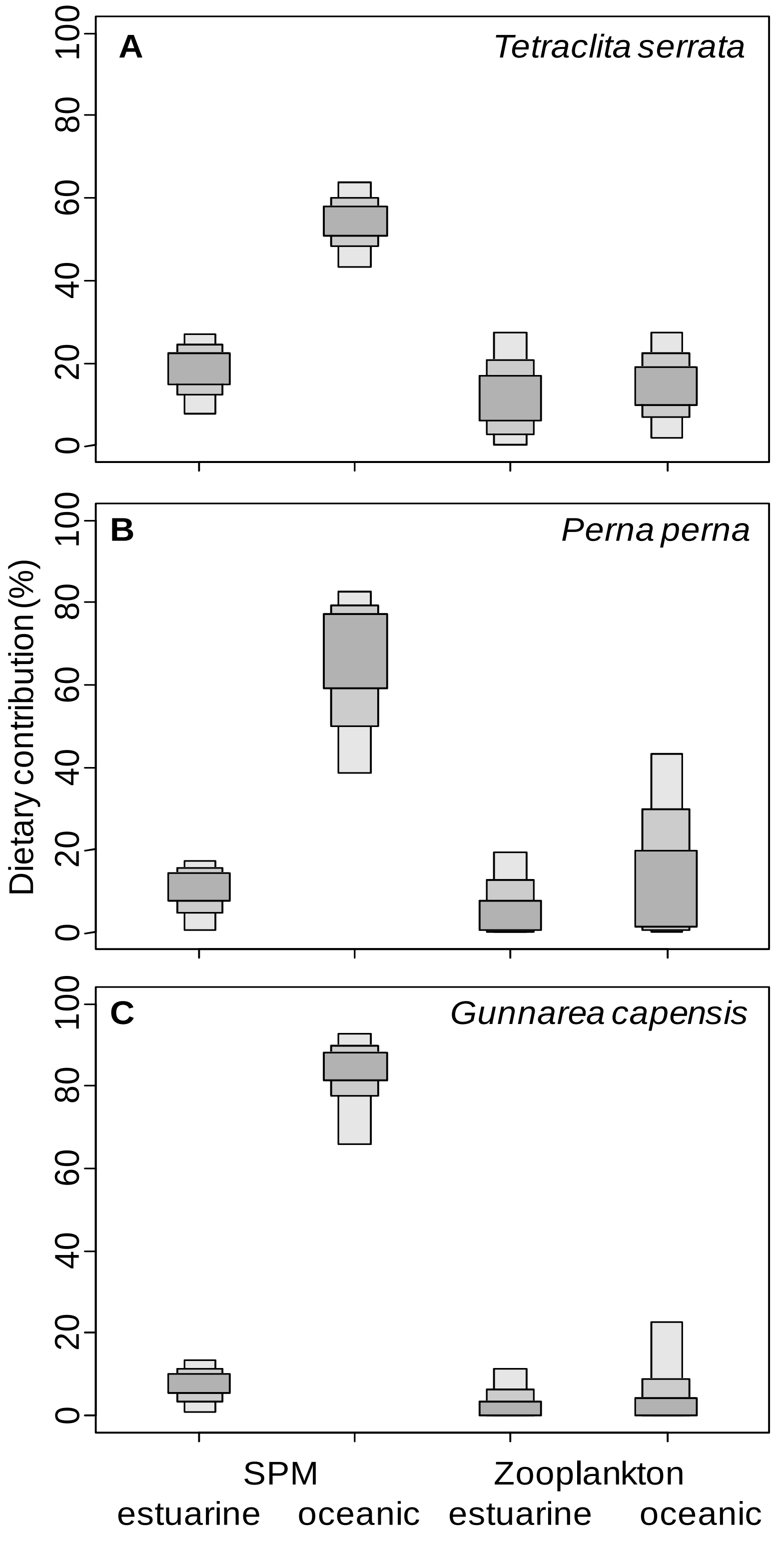

Figure 3.6 Dietary contribution (%) of potential suspended particulate matter (SPM) and zooplankton food sources for the barnacle *Tetraclita serrata*, mussel *Perna perna*, and polychaete *Gunnarea capensis* along the rocky shore environment in the Kariega region during July 2009. Results determined by a SIAR mixing model using carbon and nitrogen isotopic signatures of consumer tissues. Contribution of each food source to consumer diet is indicated by 95, 75, and 50 % credibility intervals.

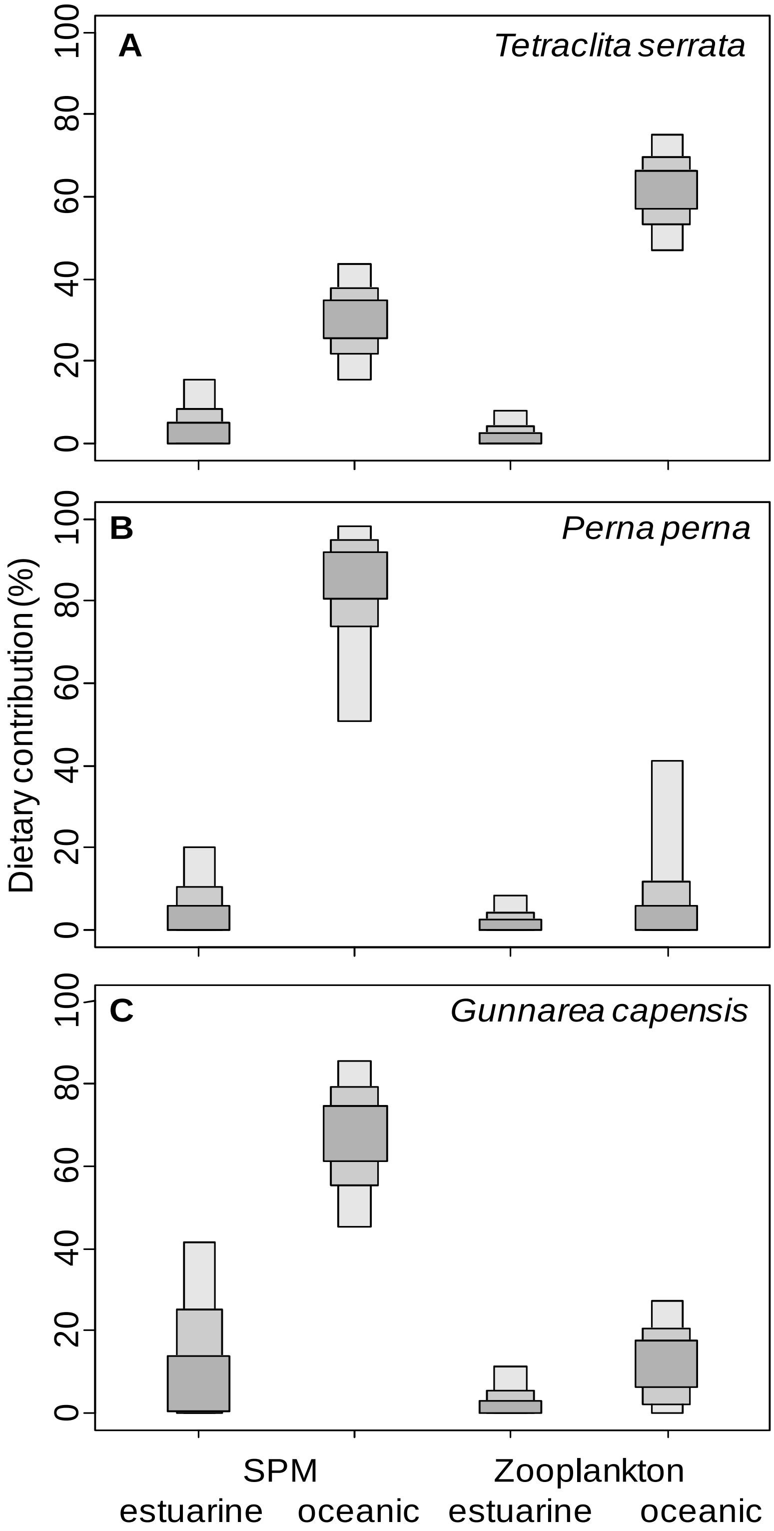

Figure 3.7 Dietary contribution (%) of potential suspended particulate matter (SPM) and zooplankton food sources for the barnacle *Tetraclita serrata*, mussel *Perna perna*, and polychaete *Gunnarea capensis* along the rocky shore environment in the Great Fish region during March 2009. Results determined by a SIAR mixing model using carbon and nitrogen isotopic signatures of consumer tissues. Contribution of each food source to consumer diet is indicated by 95, 75, and 50 % credibility intervals.

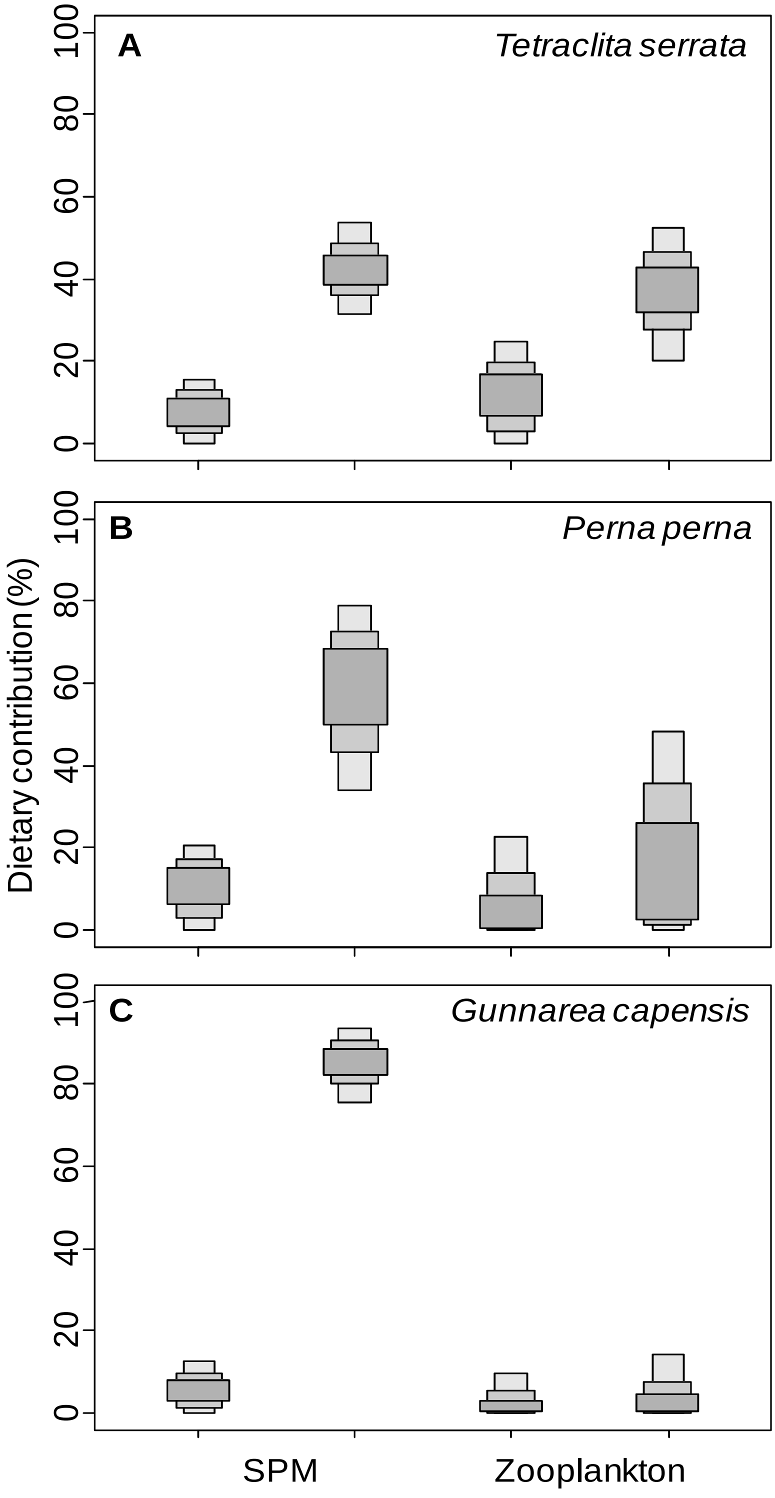

Figure 3.8 Dietary contribution (%) of potential suspended particulate matter (SPM) and zooplankton food sources for the barnacle *Tetraclita serrata*, mussel *Perna perna*, and polychaete *Gunnarea capensis* along the rocky shore environment in the Great Fish region during July 2009. Results determined by a SIAR mixing model using carbon and nitrogen isotopic signatures of consumer tissues. Contribution of each food source to consumer diet is indicated by 95, 75, and 50 % credibility intervals.

3.4 Discussion

3.4.1 $\delta^{13}C$ and $\delta^{15}N$ signatures of suspended particulate matter

The isotopic signatures of the suspended particulate matter (SPM) showed significant regional variability for carbon and distinct temporal changes for nitrogen in the Kariega and Great Fish regions (Table 3.1; Figures 3.1 to 3.4). Distinct regional variation in March and July δ^{13}C signatures clearly reflected the dynamic nature of SPM in coastal waters due to an array of environmental factors (i.e. temperature and nutrient status), and the interplay between estuarine outflows and the adjoining marine ecosystem (Vizzini & Mazzola, 2006; Lefebvre et al., 2009). The δ^{13}C and δ^{15}N signatures of the SPM recorded during this study were similar to those recorded in previous studies in the Kariega (-15.0 to -13.0 ‰) and Great Fish regions (-16.0 to -13.0 ‰; Hill et al., 2006; Allan et al., 2010). River discharge can play an important role in influencing the availability of food sources and the amount of decomposition in coastal waters (Berg & Newell, 1986). More enriched SPM signatures in the Kariega region compared to the Great Fish region were observed during this investigation (Tables 3.1 and 3.2). The Kariega region, with its low freshwater inputs and long water residence time (~2 weeks; Jennings, 2005), is possibly predisposed to greater bacterial degradation of its salt marsh plants and macrophytes. Aquatic vegetation that has been subject to substantial decomposition may contribute to a greater variability in carbon sources and more enriched isotopic signatures, as the carbon-pool could comprise an array of fresh plant fragments, detritus and bacterial sources (Brewer, 1994).

The only significant spatial trend was the highly depleted δ^{13}C signals recorded at the estuarine site in the Kariega region in both months and in the Great Fish region in July (Figures 3.1, 3.2 and 3.4). The carbon values in the SPM at the estuarine site in the Kariega Estuary fell within the range of published literature on isotopic data for the depleted riverine phytoplankton and the riparian vegetation (Paterson & Whitfield, 1998; Cloern et al., 2002; Vorwerk & Froneman, 2009). The lack of a temporal distinction at the estuarine site possibly reflects the general homogeneity in river flows of the Kariega Estuary throughout the year (Grange & Allanson, 1995).

The highly depleted δ^{13}C values at the estuarine site in the lower reaches of the Great Fish Estuary closely reflect those recorded in the riparian vegetation, riverine phytoplankton and reeds or sedges (-29 to -25 ‰; Figure 3.4). However, due to the low retention time of freshwater within the estuary (<1 day; Jennings, 2005), it has been suggested that the δ^{13}C-signal rather reflects the riverine or estuarine phytoplankton (-30.0 to -24.0 ‰; Figure 3.4) transported via the Orange River Basin transfer-scheme (Vorwerk & Froneman, 2009). The

SPM at the estuarine site in the Great Fish Estuary showed temporal variability, and along with the southern sites in March, had δ^{13}C signatures more representative of temperate marine phytoplankton (-24.0 to -18.0 ‰; Figure 3.3). As result of the large plume that is formed in the adjacent marine environment of the Great Fish Estuary (Grange et al., 2000), intense mixing of riverine and marine waters would occur. This would cause a dilution effect of the estuarine water by the nearshore marine waters (Frances & Guerrero, 2008) and thereby possibly allow a marine phytoplanktonic signal to be observed at the estuarine and southern sites in March. Hill et al. (2006) recorded a spatial pattern in carbon-depletion from nearshore to offshore waters. The signatures found within the estuarine and southern sites in the Great Fish region during this study are thus likely representative of the marine phytoplankton up to four kilometres from the coastline.

3.4.2 δ^{13}C and δ^{15}N signatures of consumers

The distinct temporal and regional changes observed in the δ^{13}C and δ^{15}N signatures in the barnacles, mussels and polychaetes reflected the variations observed in the SPM and highlight the diverse nature of water column particulates in providing food to consumers (Table 3.7; Figures 3.1 to 3.4). All three suspension-feeders showed ^{13}C-enriched signals relative to the riparian vegetation, salt marsh plants (*C. diffusa* and *S. perennis*), phytoplankton, reeds and sedges, and ^{13}C-depleted values compared to the seagrass (Tables 3.2 and 3.3; Figures 3.1 to 3.4). Since all three consumers had enriched carbon values compared to the terrestrial plants in the Kariega and Great Fish regions, terrestrial material appears to be a limited source of nutrition for the suspension-feeders. McLeod & Wing (2009) also recorded limited inclusion of terrestrial organic matter (TOM) into filter-feeder diets in a coastal New Zealand fjord, since these invertebrates consistently showed enriched isotopic signatures relative to the TOM and a higher reliance on marine algae.

Changes in consumer diet across space and time can be indicated by the δ^{13}C-range in the tissues of consumers (Schaal et al., 2010). The suspension-feeding invertebrates showed slightly enriched δ^{13}C signatures in the Kariega region compared to the Great Fish region, possibly reflecting greater variability in the SPM signatures and increased contribution of detrital food sources in the Kariega region (Table 3.2; Figures 3.1 and 3.2; Dubois et al., 2007). Considering the published literature for the primary producers in the Kariega region, consumer carbon signatures were similar to those recorded in the coastal SPM (current study), and identified the benthic algae, macroalgae *G. pristoides*, salt marsh plant *S. virginicus* and zooplankton as potential sources (Table 3.2; Figures 3.1 and 3.2). The importance of macroalgal detritus as major food source to rocky shore suspension-feeders is well recognised (McLeod & Wing, 2009; Riera et al., 2009; Schaal et al., 2010). When considering the proportion of SPM relative to zooplankton potential food sources, SIAR identified that oceanic SPM contributed most to the diets of all three suspension-feeders during March and July in the Kariega region (Figures 3.5 and 3.6).

Slightly depleted consumer $\delta^{13}C$ signatures in the Great Fish region, relative to the Kariega region, could signify greater contributions of phytoplankton and terrestrial sources to the diets of the suspension-feeders (Table 3.3; Figures 3.3 and 3.4). Vorwerk (2006) also noted the importance of riverine phytoplankton in the diets of suspension-feeders in the Great Fish region due to the high freshwater outflows causing a mixture of POM in the coastal environment from estuarine and marine origins. River hydrology can influence the $\delta^{13}C$ and $\delta^{15}N$ signatures of consumers through the amount of TOM and the increase of bacterial decomposition of algae into nutrient-rich detritus (Vizzini & Mazzola, 2006; Marín Leal et al., 2008). High river flows are typically associated with elevated terrestrial fragments and thus more depleted $\delta^{13}C$ signals in the consumers that utilise TOM for nutrition. Conversely, low freshwater flows can lead to enriched $\delta^{15}N$ signals due to an increase in algal degradation by heterotrophic bacteria (Marín Leal et al., 2008; Schaal et al., 2009). The slightly depleted carbon values in the suspension-feeders in the Great Fish region during July, when reduced flows were recorded, can possibly be attributed to an elevated utilisation of TOM due to an increase in bacterial decomposition of the vegetative materials (Figure 3.4). Although ^{13}C-depleted values closely reflecting oceanic phytoplankton were recorded in the SPM at the estuarine and southern sites in the Great Fish region in March, the $\delta^{13}C$ signatures in all three suspension-feeders remained enriched relative to the SPM, thus illustrating that these consumers assimilated a variety of sources within the water column (Figure 3.3). SIAR also identified that the particles within the oceanic SPM made up the bulk of the dietary contribution to consumer diets in the Great Fish region (Figures 3.7 and 3.8). The temporal hypothesis predicting distinct differences in isotopic signatures through time was supported in the Kariega and Great Fish regions in the barnacles, whose $\delta^{13}C$ signatures were most depleted during July. The 2009 data supported the regional hypothesis stating that intraspecific changes occur in carbon signatures between the Kariega and Great Fish regions, since the mussels and polychaetes in the Great Fish region had more depleted signatures than those recorded in the Kariega region.

Interspecific variation in isotopic signatures has been recorded among suspension-feeding mussels (*Mytilus edulis*), barnacles (*Elminius modestus*) and polychaetes

(*Pomatoceros lamarcki*) in an oyster culture ecosystem in the Bay of Veys, and identified that co-occurring consumers showed distinct signatures within the same trophic guild as result of diverse feeding mechanisms and the assimilation of similar food sources in different proportions (Dubois et al., 2007). Intraspecific variation in the isotopic signatures of the suspension-feeders was small in March and July, which suggests the dominance of mixed-diets in the Kariega and Great Fish regions (Table 3.6). Schaal et al. (2010) examined an intertidal rocky shore food web in the Bay of Brest (France), and recorded unchanged isotopic signatures in consumers (i.e. suspension-feeding crustaceans, bivalves and annelids) despite the seasonal variation in dominant food sources. These authors attributed the seasonal stability in isotopic signatures of the animals to the presence of an array of food sources and the ability of the consumers to maintain mixed-diets (Schaal et al., 2010). In my study, the range in $\delta^{13}C$ values in all three suspension-feeders (-16.5 to -14.4 ‰) was narrow, indicating that these invertebrates probably consume similar food sources. However, the barnacles had the most depleted carbon signatures, the mussels intermediate values and the polychaetes the most enriched (Table 3.6; Figures 3.1 to 3.4). The significant carbon enrichment observed in the barnacles from the estuarine site compared to the coastal sites in the Kariega region in March is possibly due to an increased contribution of estuarine detritus and microphytobenthos to their diets (Figure 3.2). Large contributions of small crustaceans (i.e. copepods) have been recorded in the gut contents of barnacles (~18 %; Hunt & Alexander, 1991). The carbon signatures of the barnacles in both regions during July closely reflected that of the zooplankton, and probably highlight substantial inputs of zooplankton in their diet (Figures 3.2 and 3.4). Zooplankton densities peak during winter in the Kariega (~3788 ind.m^{-3}) and Great Fish regions (~4860 ind.m^{-3}) mainly as result of the copepods *Calanus agulhensis* and *C. simillimus* and their nauplii (Vorwerk, 2006). SIAR also identified that oceanic zooplankton contributed substantially to the diet of barnacles compared to the mussels or polychaetes (Figures 3.5 to 3.8). Barnacles therefore appear to demonstrate generalist feeding behaviour due to their non-selective assimilation of plant and animal sources (Dubois et al., 2007; Riisgard & Larsen, 2010). Mussels, however, are able to feed selectively via mucous or ciliary processes on a wide array of particulates ranging from detritus and microflagellates to phyto- and mesozooplankton (Schleyer, 1981; Davenport et al., 2000; Espinosa et al., 2008; Lefebvre et al., 2009). Phytoplankton and microphytobenthos have been identified as the major food sources for intertidal bivalves, although the relative contribution of these may vary depending on the time of year or location (Riera et al., 1999; Page & Lastra, 2003; Marín Leal et al., 2008). The intermediate $\delta^{13}C$

signatures recorded in the mussels relative to the barnacles and polychaetes during the current study probably arise from the combined assimilation of phytoplankton, TOM, macroalgal detritus and microphytobenthos. Results from SIAR indicated a consistently large contribution of oceanic SPM to mussel diets with smaller inputs from oceanic zooplankton, particularly during July in both regions, which may be responsible for maintaining their intermediate δ^{13}C signatures (Figures 3.5b to 3.8b).

The consistently more δ^{13}C-enriched values in the polychaetes relative to the other consumers can be ascribed to their preference for smaller food particles such as microalgae, detritus and flagellates (2-12 µm), and the entrapment of a variety of particles amongst their tubes (i.e. sediments, microphytobenthos and macroalgal detritus; Table 3.6; Davies et al., 1989; Phillips & Pernet, 1996; Lefebvre et al., 2009). The higher abundance of enriched organic particles in close association with the polychaetes could therefore increase the assimilation of these particles in their diet (Figures 3.2 to 3.4; Lefebvre et al., 2009), leading to more enriched signatures than the barnacles and mussels. The results for this study therefore opposed the interspecific variation hypothesis for the δ^{13}C signatures that suggested no changes in isotopic signatures among species, as the barnacles, mussels and polychaetes in general showed depleted, intermediate, and enriched values, respectively.

The distinct δ^{15}N-ranges observed among suspension-feeding invertebrates can identify the degree of omnivory and the likelihood of resource partitioning within the trophic group (Grall et al., 2006; Schaal et al, 2010; Schaal et al., 2011). No significant spatial or temporal differences were recorded in the δ^{15}N values for all three suspension-feeders (Table 3.8). The barnacles, however, showed the most enriched δ^{15}N signatures relative to the other suspension-feeders in both regions in March and July (Table 3.6). Schaal et al. (2010) recorded similar isotopic differences in intertidal rocky shore barnacles (~10.9 ‰) and mussels (~8.0 ‰) in the Bay of Brest, and attributed the enrichment in barnacles to an increased assimilation of zooplankton in their diet. SIAR also showed that zooplankton made significant contributions to barnacle diet, particularly in the Great Fish region (Figures 3.7a and 3.8a). This may result from higher densities of copepods in the Great Fish region compared to the Kariega region (Vorwerk, 2006). Suspension-feeding bivalves can preferentially select microalgae with ^{15}N-depleted values, such as flagellates over diatoms, which are then reflected in the consumer tissues (Bougrier et al., 1997). Barnacles therefore showed a higher degree of omnivory than mussels on the Eastern Cape rocky shore. The intermediate nitrogen values recorded in the polychaetes probably reflect their feeding in close proximity to the trapped organic particulates amongst their tubes, resulting in contributions of both water column (i.e. phytoplankton) and sediment food sources to their diet (i.e. microphytobenthos and macroalgal detritus; Phillips & Pernet, 1996; Lefebvre et al., 2009). Results from SIAR reflect polychaete selection of water column particulates of plant origin, as they demonstrated very limited consumption of zooplankton (Figures 3.5c to 3.8c). In addition, ^{15}N-enriched signatures in the barnacle and polychaete tissues relative to the mussels may have resulted from the consumption of macroalgae with enriched nitrogen values (i.e. *Ulva* spp.) or highly decomposed microalgal detritus (Alfaro, 2009; Dubois et al., 2007). Lefebvre et al. (2009) attributed nitrogen isotopic differences among benthic suspension-feeders from an estuarine and marine environment along the coast of Normandy to variations in their diet. Because there are significant distinctions in nitrogen among the three suspension-feeders, the interspecific variation hypothesis for the δ^{15}N signatures predicting no isotopic differences would occur among the barnacles, mussels and polychaetes was not supported.

The contrasting nature of the Kariega and Great Fish regions (i.e. hydrology and vegetation) contributes to regional and seasonal diversity in food sources and the amount of particulates outwelling from each estuary (Vorwerk, 2006; Vorwerk & Froneman, 2009). In the Kariega and Great Fish regions, the suspension-feeders located at the southern sites were characterised by slightly depleted δ^{13}C values during both months, which could mean increased contributions of terrestrial sources to consumer diets (Table 3.4). However, the lack of significant small-scale spatial trends in the isotopic signatures of the barnacles, mussels, and polychaetes suggests that the suspension-feeding invertebrates exhibit enhanced trophic plasticity and maintain mixed-diets depending on the region and seasonal contributions of the available food sources (Machas et al., 2003). As no significant differences were noted in the isotopic signatures between northern and southern populations, the data collected in 2009 did not support the hypothesis that predicted southern-located populations of barnacles, mussels and polychaetes show a higher dependence on terrestrially-derived carbon.

3.5 Summary

The δ^{13}C signatures in the barnacles, mussels and polychaetes changed regionally and temporally in this study of the Kariega and Great Fish regions, although no such differences were noticed for the δ^{15}N values. As a result of differences in vegetation and freshwater outflow to the marine environment within the Kariega and Great Fish Estuary systems, consumers in the Kariega region had slightly enriched δ^{13}C values relative to the Great Fish region, possibly due to a higher incidence of microbial degradation of benthic microalgae and macroalgal detritus. The Great Fish region is characterised by more terrestrial influences, and substantial riverine phytoplankton inputs can potentially contribute to consumer diets (reflected in the slightly depleted $\delta^{13}C$ values in the consumers compared to the Kariega region). The 2009 data supported the regional hypothesis predicting intraspecific changes in carbon signatures between the Kariega and Great Fish regions, since the suspension-feeders in the Great Fish region had more depleted signatures than those recorded in the Kariega region. No clear trend was observed between sample dates in suspension-feeder isotopic signatures in both regions for the mussels and polychaetes. However, the temporal hypothesis predicting differences in isotopic signatures through time was supported in the Kariega and Great Fish regions by the barnacles, whose $\delta^{13}C$ signatures were more depleted during July. Lower river flows in winter could have resulted in increased bacterial decomposition of TOM in the Great Fish region, therefore increasing the assimilation of TOM in suspension-feeder diets.

The barnacle *Tetraclita serrata*, mussel *Perna perna*, and polychaete *Gunnarea capensis* are all co-occurring suspension-feeders, presumably consuming similar food sources. The proportional contribution of various food sources to suspension-feeder diets, however, can vary over space and time, leading to distinct isotopic signatures in consumer tissues. Barnacles do not feed selectively and the size of the particles consumed is largely a function of body size (Turner et al., 2001). Barnacles are therefore not able to preferentially consume the more enriched organic materials from the water column (Dubois et al., 2007; Riisgard & Larsen, 2010). In contrast, mussels display selective feeding and have the ability to accept particles of known size and character (Schleyer, 1981). Mussels can consume zooplankton, although they are known to preferentially assimilate phytoplankton, microphytobenthos and macroalgal detritus (Riera et al., 1999; Page & Lustra, 2003). As zooplankton forms a substantial contribution to the diets of barnacles, it is plausibly an important factor responsible for their enriched nitrogen values. In comparison, the depleted $\delta^{15}N$ signatures recorded in the mussel, *P. perna*, possibly reflect their stronger preference for autotrophic food. The barnacle, *T. serrata*, can thus be described as a generalist feeder with a higher incidence of omnivory compared to the mussel *P. perna* and polychaete *G. capensis*. The suspension-feeding polychaete, *G. capensis*, also feeds selectively and prefers smaller particles such as microalgae, flagellates and detritus (reflected by their enriched $\delta^{13}C$ values). However, their physical habitat also plays a role in providing easy access to enriched organic particles that become trapped amongst their tubes. The results for this study therefore opposed the interspecific variation hypothesis on the $\delta^{13}C$ signatures that predicted no changes in isotopic signatures among species, as the barnacles, mussels and polychaetes in general showed depleted, intermediate, and enriched values, respectively. In addition, the interspecific variation hypothesis for the $\delta^{15}N$ signatures predicting that no isotopic differences would occur among the consumers was not supported.

Suspension-feeding invertebrate populations situated downstream (i.e. south) of the estuary mouth showed somewhat depleted carbon signals in both regions, which could imply an elevated contribution of terrestrial carbon in their diets relative to those populations situated upstream (i.e. north). Even so, this pattern along with other local spatial trends was not significant, so my data did not support the hypothesis that barnacles, mussels and polychaetes situated south of the estuary mouth show a greater dependence on terrestrially-derived carbon. The contrasting nature of the Kariega and Great Fish regions appear to contribute to the regional and temporal diversity of food sources and the amount of particulates outwelling from each estuary. However, the distinct changes in isotopic signatures in the suspension-feeders through time in both regions appear to result from seasonal changes in food sources, species-specific feeding mechanisms and food partitioning among the invertebrates.

CHAPTER 4

SUMMARY DISCUSSION

Coastal transition zones (CTZs) form important interlinking regions where marine ecosystems, rivers and estuarine environments significantly influence each other (Levin et al., 2001; Moberg & Rönnbäck, 2003). Coastal rocky shores are key habitats that sustain a variety of primary producers and invertebrates within the CTZ (Peshcak, 2005). Due to the dynamic nature of coastal ecosystems, suspension-feeders on rocky shores can be influenced by an array of locally-produced (i.e. nearshore marine phytoplankton and coastal macroalgae) and imported food sources (i.e. riverine or estuarine phytoplankton and detritus outwelling; Kang et al., 2006). Estuaries are regarded as productive systems, and the export of organic particles can significantly contribute to the diets of adjacent rocky shore suspension-feeders (Dame & Allen, 1996; Perissinotto et al., 2003; Vorwerk & Froneman, 2009). Increasing human impacts on South African rivers and estuaries and the regional and seasonal changes in hydrodynamics and vegetation composition, can influence water seston characteristics (Peschak, 2005). Investigation into the potential food sources for suspension-feeders in the nearshore marine habitat bordering estuaries is therefore essential. The key aim of this study was to assess the spatial and temporal changes in the diets of the indigenous coastal suspension-feeding barnacle *Tetraclita serrata*, mussel *Perna perna* and polychaete *Gunnarea capensis*. Fatty acid profiles and stable isotope ratios were used to examine the intra- and inter-specific changes in the signatures of these filter-feeders in the adjacent marine environment of the freshwater-restricted Kariega and freshwater-dominated Great Fish Estuaries during March and July 2009.

Distinct temporal changes in the suspended particulate matter (SPM) fatty acid and stable isotope profiles were observed in the Great Fish region, whereas there were few such patterns evident in the Kariega region. The observed spatial pattern appears to be related to differences in the vegetation and hydrology between the two systems. The Kariega Estuary receives consistently low riverine inputs, and subsequently the degree of freshwater outflow to the marine environment remains low throughout the year (Grange et al., 2000). SPM in the Kariega region showed slightly enriched carbon values compared to the Great Fish region and large contributions of bacterial fatty acids (BFAs) mainly ascribed to the longer water residence time and substantial bacterial decomposition within the Kariega Estuary (~2 weeks;

Jennings, 2005). The small inputs of higher plant fatty acids are possibly due to the low input of terrestrial plants as result of attenuated freshwater outflow. In addition, the significantly depleted carbon values at the estuarine site in the Kariega region appeared to be largely ascribed to the presence of autochthonous estuarine phytoplankton rather than terrestrial inputs (Appendix 2.1). This is mainly due to the higher levels of the marker 16:1n-7 (2.9-5.0 % TFA) compared to HPFAs (0.0-2.2 % TFA) that were recorded at the estuarine site in March and July (Appendix 2.1). The Great Fish region, in contrast, received equal contributions of riverine phytoplankton (16:1n-7, 3.6 % TFA) and terrestrial inputs (3.6 % TFA) during summer (Appendix 2.1). The significantly depleted carbon signatures at the estuarine site in the Great Fish region during July were associated with the distinctly larger levels of HPFAs (14.3 % TFA) and the phytoplankton marker 16:1n-7 (7.1 % TFA; Appendix 2.1). The Great Fish Estuary is characterised by dynamic changes in seasonal hydrology patterns which, along with greater terrigenous influences and slightly depleted carbon signatures, are likely responsible for significant differences in SPM and total chlorophyll-*a* concentration, and fatty acid and isotopic signatures over time (Grange & Allanson, 1995).

Distinct differences between the Kariega and Great Fish regions were observed in the fatty acid and isotopic signatures in the mussels and polychaetes (Table 3.7; Figures 2.7 and 2.8), which likely reflected regional changes in the SPM detritus and in the contribution of bacterial and phytoplankton sources to their diets. Detritus and bacteria form a large part of the seston within the Kariega Estuary, while allochthonous riverine phytoplankton dominates the seston composition in the Great Fish Estuary (Grange & Allanson, 1995). The outwelling of these particulates from the Kariega and Great Fish Estuaries to the adjoining nearshore environment may therefore likely contribute to consumer diets. A study examining the isotopic signatures in suspension-feeders from two distinct habitats along the northwest coast of France also identified large contributions of detritus and microphytobenthos to the diets of the consumers in the estuarine environment with low river flows, while phytoplankton was the major food source to the filter-feeders in the intertidal rocky shore bordering an estuary with substantial freshwater outflow (Lefebvre et al., 2009). Although carbon isotopes distinguished between the Kariega and Great Fish polychaetes (Table 3.7), fatty acid analyses contrasted the isotopic results and showed no regional differences in the polychaete fatty acid profiles (Table 2.3). Carbon and nitrogen signatures in primary producers and consumers have been documented to vary widely with seasonal or regional changes (Hill & McQuaid, 2008; Allan et al., 2010; Schaal et al., 2010; Lebreton et al., 2011). As a result, δ^{13}C and

δ^{15}N ratios may display a greater sensitivity in fluctuating environments than fatty acid profiles (Allan et al., 2010). The Kariega and Great Fish polychaetes displayed little omnivory and had consistent contributions in their diets of food sources from plant origins (i.e. microalgae and detritus; Figures 2.15 and 2.19). The results from SIAR also showed that polychaetes consumed plant food sources rather than animal nutrients such as zooplankton (Figures 3.5c to 3.8c). The barnacles and mussels displayed a higher degree of omnivory and likely a wider consumption of possible carbon sources than the polychaetes, likely responsible for the significant temporal differences recorded in their carbon isotopic and fatty acid signatures (Tables 2.3 and 3.7). However, the lack of a regional distinction in the polychaete fatty acid profiles could possibly be ascribed to their consistent preference for autotrophs. The large proportions of diatoms (20:5n-3; 15.1-22.2 % TFA) and BFAs (8.7-13.6 % TFA) in polychaete diets in the Kariega and Great Fish regions significantly influenced polychaete signatures (Figure 2.19), and probably prevented a measurable difference between the Kariega and Great Fish fatty acid profiles.

The interspecific trends in the isotopic and fatty acid signatures complemented each other in the identification of potential food sources in the diets of the barnacles, mussels and polychaetes. While stable isotopes were able to provide information on the contributions of potential food sources within each region, fatty acid biomarkers could also identify temporal changes in food source contributions and differentiate among potential food constituents. Due to the slightly enriched carbon signatures in consumers and SPM in the Kariega region, benthic macroalgae, detritus, the salt marsh plant *Spartina maritima* and zooplankton were identified as potentially significant food sources to suspension-feeders (Figures 3.1 and 3.2). The more depleted carbon signatures in the Great Fish region possibly highlight, in addition to benthic macroalgae and the salt marsh plant *Sporobolus virginicus*, an input of allochthonous riverine phytoplankton and terrestrial organic matter (TOM) to consumer diets (Figures 3.3 and 3.4). Distinct spatial changes in the isotopic signatures in consumers from two different areas in a basin along the southern coast of Italy were suggested to result from differences in hydrodynamic regimes, which in turn affected the distribution and transfer of organic matter (Vizzini & Mazzola, 2006). Specifically, the central basin with limited hydrological dispersion had significant vegetative bacterial decomposition leading to more enriched signatures in the suspension-feeders, while the southern basin was exposed to large volumes of water exchange and export of vascular material, limited bacterial decomposition and thus more depleted consumer signatures due to the higher contribution of phytoplankton in their diets (Vizzini & Mazzola, 2006).

Fatty acid profiles identified the potential key food sources for all three filter-feeders during March as phytoplankton (i.e. diatoms), macroalgae and detritus and during July as phytoplankton (i.e. flagellates), zooplankton and detritus. Stable isotopes revealed that benthic macroalgae was a key food source for the suspension-feeders in the Kariega and Great Fish regions during both sampling times (Figures 3.1 to 3.4). Similar to the fatty acid results, which identified the copepod marker 20:1n-9 as influential to SPM proportions during July in both regions (Figure 2.5), stable isotopes showed that zooplankton became important during July (Figures 3.2 and 3.4) and strongly influenced barnacle signatures (Figure 2.19). The SIAR results, however, illustrated that the barnacles in the Great Fish region may potentially rely on zooplankton nutrients during both months (Figures 3.7a and 3.8a). Although isotopic signatures of the consumers remained slightly ^{13}C-enriched compared to the riverine and marine phytoplankton (Figures 3.1 to 3.4), the more depleted carbon values in the Great Fish region compared to the Kariega region possibly highlight a greater contribution of phytoplankton to consumer diets. However, the monounsaturated fatty acid 16:1n-7 illustrated a consistent availability of phytoplankton to the suspension-feeders in both the Kariega and Great Fish regions, while the polyunsaturated fatty acids 20:5n-3 and 22:6n-3 identified a possible shift in the phytoplankton communities when these biomarkers became important in July (Figure 2.4). The significance of phytoplankton as the major food source for suspension-feeders is well documented (Desvilletes et al., 1997; Kharlamenko et al., 2001), and the presence of diatoms and dinoflagellates are characteristic of the coastal waters along the eastern shores of South Africa (Machu et al., 2005; Allan et al., 2010).

Both the fatty acid and stable isotope analyses supported the published literature on the species-specific feeding patterns and potential food sources in the barnacles, mussels and polychaetes. Carbon isotopes were able to separate the suspension-feeders into slightly depleted (barnacles; -16.5 to -16.1 ‰), intermediate (mussels; -15.8 to -15.0 ‰) and enriched (polychaetes; -15.0 to -14.4 ‰) consumers, but did not provide conclusive evidence on their preferences for specific phytoplankton. Fatty acid analyses, however, highlighted that barnacles and mussels relied largely on dinoflagellates, whereas polychaetes relied on diatoms (Figure 2.19). Stable isotopes were not able to provide any information on the bacterial sources consumed, since isotopic ratios of bacteria generally reflect those of their substrate (Lebreton et al., 2011). In contrast, fatty acid markers showed that the three species had consistent BFA levels in March and July, and large proportions of saturated fatty acids with 14 to 18 carbons, suggesting that bacterial and detritus food sources played an important role in their diets (Freites et al., 2002). Suspension-feeding invertebrates consume bacterial food and algal detritus since they provide rich organic nutrition, and may even be important supplementary food sources when phytoplankton becomes limited during winter (Meziane et al., 1997; Lefebvre et al., 2009). It is important to note that although specific marker fatty acids can be used to identify possible food constituents (Dalsgaard et al., 2003), factors such as endogenous fatty acid biosynthesis, lipid metabolism and selective deposition should also be considered when attempting to estimate the importance of primary sources in consumer diets (Iverson et al., 2004; Thiemann et al., 2004). As lipid metabolisation can cause consumer signatures to differ from assimilated food sources, a thorough understanding of species- and tissue-specific lipid metabolisation and deposition is therefore crucial, and requires further investigation.

The degree of omnivory in the suspension-feeders was illustrated by both the fatty acid and stable isotope results. All three filter-feeders demonstrated herbivory in the Kariega and Great Fish regions during March and July since they all had substantial amounts of microalgal markers. Fatty acid analyses identified low levels of 18:2n-6 and 18:3n-3 in the tissues of the barnacles and polychaetes, suggesting that TOM is not an important food source for these suspension-feeders. Isotopic analyses also verified the limited assimilation of TOM in the polychaetes due to their enriched carbon values. Depleted carbon signals in the barnacles could indicate the contribution of TOM to their diets, although insignificant levels of the terrestrial markers (18:2n-6 and 18:3n-3) in their tissues probably indicate the contrary. Suspension-feeders do not generally consume higher plants unless they have undergone substantial microbial degradation (Deegan & Garritt, 1997; Marín Leal et al., 2008; McLeod & Wing, 2009). The intermediate carbon values recorded in the mussels possibly reflected fairly equal contributions of enriched and depleted food sources in their diets (Espinosa et al., 2008; Kharlamenko et al., 2001). In contrast to the barnacles and polychaetes, the mussels had significant proportions of terrestrial markers (>2.5 %) in their tissues, thus identifying TOM as a food source for this suspension-feeder. The results from the current investigation therefore highlighted that rocky shore suspension-feeders probably maintain trophic plasticity as result of distinct changes in food delivery between the Kariega and Great Fish regions during summer and winter.

Barnacles can consume large amounts of zooplankton (i.e. copepods; Hunt & Alexander, 1991; Turner et al., 2001). The extent of omnivory by the barnacles was evident by the presence of the copepod marker 20:1n-11, high omnivory ratios (18:1n-9/18:1n-7; ~2.1), significantly enriched nitrogen signatures (~10.6 ‰) and substantial dietary contributions of oceanic zooplankton (mean = 10-61 %, SIAR) in both the Kariega and Great Fish regions. Moderate contributions of the copepod marker 20:1n-11, intermediate omnivory ratios (~1.3) and dietary contributions of oceanic zooplankton (mean = 10-15 %, SIAR) identified zooplankton as a food source for the mussels. The barnacles and mussels showed the most elevated omnivory ratios (1.0 to 4.8) during winter in the Kariega and Great Fish regions, which also coincided with the greatest abundances of zooplankton in the two regions (Vorwerk, 2006). However, although mussels are known to ingest zooplankton and can be omnivorous feeders, their depleted nitrogen signatures (~7.9 ‰) relative to the barnacles and polychaetes possibly suggest a stronger preference for autotrophic food (Davenport et al., 2000). In particular, the markers 16:1n-7 and 18:1n-7 were identified to significantly influence mussels fatty acid signatures during both months (Figure 2.19). Polychaetes mainly consumed plant-based food sources (i.e. microalgae, macroalgae and detritus; high levels of *i*-18:0, 18:1n-9, 18:4n-3 and 20:5n-3; Figure 2.19). Evidence in support of this was apparent from small 20:1n-11 levels, low carnivory ratios (~0.4), and enriched carbon signatures (~9.1 ‰) relative to the barnacles and mussels, and small dietary contributions of zooplankton (mean = <10 %, SIAR). Studies examining resource partitioning among suspension-feeding invertebrates suggested that due to their species-specific feeding mechanisms, including the non-selective feeding of barnacles and the ability to preferentially select particles by mussels and polychaetes, distinct differences in fatty acid and isotopic signatures can result among co-occurring consumers (Machas et al., 2003; Grall et al., 2006; Vizzini & Mazzola, 2006). The data obtained during this investigation are thus consistent with the published literature.

Fatty acid and isotopic data on the consumers did not support one another regarding differences in diet from the north to south in each region. Although southern-located animals did show slightly depleted carbon signals compared to those in the north in both regions, possibly suggesting an increased input of TOM in their diets, stable isotopes did not significantly delineate between northern and southern suspension-feeders. In contrast, invertebrate fatty acid signatures clearly distinguished northern from southern barnacles, mussels and polychaetes in the Kariega region during March (Figures 2.16 to 2.18). Phytoplankton assemblages appeared to influence this separation in the barnacles and mussels, with elevated contributions of diatoms north of the estuary mouth, and of dinoflagellates in the south. Detritus and BFAs also appeared important in the diets of southern-located animals, with one to five percent greater BFA proportions relative to the north, and especially greater levels of 14:0, 16:0, *i*-16:0 and *i*-18:0 in the polychaetes south of the estuary mouth. Both techniques, therefore, disputed the hypothesis predicting higher dependencies on terrestrially-derived food sources by southern-located animals compared to those located north of the estuary mouth in both regions.

4.1 Final conclusions

This study documents the temporal and spatial changes in the diets of three indigenous suspension-feeders in the nearshore marine coastal habitat of the Eastern Cape coastline of South Africa. The distinct changes in the fatty acid and stable isotope signatures in all three filter-feeders in the Kariega and Great Fish regions are likely due to differences in the regional vegetation and hydrology of the systems, and interspecific resource partitioning among the species. The Kariega Estuary has eelgrasses and salt marsh vegetation and minor terrestrial inputs, whereas the Great Fish Estuary has reeds and sedges along its banks and increased terrestrial plant influences (Grange et al., 2000). River discharge can play an important role in influencing the availability and outwelling of vegetative material from estuaries, and the amount of decomposition of the algal components to detritus (Berg & Newell, 1986). Significant temporal differences in trophic signatures in all three suspension-feeders were observed between the Kariega and Great Fish regions. The Kariega region is possibly predisposed to more bacterial degradation of its salt marsh plants and submerged macrophytes due to low freshwater inputs (Grange & Allanson, 1995; Marín Leal et al., 2008). The suspension-feeders had greater contributions of detritus and BFAs in their tissues and more enriched carbon signatures in the Kariega region, reflecting the substantial contributions of these food sources to their diets. Suspension-feeders in the Great Fish region, however, with slightly more depleted carbon signatures, appear to have a greater input of riverine phytoplankton and terrestrial material in their diets.

The contrasting hydrology and vegetation composition of the Kariega and Great Fish regions are likely important contributing factors in the spatial and temporal variety of carbon sources and the amount of estuarine particulate outwelling (Vorwerk, 2006; Vorwerk & Froneman, 2009). The lack of significant small-scale spatial trends in the fatty acid and isotopic signatures of the barnacles, mussels, and polychaetes suggested that the suspension-feeders had very flexible diets depending on the region and seasonal contributions of available food sources (Machas et al., 2003). The barnacle, *Tetraclita serrata*, appears to be a non-selective generalist feeder utilising phytoplankton, macroalgae, bacteria, detritus and zooplankton (Turner et al., 2001; Dubois et al., 2007; Riisgard & Larsen, 2010). The mussel, *Perna perna*, can selectively consume a wide array of particulates, supported by their intermediate carbon ratios. Although they do consume zooplankton (Espinosa et al., 2008), the mussels showed a stronger preference towards the consumption of autotrophic food and TOM than the barnacles. The polychaete, *Gunnarea capensis*, showed less feeding on heterotrophic sources than the barnacles, and preferred the smaller particulates (i.e. microalgae and detritus). The distinct changes in trophic signatures in the suspension-feeders through time in the Kariega and Great Fish regions therefore appeared to largely result from changes in seasonal vegetative inputs, species-specific feeding mechanisms (i.e. selective *versus* unselective feeding), food partitioning among the invertebrates (i.e. consumption of phytoplankton, detritus, bacteria, TOM and zooplankton in different proportions), and possible distinctions in metabolism (Machas et al., 2003; Iverson et al., 2004; Grall et al., 2006).

Fatty acid and stable isotope trophic markers were successfully applied to determine regional and temporal changes in the diets of rocky shore suspension-feeders adjacent to two contrasting estuaries in the Eastern Cape of South Africa. Many studies have suggested that rocky shore food webs are notably linked to terrestrial habitats (Finlay et al., 2002; Kang et al., 2006; Riera et al., 2009; Schaal et al., 2010). However, the regional and temporal changes in the connections between rivers and the ocean remain poorly resolved. Since coastal rocky shores are influenced by variable hydrodynamic factors that can affect food sources on temporal and spatial scales, further investigation into the processes contributing to the dynamics of local populations, communities and ecosystems, and the factors responsible for the fluxes of material among these environments, is fundamental.

4.2 Future research

Estuaries have a highly diverse nature, represent suitable habitat for a variety of species and are continuously utilised by man (i.e. water abstraction). Within South Africa, numerous studies in the CTZ (i.e. on the ecological impacts of hydrodynamics, biological components, estuarine trophic relationships, and physico-chemical and biological characteristics in the river-estuary interface zone) have aided in providing a good foundation of knowledge for estuaries and their connectivity with the nearshore marine habitat (Berry & Schleyer, 1983; McQuaid & Branch, 1985; Bustamante & Branch, 1996; Froneman, 2001; Vorwerk, 2006; Richoux & Froneman, 2007). Further investigation into this dynamic zone is therefore required to assess the influences of altered river flows on nearby coastal feeding dynamics and to broaden our knowledge on the potential responses of these ecosystems to human impacts.

Follow-up studies within the Kariega and Great Fish regions could assist in broadening our limited knowledge on the feeding dynamics of consumers along the eastern coastline of South Africa. For this investigation, fatty acids were able to identify some of the primary carbon sources while stable isotopes revealed a different degree of omnivory in all three indigenous suspension-feeders. Examining the abundance and transport of potential carbon sources for consumers between estuaries and the ocean is therefore vital. Further research incorporating longer time scales (i.e. annual studies integrating all seasons) and consumers from different trophic levels may thus provide further insight into the food web ecology of these complex coastal ecosystems.

The highly variable nature of SPM in aquatic systems was confirmed by this study. Ongoing research into the dynamics of coastal SPM can provide a better understanding of the factors involved in causing the large variability observed within the SPM from estuarine and marine environments. In addition, follow-up studies assessing the north-south distinctions in isotopic and fatty acid signatures of consumers could possibly elucidate how the flow dynamics along coastlines create changes in available food sources.

A substantial amount of aquatic food web studies have used stable isotopes as a tool to assess trophic relationships and potential food sources to consumers. However, research into species-specific fractionation values for marine suspension-feeders has been limited. Considering the variability in fractionation values within the suspension-feeding trophic guild, further laboratory-based evaluations to assess consumer-diet discrimination values of local species (i.e. the barnacle *T. serrata*, mussel *P. perna* and polychaete *G. capensis*) may be necessary.

Since the barnacle, *T. serrata*, displays greater omnivory compared to the mussels and polychaetes, a thorough study examining the potential food sources of this suspension-feeder could provide further insight on their particle assimilation and position in the food web.

Tube-building rocky shore polychaetes have been identified to obtain food from two distinct ecotones in close association with one another (i.e. benthic sediments and water column; Lefebvre et al., 2009). A study examining the relationship between the feeding mode and physical habitat of the polychaete, *G. capensis* could present information on the degree to which the polychaetes feed from the water column or in close association with the sediments, and whether any specific factors are responsible for influencing their degree of feeding from each ecotone.

Since there is a general trend to focus on single level trophodynamic research, further study into multiple trophic levels would be more useful in evaluating the relationships within aquatic food webs over larger spatial and seasonal scales. Although suspension-feeders comprise the most abundant organisms on rocky shores, other functional groups such as herbivores, detritivores, scavengers and predators have important roles in the food web dynamics. Therefore, by obtaining information on entire rocky shore trophic webs, a more comprehensive representation of the spatial and temporal patterns in potential food sources can be obtained which will allow for objective management of coastal marine habitats.

The EFAs are generally obtained from a consumer's diet. However, various other processes such as nutrient status and physiological state of primary producers and distinctions in consumer metabolism have been identified to influence fatty acid signatures. In particular, reproduction and growth dynamics have been recognised to influence fatty acid signatures in consumers (Richoux et al., 2005; Kang et al., 2006). Studies investigating the ontogenetic and growth dynamics of consumers in conjunction with spatial and temporal questions along the coastal rocky shores of South Africa could therefore be relevant. These studies may highlight the degree at which variation in food sources, compared to species-specific physiological function, influence fatty acid and isotopic signatures in consumers.

REFERENCES

Able, K.W. 2005. A re-examination of fish estuarine dependence: evidence for connectivity between estuarine and ocean habitats. *Estuarine, Coastal and Shelf Science* **64**: 5-17.

Achituv, Y., Brickner, I. & Erez, J. 1997. Stable carbon isotope ratios in Red Sea barnacles (Cirripedia) as an indicator of their food source. *Marine Biology* **130**: 243-247.

Ackman, R.G. & Hooper, S.N. 1973. Long-chain monoethylenic and other fatty acids in heart, liver and blubber lipids of two harbour seals (*Phoca vitulina*) and one grey seal (*Halichoerus grypus*). *Journal of the Fisheries Research Board of Canada* **31**: 333-341.

Ackman, R.G., Sebedio, J-L. & Kovacs, M.I.P. 1980. Role of eicosenoic and docosenoic fatty acids in freshwater and marine lipids. *Marine Chemistry* **9**: 157-164.

Alfaro, A.C. 2006. Evidence of cannibalism and bentho-pelagic coupling within the life-cycle of the mussel, *Perna canaliculus*. *Journal of Experimental Marine Biology and Ecology* **329**: 206-217.

Alfaro, A.C. 2009. Diet of the pulmonate gastropod Onchidella nigricans in the intertidal rocky shore, New Zealand. *Animal Biology* **59**: 231-240.

Alfaro, A.C., Thomas, F., Sergent, L. & Duxbury, M. 2006. Identification of trophic interactions within an estuarine food web (northern New Zealand) using fatty acids biomarkers and stable isotopes. *Estuarine, Coastal and Shelf Science* **70**: 271-286.

Allan, E.L., Ambrose, S.T., Richoux, N.B. & Froneman, P.W. 2010. Determining spatial changes in the diet of nearshore suspension-feeders along the South African coastline: Stable isotope and fatty acid signatures. *Estuarine, Coastal and Shelf Science* **87**: 463-471.

Allanson, B.R. & Read, G.H.L. 1987. The response of estuaries along the south eastern coast of southern Africa to marked variation in freshwater. *Institute for freshwater studies, Rhodes University, Grahamstown, Special Report* No. 2/78. pp 40.

Allanson, B.R. & Read, G.H.L. 1995. Further comment on the response of Eastern Cape Province estuaries to variable freshwater inflows. *South African Journal of Aquatic Science* **21**: 56-70.

Baird, D., Winter, P.E.D., and Wendt, G. 1987. The flux of particulate material through a well-mixed estuary. *Continental Shelf Research* **7**: 1399-1403.

Bate, G.C., Whitfield, A.K., Adams, J.B., Huizinga, P. & Wooldridge, T.H. 2002. The importance of the river-estuary interface (REI) zone in estuaries. *Water SA* **28**(3): 271-280.

Beal, L.M., Chereskin, T.K., Lenn, Y.D. & Elipot, S. 2006. The sources of mixing characteristics of the Agulhas Current. *Journal of Physical Oceanography* **36**: 2060-2074.

Berg, J.A. & Newell, R.I.E. 1986. Temporal and spatial variations in the composition of seston available to the suspension feeder *Crassostrea virginica*. *Estuarine, Coastal and Shelf Science* **23**: 375-386.

Berry, P.F. & Schleyer, M.H. 1983. The brown mussel *Perna perna* on the Natal coast, South Africa: utilization of available food and energy budget. *Marine Ecology Progress Series* **13**: 201-210.

Bodin, N., Le Loc'h, F. & Hily, C. 2007. Effect of lipid removal on carbon and nitrogen stable isotope ratios in crustacean tissues. *Journal of Experimental Marine Biology and Ecology* **341**: 168-175.

Bougrier, S., Hawkins, A.J.S. & Héral, M. 1997. Preingestive selection of different microalgal mixture in *Crassostrea gigas* and *Mytilus edulis*, analysed by low cytometry. *Aqualculture* **150**: 123-124.

Branch, G. & Branch, M. 1983. *The Living Shores of Southern Africa*. C. Struik Publishers, Cape Town. 272 pp.

Branch, F.M., Odendaal, F. & Robinson, T.B. 2010. Competition and facilitation between the alien mussel *Mytilus galloprovincialis* and indigenous species: moderation by wave action. *Journal of Experimental Marine Biology and Ecology* **383**: 65-78.

Brett, M.T. & Müller-Navarra, D. 1997. The role of highly unsaturated fatty acids in aquatic foodweb processes. *Freshwater Biology* **38**: 483-499.

Brewer, R. 1994. *The science of ecology – Second Edition*. Saunders College Publishing, Orlando. 773 pp.

Budge, S.M., Iverson, S.J. & Koopman, H.N. 2006. Studying trophic ecology in marine ecosystems using fatty acids: a primer on analysis and interpretation. *Marine Mammal Science* **22**(4): 759-801.

Budge, S.M. & Parrish, C.C. 1998. Lipid biogeochemistry of plankton, settling matter and sediments in Trinity Bay, Newfoundland. II. Fatty acids. *Organic Geochemistry* **29**:1547-1674.

Budge, S.M., Parrish, C.C. & McKenzie, C.H. 2001. Fatty acid composition of phytoplankton, settling particulate matter and sediments at a sheltered bivalve aquaculture site. *Marine Chemistry* **76**: 285-303.

Bustamante, R.H. 1994. Patterns and causes of intertidal community structure around the coast of southern Africa. Ph.D. thesis, University of Cape Town, South Africa.

Bustamante, R.H. & Branch, G.M. 1996. The dependence of intertidal consumers on kelp-derived organic matter on the west coast of South Africa. *Journal of Experimental Marine Biology and Ecology* **196**: 1-28.

Bustamante, R.H., Branch, G.M., Eekhout, S., Robertson, B., Zoutendyk, P., Schleyer, M., Dye, A., Hanekom, N., Keats, D., Jurd, M. & McQuaid, C. 1995. Gradients of intertidal primary productivity around the coast of South Africa and their relationships with consumer biomass. *Oecologia* **102**: 189-201.

Canuel, E.A. 2001. Relations between river flow, primary production and fatty acid composition of particulate organic matter in San Francisco and Chesapeake Bays: a multivariate approach. *Organic Geochemistry* **32**: 563-583.

Carabel, S., Godínez-Domínguez, E., Verísimo, P., Fernández, L. & Freire, J. 2006. An assessment of sample processing methods for stable isotope analyses of marine food webs. *Journal of Experimental Marine Biology and Ecology* **336**: 254-261.

Chavez, F.P., Barber, R.T., Kosro, P.M., Huyer, A., Ramp, S.R., Stanton, T.P. & De Mendiola, B.R. 1991. Horizontal transport and the distribution of nutrients in the coastal transition zone off northern California: Effects on primary production, phytoplankton biomass and species composition. *Journal of Geophysical Research* **96**(C8): 14,833-14,848.

Cloern, J.E., Canuel, E.A. & Harris, D. 2002. Stable carbon and nitrogen isotope composition of aquatic and terrestrial plants of the San Francisco Estuarine Bay system. *Limnology and Oceanography* **47**: 713-729.

Connolly, R.M., Gorman, D. & Guest, M.A. 2005. Movement of carbon among estuarine habitats and its assimilation by invertebrates. *Oecologia* **144**: 684-691.

Copeman, L.A. & Parrish, C.C. 2003. Marine lipids in a cold coastal ecosystem: Gilbert Bay, Labrador. *Marine Biology* **143**: 1213-1227.

Costanza, R., D'Arge, R., De Groots, R., Farber, S., Grasso, M., Hannon, B., Limburg, K., Naeem, S., O'Neill, R.V., Paruelo, J., Raskin, R.G., Sutton, P. & Van Der Belt, M. 1997. The value of the world's ecosystem services and natural capital. *Nature* **387**: 253-260.

Cravo, A., Madureira, M., Felícia, H., Rita, F. & Bebianno, M.J. 2000. Impact of outflow from the Guadiana River on the distribution of suspended particulate matter and nutrients in the adjacent coastal zone. *Estuarine, Coastal and Shelf Science* **70**: 63-75.

Dalsgaard, J., St. John, M., Kattner, G., Muller-Navarra, D. & Hagen, W. 2003. Fatty acid trophic marker in the pelagic marine environment. In: *Advances in Marine Biology*. Eds.

A.J. Southward, P.A. Tyler, C.M. Young & L.A. Fuiman. Academic Press, Amsterdam. pp 225-340.

Dame, R.F. & Allen, D.M. 1996. Between estuaries and the sea. *Journal of Experimental Marine Biology and Ecology* **200**: 169-185.

Dannheim, J., Struck, U. & Brey, T. 2007. Does sample bulk freezing affect stable isotope ratios of infuanal macrozoobenthos? *Journal of Experimental Marine Biology and Ecology* **351**: 37-41.

Davenport, J., Smith, R.J.J.W & Packer, M. 2000. Mussels *Mytilus edulis*: significant consumers and destroyers of mesozooplankton. *Marine Ecology Progress Series* **198**:131-137.

Davies, B.R. & Day, J.A. 1998. *Vanishing waters*. University of Cape Town Press, Cape Town. 487 pp.

Davies, B.R., Stuart, V. & de Villiers, M. 1989. The filtration activity of a serpulid polychaete population (*Ficopomatus enigmaticus* (Fauvel) and its effects on water quality in a coastal marina. *Estuarine, Coastal and Shelf Science* **29**: 613-620.

Deegan, L.A. & Garritt, R.H. 1997. Evidence for spatial variability in estuarine food webs. *Marine Ecology Progress Series* **147**: 31-47.

Desvilettes, C.H., Bourdier, G., Amblard, C.H. & Barth, B. 1997. Use of fatty acids for the assessment of zooplankton grazing on bacteria, protozoans and microalgae. *Freshwater Biology* **38**: 629-637.

Dijkstra, H.A. & De Ruijter, W.P.M. 2001. On the physics of the Agulhas Current: Steady retroflection regimes. *Journal of Physical Oceanography* **31**: 2971-2985.

Drazen, J.C., Phleger, C.F., Guest, M.A. & Nichols, P.D. 2008. Lipid, sterols and fatty acids of abyssal polychaetes, crustaceans, and a cnidarians from the northeast Pacific Ocean: food web implications. *Marine Ecology Progress Series* **372**: 157-167.

Dubois, S., Jean-Louis, B., Bertrand, B. & Lefebvre, S. 2007. Isotope trophic-step fractionation of suspension-feeding species: implications for food partitioning in coastal systems. *Journal of Experimental Marine Biology and Ecology* **351**:121-128.

Dunton, K.H. 2001. δ^{15}N and δ^{13}C measurements of Antarctic Peninsula fauna: trophic relationships and assimilation of benthic seaweeds. *American Zoologist* **41**: 99-112.

DWAF, D.O.W.A.A.F. 2004. DWAF Report No. PBV000-00-10310. Thukela Bank: Impacts of flow scenarios on prawn and fish catch report – Reserve Determination Study: Thukela River System. Prepared by IWR Source-to sea as part of the Thukela Water Project Decision Support Phase.

Erlandsson, J, McQuaid, C.D. & Kostylev, V.E. 2005. Contrasting spatial heterogeneity of sessile organisms within mussel (*Perna perna L.*) beds in relation to topographic variability. *Journal of Experimental Marine Biology and Ecology* **314**: 79-97.

Espinosa, E.P., Allam, B. & Ford, S.E. 2008. Particle selection in the ribbed mussel *Geukensia demissa* and the eastern oyster *Crassostrea virginica*: effect of microalgae growth stage. *Estuarine, Coastal and Shelf Science* **79**: 1-6.

Falk-Peterson, S., Hagen, W., Kattner, G., Clarke, A. & Sargent, J. 2000. Lipids, trophic relationships and biodiversity in Arctic and Antarctic krill. *Canadian Journal of Fisheries and Aquatic Science* **57**: 178-191.

Farkas, T. & Herodek., S. 1964. The effect of environmental temperature on the fatty acid composition of crustacean plankton. *Journal of Lipid Research* **5**: 369-373.

Finlay, J.C., Khandwala, S. & Power, M.E. 2002. Spatial scales of carbon flow in a river food web. *Ecology* **83**: 1845-1859.

Folch, J., Lees, M. & Stanley, G.H.S. 1957. A simple method for the isolation and purification of total lipides from animal tissues. *Journal of Biological Chemistry* **226**:497-509.

Forrest, B.M., Gillespie, P.A., Cornelisen, C.D. & Rogers, K.M. 2007. Multiple indicators reveal river plume influence on sediments and benthos in a New Zealand coastal embayment. *New Zealand Journal of Marine and Freshwater Research* **41**: 13-24.

France, R.L. 1995. Carbon-13 enrichment in benthic compared to planktonic algae: foodweb implications. *Marne Ecology Progress Series* **124**: 307-312.

Frances, P. & Guerrero, A.G. (Eds.) 2008. *Ocean*. Dorling Kindersley, London. pp 512.

Freites, L., Fernández-Reiríz, M.J. & Labarta, U. 2002. Fatty acid profiles of *Mytilus galloprovincialis* (Lmk) mussel of subtidal and rocky shore origin. *Comparative Biochemistry and Physiology Part B* **132**: 453-461.

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

Froneman, P.W. 2001. Seasonal changes in zooplankton biomass and grazing in a temperate estuary, South Africa. *Estuarine, Coastal and Shelf Science* **52**: 543-553.

Froneman, P.W. 2002. Trophic cascading in an oligrotrophic temperate estuary, South Africa. *Journal of Plankton Research* **24**: 807-816.

Froneman, P.W. 2004. Food web dynamics in a temperate temporarily open/closed estuary (South Africa). *Estuarine, Coastal and Shelf Science* **59**: 87-95.

Froneman, P.W. & McQuaid, C.D. 1997. Preliminary investigation of the ecological role of microzooplankton in the Kariega Estuary, South Africa. *Estuarine, Coastal and Shelf Science* **45**: 689-695.

Fry, B. & Sherr, E. 1984. δ^{13}C measurements as indicators of carbon flow in marine and freshwater ecosystems. *Contributions in Marine Science* **27**: 13-47.

García-Alonso, J., Müller, C.T. & Hardege, J.D. 2008. Influence of food regimes and fatty acid composition in the rag worm. *Aquatic Biology* **4**: 7-13.

Garcia-Soto, C., de Madariaga, I., Villate, F. & Orive, E. 1990. Day-to-day variability in the plankton community of a shallow coastal embayment in response to changes in river runoff and water turbulence. *Estuarine, Coastal and Shelf Science* **31**: 217-229.

Garrett,R.H. & Grisham, C.M. 1999. *Biochemistry – Second Edition*. Saunders College Publishing, Orlando. 1127 pp.

Graeve, M., Kattner, G. & Hagen, W. 1994. Diet-induced changes in the fatty acid composition of Arctic herbivorous copepods: experimental evidence of trophic markers. *Journal of Experimental Marine Biology and Ecology* **182**: 97-110.

Graeve, M., Kattner, G. & Piepenburg, D. 1997. Lipids in Arctic benthos: does the fatty acid and alcohol composition reflect feeding and trophic interactions? *Polar Biology* **18**: 53-61.

Grall, J., Le Loc'h, F., Guyonnet, B. & Riera, P. 2006. Community structure and food web based on stable isotopes (^{15}N and ^{13}C) analysis of a North Eastern Atlantic mearl bed. *Journal of Experimental Marine Biology and Ecology* **338**: 1-15.

Grange, N. & Allanson, B.R. 1995. The influence of freshwater inflow on the nature, amount and distribution of seston in estuaries of the Eastern Cape, South Africa. *Estuarine, Coastal and Shelf Science* **40**: 403-420.

Grange, N., Whitfield, A.K., De Villiers, C.J. & Allanson, B.R. 2000. The response of two South African east coast estuaries to altered river flow regimes. *Aquatic Conservation: Marine and Freshwater Ecosystems* **10**: 155-177.

Hall, D., Lee, S.Y. & Meziane, T. 2006. Fatty acids as trophic tracers in an experimental estuarine food chain: tracer transfer. *Journal of Experimental Marine Biology and Ecology* **336**: 42-53.

Hama, T. 1991. Production and turnover rates of fatty acids in marine particulate matter through phytoplankton photosynthesis. *Marine Chemistry* **33**: 213-227.

Hammer, Ø., Harper, D.A.T. & Ryan, P.D. 2001. PAST: palaeontological statistics software package for education and data analysis. Palaeontologia Electronica 4, p 9. Available at: palaeo-electronica.org/2001_1/past/issue1_01.htm.

Harrison, T.D., Hohls, D.R., Meara T.P. and Webster, M.S. 2001. National Summary Report. *South African Estuaries: Catchment land-cover.* Prepared by the Division of Water, Environment and Forestry Technology (CSIR) as part of the National Land-Cover Database Project (NLC), for the Directorate Environmental Information and Statistics (DEAT). pp 30.

Henninger, T.O., Froneman, P.W., Richoux, N.B. & Hodgson, A.N. 2009. The role of macrophytes as refuge and food source for the estuarine isopod *Exosphaeroma hylocetes* (Barnard, 1940). *Estuarine, Coastal and Shelf Science* **82**: 285-293.

Herman, P.M.J, Middelburg, J.J., Widdows, J., Lucas, C.H. and Heip, C.H.R. 2000. Stable isotopes as trophic tracers: combining field sampling and manipulative labelling of food resources for macrobenthos. *Marine Ecology Progress Series* **204**: 79-92.

Hickman, C.P., Roberts, L.S. & Larson, A. 2001. *Integrated principles of zoology – Eleventh Edition.* McGraw-Hill, New York. 899 pp.

Hill, J.M. & McQuaid, C.D. 2008. δ^{13}C and δ^{15}N biogeographic trends in rocky intertidal communities along the coast of South Africa: evidence of strong environmental signatures. *Estuarine, Coastal and Shelf Science* **80**: 261-268.

Hill, J.M., McQuaid, C.D. & Kaehler, S. 2006. Biogeographic and nearshore-offshore trend in isotope ratios of intertidal mussels and their food sources around the coast of southern Africa. *Marine Ecology Progress Series* **318**: 63-73.

Hiscock, K. & Marshall, C. 2006. Dossier on Ecosystem Structure and Functioning – characterization and importance for management: rocky shores. In: Hiscock, K., Marshall, C., Sewell, J. & Hawkins, S.J., 2006. The structure and functioning of marine ecosystems: an environmental protection and management perspective. Report to English Nature from the Marine Life Information Network (MarLIN). Plymouth: Marine Biological Association of the UK. [English Nature Research Reports, ENRR No. 699]

Hobson, K.A., Schell, D.M., Renouf, D. & Noseworthy, E. 1996. Stable carbon and nitrogen isotope fractionation between diet and tissues of captive seals: implications for dietary reconstructions involving marine mammals. *Canadian Journal of Fisheries and Aquatic Science* **53**: 528-533.

Hodgson, A.N. 1987. Distribution and abundance of the macrobenthic fauna of the Kariega estuary. *South African Journal of Zoology* **22**(2): 153-162.

Howell, K.L., Pond, D.W., Billit, D.S.M. & Tyler, P.A. 2003. Feeding ecology of deep-sea seastars (Echinodermata: Asteroidea): a fatty-acid biomarker approach. *Marine Ecology Progress Series* **255**: 193-206.

Hudson, I.R., Pond, D.W., Billett, D.S.M., Tyler, P.A., Lampitt, R.S. & Wolff, G.A. 2004. Temporal variations in fatty acid composition of deep-sea holothurians: evidence of bentho-pelagic coupling. *Marine Ecology Progress Series* **281**: 109-120.

Hunt, M.J. & Alexander, C.G. 1991. Feeding mechanisms in the barnacle *Tetraclita squamosa* (Bruguière). *Journal of Experimental Marine Biology and Ecology* **154**: 1-28.

Iverson, S.J., Field, C., Bowen, W.D. & Blanchard, W. 2004. Quantitative fatty acid signature analysis: a new method of estimating predator diets. *Ecological Monographs* **74**: 211-235.

Jacob, U., Mintenbeck, K., Brey, T., Knust, R. & Beyer, K. 2005. Stable isotope food web studies: a case for standardized sample treatment. *Marine Ecology Progress Series* **287**:251-253.

Jeffries, H.P. 1970. Seasonal composition of temperate plankton communities: fatty acids. *Limnology and Oceanography* **15**: 419-426.

Jennings, M.E. 2005. *Nutrient dynamics in and offshore of two permanently open South African estuaries with contrasting freshwater inflow*. M.Sc., Rhodes University, Grahamstown. 155 pp.

Jennings, S., Reñones, O., Morales-Nin, B., Polunin, N.V.C., Moranta, J. & Coll, J. 1997. Spatial variation in the N and C stable isotope of plants, invertebrates and fishes on Mediterranean reefs: implications for the study of trophic pathways. *Marine Ecology Progress Series* **146**: 109-116.

Jerling, H.L. & Wooldridge, T.H. 1995. Feeding of two mysid species on plankton in a temperate South African Estuary. *Journal of Experimental Marine Biology and Ecology* **188**: 243-259.

Jørgensen, C.B. 1996. Bivalve filter feeding revisited. *Marine Ecology Progress Series* **142**:287-302.

Kanazawa, A. & Koshio, S. 1994. Lipid nutrition of the spiny lobster *Panuliris japonicus* (Decapoda, Panuliridae): a review. *Crustaceana* **67**: 226-234.

Kang, C-K., Lee, Y-W., Choy, E.J., Shin, K-K., Seo, I-S. & Hong, J-S. 2006. Microphytobenthos seasonality determines growth and reproduction in intertidal bivalves. *Marine Ecology Progress Series* **315**: 113-127.

Kharlamenko, V.I., Kiyashko, S.I., Imbs, A.B. & Vyshkvartzev, D.I. 2001. Identification of food sources of invertebrates from the seagrass *Zostera marina* community using carbon and sulfur stable isotope ratio and fatty acid analyses. *Marine Ecology Progress Series* **220**: 103-117.

Kharlamenko, V.I., Kiyashko, S.I., Rodkina, S.A. & Imbs, A.B. 2008. Determination of food sources of marine invertebrates from a subtidal sand community using analyses of fatty acids and stable isotopes. *Ecology* **34**: 101-109.

Kosro, P.M., Huyer, A., Ramp, S.R., Smith, R.L., Chavez, F.P., Cowles, T.J., Abbott, M.R., Strub, P.T., Barber, R.T., Jessen, P. & Small, L.F. 1991. The structure of the transition zone between coastal waters and the open ocean off northern California, winter and spring 1987. *Journal of Geophysical Research* **96**(C8): 14,707-14,730.

Kupfer, A., Langel, R., Scheu, S., Himstedt, W. & Maraun, M. 2006. Trophic ecology of a tropical aquatic and terrestrial food web: insights from stable isotopes (^{15}N). *Journal of Tropical Ecology* **22**: 469-476.

LaBarbera, M. 1984. Feeding currents and particle capture mechanisms in suspension-feeding animals. *American Zoologist* **24**: 71-84.

Labarta, U., Fernández-Reiríz, M.J. & Babarro, J.M.F. 1997. Differences in physiological energetic between intertidal and raft cultivated mussels *Mytilus galloprovincialis*. *Marine Ecology Progress Series* **152**: 167-173.

Largier, J.L. 1993. Estuarine fronts: how important are they? *Estuaries* **16**: 1-11.

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

Lebreton, B., Richard, P., Galois, R., Radenac, G., Pfléger, C., Guillou, G., Mornet, F. & Blanchard, G.F. 2011. Trophic importance of diatoms in an intertidal *Zostera noltii* seagrass bed: evidence from stable isotope and fatty acid analysis. *Estuarine, Coastal and Shelf Science* (2011), doi:10.1016/j.ecss.2010.12.027.

Lefebvre, S., Marìn Leal, J.C., Dubois, S., Orvain, F., Blin, J-L., Bataillé, M-P., Ourry, A. and Galois, R. 2009. Seasonal dynamics of trophic relationships among co-occurring suspension-feeders in two shellfish culture dominated ecosystems. *Estuarine, Coastal and Shelf Science* **82**: 415-425.

Lesser, M.P., Shumway, S.E., Cucci, T., Smith, J. 1992. Impact of fouling organisms on mussel rope culture: interspecific competition for food among suspension-feeding invertebrates. *Journal of Experimental Marine Biology and Ecology* **165**: 91-102.

Levin, L.A., Boesch, D.F., Covich, A., Dahm, C., Erséus, C., Ewel, K.C., Kneib, R.T., Moldenke, A., Palmer, M.A., Snelgrove, P., Strayer, D. & Weslawski, J.M. 2001. The function of marine critical transition zones and the importance of sediment biodiversity. *Ecosystems* **4**: 430-451.

Levinton, J.S. 2001. *Marine biology: function, biodiversity and ecology*. Oxford University Press, New York. 515 pp.

Little, C. & Kitching, J.A. 1996. *The biology of rocky shores*. Oxford University Press, New York. pp 240.

Lorenzen, C. & Jeffrey, S.W. 1980. Determination of chlorophyll in sea water. *Unesco Technical Papers in Marine Science* **35**: 1-20.

Lutjeharms, J.R.E. 2007. Three decades of research on the greater Agulhas Current. *Ocean Science* **3**: 129-147.

Lutjeharms, J.R.E., Cooper, J. & Roberts, M. 2000. Upwelling at the inshore edge of the Agulhas Current. *Continental Shelf Research* **20**: 737-761.

Machas, R., Santos, R. & Peterson, B. 2003. Tracing the flow of organic matter from primary producers to filter feeders in Ria Formosa Lagoon, southern Portugal. *Estuaries* **26**: 846-856.

Machu, E., Biastoch, A., Oschlies, A., Kawamiya, M., Lutjeharms, J.R.E. & Garçon, V. 2005. Phytoplankton distribution in the Agulhas system from a coupled physical-biological model. *Deep-Sea Research I* **52**: 1300-1318.

Maksymowska, D., Richard, P., Piekarek-Jankowska, H. & Riera, P. 2000. Chemical and isotopic composition of the organic matter sources in the Gulf of Gdansk (southern Baltic Sea). *Estuarine, Coastal and Shelf Science* **51**: 585-598.

Mann, K.H. 1988. Production and use of detritus in various freshwater, estuarine, and coastal marine ecosystems. *Limnology and Oceanography* **33**(4, part 2): 910-930.

Marchinko, K.B. 2007. Feeding behaviour reveals the adaptive nature of plasticity in barnacle feeding limbs. *Biological Bulletin* **213**: 12-15.

Maree, C. & Casey, N.H. 1993. *Livestock production systems*. Agri-Development Foundation, Pretoria. 403 pp.

Marieb,E. N. & Hoehn, K. 2010. *Human anatomy and physiology – Eighth Edition*. Pearson Benjamin Cummings, San Francisco. 1114 pp.

Marín Leal, J.C., Dubois, S., Orvain, F., Galois, R., Blin, J-L., Ropert, M., Bataillé, M-P., Ourry, A. & Lefebvre, S. 2008. Stable isotopes (δ^{13}C, δ^{15}N) and modelling as tools to estimate trophic ecology of cultivated oysters in two contrasting environments. *Marine Biology* **53**: 673-688.

Mayzaud, P.,Chanut, J.P. & Ackman, R.G. 1989. Seasonal changes of the biochemical composition of marine particulate matter with special reference to fatty acids and sterols. *Marine Ecology Progress Series* **56**: 189-204.

McCutchan, J.H., Lewis, W.M., Kendall, C. & McGrath, C.C. 2003. Variation in trophic shift in stable isotope ratios of carbon, nitrogen and sulfur. *Oikos* **102**: 378-390.

McLeod, R.J. & Wing, S.R. 2009. Strong pathways for incorporation of terrestrially derived organic matter into benthic communities. *Estuarine, Coastal and Shelf Science* **82**: 645-653.

McQuaid, C.D. & Branch, G.M. 1985. Trophic structure of rocky intertidal communities: response to wave action and implications for energy flow. *Marine Ecology Progress Series* **22**: 153-161.

McQuaid, C.D. & Mostert, B.P. 2010. The effects of within-shore water movement on growth of the intertidal mussel *Perna perna*: An experimental field test of bottom-up control at centimetre scales. *Journal of Experimental Marine Biology and Ecology* **384**:119-123.

Michener, R.H. & Schell, D.M. 1994. Stable isotope ratios as tracers in marine aquatic food webs. In: *Stable Isotopes in Ecology and Environmental Science*. Eds. K. Lajtha & R.H. Michener. Blackwell, Oxford, pp 138-157.

Moberg, F. Rönnbäck, P. 2003. Ecosystem service of the tropical seascape: interactions, substitutions and restorations. *Ocean and Coastal Management* **46**: 27-46.

Narváez, M., Freites, L., Guevara, M., Mendoza, J., Guderley, H., Lodeiros, C.J. & Salazar, G. 2008. Food availability and reproduction affects lipid and fatty acid composition of the brown mussel, *Perna perna*, raised in suspension culture. *Comparative Biochemistry and Physiology B* **149**: 293-302.

Nicholls, P. & Ellis, J. 2002. Fringing habitats in estuaries: the sediment-mangrove connection. *Water and Atmosphere* **10**(4): 24-25.

Noffke, A., Hertweck, G., Kröncke, I. & Wehrmann, A. 2009. Particle size selection and tube structure of the polychaete *Owenia fusiformis*. *Estuarine, Coastal and Shelf Science* **81**:160-168.

Odum, E.P. 1980. The status of three ecosystem-level hypotheses regarding salt marsh estuaries: tidal subsidy, outwelling, and detritus-based food chains. In: Kennedy, V.S. (Ed). *Estuarine Perspectives*. Academic Press, New York. pp 485 - 496.

Olsen, Y. 1999. Lipids and essential fatty acids in aquatic food webs: what can freshwater ecologists learn from mariculture? In: *Lipids in freshwater ecosystems*. Eds. M.T. Arts & B.C. Wainman. Springer-Verlag, New York. pp 161-202.

Page, H.M. & Lastra, M. 2003. Diet of intertidal bivalves in the Ria de Arosa (NW Spain): evidence from stable C and N isotope analysis. *Marine Biology* **143**: 519-532.

Parnell, A.C., Inger, A., Bearhop, S. & Jackson, A.L. 2010. Source partitioning using stable isotopes: coping with too much variation. PLoS ONE 5(3): e9672. doi:10.1371/journal.pone.0009672

Parrish, C.C. 1999. Determination of total lipid, lipid classes, and fatty acids in aquatic systems. In: *Lipids in freshwater ecosystems*. Eds. M.T. Arts & B.C. Wainman. Springer-Verlag, New York. pp 4-20.

Parsons, T.R., Maita, Y. & Lalli, C.M. 1984. *A manual of chemical and biological methods for seawater analysis.* Pergamon Press, Oxford. pp 173.

Pasquaud, S., Lobry, J. & Elie, P. 2007. Facing the necessity of describing estuarine ecosystems: a review of food web ecology study techniques. *Hydrobiologia* **588**: 159-172.

Paterson, A.W. & Whitfield, A.K. 1997. A stable carbon isotope study on the food-web in a freshwater-deprived South African estuary, with particular emphasis on the ichthyofauna. *Estuarine, Coastal and Shelf Science* **45**: 705-715.

Perga, M-E., Kainz, M., Matthews, B. & Mazumder, A. 2006. Carbon pathways to zooplankton: insights from the combined use of stable isotope and fatty acid biomarkers. *Freshwater Biology* **51**: 2041-2051.

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

Peschak, T.P. 2005. *Currents of contrast – life is southern Africa's two oceans*. Struik Publishers, Cape Town. 200 pp.

Peters, J., Renz, J. & van Beusekom, J. 2006. Trophodynamics and seasonal cycle of the copepod *Pseudocalanus acuspes* in the central Baltic Sea (Bornholm Basin): evidence from lipid composition. *Marine Biology* **149**: 1417-1429.

Phillips, N.E. & Pernet, B. 1996. Capture of large particles by suspension-feeding scaleworm larvae (Polychaeta: Polynoidae). *Biological Bulletin* **191**: 199-208.

Prins, T. & Escaravage, V. 2005. *Can bivalve suspension-feeders affect pelagic food web structure?* In: R.F. Dame & S. Olenin. The comparative roles of suspension-feeders in ecosystems. Springer, Dordrecht. pp 31-51.

Quiñones, R.A. & Montes, R.M. 2001. Relationship between freshwater input to the coastal zone and the historical landings of the benthic/demersal fish *Eleginops maclovinus* in central-south Chile. *Fisheries Oceanography* **10**: 311-328.

Raby, D., Mingelbier, M., Dodson, J.J., Klein, B., Lagadeuc, Y. & Legendre, L. 1997. Food particle size and selection by bivalve larvae in a temperate embayment. *Marine Biology* **127**: 665-672.

Ray, G.C. 2005. Connectivities of estuarine fishes to the coastal realm. *Estuarine, Coastal and Shelf Science* **64**: 18-32.

Ricciardi, A. & Bourget, E. 1999. Global patterns of macroinvertebrate biomass in marine intertidal communities. *Marine Ecology Progress Series* **185**: 21-35.

Richoux, N.B., Deibel, D., Thompson, R.J. & Parrish, C.C. 2005. Seasonal and developmental variation in the fatty acid composition of *Mysis mixta* (Mysidacea) and *Acanthostepheia malmgreni* (Amphipoda) from the hyperbenthos of a cold-ocean environment (Conception Bay, Newfoundland). *Journal of Plankton Research* **27**(8):719-733.

Richoux, N.B., Jaquemet, S., Bonnevie, B.T., Cherel, Y. & McQuaid, C.D. 2010. Trophic ecology of Grey-headed albatrosses from Marion Island, Southern Ocean: insights from stomach contents and diet tracers. *Marine Biology* **157**: 1755-1766.

Richoux, N.B. & Froneman, P.W. 2007. Assessment of spatial variation in carbon utilization by benthic and pelagic invertebrates in a temperate South African estuary using stable isotope signatures. *Estuarine, Coastal and Shelf Science* **71**: 545-558.

Richoux, N.B. & Froneman, P.W. 2008. Trophic ecology of dominant zooplankton and macrofauna in a temperate, oligotrophic South African estuary: a fatty acid approach. *Marine Ecology Progress Series* **357**: 121-137.

Riera, P., Escaravage, C. & Leroux, C. 2009. Trophic ecology of the rocky shore community associated with the *Ascophyllum nodosum* zone (Roscoff, France): a δ^{13}C vs δ^{15}N investigation. *Estuarine, Coastal and Shelf Science* **81**: 143-148.

Riera, P. & Richard, P. 1996. Isotopic determination of food sources of *Crassostrea gigas* along a trophic gradient in the estuarine bay of Marennes-Oléron. *Estuarine, Coastal and Shelf Science* **42**: 347-360.

Riera, P., Stal, L., Nieuwenhuize, J., Richard, P., Blanchard, G. & Gentil, F. 1999. Determination of food sources for benthic invertebrates in a salt marsh (Aiguillon bay, France) by carbon and nitrogen stable isotopes: importance of locally-produced sources. *Marine Ecology Progress Series* **187**: 301-307.

Riisgård, H.U & Larsen, P.S. 2010. Particle capture mechanisms in suspension-feeding invertebrates. *Marine Ecology Progress Series* **418**: 255-293.

Riley, G.A. 1970. Particulate organic matter in sea water. *Advances in Marine Biology* **8**: 1-118.

Rossi, F., Herman, P.M.J. & Middelburg, J.J. 2004. Interspecific and intraspecific variation of $\delta^{13}C$ and $\delta^{15}N$ in deposit- and suspension-feeding bivalves (*Macoma balthica* and *Cerastoderma edule*): evidence of ontogenetic changes in feeding mode of *Macoma balthica*. *Limnology and Oceanography* **49**: 408-414.

Rousch, J.M., Bingham, S.E. & Sommerfeld, M.R. 2003. Changes in fatty acid profiles of thermo-intolerant and thermo-tolerant marine diatoms during temperature stress. *Journal of Experimental Marine Biology and Ecology* **295**: 145-156.

Saito, H., Kotani, Y., Keriko, J.M., Xue, C., Taki, K., Ishihara, K., Ueda, T. & Miyata, S. 2002. High levels of *n*-3 polyunsaturated fatty acids in *Euphasia pacifica* and its role as a source of docosahexaenoic and eicosapentaenoic acids for higher trophic levels. *Marine Chemistry* **78**: 9-28.

Sanford, E. & Menge, B.A. 2001. Spatial and temporal variation in barnacle growth in a coastal upwelling system. *Marine Ecology Progress Series* **209**: 143-157.

Sanina, N.M., Goncharova, S.N. & Kostetsky, E.Y. 2004. Fatty acid composition of individual polar lipid classes from marine macrophytes. *Phytochemistry* **65**: 721-730.

Schaal, G., Riera, P. & Leroux, C. 2008. Trophic coupling between two adjacent benthic food webs within a man-made intertidal area: a stable isotopes evidence. *Estuarine, Coastal and Shelf Science* **77**: 523-534.

Schaal, G., Riera, P. & Leroux, C. 2009. Trophic significance of the kelp *Laminaria digitata* (Lamour.) for the associated food web: a between-sites comparison. *Estuarine, Coastal and Shelf Science* **85**: 565-572.

Schaal, G., Riera, P. & Leroux, C. 2010. Microscale variations in food web functioning within a rocky shore invertebrate community. *Marine Biology* (2010), doi:10.1007/s00227-010-1586-2.

Schaal, G., Riera, P., Leroux, C & Grall, J. 2010. A seasonal stable isotope survey of the food web associated to a peri-urban rocky shore. *Marine Biology* **157**: 283-294.

Schaeffer, M.B. 1972. *Conservation of biological resources of the coastal zone*. In**:** Peel Brahtz, J.F. (Eds). Coastal Zone Management: Multiple Use with Conservation. John Wiley and Sons Inc., New York. pp 35-79.

Schlacher, T.A. & Wooldridge, T.H. 1996. Ecological responses to reductions in freshwater supply and quality in South Africa’s estuaries: lessons for management and conservation. *Journal of Coastal Conservation* **2**: 115-130.

Schlechtriem, C.H., Focken, U. & Becker, K. 2003. Effect of different lipid extraction methods on $\delta^{13}C$ of lipid and lipid-free fractions of fish and different fish feeds. *Isotopes in Environmental and Health Studies* **39**(2): 135-140.

Schleyer, M.H. 1981. Microorganisms and detritus in the water column of a subtidal reef of Natal. *Marine Ecology Progress Series* **4**: 307-320.

Schmidt, K., Atkinson, A., Petzke, K-J., Voss, M. & Pond, D.W. 2006. Protozoans as a food source for Antarctic krill, *Euphasia superba*: complimentary insights from stomach content, fatty acids, and stable isotopes. *Limnology and Oceanography* **51**: 2409-2427.

Schmidt-Nielsen, K. 2007. *Animal Physiology, Adaptation and Environment – Fifth Edition*. Cambridge University Press, New York. 612 pp.

Schumann, E.H. & Pearce, M.W. 1997. Freshwater inflow and estuarine variability in the Gamtoos Estuary, South Africa. *Estuaries* **20**: 124-133.

Shi, W., Sun, M-Y., Molina, M & Hodson, R.E. 2001. Variability in the distribution of lipid biomarkers and their molecular isotopic composition in Altamaha estuarine sediments: implications for the relative contribution of organic matter from various sources. *Organic Chemistry* **32**: 453-467.

Shin, P.K.S., Yip, K.M., Xu, W.Z., Wong, W.H. & Cheung, S.G. 2008. Fatty acid as marker to demonstrating trophic relationships among diatoms, rotifers and green-lipped mussels. *Journal of Experimental Marine Biology and Ecology* **357**:75-84.

Stevens, C.J., Deibel, D. & Parrish, C.C. 2004. Copepod omnivory in the North Water Polynya (Baffin Bay) during autumn: spatial patterns in lipid composition. *Deep-Sea Research I* **51**: 1637-1658.

St. John, M.A. & Lund, T. 1996. Lipid biomarkers: linking the utilisations of frontal plankton biomass to enhanced condition of juvenile North Sea cod. *Marine Ecology Progress Series* **131**: 75-85.

Tamelander, T., Soreide, J.E., Hop, H. & Carroll, M.L. 2006. Fractionation of stable isotopes in the Arctic marine copepod *Calanus glacialis*: effects on the isotopic composition of marine particulate organic matter. *Journal of Experimental Marine Biology and Ecology* **333**:231-240.

Teegarden, G.J. 1999.Copepod grazing selection and particle discrimination on the basis of PSP toxin content. *Marine Ecology Progress Series* **181**: 163-167.

Teegarden, G.J., Campbell, R.G. & Durbin, E.G. 2001. Zooplankton feeding behaviour and particle selection in natural plankton assemblages containing toxic *Alexadrium spp*. *Marine Ecology Progress Series* **218**: 213-226.

Thiemann, G.W., Budge, S.M., Bowen, W.D. & Iverson, S.J. 2004. Comment on Grahl-Nielsen et al. (2003) ‘fatty acid composition of the adipose tissue of poler bears and of their prey: ringed seals, bearded seals, and harp seals’. *Marine Ecology Progress Series* **281**: 297-301.

Thompson, J.L., Manore, M.M. & Vaughan, L.A. 2008. *The science of nutrition – Tenth Edition.* Pearson Benjamin Cummings, San Francisco. pp. 1062.

Tieszen, L.L., Boutton, T.W., Tesdahl, K.G. & Slade, N.A. 1983. Fractionation and turnover of stable carbon isotopes in animal tissues: Implications for $\delta^{13}C$ analysis of diet. *Oecologia* **57**(1): 32-37.

Turner, J.T., Levinsen, H., Nielsen, T.G. & Hansen, B.W. 2001. Zooplankton feeding ecology: grazing on phytoplankton and predation on protozoans by copepod and barnacle nauplii in Disko Bay, West Greenland. *Marine Ecology Progress Series* **221**: 209-219.

Turpie, J. & Clark, B. 2007. *Development of a conservation plan for temperate South African estuaries on the basis of biodiversity importance, ecosystem health and economic costs and benefits.* Cape Action Plan for the Environment (C.A.P.E.) Regional Management Programme, Cape Nature. pp 125.

Underwood, G.J.C. & Kromkamp, J. 1999. Primary production by phytoplankton and microphytobenthos in estuaries. *Advances in Ecological Research* **29**: 93-154.

Van Den Meersche, K., Van Rijswijk, P., Soetaert, K. & Middelburg, J.J. 2009. Autochthonous and allochthonous contributions to mesozooplankton diet in a tidal river and estuary: integrating carbon isotope and fatty acid constraints. *Limnology and Oceanography* **54**(1): 62-74.

Van der Elst, R. & King, D. 2000. *Everyone’s guide to sea fishes of southern Africa*. Struik, Cape Town. 112 pp.

Vander Zanden, M.J. & Rasmussen, J.B. 2001. Variation in $\delta^{15}N$ and $\delta^{13}C$ trophic fractionation: implications fro aquatic food web studies. *Limnology and Oceanography* **46**: 2061-2066.

Van Rooyen, N. 2002. Geomorphology and climate of South Africa. In: *Game Ranch Management – Fourth Edition*. Ed. J. du P. Bothma. Van Schaik Publishers, Hatfield. pp 34-35.

Vizzini, S. & Mazzola, A. 2006. Sources and transfer of organic matter in food webs of a Mediterranean coastal environment: evidence for spatial variability. *Estuarine, Coastal and Shelf Science* **66**: 459-465.

Vorwerk, P.D. 2006. *A preliminary examination of selected biological links between four Eastern Cape estuaries and the inshore marine environment*. Ph.D., Rhodes University, Grahamstown. 245 pp.

Vorwerk, P.D. and Froneman, P.W. 2009. The importance of estuarine-derived carbon for the nearshore marine environment: studies on two contrasting South African estuaries. *African Journal of Aquatic Science* **34**: 137-146.

Vorwerk, P.D., Froneman, P.W., Paterson, A.W., Strydom, N.A. & Whitfield, A.K. 2008. Biological responses to a resumption in river flow in a freshwater-deprived, permanently open southern African estuary. *Water SA* **35**: 597-604.

Vorwerk, P.D., Whitfield, A.K., Cowley, P.D. & Paterson, A.W. 2001. A survey of selected Eastern Cape estuaries with particular reference to the ichthyofauna. *Ichthyological Bulletin* **72**.

Vorwerk, P.D., Whitfield, A.K., Cowley, P.D. & Paterson, A.W. 2003. The influence of selected environmental variables on fish assemblage structure in a range of southeast African estuaries. *Environmental Biology of Fishes* **66**: 237-247.

Ward, J.E., Sanford, L.P., Newell, R.I.E. & MacDonald, B.A. 1998. A new explanation of particle capture in suspension-feeding bivalve molluscs. *Limnology and Oceanography* **43**: 741-752.

Whitfield, A.K. 1998. Biology and ecology of fishes in Southern African estuaries. *Ichthyological Monograph of the J.L.B. Smith Institute of Ichthyology* **2**: 223 pp.

Winter, P.E.D., Schlacher, T.A., and Baird, D. 1996. Carbon flux between an estuary and the ocean: a case for outwelling. *Hydrobiologia* **337**: 123-132.

Wong, W.H. & Levinton, J.S. 2006. The trophic linkage between zooplankton and benthic suspension feeders: direct evidence from analyses of bivalve faecal pellets. *Marine Biology* **148**: 799-805.

Xu, X., Ji, W., Castell, J.D. & O'Dor, R. 1993. The nutritional value of dietary n-3 and n-6 fatty acids for the Chinese prawn (*Penaeus chinesis*). *Aquaculture* **118**: 277-285.

Yokoyama, H., Tamaki, A., Koyama, K., Ishihi, Y., Shimoda, K. & Harada, K. 2005. Isotopic evidence for phytoplankton as a major food source for macrobenthos on an intertidal sandflat in Ariake Sound, Japan. *Marine Ecology Progress Series* **304**: 101-116.

Zhukova, N.V. 2000. Fatty acid components of two species of barnacles, *Hesperibalanus hesperius* and *Balanus rostratus* (Cirripedia), as indicators of food sources. *Crustaceana* **73**: 513-518.

Zhukova, N.V., Kharlamenko, V.I., Svetashev, V.I. & Rodionov, I.A. 1992. Fatty acids as markers of bacterial symbionts of marine bivalve molluscs. *Journal of Experimental Marine Biology and Ecology* **162**: 253-263.