Diet of coastal filter feeders: impact of factors operating at different scales

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ABSTRACT

Benthic filter feeders have a key functional role in the dynamics of coastal food web as an intermediate trophic level and bioengineers. A wide variety of factors, operating across multiple spatial scales (*e.g*. hydrographic regime, human activities), can affect the composition of the water column and thus the availability of food for benthic populations. Food availability in turn affects the growth, reproductive rates and survival of benthic organisms, and consequently, can influence the functioning of the entire ecosystem.

This study aims to evaluate how various environmental factors may modify the diet of intertidal filter feeders living along the South African coast. Specifically, the effects of biogeography, upwelling, urbanization and freshwater input on the dietary regimes of five species of filter feeders (two mussel and three barnacle species) were investigated using fatty acid (FA) and stable isotope (SI) analyses.

Strong interspecific differences were found among the five species considered. However, all species responded to factors operating at large (100s km) and meso (10s-100s km) scales (*i.e.* biogeography and upwelling respectively). The barnacles exhibit habitat segregation and showed different FA and SI signatures from each other, while the two mussel species, an invasive and native species that co-occur in the same mussel beds, had partially overlapping diets. Differences in their diets were found only using FA analysis, while their SI signatures differed on only one occasion. This highlights the importance of using the appropriate tool, and ideally combined techniques, to investigate diets.

FA and SI signatures of all species considered changed among the three biographical provinces (west, south and east coasts of South Africa) exhibiting similar patterns that reflect the two oceanographic regimes that characterize the coastline: the eutrophic Benguela Current on the west coast and the oligotrophic Agulhas Current on the other two coasts.

Upwelling had a significant effect on FA and SI signatures, with stronger effects on the west coast than the south coast. The results indicate that benthic filter feeders at upwelling areas consumed a mix of coastal macroalgal detritus and phytoplankton, which was probably brought onshore during downwelling events. At smaller spatial

scales and using repeated sampling, the influence of upwelling on the west coast was found to be pervasive, rather than discrete, so that it may be more appropriate to categorize upwelling by referring to upwelling centres and downstream areas.

SI underlined a significant effect of urbanization on the diet of filter feeders with an enrichment in the $\delta^{15}N$ being characteristic of anthropogenic effect.

Although a large number of rivers characterize the South African coast, no distinct effect of freshwater input was found for either the SI or FA signatures of the filter feeders. This contrasts with earlier work on demersal species and suggests that freshwater input does not significantly affect food availability for intertidal filter feeders, and that other factors (*e.g.* hydrogeography) are more important in determining the diet of these populations.

These results highlight that environmental and anthropogenic factors operating at different spatial and temporal scales have a profound effect on benthic ecosystems, and that they control the relationship between primary production and primary consumers in coastal areas. Above all, this work highlights the importance of understanding the spatial and temporal scales at which different factors affect feeding regimes, and their critical role in coastal food webs.

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Acronyms

BAME = Bacterial Fatty Acid

C = Carbon

CAP = Canonical Analysis of Principal Coordinates

DHA = 22:6w3 fatty acid

EFA = Essential Fatty Acid

EPA = 20:5w3 fatty acid

FA = Fatty Acid

FAME = fatty Acid Methyl Esters

FATM = Fatty Acid Trophic Marker

GC = Gas Chromatography

LCFA = Long Chain Fatty Acid

MUFA = Monounsaturated Fatty Acid

N = Nitrogen

NMI = Non-methylene-interrupted fatty acid

P = Phosphorus

PCA = Principal component analysis

POM = Particulate Organic Matter

PUFA = Polyunsaturated Fatty Acid

SFA = Saturated Fatty Acid

SI = Stable Isotope

SPM = Suspended Organic Matter

TFA = Total Fatty Acid

TM = Trophic Marker

Acknowledgments

Ci sono esperienze che ti segnano, ti cambiano, e ti fanno vedere la vita con un'ottica diversa; dopo di quelle la tua vita non sarà più la stessa. Il mio percorso negli ultimi tre anni può essere classificato come una di questa esperienze. Ho conosciuto tante persone diverse lungo questo cammino ed ho visto posti unici, che pochi hanno la possibilità di visitare. Ho la fortuna di essere circondata da alcune persone particolarmente speciali, senza le quali non sarei mai stata in grado di iniziare, e tantomeno di arrivare alla fine di questo "viaggio".

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Un ringraziamento va a tutti i miei amici italiani, Alice, Daniela, Lorenzo, Eleonora C, Eleonora DC, Celeste, Elisa, Luana, Simona, Patrizia, che nonostante siamo a mille di

mille chilometri di distanza mi siete stati vicini in un modo totalmente inaspettato, quasi come fossimo vicini di casa. In particolare Alice, amiche da letteralmente una vita, non mi ha fatto sentire lontana da casa nemmeno per un minuto. Non posso non parlare di tutte quelle persone che mi sono state accanto nel passato e che mi hanno supportato e dato la forza di perseguire il sogno. Grazie.

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Ma come diceva un famoso cantante.. Il meglio deve ancora venire.

Qui metto alcuni dei commenti più esilaranti che sono emersi nel corso della tesi… i miei supervisori non me ne vogliano, ma credo che faranno sorridere alcuni di voi prima della lunga lettura ☺

p.s. non ci sono i miei.. la lista sarebbe stata troppo lunga ☺

- Lettere maiuscole per nomi propri. Per esempio Sud Africa, ma sud Italia e costa sud. Eastern Cape, costa est. Student's t-test, denominato da un uomo con cognome Student, mentre te sei uno studente. Probabilmente un tempo anche Student era uno studente.
- Non credo che si possa definire Mossel Bay una città dall'alto livello di urbanizzazione.. ma adesso sono seduto guardando il porto di Hong Kong ☺
- In inglese per favore ☺
- Chiedi a Charles, ma direi "to assess the effect of.." non sono sicuro di quale sia meglio, Puoi farmi sapere dopo? ☺
- Trovo questa somiglianza straordinaria! Il che mi dà estrema confidenza nei risultati.
- Ok questo si impara alla scuola elementare – non credo che serva metterlo qui…
- Titoli possibili per la tesi:
  1- How factors operating at different spatial scales can affect the diet of intertidal primary consumers? The case of filter feeders on the South African coast.
  2- Filter feeder diets along the SA coast: impact of factors operating at various spatial scales

  S- Preferisco il secondo. Poi la scelta è tua ma questo è grammaticamente corretto.. il primo non lo è. ☺ Puoi trovare l'errore?

  E-Né io né i miei colleghi di ufficio, anche di prima lingua inglese, furono capaci di trovare l'errore ☺

There are some experiences in your life that leave a mark on you, change you, and make you see life with a different point of view; after them, your life is never going to be the same. My PhD experience is one of them. I met many different people over this journey, and I visited some unique places that not many are so fortunate to see. I have been very lucky to be surrounded by some very special people and without some of them, I would never have even started, let alone arrived at the end of this journey.

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Here I left some of the funniest comment that my supervisors gave to my thesis. I really hope they won't get upset, because I think that will make many people smile before the long reading of the thesis ☺
p.s. I didn't put mine. The list would have been too long! ☺

- Capital letters for proper names. E.g. South Africa, but south Italy and south coast. Eastern Cape, east coast. Student's t-test, named after a man called Student, whereas you are a student. Presumably one day Student was a student.
- I don't think we can claim Mossel Bay has a high level of urbanization…but then I am sitting looking at the harbour of Hong Kong. ☺
- Ok well we learn that in primary school – I don't think you should put it here…
- In English please. ☺
- Ele, you are using "in particular" and "particularly" all the time… I'm not sure it's well used either so maybe ask someone to explain you how to use it… And tell me, I would like to know too… ☺
- I find the similarity extraordinary! Which gives me huge confidence in the findings.
- 3 times "respective" in the same sentence!!!! :-D
- Possible title for the thesis:
  a- How factors operating at different spatial scales can affect the diet of intertidal primary consumers? The case of filter feeders on the South African coast.
  b- Filter feeder diets along the SA coast: impact of factors operating at various spatial scales
  s- I think I prefer the second one. It is of course up to you, but make sure its grammatically correct…..the first one isn't. ☺ Can you see why?
  Neither myself nor any of the colleagues in my office, even English speakers, could identify the mistake ☺

La bella vita con il sorriso a trentasei denti
E un po' di musica che lubrifica i legamenti.. Africa
Con un amore abbastanza grande
da far pensare che l'universo
L'han fatto apposta perché voi due vi incontraste là
la la la la
La bella vita abbastanza bella da essere vita.. Africa
La bella vita abbastanza vita da essere bella.. Africa

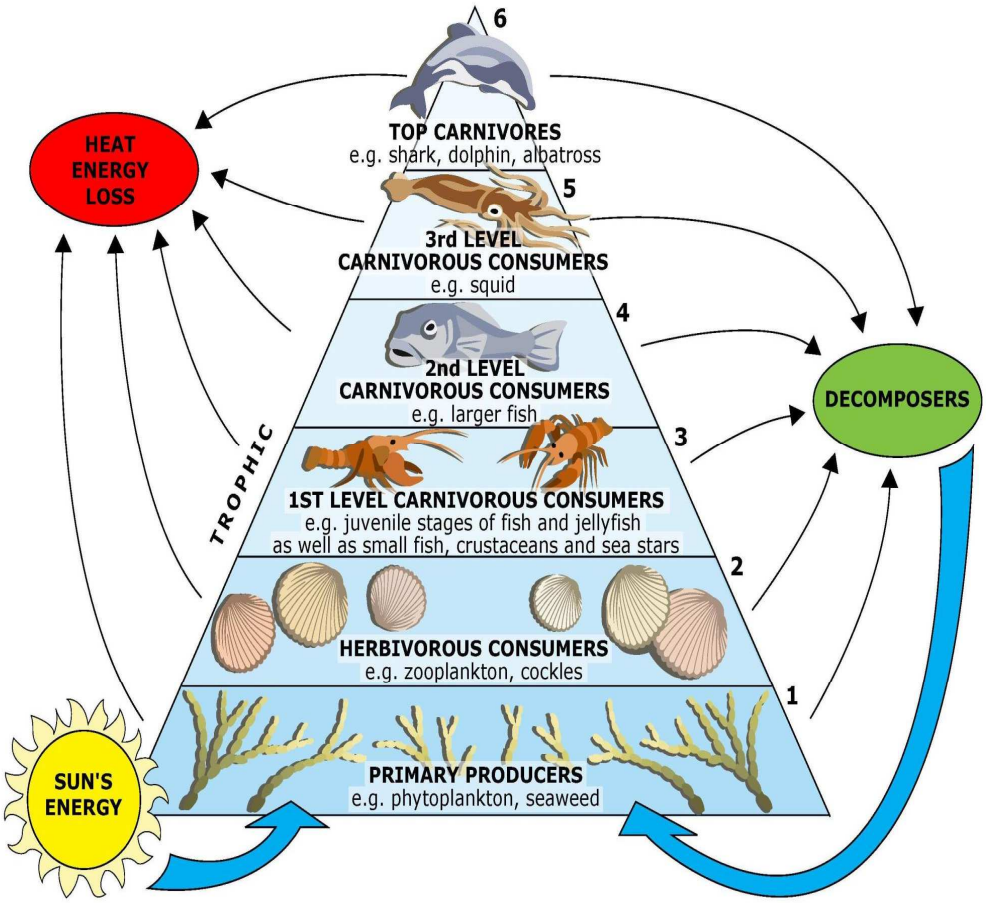
Giovanotti- La bella vita

Sicuramente esistono individui che temono I sogni, I sognatori e la capacità di sognare,
ma i sogni e i sognatori sono una presenza inestirpabile.

Il potere dei sogni- Luis Sepulveda

CHAPTER 1

General Introduction



1. General introduction

1.1. Rocky shore filter feeders

Primary consumers play a key role in coastal ecosystems because they are responsible for the transfer of organic matter from autotrophic and small heterotrophic organisms to higher predators in the food chain (Dame 1993a). They are simultaneously affected by oceanographic processes that affect supplies of larvae and food (Blanchette 2002, Menge et al. 2003) and by benthic processes which include predation, competition and physical processes (Connell 1961, Paine 1966, Dayton 1971, Nielsen 2003). Together, these processes operate across multiple spatial and temporal scales and can affect the physiology of individuals, population sizes and species distributions (Menge et al. 1997, Menge 2000, Palumbi 2003, Schiel 2004, Blanchette et al. 2006).

In the marine environment, filter feeders are a major group of primary consumers (Beukema and Cadée 1996, Gili and Coma 1998). They have a fundamental role in the re-cycling of nutrients, secondary production and food web dynamics, particularly in coastal areas (Doering et al. 1986, Smaal and Prins 1993, Polis et al. 1997). On rocky shores, benthic filter feeders also fulfil highly important ecological functions. These include ecosystem engineering, acting as key or habitat forming species (Menge et al. 1994, Jones et al. 1996, Bruno et al. 2003, Gutiérrez et al. 2003, Kelaher and Castilla 2005, Cole et al. 2011). For example, mussels are ecosystem engineers providing habitats for smaller species (Cole and McQuaid 2010), and also contributing to the maintenance of high levels of biodiversity (Borthagaray and Carranza 2007). Haven and Morales-Alamo (1966) demonstrated the importance of filter feeders in initiating sedimentation, by transforming fine suspended matter from the water column into faeces or pseudofaeces ready to be used by other species. Von Erkom Schurink and Griffiths (1991) showed that the input of mussel gametes can represent a significant energy supplement to the benthic and pelagic communities close to mussel beds. Dame (1993b) and Prins et al. (1997) underlined the key functional role that bivalves have in nutrient recycling in estuarine and coastal areas, while Officer and Smayda (1982) emphasized the importance of benthic filter feeder communities in the natural control of eutrophication in the south of San Francisco Bay. Given the ecological importance of

these organisms, understanding the fundamental factors underpinning their survival and success is imperative. Food availability is one of the most important factors for benthic filter feeders success, because it can have strong implications for individual physiology and the distributions of these populations, with consequences for higher consumers, and ultimately for the functioning of the entire ecosystem (Connell 1985, Menge 2000, Dodson et al. 2000, Lavorel and Garnier 2002, Le Bauer and Treseder 2008).

1.2. Factors influencing the diet of primary consumers over different spatial scales

Primary production is defined as organic carbon or dry matter produced annually per unit of surface area within an ecosystem (Fisher 1939, Odum 1956, Prince and Goward 1995). In the marine environment, primary producers comprise phytoplankton, micro- and macroalgae and highly specialised angiosperms such as seagrasses and mangroves (Hall et al. 2005, Burkepile and Hay 2006). Primary productivity (the rate of photosynthesis) changes significantly with latitude (Rohde 1999) and is one of the major factors responsible for the control of species richness, population density and the spatial distribution of organisms (Menge et al. 1997, Rosenzweig and Sandlin 1997, Nielsen 2003). Several other factors acting at different spatial scales also have profound effects on the distribution, quality and quantity of primary production in marine systems and consequently on the food available for coastal organisms. In marine environments, the highest productivity occurs at high latitudes and in coastal areas (Small and Menzies 1981, Antoine et al. 1996, Field et al. 1998, Huston and Wolverton 2009). Over a latitudinal gradient, the nature of the key primary producers changes significantly. For instance, in polar regions the main primary producers are phytoplankton, while cyanobacteria and microalgae are more dominant in tropical coral reefs, and kelps are important in cool temperate areas (Sullivan et al. 1993, Tribollet 2008, Reed et al. 2011). The effects of several of these factors operating at different spatial scales are discussed in the next few paragraphs.

1.2.1. Large scales

Temperature, solar radiation and nutrient availability are the most frequently identified drivers of primary productivity at large spatial scales of 100s km (Field et al. 1998, Rohde 1999). Air temperature near the planet' surface affects primary productivity by controlling the exchange of CO_2 between marine systems and the atmosphere and can strongly affect primary production (Churkina and Running 1998, Potter et al. 1999). Temperature can also affect metabolic rates and as such acts on the growth, reproduction and production of primary producers (Grime 1977, Xiong et al. 2000). Hartman (1958) used larval settlement as an index of sexual reproduction in a population of the sponge *Haliclona loosanoffi*, and he found settling occurred when the temperature reached 20 to 22 °C; while Vidal (1980) showed that the growth of two species of copepods was directly correlated to temperature increases. Another indirect effect of seawater temperature is represented by stratification of the water column, which creates a physical boundary between the warmer surface waters and the nutrient rich deep water (Bunt 1973, Behrenfeld et al. 2006). As a consequence, phytoplankton productivity can decrease as photosynthesis in the euphotic zone is restricted by nutrient limitation (Cullen et al. 1992, Beardall et al. 2001, Cermeño et al. 2008, Huertas et al. 2011).

Another major factor affecting primary productivity which also changes with latitude is solar radiation (Bondeau et al. 1999). Light limitation has a major effect on the production of primary producers. It can also determine species composition and, indirectly, the nature and abundance of primary consumers (Cloern 1987, Nemani et al. 2003, Kelble et al. 2005, Huston and Wolverton 2009). Factors such as the transparency of the water column are critical in determining the amount of solar radiation that reaches photosynthetic organisms. For example most benthic primary producers remain within the 50 m depth range, where there is sufficient solar radiation to sustain their energetic demand (Duarte 1991, Kenworthy and Fonseca 1996). In addition, the combined effect of transparency of the water column and latitudinal changes in solar radiation also determine the spatial distribution of primary producers over a latitudinal gradient (Dennison 1987, Vincent and Roy 1993), and consequently affect the quality

and quantity of primary production in these area (Sullivan et al. 1993, Tribollet 2008, Reed et al. 2011).

Nutrient availability is the other critical limit to primary productivity (Nixon 1981, Downing et al. 1999, Elser et al. 2007, Cermeño et al. 2008, Harpole et al. 2011). In marine systems, nitrogen (N) and phosphorus (P) are considered to be the predominant limiting macronutrients for primary production (Howarth 1988, Herbert 1999, Sundareshwar et al. 2003). The availability of N and P depends on the re-mineralization during biological decomposition (Howarth 1988) and the introduction of so-called new nitrogen by oceanographic processes, such as upwelling, that bring deep nutrients from the bottom to the euphotic zone (Thiel et al. 2007). However other elements, such as iron or silicon, can also have a key role in controlling primary production in the ocean (Martin et al. 1989, Peng and Broecker 1991, Dugdale et al. 1995). Hence, nutrients are derived from a variety of sources and are cycled through the marine environment in several ways, including vertical and lateral hydrodynamic processes (Lewis et al. 1986, Oschlies and Garçon 1998, Palter et al. 2005).

1.2.2. Mesoscales

At mesoscales of 10s-100s km, oceanographic processes are the main factors affecting primary production in coastal areas, consequently influencing the food available for benthic primary consumers. A few of these processes are represented by upwelling, coastal currents or freshwater input. In addition, human activities also influence primary production at these scales by altering nutrient and light regimes.

Coastal upwelling plays an important role in coastal primary production and supports large biomasses of primary and secondary consumers all over the world (Payne and Crawford 1989, Bustamante et al. 1995, Basterretxea and Arístegui 2000, Connolly et al. 2001, Menge 2000). Upwelling events bring cold, nutrient-rich water into the euphotic zone, which stimulate photosynthesis of auto- and heterotroph organisms. As a consequence, upwelling can modify the composition of the water column and thus the quantity and quality of the food available for benthic populations (Bustamante et al. 1995, Blanchette et al. 2006, Lutjeharms 2006), which subsequently can directly and

indirectly affect their metabolism and success (Connell 1985, Duggins et al. 1989, Raimondi 1990, Menge 2000). For instance, Figueiras et al. (2002) showed an increase in mussel growth in the coastal upwelling area of Rías Baixas of Galicia due to a strong increase in phytoplankton availability during the upwelling season. Similarly, Nielsen and Navarrete (2004) highlighted an increase in abundance of intertidal corticated algae at sites with high intensity upwelling events. Because of the direct impact of upwelling on productivity of primary producers, studies on these upwelling systems are important to understand trophic dynamics in coastal areas. Upwelling systems are regions dominated by diatoms, which are at the base of the food chain (Abrantes 1988, Ducklow and Harris 1993, Savidge et al. 1995). Following an upwelling event these systems usually become nutrient depleted (mainly silicon) resulting in the replacement of diatoms by dinoflagellates (Humborg et al. 2000, Martin-Jézéquel et al. 2000). For instance Allan et al. (2010), on the south coast of South Africa, showed that filter feeders in upwelling areas were characterized by diatom fatty acid trophic markers, while further downstream of the upwelling centre, the proportion of diatoms trophic makers decreased in favour of dinoflagellates. An aspect to consider in relation to upwelling is the hydrography of the upwelling centre. Specifically, depending on the hydrography of the site, upwelling can have dissimilar effects on benthic populations at the upwelling centre and at downstream sites. Some studies have shown upwelling enhances nutrient levels, and thus stimulates phytoplankton and macrophyte growth at the upwelling centre (Nielsen and Navarrete 2004, Wieters 2005); while other studies highlighted the fact that nutrients and particles upwelled can very rapidly be carried offshore during upwelling, resulting in phytoplankton-poor waters close inshore (Andrews and Hutchings 1980, Brown and Field 1986, Wieters et al. 2003).

Another important factor that operates at mesoscales is represented by currents. Several studies have highlighted the important role that currents can play in larval dispersal and recruitment, as well as in the structure and functioning of coastal ecosystems (*e.g.* Nielsen and Navarrete 2004, Blanchette et al. 2006). Others have emphasised the link between hydrogeographic regime and the nature of food available for benthic organisms (Hill and McQuaid 2006, Allan et al. 2013). In particular, Hill and McQuaid (2006) showed that intertidal filter feeders from the three biogeographic

regions around South Africa exhibited different stable isotope signatures due to the influence of the contrasting currents that dominate the different areas of the coast. Similarly Allan et al. (2013) showed a decadal shift in food sources for benthic subtidal organisms around the sub-Antarctic Prince Edward Islands from allochthonous to autochthonous due to a concomitant climate-driven shift in the position of the sub-Antarctic Front.

Freshwater input is another factor that can influence the food available for primary consumers at mesoscales (Ludwig et al. 2009). Estuaries represent the geographic area where terrestrial nutrients reach the open sea. These nutrients can originate from terrestrial vegetation, soil, industrial and farming discharges (Rabalais et al. 1996, Smith et al. 1999). Nutrients of freshwater origin can thus enhance primary production in coastal areas and therefore can affect food availability for marine communities within a few kilometres of river mouths (Gillanders and Kingsford 2002, Robins et al. 2005, Vorwerk and Froneman 2009). Simultaneously, the load of freshwater input can increase turbidity in coastal areas and therefore affect the light available for benthic and pelagic primary producers (Lehrter et al. 2009, González-Ortegón and Drake 2012), again with consequences for food available for benthic primary consumers (Chanton and Lewis 2002, Urabe et al. 2002).

Over the last few centuries there has been strong growth in human populations, particularly in coastal areas, resulting in increased impact of human activities on natural systems (Harvell et al. 1999, Halpern et al. 2008, Claudet and Fraschetti 2010). Major threats are represented by habitat destruction or fragmentation, building of artificial structures, and degradation of water quality (Mallin et al. 2000, Peters and Meybeck 2000, Bulleri and Chapman 2010). Decreased water quality in coastal areas is the result of both land-based and ocean-based human activities causing eutrophication, increased sediment loads, or chemical and oil pollution (Ryther and Dunstan 1971, Jones et al. 1985, Antizar-Ladislao 2008). For example, a number of studies have recorded negative effects on the seabed beneath and around fish cages due to an accumulation of fish extraction and pseudofaeces that caused pollution and oxygen depletion (Wu 1995, Fernandes et al. 2001). Others showed that mining and resources excretion (*i.e.*

petroleum), in coastal areas and more specifically off river mouths, represent a strong disturbance of the seabed through sedimentation and deposition of waste material (e.g. in Namibia; Sink et al. 2012). Similarly, Duarte (1995) and Verdelhos et al. (2005) have highlighted the remarkable negative effect of eutrophication in altering coastal habitats, while others revealed the positive impact of artificial reefs (Charbonnel 2002).

1.2.3. Local and small scales

At local (from one to a few km) and small (from cm to a few m) spatial scales other factors contribute to variability in coastal primary production. Common factors include local currents, wave exposure, the presence of kelp beds or tidal cycles (Fréchette and Bourget 1985, Eisma and Kalf 1987, Carter 1988, Kingsford et al. 1991, Bustamante et al. 1995). For example, local currents or topographically generated fronts may act as a barrier to food supply to the shore (Iverson et al. 1979). However, these factors are often very chaotic and difficult to predict, being specific of each area investigated and variable in time.

Several studies have shown that wave exposure can affect the growth (Blanchette et al. 2000), distribution (Westerbom and Jattu 2006) and abundance of organisms (Zardi et al. 2007a). In addition, mechanical disturbance by wave action can influence the distribution of primary production in coastal areas, by influencing the turnover rate of particulate matter in the intertidal zone (Eisma and Kalf 1987, Carter 1988, Bustamante et al. 1995).

Kelp forests can also be important. These forests support high primary productivity and they magnify secondary productivity (Duggins et al. 1989, Kelly 2005, Smale et al. 2013). Bustamante et al. (1995) highlighted the importance of kelp detritus on the South African west coast, as it represents an important component of the food for benthic populations. Similarly Schaal et al. (2009) brought attention to the high contribution of kelp-derived organic matter to the diet of filter-feeders in Northern Brittany (France).

The tidal cycle can also influence the food available for benthic populations. Fréchette and Bourget (1985) showed a depletion of particulate organic matter (POM) concentration over an immersed mussel bed passing from high to low tide.

1.3. Diet analyses

Changes in food quality and quantity of food available are difficult to measure directly and studying the diet of organisms represents a more direct way of investigating what is actually ingested and assimilated by organisms (Jeffries 1975, DeNiro and Epstein 1978, Kelly and Scheibling 2012). The commonest approach used in the past to study the diet of organisms was the analysis of stomach contents. This technique provides information on the size and taxonomy of food ingested at the time of sampling (Hyslop 1980). However, it does not provide integrated information on the food assimilated, and only constitutes a snapshot of the diet. There are also problems of differential rates of prey digestion and the accumulation of undigested food items in the stomach. Therefore, the analysis of stomach content is not necessarily a good representation of what animals are feeding on (Reñones et al. 2002, Ruiz-Cooley et al. 2006). In addition, food studies based on stomach-content analyses alone require a large numbers of samples and often involve problems with the identification of prey, especially in the case of detritivores (Cocheret de la Morinière et al. 2003). For example Harrigan et al. (1989) conducted a study on the diet of the fish *Lutjanus griseus* (the gray snapper) from mangrove and seagrass habitats using stomach content and stable isotope analyses. The first technique revealed similar diets between habitats, whereas stable isotopes indicated different diets between mangrove and seagrass habitats.

In the last few decades, there has been increased use of other techniques that can provide information on diets, specifically stable isotope and fatty acid analyses. These techniques are important tools for understanding trophic relationships within natural ecosystems as they provide time-integrated information on the assimilated diet and they can be used to trace the flux of organic matter from producers to consumers (Peterson and Fry 1987, Hobson and Welch 1992, Dalsgaard et al. 2003, Kelly and Scheibling 2012).

Stable isotope (SI) analysis is based on the analysis of the ratios of the stable isotopes of carbon ($^{13}C/^{12}C$) and nitrogen ($^{15}N/^{14}N$). This method relies on the fact that the carbon and nitrogen isotope ratios of an organism reflect the isotopic values of the food consumed (DeNiro and Epstein 1978, 1981) after fractionation through feeding, food processing and excretory processes (Peterson and Fry 1987, Owens 1988). Hence, $\delta^{15}N$ indicates the trophic level of an organism (Kling et al. 1992, Hansson et al. 1997, Vander Zanden et al. 1997), while $\delta^{13}C$ provides information on the food source assimilated (Tieszen et al. 1983, Kaehler et al. 2000). In addition, SI can be used to study within- and among- population variation in trophic regimes (Vander Zanden et al. 2000, Overman and Parrish 2001, Bearhop et al. 2004) as well as individual diet specialization (Matthews and Mazumder 2004). For example, Vander Zanden et al. (2000) with the use of $\delta^{15}N$, found the same species of lake trout (*Salvelinus namaycush)* collected in different lakes in Ontario and Quebec occupied different trophic levels.

Fatty acid (FA) techniques can also be used to study foraging strategy and food web dynamics. Studies have examined the FA composition of a single species, in order to acquire information on the spatial and temporal variation in diet among individuals and within populations. In addition, lipid analyses can give insights to assess FA synthesis pathways, metabolism and reproduction strategies of organisms (Iverson et al. 1997, Freites et al. 2002, Budge et al. 2006). FA can also be used as trophic markers (fatty acid trophic markers, FATM). Some FA or preferably group of FA are specific to particular species or taxa, and can be used to identify the food source (Pascal and Ackman 1975, Bergé and Barnathan 2005, Kelly and Scheibling 2012). The FA composition of an organism can also provide information on the quality of food available (Jónasdóttir 1994, Cotonnec et al. 2001). Thus, FATM can be attributed to one or a few-similar prey types, from which it is possible to assess their importance in an organism's diet (Parrish et al. 2000, Dalsgaard et al. 2003).

SI and FA are also used in the modelling field (Bearhop et al. 2004, Iverson et al. 2004). Both techniques can be related to statistical models that can provide a quantitative estimation of each prey in the diet of the predators. A critical difference between the two techniques is the different integration time of the elements (*i.e.* FA vs SI). Previous

studies showed that the turnover time for the SI of the adductor muscle of mussels is about 9 months (Hill and McQuaid 2009), whereas the turnover for FA is less than a month (Pirini et al. 2007), indicating that SI provide a more integrated and conservative information than FA.

1.4. Study aims and thesis overview

In the last few decades, research focused on how meso and large scale processes affect benthic–pelagic linkages, with examination of the consequences for diversity, biology and food availability in coastal environments, and ultimately for the functioning of entire ecosystems (Abbott and Zion 1987, Ellis et al. 2000, Thrush et al. 2000, Russell et al. 2005). In particular, food availability can affect benthic populations, with strong consequences on food web dynamics and ecosystem structure. However, very little information is available on the effect of factors operating at different spatial and temporal scales on food availability and thus on the diet of intertidal consumers. The present work aims to increase the current understanding of the effect of factors operating from large to local spatial scales and at temporal scale on the dietary regime of benthic filter feeders. By using two complementary techniques, SI and FA analyses, this work examines the potential effect of upwelling, biogeography, urbanization and freshwater input on the dietary regimes of five intertidal filter feeders that co-exist on rocky shores: two mussels and three barnacles' species. This aim is addressed in five working chapters. A preliminary study investigated the diet of two species of mussels, an invasive and a native species that co-occur on the South African coast. This was conducted in order to assess if the two species display differences in FA and SI (Chapter 2). The four following chapters assessed the effects of urbanization (Chapter 3), freshwater input (Chapter 4), biogeography (Chapter 5) and upwelling (Chapter 5 and 6) on the FA and SI signatures of benthic filter feeders. Each of these studies was conducted at several sites along the South African coast and tested the effect of the specific factor on several species of filter feeders, in order to assess within-species variability. Chapter 7 provides a synthesis of the main findings provided in the previous chapters.

1.5. Study area

The South African coast can be divided into three biogeographic provinces that correspond to the three coasts: west, south and east, with transitional areas between the main provinces (Emanuel et al. 1992, McQuaid and Payne 1998, Harrison 2002, Bolton et al. 2004). The cool-temperate west coast extends from the Namibian border to the Cape of Good Hope, the warm-temperate south coast ranges from Cape Agulhas to Port St. Jones, while the subtropical east coast spreads from Port St. Jones to the Mozambique border (Harrison 2002, Teske et al. 2006).

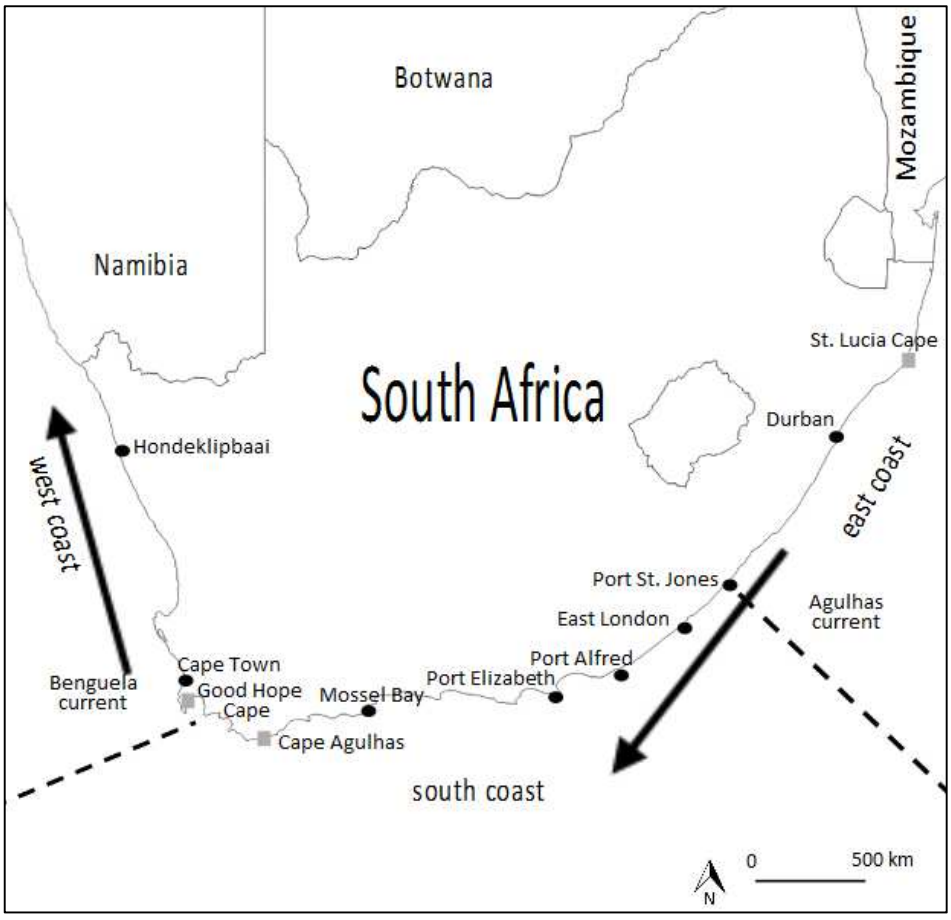


Fig 1.1 Map of South Africa illustrating the three biogeographic provinces.

Each of the three biogeographic provinces are characterized by different hydrographic conditions. The west coast is dominated by the Benguela Current, eutrophic cold water (11-16 °C; Fig 1.2, Fig 1.3) with Southern Ocean origins which flows from south to north, and that upwells at several locations on the west coast (Andrews and Hutchings 1980, Demarcq 2009). The Benguela is a highly productive system that supports a large

diversity of primary producers and consumers (Shannon et al. 1983, Shannon and Nelson 1996, Fennel 1999). The south coast and east coasts are dominated by the oligotrophic warm water Agulhas Current (22-26 °C, Fig 1.2, Fig 1.3; Probyn et al. 1994, Lutjeharms 2006, Backeberg et al. 2008). This water originates from the Mozambique Channel and flows from the north-east part of the coast towards the south-west. The limit between these two provinces located in the region of East London, is not as distinct as the one differentiating the west coast from the south coast. It seems that the main difference between the east and south provinces is related to dissimilarity in water temperature with the south coast being cooler than the east coast. This is probably due to the presence of a wide continental shelf in the region of East London that pushes the Agulhas Current further offshore, and thus the coastal water on the south coast becomes slightly cooler (Lutjeharms 2006).

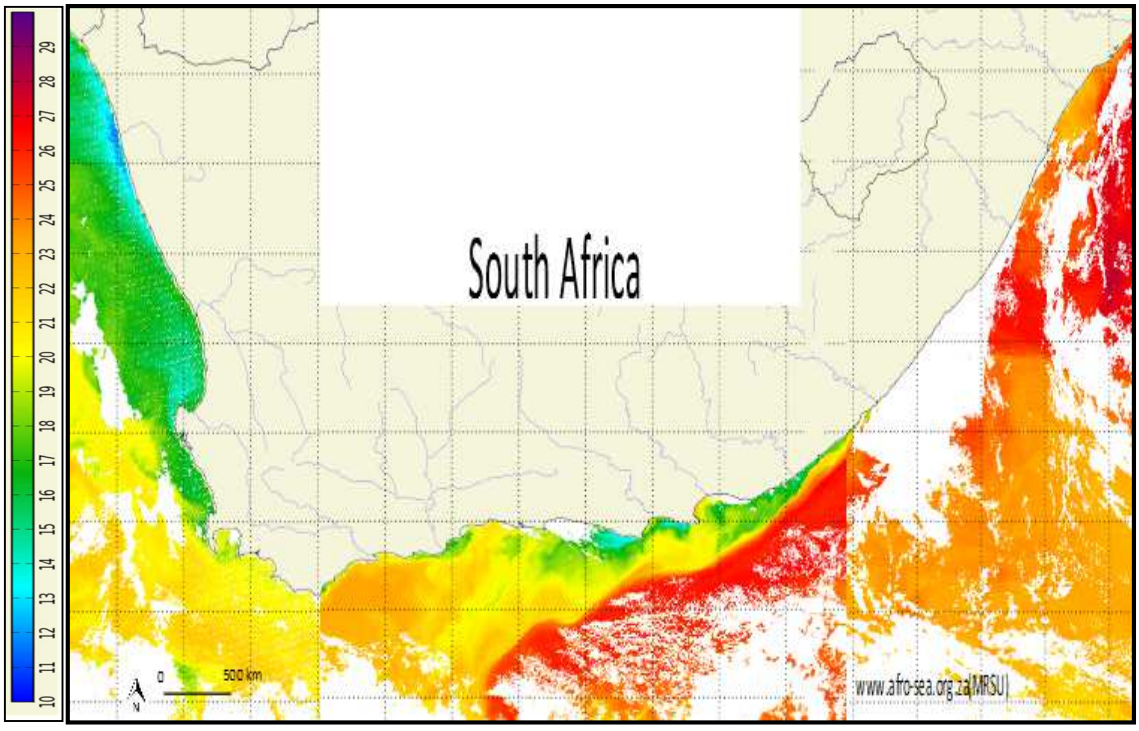


Fig 1.2 Marine remote sensing unit (MRSU) satellite image of sea surface temperature (SST) on the South African coast on the 22th of December 2013.

The three coasts experience events of upwelling that differ profoundly in their intensity and frequency. The Benguela Current on the west coast enhances wind-driven upwelling events that occur over the summer season along the Cape Peninsula, and become more frequent moving northward to around the region of Hondeklipbaai (Fig 1.1.; Andrews

and Hutchings 1980). This system, is one of the major upwelling systems of the world's oceans (Carr and Kearns 2003, Chavez and Messié 2009). The south coast supports a semi-permanent cell of continuous upwelling in the region of Port Alfred (Fig 1.1). Although this coast experiences more frequent events of upwelling over the year than the west coast, the upwelling events are much less intense (Schumann et al. 1982, Lutjeharms et al. 2000). The east coast has an upwelling cell off Cape St. Lucia (Fig 1.1), however upwelling events there, are very rare and weak (Lutjeharms 2006).

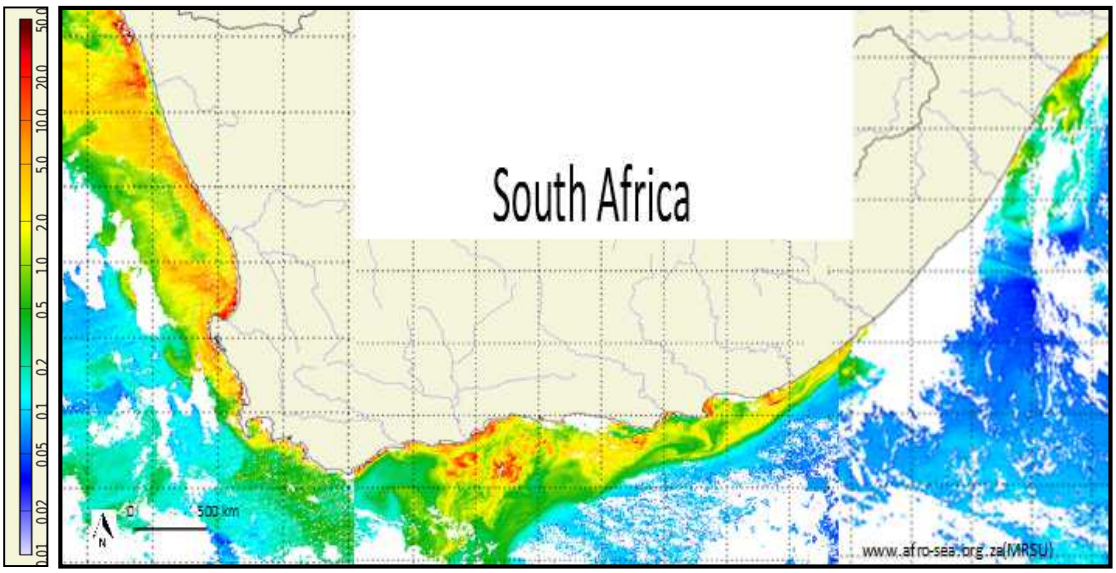


Fig 1.3 MRSU satellite image of chlorophyll *a* (mg m^{-3}) on the South African coast on the 22th of December 2013.

The South African coast supports only a few cities with high levels of urbanization. Cape Town is located close to the conjunction of the west and south coasts and is the coastal city with the highest population (~ 4 million), followed by Durban on the east coast (~ 3 million; "Census 2011- Cape Town" 2011, "Census 2011- Durban" 2011). The west coast has only a few very small towns and besides Cape Town and Durban, most of the larger towns or cities with relatively high levels of urbanization are present on the south coast. In particular Port Elizabeth, East London and Mossel Bay, although small by the standards of other countries, have the highest numbers of inhabitants (range between 1 million and 120000 ; "Census 2011- East London" 2011, "Census 2011- Mossel Bay" 2011, "Census 2011- Port Elizabeth" 2011). In addition, the wastewater of these cities runs straight into the ocean due to poor infrastructure for the recycling of wastewater

and the existence of high density of informal settlements. None of these three cities extends far inland, but all occupy long stretches of the coast (between 13 and 30 km).

South Africa is a semi-arid country and the highest numbers of rivers with temporarily or permanently open estuaries are found on the south and especially on the east coasts, therefore the majority of rivers flow into the Indian Ocean (Whitfield and Bate 2007). The east coast has a subtropical climate and rainfall varies seasonally, with 80% of the annual total precipitation (900-1000 mm) occurring in the summer months (Cooper 1993). Most of the estuaries of this area are temporarily closed during the winter dry season due to low river discharge (Whitfield and Bate 2007).

The present study was conducted on the intertidal rocky shore of South Africa. The intertidal zone represents the transition between the terrestrial and the marine environments (Levin et al. 2001). The principal characteristic of this zone is that it is affected by tidal cycles, so that this area is alternately immersed in water and exposed to air (Little et al. 1996). Tidal ranges vary enormously around the world, but along the coast of South Africa, the range is approximately 2 m. The intertidal shore can be classified as exposed or sheltered based on wave exposure. Sheltered shore are usually within bays or enclosed areas, while exposed areas include, for example, capes or headlands. Water turnover on exposed shores is about seven times greater than on sheltered shores and the degree of wave exposure can differ at small scales depending on several aspects for example geomorphology or hydrographic of the specific area (Bustamante and Branch 1996b). In the present study, the sites chosen for the comparison all had similar wave exposure and within the South African context would be regarded as moderately exposed.

1.6. Study species

The species studied were five species of filter feeders, chosen because they are widely distributed around the South African coast. These were two mussels, *Perna perna* (Linnaeus) and *Mytilus galloprovincialis* (Lamarck), and three barnacles, *Chthamalus dentatus* (Krauss), *Octomeris angulosa* (Sowerby) and *Tetraclita serrata* (Darwin).

P. perna is native to South Africa and characteristic of the east and south coasts and its size range varies between 80 and 125 mm (Branch et al. 2007). On the east coast it is the dominant mussel species, while on the south coast it occupies the low and mid mussel zones (Zardi et al. 2006, 2008).

M. galloprovincialis, the Mediterranean blue mussel, was accidentally introduced to South Africa around 1970 (Grant and Cherry 1985) and is the most successful mussel species on intertidal shores on the South African coast (Robinson et al. 2005, Griffiths et al. 2009). This species co-exists with *P. perna* on the south coast (Bownes and McQuaid 2006), where it is mostly present on the mid and upper mussel zone (Fig 1.4). Adult size range varies between 60 and 140 mm (Branch et al. 2007).

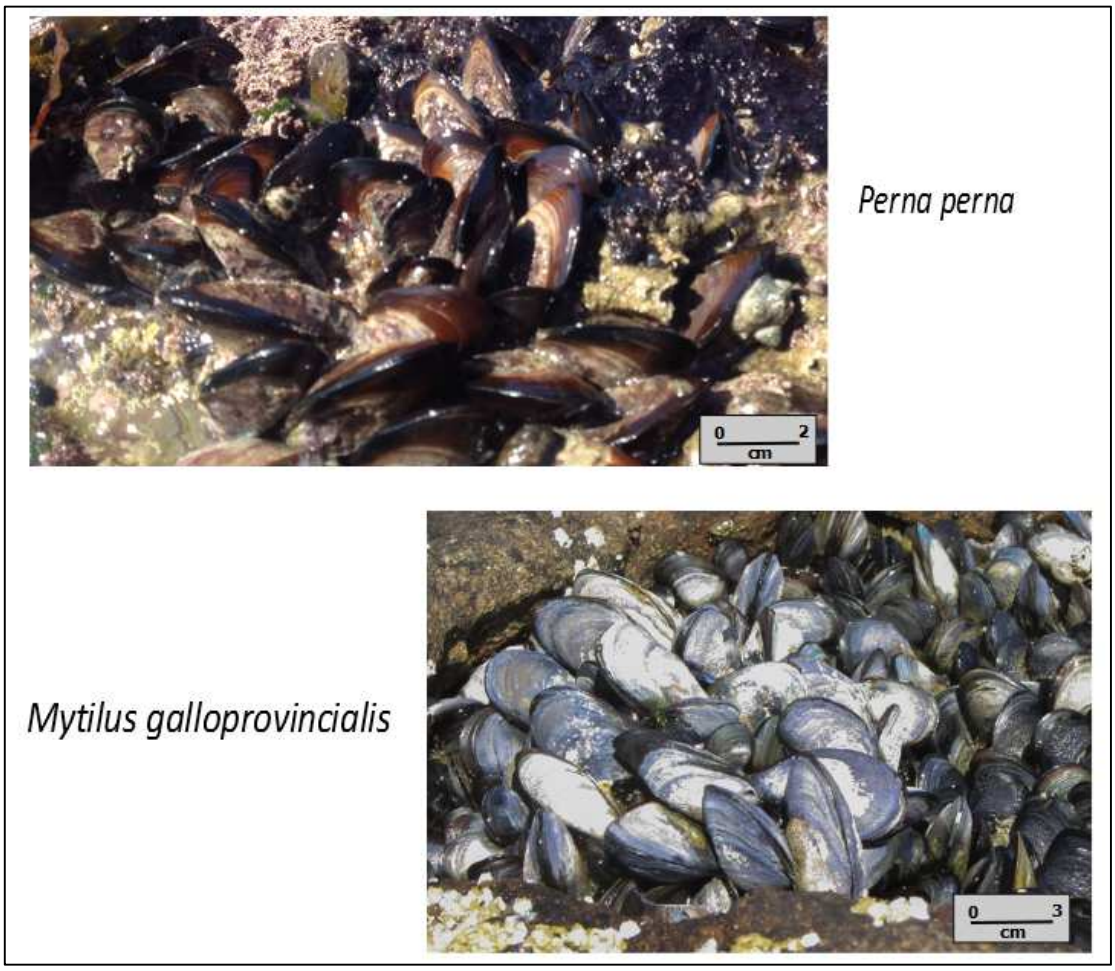


Fig 1.4 The two mussels species used in this study: *Perna perna* and *Mytilus galloprovincialis*.

C. dentatus is a small species of barnacle (size range 5 - 10 mm), with a wide geographic distribution which covers the entire South African coastline. It is common in the upper intertidal zone (Fig 1.5; Dye 1998, Branch et al. 2007).

O. angulosa is a barnacle that occurs in the mid and low intertidal zones, characteristic of wave-exposed areas on all three coasts of South Africa. Its size range is between 10 and 25 mm and individuals can form extensive aggregations (Fig 1.5; Boland 1997).

T. serrata is a volcano shaped barnacle common along the whole coast of South Africa. It occurs on the mid intertidal shore and preferentially in more sheltered areas than *O. angulosa*. Adult size is about 20 mm (Fig 1.5; Boland 1997a, Branch et al. 2007).

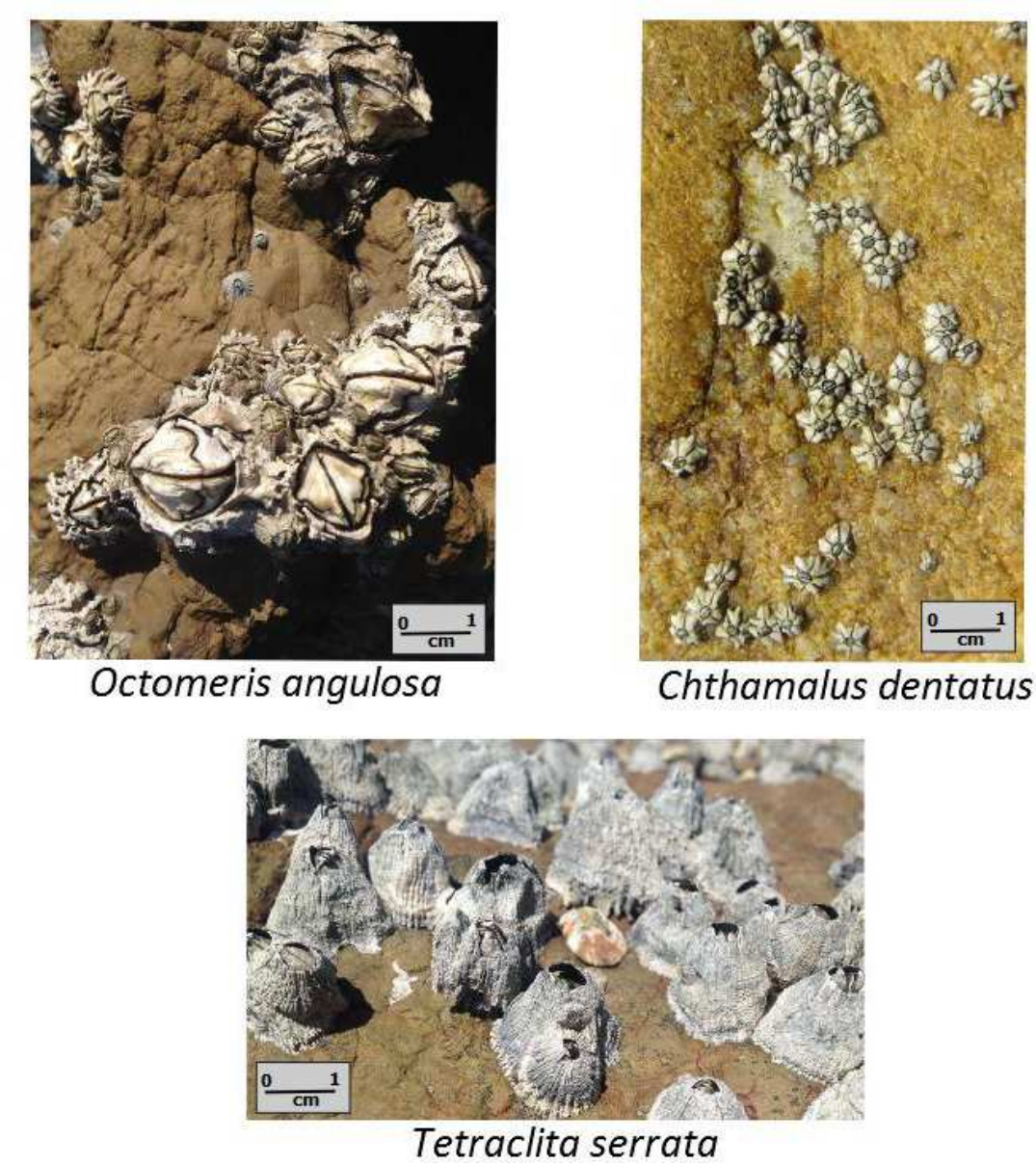


Fig 1.5 The three barnacle species of this study: *Octomeris angulosa, Chthamalus dentatus* and *Tetraclita serrata.*

CHAPTER 2

Diet partitioning between an invasive and an indigenous species of mussels

Perna perna and *Mytilus galloprovincialis- south coast of South Africa*

2. Diet partitioning between an invasive and an indigenous species of mussel

2.1. Introduction

The intentional or accidental introduction of exotic species represents an important stressor for coastal marine ecosystems (Grosholz 2002, Bax et al. 2003, Carlton 2009, Vilà et al. 2009). Species invasions can have profound ecological impacts, including changes in patterns of distribution, abundance and diversity of native species (Benedetti-Cecchi et al. 2006, Claudet and Fraschetti 2010). Often non- endemic species become predominant in term of abundance and lead to ecosystem homogenization by reducing food-web complexity (Stachowicz et al. 1999). Hence non-endemic species can drastically alter ecosystem functioning. Although many studies have tried to determine the factors responsible to the susceptibility of a community to invasion, habitats appear to vary in their resistance to invasions. In the marine environment, it seems that temperature, predation, competition for space or exposure to wave action are potential factors responsible for invasions success (deRivera et al. 2005, Riel et al. 2006, Stachowicz and Byrnes 2006, Sousa et al. 2008). For example Rius and McQuaid (2009) in a study conducted on two species of mussel (a native and an invasive species) showed the invasive species was less resistant to wave action compared to the native. Schneider and Helmuth (2007) indicated in another study that both the local and geographic distributions of a native and an invasive intertidal mussel species were mainly driven by physiological stress associated with aerial exposure. An aspect that may have been underestimated, however, is if the food environment can affect invasive species success. Coastal ecosystems are profoundly influenced by the physical processes that affect the water column and drive food and nutrients delivery to benthic populations (Rohde 1999, Huston and Wolverton 2009, Smith et al. 2009). The availability and quality of food affect the metabolism, biomass and survival of heterotrophic organisms, with consequences on ecosystem functioning (Raimondi 1990). Changes in food sources or food availability can compromise the survival of indigenous benthic organisms and thus facilitate invasive species success. This could have important consequences for the rest of the ecosystem by modifying the structure and dynamics of benthic rocky shore communities (Connolly et al. 2001). Hence, evaluating the relationship between species invasion and

food availability could contribute to understand how and why colonization by invasive species succeeds.

Mytilus galloprovincialis is one of the most widely spread marine invasive species, occurring on all continents except Antarctica (Hockey and van Erkom Schurink 1992, Branch and Steffani 2004, Robinson et al. 2005) and it was accidently introduced on the South African coast in the late 1970s (Grant and Cherry 1985). In South Africa, *M. galloprovincialis* has largely replaced the native mussel species *Aulacomya ater* on the west coast, and coexists with the native *Perna perna* on the south coast (Hockey and van Erkom Schurink 1992, Robinson et al. 2005, Griffiths et al. 2009). Where the two species co-occur on the south coast, *M. galloprovincialis* usually occupies the mid and upper mussel zone, which is less exposed to wave action and sand stress, while *P. perna* is limited to the mid and low mussel zones, where desiccation stress is lower (Robinson et al. 2005, Bownes and McQuaid 2006, Zardi et al. 2008). Several studies have identified possible explanations for the success of *M. galloprovincialis* in South Africa (Zardi et al. 2006, 2008, Rius and McQuaid 2009), however no information on the effects of food availability exist. The two species are filter feeders with a similar size range and as such, it is possible to hypothesize that they have a similar broad diet. However, it is well accepted that filter feeders can be selective (Drenner et al. 1984, Baker and Levinton 2003, Heidman et al. 2012) and can differ in their filtering efficiency (Tenore and Dunstan 1973), however no studies have investigated diet differences between *P. perna* and *M. galloprovincialis*.

The present study aims to investigate if the diets of the invasive *Mytilus galloprovincialis* and the indigenous *Perna perna* differ where they co-occur in mixed-species mussel beds. In particular, the study aimed to assess if the two species rely on different food by using fatty acid (FA) and stable isotope (SI) approaches.

2.2. Materials and Methods

2.2.1. Study area and samples collection

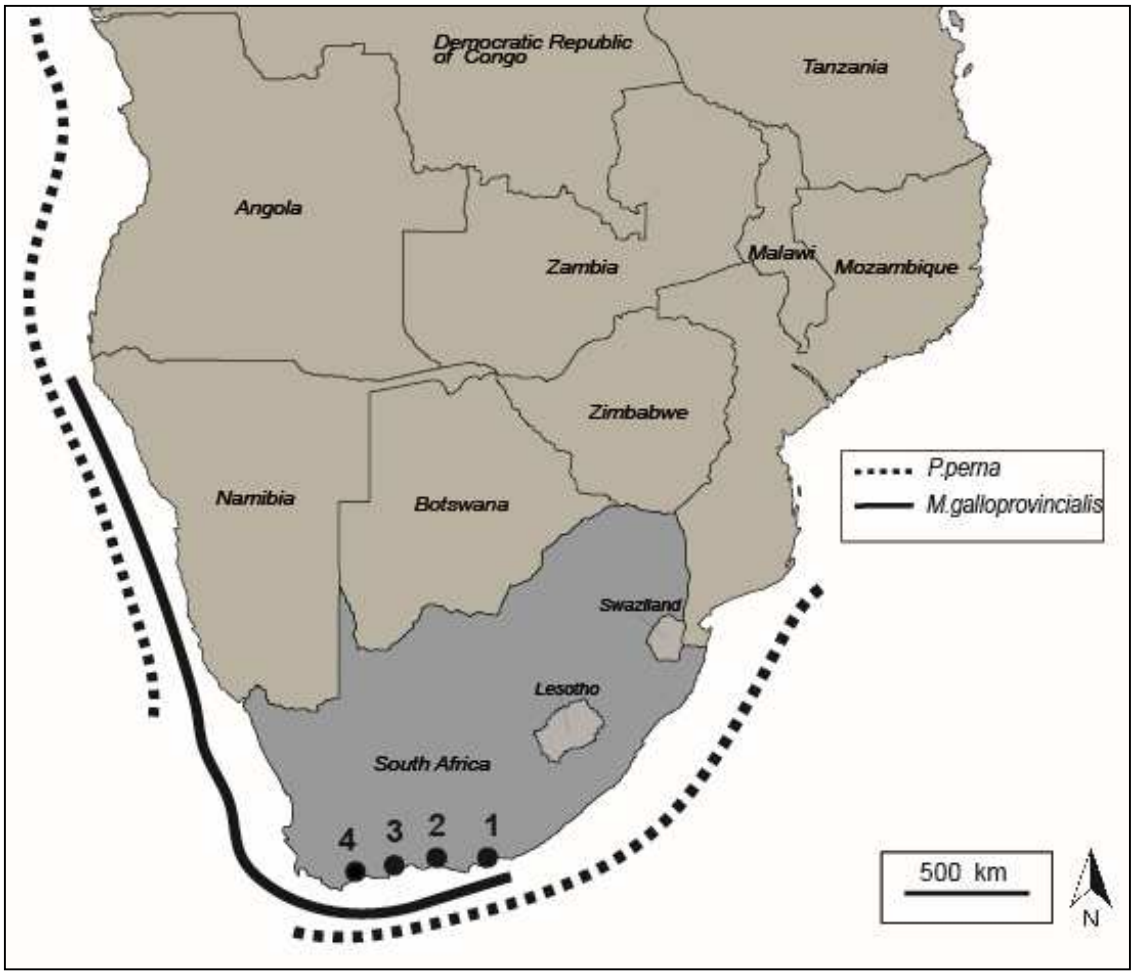


Fig 2.1 Geographic distribution of *Mytilus galloprovincialis* and *Perna perna* along the Southern African coastline and the four sampling sites: Port Elizabeth (site 1), Brenton on Sea (site 2), Mossel Bay (site 3) and Jongensfontein (site 4).

The study was conducted along the South African south coast where the two species of mussels co-occur (Fig 2.1, 34.4-33.3 S° 21.3-26.5 E). *P. perna* is present on the south and east coasts of South Africa, along the Namibian coast and in southern Mozambique, whereas the introduced *M. galloprovincialis* occurs on the South African south and west coasts, extending northwards into Namibia (Fig 2.1). *P. perna* was never present on the South African west coast, even before the introduction of *M. galloprovincialis*.

In order to compare the diets of the two mussel species, samples were collected at four sites where they co-occur in June 2012: Port Elizabeth (site 1), Brenton on Sea (site 2), Mossel Bay (site 3) and Jongensfontein (site 4). Port Elizabeth and Mossel Bay

are sites characterized by relatively high levels of urbanization and industrial activity along the coast, with human populations of 1.3 million and 117 840 for Port Elizabeth and Mossel Bay, respectively ("Census 2011- Mossel Bay" 2011, "Census 2011- Port Elizabeth" 2011). Brenton on Sea experiences sporadic and relatively weak seasonal upwelling events (Schumann et al. 1982) and Jongensfontein is not exposed to either anthropogenic, upwelling or riverine influences. At each site, samples of the two species were taken at two locations, separated by 1 - 3 km, from mixed species mussel beds at the same height on the shore. Three replicates of each species were collected for the FA analyses and five replicates for the SI analyses. The adductor muscle of each replicate was chosen for the comparison due to its low turnover rate (Gorokhova and Hansson 1999). Live animals were transported on ice to the laboratory (2 - 3 h) in order to decrease their metabolic rates and thus potential degradation of their tissues. Each replicate was dissected; the tissue was washed with distilled water in order to remove sand and shell fragments, and placed in a 2 ml cryotube. The samples were then flash frozen in liquid nitrogen to stop degradation of lipids and then transferred to a -80 °C freezer until processing.

2.2.2. Diet analysis

2.2.2.1. Isotopic analysis

In the laboratory, samples for SI analyses were dried at 60 °C for 48 h. The samples were ground into a fine powder with ball mills and 1 mg subsamples were placed into tin foil capsules. Samples were analysed for stable isotope ratios of carbon ($^{13}C/\ ^{12}C$) and nitrogen ($^{15}N/\ ^{14}N$) using a continuous flow Isotopic Ratio Mass Spectrometer (Europa Scientific 20 - 20 IRMS linked to ANCA SL Prep Unit) at the IsoEnvironmental Laboratory, Rhodes University, Grahamstown, South Africa. Results are expressed in standard unit notation as:

$$\delta X = ([R\ sample/R\ standard] - 1) \times 1000$$

where X is ^{13}C or ^{15}N, R is the ratio of $^{13}C/ ^{12}C$ or $^{15}N/ ^{14}N$. Beet sugar, ammonium sulphate and casein were used as standard, calibrated against multiple International Atomic Energy reference standards. Measurement precision for both carbon and nitrogen was ± 0.05 ‰.

2.2.2.2. Fatty acid analysis

Mussel samples were lyophilized (VirTis BenchTop K) for 24 h. Subsequently they were stored in a -80 °C freezer until processed. Total lipids were extracted and trans-esterified using a modified Indarti one step procedure (Indarti et al. 2005) within six months of collection. Samples were homogenized into a four mL fresh solution of a mixture of methanol, concentrated sulphuric acid, and chloroform containing 0.01 % of an anti-oxidant, BHT (butylated hydroxytoluene) (1.7:0.3:2.0 v/v/v), and closed under nitrogen in lipid clean test tubes. The extraction and transesterification reactions occurred at 100 °C for 30 min. The FA methyl esters (FAME) hence formed were then stored at -80 °C until Gas Chromatography (GC) analyses. FAME composition of each sample was determined by GC (Agilent Technologies 7890A, at the National Research Foundation (NRF) Fatty Acid Facility at Rhodes University, Grahamstown, South Africa) equipped with a ZB-Waxplus capillary column (ZB-Waxplus 320 column), with helium as the carrier gas at a flow rate of 1.664 ml min^{-1}. The injector was at a temperature of 250 °C. The flame ionization detector was set at 260 °C, and the oven was initially set at 70 °C. After one min, the oven temperature was increased by 40 °C min^{-1} until 170 °C and then raised to 250 °C at a rate of 2.5 °C min^{-1} and held for 4.5 min. Peaks were integrated using GC ChemStation software (Agilent Technologies, version B.04.02), identified by comparison with retention times of external standards (37 component fatty acid methyl ester mix Supelco, marine PUFA no. 1 Supelco, menhaden oil PUFA no. 3, bacterial acid methylesters mix Supelco), as well as by mass spectrometry analyses (Agilent Technologies 7000 GC/MS Triple Quad; Agilent Mass Hunter (MS), version B.05.00) using the NIST library. Each FA was measured as a proportion of the total fatty acid (TFA) composition (weight % of TFA) and peak areas were corrected according to the FID response to FA chain length (Ackman 2002). FA are reported using a shorthand notation of A:Bw*x*, where A indicates the number of carbon atoms, B is the number of double

bonds and *x* indicates the position of the first double bond relative to the terminal methyl group (Budge et al. 2006).

Amongst the common FA trophic markers (FATM), it is possible to find bacterial FA (BAME) which are the sum of *iso*- and *anteiso*-branched chain FA and unbranched 15:0 and 17:0 FA (Wakeham and Beier 1991, Volkman et al. 1998, Budge et al. 2001). The FA 16:1w7 and 20:5w3 (EPA) are characteristic of diatoms, while the FA 22:6w3 (DHA) and 18:4w3 are prevalent in dinoflagellates (Parrish et al. 2000, Dalsgaard et al. 2003). Macroalgae are usually rich in w-6 FA and more specifically kelps are characteristically 20:4w6 enriched (Hanson et al. 2010) while microalgae are rich in w-3 FA (Dalsgaard et al. 2003). Non-methylene-interrupted FA (NMI) are synthesised *de novo* by marine filter feeders from other FA such as 16:1w7 and 18:1w9, which are very abundant in their food (Pirini et al. 2007, Barnathan 2009). Essential FA for filter feeders are 20:4w6, 20:5w3 and 22:6w3 (Alkanani et al. 2007).Vascular plants are characterized by long chain (LCFA, > 24 atoms of carbon) of saturated FA (SFA) and 18:2w6 and 18:3w3 (Parrish et al. 2000, Dalsgaard et al. 2003, Kelly and Scheibling 2012). In order to assess if the specimens of this study had long chain FA, a few tests were run for 60 min in the GC machine (compared to the standard time of 40 min), however no LCFA were recorded in this work.

2.2.3. Data analysis

2.2.3.1. Stable isotopes

To examine possible differences in $\delta^{13}C$ and $\delta^{15}N$ signatures of the native and invasive mussels, a mixed model design consisting of three factors was performed: species (two levels, fixed), site (four levels, random and crossed with species) and location (two levels, random, nested in site and crossed with species). ANOVA, followed by Tukey HSD *post-hoc* tests, was performed in order to assess significant effects. Levene's test was used to test for homogeneity of variances. All analyses were performed using STATISTICA v12 (StatSoft Inc. 2012).

2.2.3.2. Fatty acids

The FA composition of the two mussel species was compared with the same design as for the SI analyses. A Multivariate Permutation Analysis (PERMANOVA; Anderson 2001) on Bray-Curtis dissimilarities was used in order to assess differences among factors. Each term in the analysis was tested using > 9999 permutations as the relevant permutable units (Anderson and Braak 2003). Principal component analysis (PCA), a non-constrained explorative multivariate analysis, was used to explore differences in FA signatures among species. PCA describes relationships among variables by reducing a large number of variables to a few components. These components are calculated to maximise the projected variance of the samples by combining correlated variables into new components (Clarke and Gorley 2006). Proportions of FA were transformed with a square root function prior to statistical analyses. The combined results of the PCA and SIMPER (similarity percentage, PRIMER) were used to assess which FA were influencing the principal components of the PCA and the differences among groups of samples. Only FA forming more than 1 % TFA were considered for the analyses. The analyses were conducted using the PRIMER v6 and PERMANOVA+ add-on package of PRIMER v6 (Clarke and Gorley 2006, Anderson et al. 2008).

2.3. Results

2.3.1. Stable isotopes

ANOVA highlighted significant effects of the factors species, site and their interaction for both isotopic elements, with no significant effects of location for either species (Table 2.1). However, Tukey HSD *post-hoc* test showed the only difference between the two species was at site 2 (Fig 2.2; Tukey HSD, $p < 0.001$), where *P. perna* was significantly enriched in $\delta^{15}N$ and depleted in $\delta^{13}C$ compared to *M. galloprovincialis:* $\delta^{15}N$ = 9.04 ‰ and 8.53 ‰, $\delta^{13}C$ -16.28 ‰ and -15.74 ‰ for *P. perna* and *M. galloprovincialis* respectively.

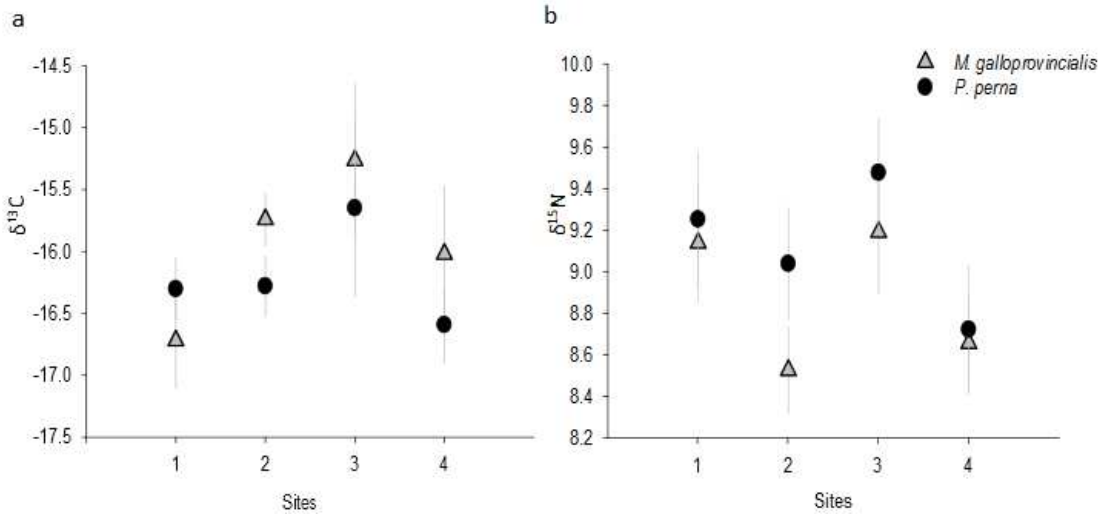


Fig 2.2 Stable isotope nitrogen (a) and carbon (b) signatures (mean ± SD; n = 5) of *M. galloprovincialis* and *P. perna* at four sites along the South African south coast where the two species co-occurred. Site 1: Port Elizabeth, 2: Brenton on Sea, 3: Mossel Bay and 4: Jongensfontein. Values indicate means and error bars represent standard deviation.

Considering the two species together, strong dissimilarities in $\delta^{15}N$ and $\delta^{13}C$ signatures were found among sites (Table 2.1). At sites 1 and 3, both species had higher $\delta^{15}N$ than *M. galloprovincialis* from site 2 and 4 (Fig 2.2, b; Tukey HSD, $p < 0.01$). In addition $\delta^{13}C$ of *M. galloprovincialis* at site 2 was not significantly different from $\delta^{13}C$ of either species at sites 3 and 4 (Fig 2.2, a; Tukey HSD, $p > 0.05$), whereas it was higher than for both species at site 1 (Fig 2.2, a; Tukey HSD, $p < 0.01$). *P. perna* at site 2 did not have a different $\delta^{15}N$ or $\delta^{13}C$ signature from specimens of either species at sites 1, 3 and 4 (Fig 2.2; Tukey HSD, $p > 0.05$ for both elements).

Table 2.1 Results of ANOVA performed on stable isotope values for two species of mussels (*M. galloprovincialis* and *P. perna*) co-occurring in the same sites. Sp = Species, Si = Site, Loc = Location; df = degrees of freedom, MS = mean square; * *p*, 0.05; ** *p*, 0.01; *** *p*, 0.001.

	$\delta^{13}C$				$\delta^{15}N$			
	df	**MS**	**F**	**p**	**df**	**MS**	**F**	**p**
Species (Sp)	1	1.46	11.47	**	1	0.96	10.33	**
Site (Si)	3	4.20	33.07	***	3	1.84	19.73	***
Loc (Si)	4	0.06	0.61		4	0.05	0.54	
Sp x Si	3	1.08	8.49	***	3	0.26	2.80	*
Sp x Loc (Si)	4	0.05	0.38		4	0.17	1.83	
Error	64	0.13			64	0.09		

2.3.2. Fatty acid composition

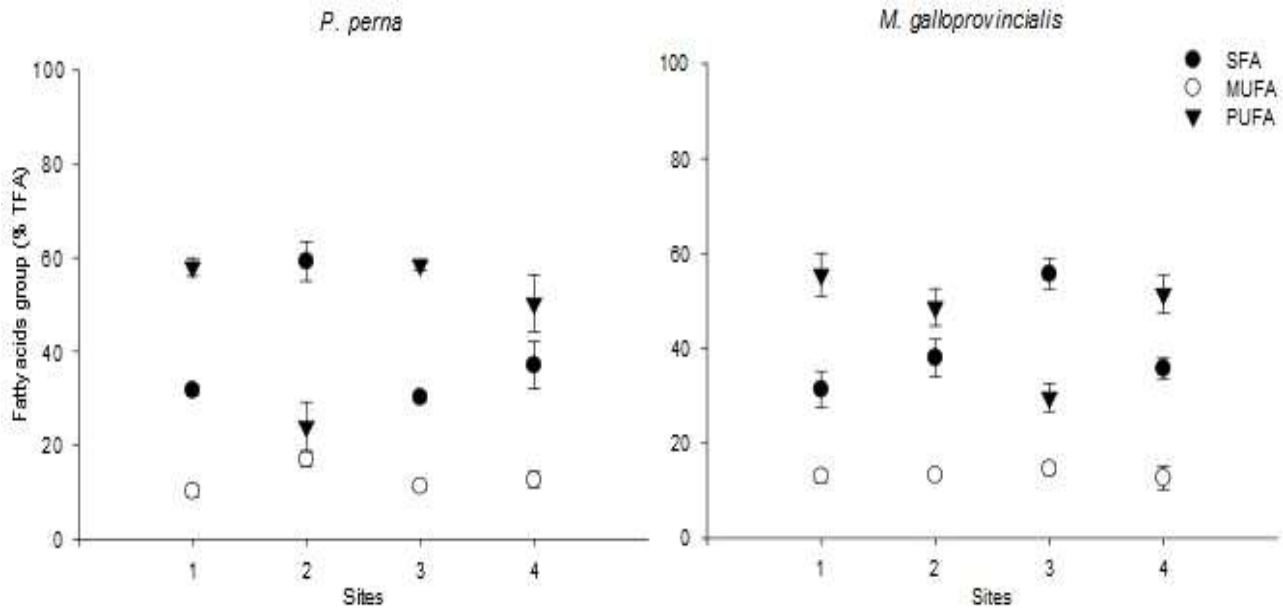


Fig 2.3 Saturated fatty acids (SFA), monounsaturated fatty acids (MUFA) and polyunsaturated fatty acids (PUFA) (mean ± SD) of *P. perna* (a) and *M. galloprovincialis* (b) collected at four sites on the South African south coast. TFA: total fatty acids.

The two mussel species had similar FA composition, with thirty-two FA contributing > one % of TFA in each species (Table 2.2). In both species, NMI FA formed 10 to 20 % of TFA (Table 2.2). Polyunsaturated FA (PUFA) contributed the highest percentage of TFA at all sites and in both species (approximately 50 % or more), with the exception of site 2 for *P. perna* and site 3 for *M. galloprovincialis*, where they comprised 24.02 % and 29.64 % of TFA, respectively (Fig 2.3; Table 2.2). In both species, the contribution of MUFA was between 10 and 16 % of TFA (Fig 2.3; Table 2.2)

Table 2.2 Fatty acid composition of *P. perna* and *M. galloprovincialis* (% of TFA) at four sites on the South African south coast. The values are percentages expressed as mean ± standard deviation. Only FA contributing >1 % of TFA are displayed below.

	Perna perna				***Mytilus galloprovincialis***			
TFA	**Site 1 Port Elizabeth**	**Site 2 Brenton on Sea**	**Site 3 Mossel Bay**	**Site 4 Jongensfontein**	**Site 1 Port Elizabeth**	**Site 2 Brenton on Sea**	**Site 3 Mossel Bay**	**Site 4 Jongensfontein**
14:0	2.20 ± 0.31	4.08 ± 0.71	1.71 ± 0.45	2.00 ± 0.32	0.98 ± 0.21	1.21 ± 0.39	1.19 ± 0.44	0.88 ± 0.11
16:0	16.42 ± 0.56	33.65 ± 2.38	16.87 ± 1.00	20.73 ± 2.91	17.96 ± 3.01	22.70 ± 1.98	30.88 ± 2.54	20.75 ± 1.27
16:1w7	2.73 ± 0.30	3.06 ± 0.52	2.65 ± 0.50	2.56 ± 0.34	1.93 ± 0.54	1.91 ± 1.17	0.84 ± 0.37	1.53 ± 0.55
C18:0	7.36 ± 0.45	12.87 ± 1.96	6.75 ± 0.93	8.65 ± 1.33	7.25 ± 1.42	8.67 ± 1.84	15.02 ± 1.25	8.68 ± 0.99
18:1w9	0.89 ± 0.15	1.98 ± 0.49	1.28 ± 0.30	1.38 ± 0.15	1.12 ± 0.32	1.21 ± 0.46	1.93 ± 0.27	1.04 ± 0.33
18:1w7	1.73 ± 0.88	2.06 ± 0.24	1.80 ± 0.40	2.04 ± 0.35	2.14 ± 0.14	1.71 ± 0.50	1.46 ± 0.46	1.64 ± 0.23
18:2w6	2.53 ± 0.28	1.17 ± 0.40	2.99 ± 0.66	2.41 ± 0.44	1.49 ± 0.40	1.39 ± 0.81	0.47 ± 0.38	1.17 ± 0.17
18:3w3	1.14 ± 0.13	0.40 ± 0.47	1.14 ± 0.24	0.77 ± 0.28	0.59 ± 0.28	0.71 ± 0.25	0.71 ± 0.21	0.46 ± 0.17
18:4w3	0.93 ± 0.17	0.34 ± 0.58	1.08 ± 0.24	0.58 ± 0.21	0.74 ± 0.30	0.58 ± 0.18	0.50 ± 0.38	0.48 ± 0.17
20:1w11	1.78 ± 0.28	1.40 ± 0.24	1.93 ± 0.32	1.58 ± 0.29	2.43 ± 0.30	2.42 ± 0.71	1.38 ± 0.20	2.73 ± 0.44
20:1w9	2.75 ± 0.18	7.02 ± 1.02	2.95 ± 0.24	4.27 ± 1.01	4.57 ± 0.70	5.53 ± 1.27	8.34 ± 0.50	5.03 ± 1.00
20:1w7	0.35 ± 0.53	1.45 ± 0.23	0.66 ± 0.22	0.76 ± 0.29	0.87 ± 0.18	0.52 ± 0.14	0.69 ± 0.41	0.76 ± 0.33
20:2 NMI1	6.00 ± 0.87	2.38 ± 0.62	5.67 ± 0.83	4.14 ± 0.64	5.68 ± 0.58	5.50 ± 0.99	2.33 ± 0.30	5.82 ± 1.10
20:2 NMI2	0.79 ± 0.54	1.17 ± 0.46	0.44 ± 0.20	0.63 ± 0.23	1.08 ± 0.41	1.37 ± 0.68	0.82 ± 0.40	1.43 ± 0.37
20:4w6	5.36 ± 0.57	1.97 ± 0.51	7.01 ± 1.54	4.96 ± 0.72	6.58 ± 0.36	4.90 ± 1.38	2.46 ± 0.48	5.14 ± 0.66
20:5w3	7.60 ± 0.71	1.53 ± 0.62	6.47 ± 0.50	5.39 ± 1.61	9.40 ± 2.12	7.39 ± 1.57	1.38 ± 0.34	7.36 ± 1.15
22:2w6	2.20 ± 0.43	1.18 ± 0.40	2.66 ± 0.62	2.00 ± 0.44	0.90 ± 0.48	0.77 ± 0.31	2.39 ± 1.26	1.16 ± 0.29
22:2 NMI1	5.98 ± 0.67	2.59 ± 0.91	6.09 ± 0.82	5.32 ± 0.78	6.35 ± 0.55	4.96 ± 0.82	3.61 ± 1.63	5.17 ± 0.81
22:2 NMI2	1.05 ± 0.31	2.00 ± 1.35	1.22 ± 0.72	1.47 ± 0.50	0.91 ± 0.75	0.37 ± 0.50	6.01 ± 1.54	1.68 ± 1.79
22:3 NMI	1.10 ± 0.38	0.44 ± 0.71	1.87 ± 0.09	1.44 ± 0.33	1.42 ± 0.17	1.71 ± 0.17	0.63 ± 0.12	1.78 ± 0.40
22:4w6	1.68 ± 0.16	0.31 ± 0.36	1.98 ± 0.78	1.42 ± 0.48	0.80 ± 0.08	0.96 ± 0.99	0.84 ± 0.42	0.59 ± 0.07
22:5w6	1.33 ± 0.24	0.54 ± 0.28	1.22 ± 0.09	1.25 ± 0.16	1.28 ± 0.14	0.85 ± 0.36	0.74 ± 0.29	0.56 ± 0.11
22:5w3	2.67 ± 0.42	0.90 ± 0.49	2.08 ± 0.28	2.04 ± 0.32	1.42 ± 0.25	1.54 ± 0.36	0.97 ± 0.37	1.29 ± 0.26
22:6w3	17.31 ± 1.64	6.97 ± 2.08	16.36 ± 1.49	16.35 ± 3.50	16.49 ± 2.70	15.54 ± 2.36	4.94 ± 0.89	16.93 ± 3.70
BAME	5.77 ± 0.64	8.65 ± 0.95	4.95 ± 0.41	5.72 ± 0.74	5.19 ± 1.40	5.46 ± 0.80	8.62 ± 0.26	5.46 ± 0.51
SFA	31.75 ± 1.05	59.25 ± 4.23	30.27 ± 0.94	37.11 ± 4.99	31.38 ± 3.81	38.04 ± 3.91	55.71 ± 3.14	35.76 ± 2.11
MUFA	10.22 ± 1.31	16.98 ± 1.53	11.27 ± 0.64	12.59 ± 1.74	13.07 ± 1.22	13.30 ± 0.68	14.65 ± 1.16	12.73 ± 2.44
PUFA	58.02 ± 1.94	24.02 ± 5.22	58.45 ± 1.11	50.30 ± 5.98	55.56 ± 4.49	48.66 ± 3.92	29.64 ± 2.99	51.50 ± 3.95

Despite these similarities in broad FA composition, PERMANOVA highlighted strong dissimilarities between species ($p < 0.001$) and among sites within species ($p < 0.01$), whereas no location effect was recorded for either species ($p > 0.05$). *P. perna* and *M. galloprovincialis* were dissimilar from each other at all sites (PERMANOVA *post-hoc* pair wise test, $p < 0.01$). PERMANOVA *post-hoc* pair wise tests showed that the FA composition of *P. perna* at site 2 was significantly different from the other three sites ($p < 0.001$), which did not differ among each other ($p > 0.05$). The same was observed for *M. galloprovincialis* but for site 3, which had different FA signatures from conspecifics

collected at sites 1, 2 and 4 ($p < 0.001$), which again, did not differ among each other. These results were confirmed by the principal component analysis (PCA; Fig 2.4).

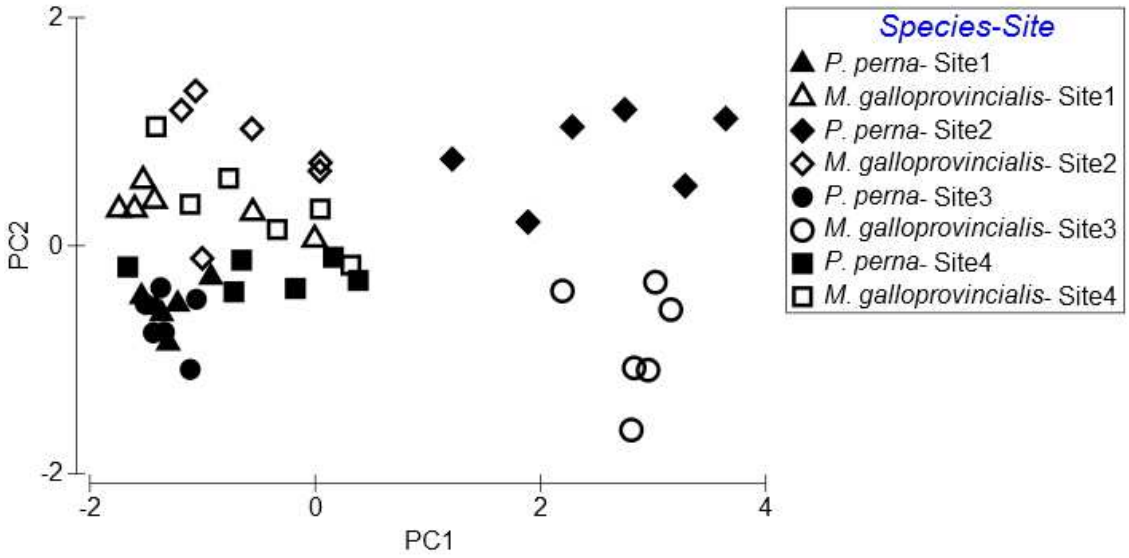


Fig 2.4 PCA of fatty acid composition of the mussels *P. perna* and *M. galloprovincialis* collected at four sites on the South African south coast. PC1 explained 61.5 % of the total variance and PC2 a further 10.7%.

PC1 which explained 61.5 % of the total variance, highlighted remarkable dissimilarities between *P. perna* from site 2 and *M. galloprovincialis* from site 3 on one hand and the remaining samples on the other hand. The samples on the negative part of PC1 had a higher proportion of 16:1w7 and PUFA such as 18:4w3, 20:4w6, 20:5w3, 22: 2NMI and 22:6w3, while the samples on the positive part of PC1 were enriched in SFA and MUFA (14:0, 16:0, 18:0, 18:1w9, 20:1w7, 20:1w9, BAME). This agrees with the general observations described earlier, with *P. perna* from site 2 and *M. galloprovincialis* from site 3 having more SFA than PUFA. PC2 identified dissimilarities between species, separating *P. perna* from site 2 and *M. galloprovincialis* from sites 1, 2 and 4 from specimens at the other sites (Fig 2.4). Specimens from the positive part of PC2 were characterized by 14:0, 16:0, 16:1w7, 20-MUFA, 20:5w3, 20:2NMI1, 20:2NMI2 and 22:6w3; while the samples in the negative part were enriched in 18:3w3, 18:4w3, 20:PUFA-w6, 22:2NMI1, 22:2NMI2, 22:5w3 and 22: PUFA-w6. However, PC2 explained only 10.7 % of the total variance, therefore caution should be taken in its interpretation. To identify differences between the two species a SIMPER analysis was performed on specimens of *P. perna* at sites 1, 3 and 4, and *M. galloprovincialis* at sites 1, 2 and 4. The

dissimilarities were mainly driven by w-6 PUFA that were in higher proportions in *P. perna*, while *M. galloprovincialis* was enriched in 16:0, 18:0, 20-MUFA and 20:5w3.

2.4. Discussion

The present study showed dissimilarities between *P. perna* and *M. galloprovincialis* at sites where the two species co-occurred, with individuals of the two species separated by only a few centimeters. However, these differences were found only in the FA composition, while their SI signatures differed only at site 2. With the exception of two cases, the FA signatures of both species had high values of PUFA. Some PUFA are particularly important for organisms for their essential role in membrane structure and function (Kanazawa et al. 1979, Volkman et al. 1989, Alkanani et al. 2007). Having a diet enriched in PUFA is an indication of better food quality compared for example to detritus which is poor in PUFA (Dalsgaard et al. 2003, Iverson 2009). The findings of this study indicate the two species were feeding on good food quality. However, some dissimilarities were found: *P. perna* was characterized by w-6 PUFA, which are more abundant in macroalgae, while no specific FATM for *M. galloprovincialis* were identified. These dissimilarities can be reconciled through two possible explanations: differences in their metabolic pathways or in their diets, most probably related to selectivity. Indeed the two mussels may have different FA pathways, possibly with different metabolic rates, which can lead to different FA profiles despite feeding on the same food sources. Filter feeders can acclimate to changes in their feeding environment by modifying physiological processes (Iglesias et al. 1992, 1996, Urrutia et al. 1997). For instance filter feeders can maximize energy uptake in the presence of high turbidity and/or low quality of food by increasing filtration rates and pseudofaeces production (Iglesias et al. 1992). In addition, mussels filter material using their gills (Foster-Smith 1975) and two pairs of labial pulps (Ward et al. 1998) and they can regulate filter-rejecting mechanisms depending on particle concentrations (Widdows et al. 1979, Leverone 1995). Zardi et al. (2008) showed *M. galloprovincialis* have longer labial palps that *P. perna*, suggesting a greater, or at least different ability to sort particles. Conceivably this can drive differences in their diet and thus mirrored in their FA composition. It was suggested that selectivity could be important for invasive species success if they are able to feed on better quality food than native species (Meng and Orsi 1991, Baker and Levinton 2003). Baker and Levinton (2003), in a study conducted on three native (*Margaritifera margaritifera*, *Amblema plicata* and *Pyganodon cataracta*) and an invasive (*Dreissena polymorpha*) species of freshwater mussels in

North America, showed the invasive species exhibited a selective diet focusing on highly nutritious species of phytoplankton. This suggests that the ability to select better food quality or quantity combined with other physical stressors (*i.e.* thermal stress or competition for space) can determine the success of the invasive over the native species. In the present study, this hypothesis was only partially supported as the two mussel species were different from each other, but it was not possible to identify specific diet trophic markers. However, this study was conducted at only four sites and on one occasion. Further investigations comparing the diet of *P. perna* and *M. galloprovincialis* in different feeding environments are required to clarify this pattern. A similar FA pattern was found for both species and at all sites except for *P. perna* from site 2 and *M. galloprovincialis* from site 3. A low proportion of PUFA indicates that specimens are exposed to a low quality of food, starving or in an unhealthy state for other reasons (*i.e.* disease, pollution; Müller-Navarra and Lampert 1996, Wacker and von Elert 2001, Dalsgaard et al. 2003). Thus, this indicates that those specimens were in an unfavorable environment in contrast to specimens of the other species from the same mussel bed. It is difficult to explain these differences with the available data. The fact that the species were collected in the same mussel bed, only a few centimeters apart, suggests that the food available was the same for the two mussels and that other factors influenced them. However, it is not possible to provide a plausible explanation for dissimilarities between *M. galloprovincialis* at site 3 and *P. perna* at site 2 with the conspecifics elsewhere.

M. galloprovincialis is a successful invasive species along the South African coast. From this study, it emerges that the two species were slightly different from each other in term of FA signatures, however both seemed to exhibit a diet characterized by good food quality. This suggests that good food quality was not a limiting resource. However, the dissimilarities in FA of *P. perna* at site 3 and of *M. galloprovincialis* at site 2 highlight the need for further studies to assess if the differences observed were unusual or if it is a common pattern due to selective feeding behaviour or to species-specific metabolisms.

CHAPTER 3

Does proximity to urban centres affect the dietary regime of filter feeders?

Urbanized centre- South Africa

3. Does proximity to urban centres affect the dietary regime of filter feeders?

3.1. Introduction

Human activities can directly and indirectly affect natural systems at local and global scales (Chapman et al. 1995, Hooper et al. 2005, Claudet and Fraschetti 2010, Bode et al. 2014). Major threats that can affect coastal marine environments include habitat destruction and degradation of water quality (Lotze et al. 2006, Airoldi and Beck 2007, Boero and Bonsdorff 2007, Bulleri and Chapman 2010). A decrease in water quality in coastal areas can result from both land-based and ocean-based human activities, most commonly through increased sediment loads, water turbidity and chemical or oil pollution (Vitousek et al. 1997, Lillebø et al. 2005, Halpern et al. 2008, Mangialajo et al. 2008).

Nutrients derived from urban and agricultural wastewater are identified as one of the causes of change in the structure and composition of marine food web dynamics because of their impact on nutrient cycles in rivers, estuaries and coastal waters (Richardson and Jørgensen 1996, Paerl 1997, McClelland and Valiela 1998, Castro et al. 2007). Nutrient availability is one of the main drivers that enhances primary production (Howarth 1988). However, an excess of nutrients can lead to eutrophication which, in coastal areas, can potentially create serious and perhaps irreversible environmental changes (Nixon 1995, Connell et al. 2008, Gorman et al. 2009). One effect of eutrophication is the bacterial remineralisation of large amounts of organic matter which depletes the water of its oxygen (Rosenberg and Loo 1988, D'Avanzo and Kremer 1994, Karlson et al. 2002). The resultant phenomenon of anoxia can cause mass mortality of fishes and benthic invertebrates in coastal areas (Borum and Sand-Jensen 1996, Bode et al. 2014). Other studies have highlighted the indirect effect of eutrophication on the productivity of benthic primary producers due to the attenuation of light penetration in the water column caused by phytoplankton blooms (Cambridge et al. 1986). Some of these blooms are due to toxic algae (Harmful Algal Blooms- HABs) that can consequently cause mass mortality of other organisms present in the water (Landsberg 2002). Other works showed how changes in nutrient availability for primary producers can shift community composition, by increasing the size and density of some

benthic primary consumers to the detriment of other species (Blumenshine et al. 1997, Persson and Svensson 2006). Most of these effects are usually observed in semi-enclosed areas such as bays, where the recirculation of the water is limited compared to the open coast (Le Pape et al. 1996, Wang et al. 1999). Nevertheless these changes often remain unnoticed because other factors can mitigate the effect of human activities (Lillebø et al. 2005). Some studies have hypothesised that bivalve populations can mitigate the effects of eutrophication by facilitating the removal of particles and so increasing the clarity of coastal waters (Soto and Mena 1999, Rise 2001). For example Soto and Mena (1999) showed that it was possible to decrease the impact of salmon farming in a southern Chilean lake by using a freshwater mussel species (*Diplodon chilensis)* to filter and clear particulates and dissolved nutrients. Lillebø et al. (2005) in a study conducted in a Portuguese estuary showed how hydrodynamic changes can reverse eutrophication processes, represented in this case by a decrease of freshwater input to the sea after minimizing a sluice opening. Bode et al. (2014) suggested that the effects of anthropogenic inputs are not always visible because some coastal ecosystems are already highly productive (*e.g.* upwelling systems). Consequently, because of processes that regulate coastal dynamics, anthropogenic effects are not always obviously detectable. It is thus important to investigate these processes using appropriate techniques that integrate signals of changes over a period of time, and are therefore able to detect underlying anthropogenic effects. In particular, assessing the relevance of anthropogenic effects on the food sources available for organisms in coastal areas is fundamental to evaluating possible consequences of increasing anthropogenic pressure on coastal ecosystem food webs and functioning (Grimm et al. 2000).

McClelland et al. (1997) drew attention to the use of $\delta^{15}N$ to track the assimilation of anthropogenic nitrogen into food webs, hence providing a method to detect eutrophication. Differences in $\delta^{15}N$ are usually used to identify the trophic level to which an organism belongs, but they can also reflect variations in the source of nitrogen consumed by the primary producers (McClelland and Valiela 1998, Aguiar et al. 2003, Žvab Rožič et al. 2014). Nitrogen from human wastewater is generally enriched in heavy isotopes because of the high degree of fractionation associated with nitrification occurring in these waters after discharge (Mariotti et al. 1981, Heikoop et al. 2000,

Carmichael and Valiela 2005, Cole et al. 2005). Consequently, a higher proportion of heavy isotopes can provide an indicator of anthropogenic input into a particular system.

Fatty acids (FA) can also be used to study trophic ecology and food web dynamics by examining specific FA which are transferred almost unaltered from producers to consumers (fatty acid trophic markers, FATM; Dalsgaard et al. 2003). They can also provide information about food quality, for example, 22:6w3 and 20:5w3 both indicate good food quality when present in high concentrations (Dalsgaard et al. 2003). Although this technique has not frequently been applied to the evaluation of anthropogenic effects, several studies have highlighted its importance in identifying anthropogenic food sources. For instance, Sakdullah and Tsuchiya (2009) showed that the dietary regime of estuarine fishes close to wastewater discharges had high proportions of 18:1w9 and 18:2w6 FA, which are abundant in urban waste discharges to the sea (Quéméneur and Marty 1994, Rieley et al. 1997).

Most studies of anthropogenic effects have been conducted in areas with high human population densities (*e.g.* Europe, North America), and very little or no information is present for less developed areas. Along the South African coast there are several cities with relatively high levels of urbanization that exist on an otherwise sparsely populated coastline. Several studies investigating anthropogenic effects on bivalves have focussed on the effect of heavy metals or eutrophication (El-Shenawy 2004, Verdelhos et al. 2005), however, to the present knowledge no studies have yet investigated the effects of anthropogenic input on primary consumers diet in intertidal habitats. This is important as anthropogenic input can affect primary productivity and thus food availability and quality for higher trophic level organisms. In view of the fundamental role that benthic filter feeders have in coastal areas, the present study aims to investigate how the presence of large cities with a relatively high level of urbanization (population higher > 100 000 inhabitants) along the South African south coast affects the dietary regime of several species of intertidal filter feeders. Specifically the dietary regime of three barnacle and one mussel species were assessed and compared using FA and stable isotope (SI) techniques.

3.2. Materials and Methods

3.2.1. Study area and sample collection

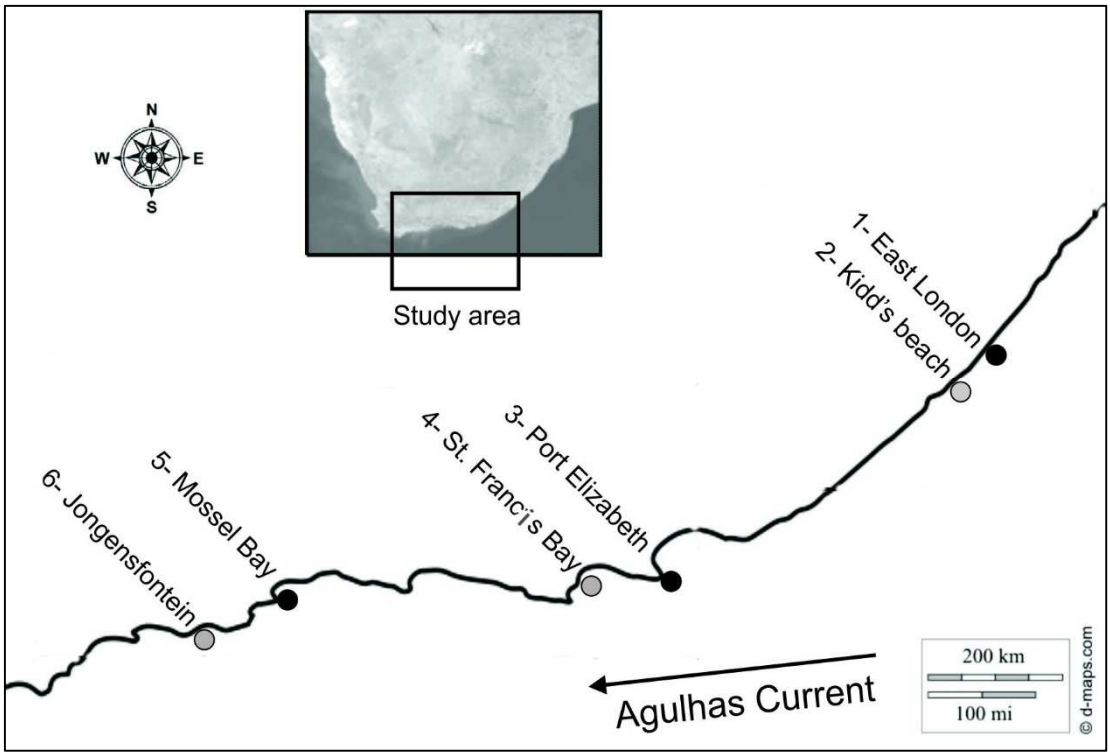


Fig 3.1 Map of the South African south coast showing sampling sites potentially influenced by urbanisation (black dots) and sites far from urbanized area (grey dots).

The study was conducted along the South African south coast (Fig 3.1, latitude 34.3-33.2 S° 27.6-21.2 E). The south coast has a temperate climate with mainly cool-water species which extend from the Cape of Good Hope to Port St Johns (Stephenson and Stephenson 1972, Harrison 2002). This coast is characterized by the Agulhas Current, a fast-flowing current carrying oligotrophic water southwards from the Mozambique channel (Probyn et al. 1994, Lutjeharms et al. 2000, Lutjeharms 2006).

In order to test the potential effect of urbanization on the diet of intertidal filter feeders, tissue samples were collected at three sites in cities: East London (site 1), Port Elizabeth (site 3) and Mossel Bay (site 5); and three control sites far (> 30 km) from large urbanized areas: Kidd's beach (site 2), St. Francis Bay (site 4) and Jongensfontein (site 6). The three cities are characterized by a relatively high level of urbanization and industrial activities. In particular the populations are estimated at 267 000, 1.3 million and 117 840 for East London, Port Elizabeth and Mossel Bay, respectively ("Census 2011- East London" 2011, "Census 2011- Mossel Bay" 2011, "Census 2011- Port Elizabeth"

2011). East London is located at the conjunction of two rivers, the Buffalo River and the Nahoon River, which both flow through the city, while Port Elizabeth and Mossel Bay are not located on any river mouth. In addition Port Elizabeth and Mossel Bay are in bays (Algoa Bay and Mossel Bay respectively), while East London is located on the open coast. The filter feeders investigated during this study were the indigenous mussel *Perna perna*, and three species of barnacle: *Chthamalus dentatus*, *Tetraclita serrata* and *Octomeris angulosa*.

Sampling was carried out in June 2012. At each site, samples of each species were taken at two locations, separated by 1 - 3 km. Specimens were collected in their corresponding microhabitats where they naturally occur, on the same rocky shore. Hence, *P. perna* was collected from the low intertidal zone, *C. dentatus* from the upper intertidal zone, *T. serrata* from sheltered areas and *O. angulosa* from exposed areas. Three replicates of each species were used for the FA analyses and five for the SI analyses. Because of the size of the barnacle *C. dentatus*, several animals were pooled together for each replicate (between 8 and 10 animals), while replicates of *T. serrata* and *O. angulosa* comprised the whole body of a single individual. For *P. perna*, the adductor muscle of one individual was analysed due to its low turnover rates (Gorokhova and Hansson 1999). Samples for FA and SI analyses were processed as described in Chapter 2 (Chapter 2; paragraph 2.2.1 and 2.2.2).

3.2.2. Data analysis

3.2.2.1. Stable isotopes

To examine the differences in tissue of $\delta^{13}C$ and $\delta^{15}N$ signatures between urbanized and non- urbanized areas, a mixed model design was used, consisting of the factors: city (two levels, fixed), site (three levels, random and nested in city), location (two levels, random and nested in site) and species (four levels, fixed and crossed with all the other factors). ANOVA followed by Tukey HSD *post hoc* tests was performed in order to assess differences among the different factors. Levene's test was used to test for homogeneity of the variances. The ANOVA and the *post hoc* tests were performed using STATISTICA v12 (StatSoft Inc. 2012).

3.2.2.2. Fatty acids

The FA composition of species from urbanized and non-urbanized areas was compared using a PERMANOVA based on a Bray-Curtis dissimilarities matrix with the same design as for the SI data analyses. Principal component analysis (PCA) and SIMPER were also performed as described in Chapter 2 (paragraph 2.2.3.2.).

3.3. Results

3.3.1. Differences among species

SI and FA analyses showed differences among species across all sites (ANOVA, $p < 0.001$ and PERMANOVA, $p < 0.001$). A strong dissimilarity was found between mussels and the three barnacle species in terms of their SI signatures (Fig 3.2 a). *P. perna* showed higher $\delta^{13}C$ (-14.4 to -17.5 ‰) and lower $\delta^{15}N$ (6 to 9 ‰) compared to the three barnacles species (-15.8 to -20.6 ‰ for $\delta^{13}C$ and 9.2 to 13.36 ‰ for $\delta^{15}N$). Amongst barnacles, no significant differences were found between *C. dentatus* and *T. serrata* at all sites considered, whereas *O. angulosa* differed from the two other species for both isotopes at sites at sites 3, 4, 5 and 6 (Tukey HSD, $p < 0.001$).

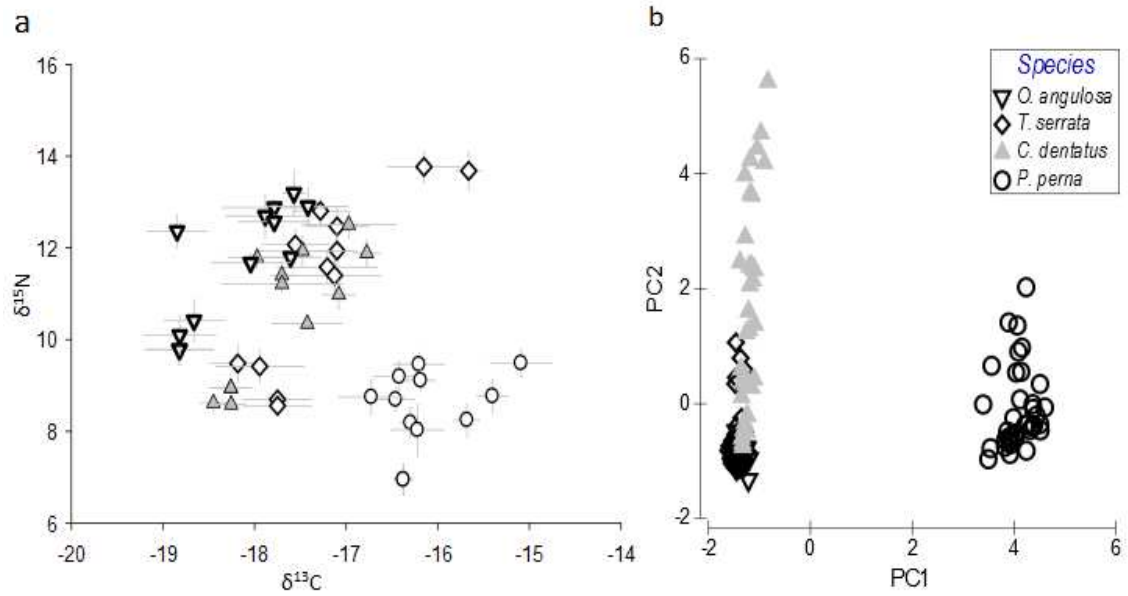


Fig 3.2 Stable isotope signatures (a) and principal component analysis (PCA) performed on the fatty acid composition (b) of one species of mussel *P. perna* and three species of barnacle *O. angulosa*, *C. dentatus* and *T. serrata* from six sites on the South African south coast. PC1 explained 60 % of the total variance and PC2 21 %.

A similar pattern was observed using the FA composition of the four filter feeder species (Fig 3.2 b). PCA separated mussels and barnacles along axis 1, which explained 60 % of the variance. SIMPER showed the differences between these two groups were mainly due to the non-methylene-interrupted fatty acids (NMI) and the following FA: 18:2w6, 20:1w9, 20:4w6, 22:4w6, 22:5w6 and 22:5w3, which were more abundant in mussels than barnacles, while levels of 20:5w3 (EPA), monounsaturated FA (MUFA) and

saturated (SFA) FA were higher in barnacles than mussels. These FA contributed 55 % to the dissimilarities (for full list of fatty acids see Appendix a). PERMANOVA highlighted differences between *C. dentatus* and the other two barnacle species (PERMANOVA *post hoc* pair-wise test, $p < 0.001$). Further analyses using SIMPER showed that *C. dentatus* had higher concentrations of SFA (16:0, 18:0, 20:0), bacterial FA (BAME) and 20:1w11 FA, compared to the other two species; whereas *O. angulosa* and *T. serrata* were characterized by higher values of 14:0, 16:1w7, 18:4w3, 20:4w3, 20:5w3 and 22:6w3. Again, these FA contributed 55 % to the dissimilarities. In view of these dissimilarities among species, each filter feeder was considered separately for subsequent analyses.

3.3.2. Effects of urbanisation

3.3.2.1. Stable isotope composition

$\delta^{13}C$ of all species taken individually showed no significant effect of the factors city or location (ANOVA, $p > 0.05$; Fig 3.3). However, the ANOVA revealed that $\delta^{13}C$ values differed among sites (ANOVA, $p < 0.01$). Although overall each species differed significantly in their $\delta^{13}C$ signatures, they showed similar pattern of rank order among sites. $\delta^{13}C$ increased from site 1 to 2, decreased from sites 2 to 3 and remained constant afterwards, between site 3 and 6 (Tukey HSD, $p < 0.01$).

Table 3.1 ANOVA results preformed on the $\delta^{15}N$ of filter feeders at six sites along the South African south coast. n = 5 per location for each species. Ci = City, Si = Site, Loc = Location, df = degrees of freedom, MS = mean square; * *p*, 0.05; ** *p*, 0.01; *** *p*, 0.001.

δ 15 N	***P. perna***				***C. dentatus***				***T. serrata***				***O. angulosa***			
	df	**MS**	**F**		**df**	**MS**	**F**		**df**	**MS**	**F**		**df**	**MS**	**F**	
Ci	1	11.96	88.53	***	1	0.47	8.66	**	1	0.98	6.34	*	1	0.40	0.33	*
Si (Ci)	4	8.14	60.26	***	4	27.40	503.51	***	4	32.66	210.83	***	4	16.01	13.11	***
Loc (Si (Ci))	6	0.17	1.25		6	0.37	6.84		6	0.27	1.73		6	1.71	1.40	
Error	48	0.14			48	0.05			48	0.15			48	1.22		

$\delta^{15}N$ was significantly higher at city sites compared to their corresponding control sites for *P. perna* and *C. dentatus* (Fig 3.4; Table 3.1) but not for *T. serrata* and *O. angulosa* at site 2 (Tukey HSD, $p < 0.05$; Fig 3. c and d). Indeed *T. serrata* at site 1 and 2 were not significantly different (Tukey HSD, $p > 0.05$; Fig 3. c), while *O. angulosa* at site 1 was $\delta^{15}N$ depleted compared to site 2 (Tukey HSD, $p < 0.01$; Fig 3. d).

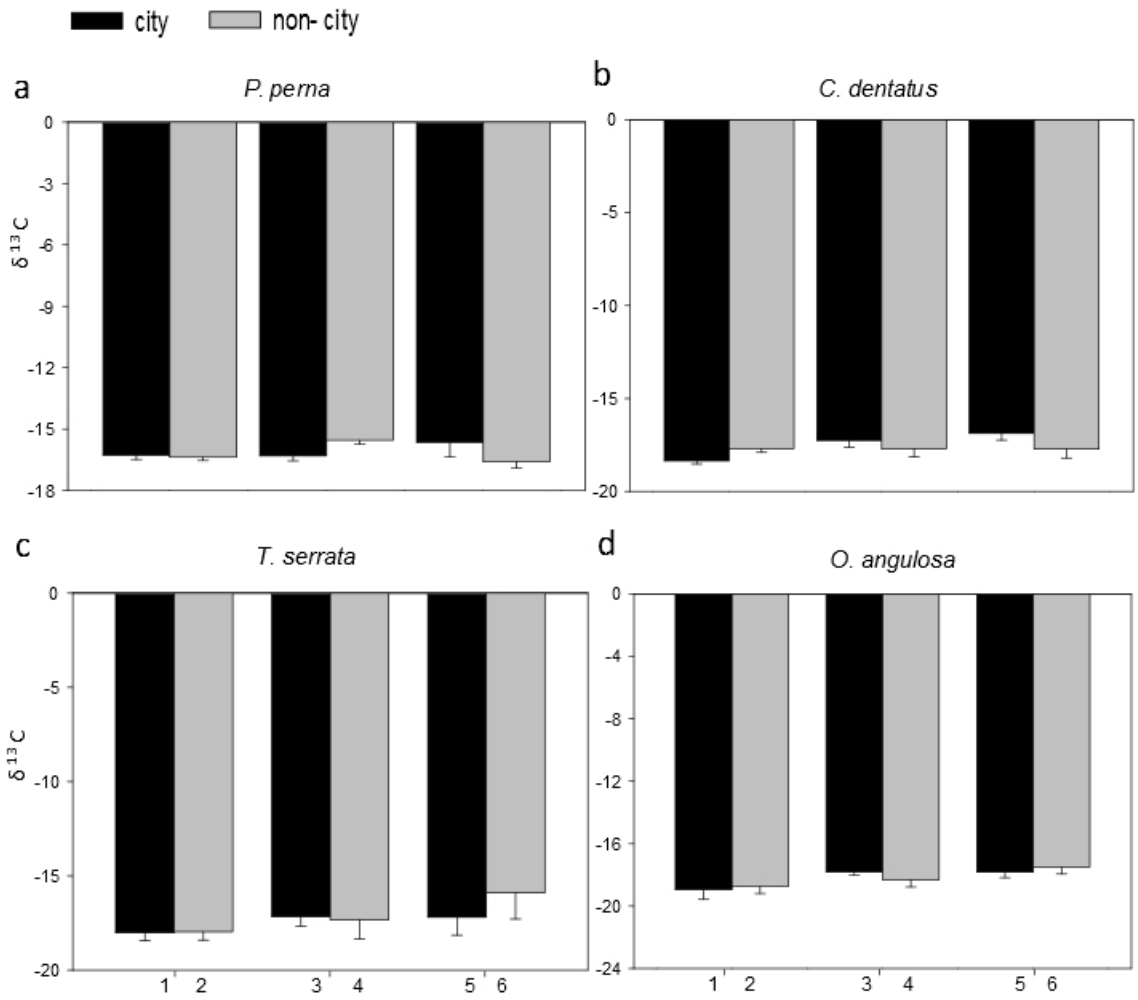


Fig 3.3 Stable isotope carbon signatures (mean + SD; n = 10) for filter feeders at city (black) and control sites (grey) along the South African south coast. a) *P. perna* b) *C. dentatus* c) *T. serrata* d) *O. angulosa*.

A clear geographic trend was visible with increasing $\delta^{15}N$ from east to west considering sites under the same conditions (city sites or control sites, Tukey HSD, $p < 0.01$; Fig 3.4). In addition, site 1 and 2 showed significantly lower $\delta^{15}N$ signatures compared to the other four sites (Fig 3.). No location effects were recorded for any species (Table 3.1).

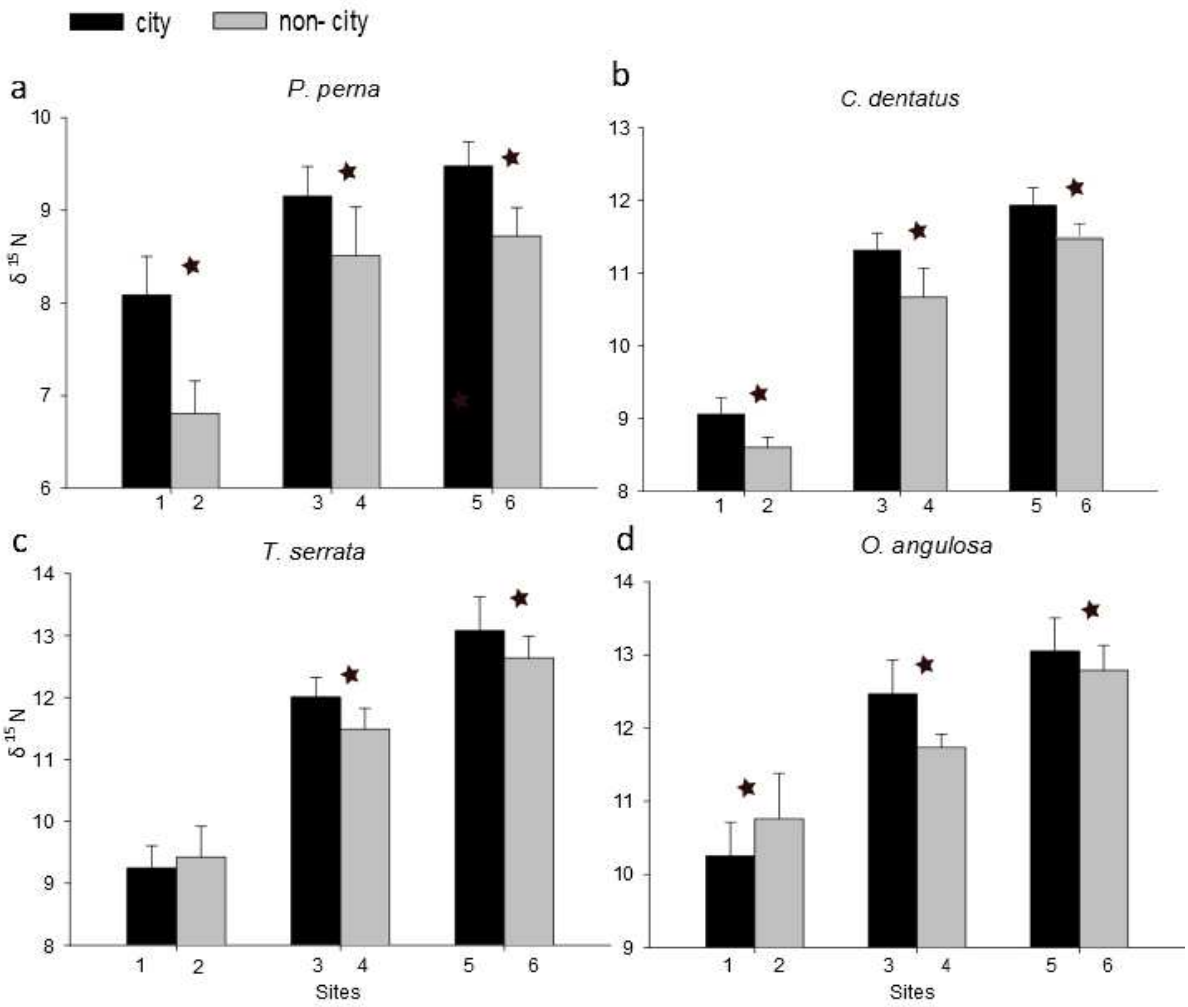


Fig 3.4 Stable isotope signatures (mean + SD; n = 10) for filter feeders at city (black) and control sites (grey) along the South African south coast. a) *P. perna* b) *C. dentatus* c) *T. serrata* d) *O. angulosa*. Stars indicate significant differences between city and non-city sites (ANOVA, $p < 0.05$).

3.3.2.2. Fatty acid composition

FA signatures of all barnacle species were not affected by proximity to a city (PERMANOVA, $p > 0.05$), while mussel FA signatures showed differences between city and control sites (PERMANOVA, $p < 0.05$, Fig 3.5). For mussels, the differences were mainly driven by 18:2w6, 18:3w3, 18:4w3, 20:2 NMI1, 20:4w6, 20:5w3 and 22:6w3, which were more abundant at city sites, while control sites were enriched in 16:0, 18:0, 18-MUFA, 20:1w7, 20:1w9 and 22:2 NMI2 (SIMPER). PERMANOVA showed no significant effects of the factors site or location on the FA signatures of any species ($p > 0.05$).

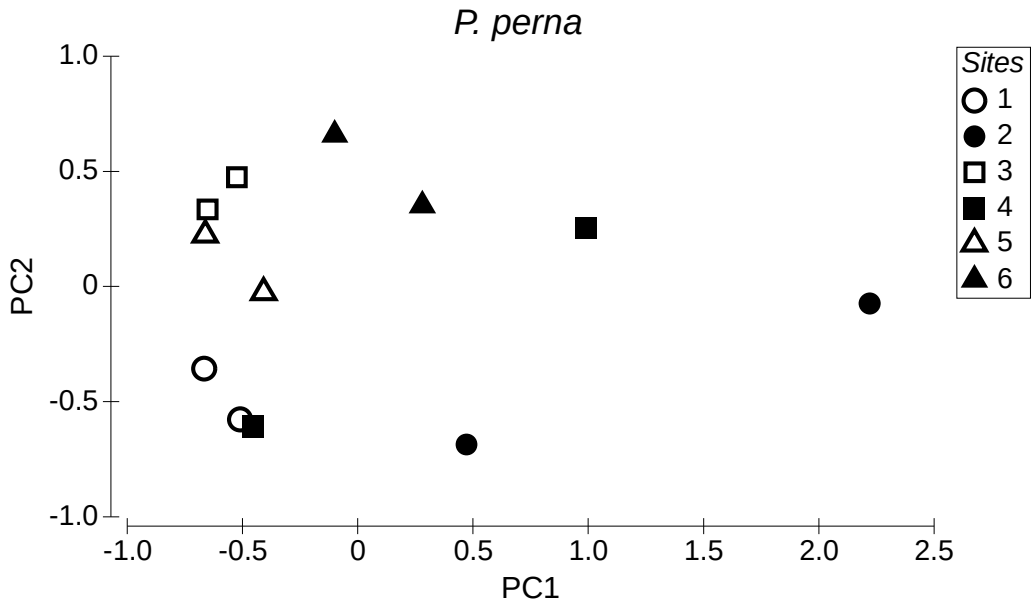


Fig 3.5 Principal component analysis (PCA) of transformed proportional FA profiles of *P. perna* collected in city (white) and control sites (black) along the south coast of South Africa. Each sample represents the average values of three replicates at a single location. PC1 explained 60 % of the total variance and PC2 16 %.

3.4. Discussion

SI and FA analyses showed strong differences between mussels and barnacles and among barnacle species. Explanations for these differences are likely to be related to the ecology, the metabolism or the physical size of the species considered. Within species, changes in body size can influence consumer diets (Zhukova 2000, Tallis 2009). Mussels in the present study were larger in size than the barnacles (mean 60 mm and 20 mm respectively) and *C. dentatus* was much smaller (max 10 mm) than the other two species of barnacles (max 25 mm for both), suggesting they might feed on different size particles. In addition, mussels are active feeders while barnacles are passive feeders (Nixon et al. 1971, Rubenstein and Koehl 1977). These two characteristics (size and filtration mechanisms) can influence the type and the size of the particles ingested. Hence mussels can filter particles of up to 2.4 mm while barnacles filter only up to 1 mm, with a critical minimum size of 1 - 3 µm for both (Jørgensen 1955, Southward 1987, Trager et al. 1990, Wang and Fisher 1996, Alfaro 2006). Small- scale variability in species habitats can also influence the type of food available to each species and thus modify their FA and SI profiles (Budge et al. 2002, Khotimchenko et al. 2005, Guest et al. 2010, McQuaid and Mostert 2010). For example Barnes & Powell (1953) found barnacles from the lower intertidal grew faster than those located higher on the shore; while Strathmann et al. (1981) showed cyprid larvae of *Balanus cariosus* prefer to settle on the lower intertidal shore where food availability is higher. Along the South African coast, *T. serrata* is usually associated with relatively sheltered shores and replaced by *O. angulosa* in very wave exposed areas (Boland 1997a, 1997b), while *C. dentatus* is generally found in the upper intertidal zone and *P. perna* in the lower intertidal (Dye 1998). Differences in food particle availably at different heights on the shore could therefore also have been a factor driving FA and SI dissimilarities among species in this comparison. Another point to consider is that only the adductor muscle of *P. perna* was taken for the present investigation, while for the barnacles, the entire body was used. Animal tissues exhibit different levels of metabolic activity that depend on the function of the organ itself. For example, gonads have a faster turnover rate compared to muscle (Ezgeta-Balić et al. 2014). Therefore, the different metabolic rates of the tissues chosen in this comparison (adductor vs whole body) perhaps could have driven FA and SI

dissimilarities between barnacles and mussels, but would not explain the differences among the barnacle species.

$\delta^{15}N$ values of filter feeders near urbanized areas were significantly higher than at control sites, and in addition, there was a clear geographic trend in nitrogen SI signatures from east to west. Previous studies highlighted the fact that urbanised areas can have an impact on the nitrogen signature of food sources, and subsequently on higher consumers within the food web by increasing the amount of the heavy isotope through fractionation associated with nitrification in these environments (McClelland and Valiela 1998, Castro et al. 2007, Pastor et al. 2014). Therefore, it is hypothesised that the significant increase of $\delta^{15}N$ observed in the present study indicates a potential influence of anthropogenically derived nitrogen linked to wastewater input from domestic and industrial sewage. lsen et al. (2010) in a seagrass habitat of Puerto Rico, also found elevated $\delta^{15}N$ signatures in primary consumers that were linked to the incorporation of nitrogen from wastewater sources. Abreu et al. (2006) highlighted an increase of 3.5‰ $\delta^{15}N$ in primary producers and consumers from a polluted lagoon system compared to organisms from a non-polluted system. However in the present study, while this effect was observed for all the species in Mossel Bay and Port Elizabeth (sites 3 and 5), it was only observed for *P. perna* and *C. dentatus* in site 1 (East London). The topography of the area could have contributed to the dissimilarities among cities. Indeed East London is located on the open coast whereas Port Elizabeth and Mossel Bay are situated in bays. Archambault et al. (1999) found that *chl a* concentration and mussel growth were significantly greater inside a bay than outside and suggested that this was due to retention of nutrients within the bay. The same hypothesis can also explain the present results, with retention of anthropogenically-associated nutrients in the bays of Mossel Bay and Port Elizabeth compared to East London. Therefore, the different environmental and/or hydrodynamic conditions among these cities could be responsible for the differences in $\delta^{15}N$.

$\delta^{13}C$ did not differ between city and control sites. It has been demonstrated that $\delta^{13}C$ differentiates among food sources (Peterson and Fry 1987) and this is reflected in consumer signatures (Peterson 1999). The lack of differences in this study suggests that filter feeders in city and control sites consume the same food sources, with the values

found here corresponding to a typical signature of macroalgal detritus (Hill and McQuaid 2006).

Strong differences in SI signatures were found among sites. In particular, $\delta^{15}N$ increased from east to west following a geographic gradient. This pattern most probably reflects the isotopic gradient from oligotrophic to eutrophic conditions described by Hill and McQuaid (2006). They suggested that the distinct geographic pattern in $\delta^{15}N$ signatures in mussels species along the south coast of South African is driven by the reliance on recycled nitrogen in oligotrophic waters (Miyake and Wada 1967). Sites 1 and 2 differed in their carbon signatures from each other and from all other sites. Specifically, $\delta^{13}C$ at site 1 was low compared to the other sites. These differences could be driven by the presence of a cell of continuous upwelling at Port Alfred (Schumann et al. 1982, Bustamante et al. 1995) as the signature of upwelling generally propagates eastwards, towards site 1 and 2. Upwelling events bring cold, nutrient-rich water into the nearshore environment, and thus enhance intertidal primary production (Nelson and Hutchings 1983, Basterretxea and Arístegui 2000, Blanchette et al. 2006). In addition, it was shown that $\delta^{13}C$ values of benthic consumers at upwelling sites are depleted compared to specimens from non-upwelling sites (see Chapter 5), while a study conducted on filter feeders in this area also demonstrated that specimens from an upwelling area had low $\delta^{13}C$ values compared to specimens from sites downstream of the upwelling cell (Allan et al. 2010). The eastward influence of the Port Alfred upwelling cell could explain the particularly depleted values for site 1, but not the fact that $\delta^{13}C$ values were most enriched at site 2, which lies between site 1 and Port Alfred.

FA results partially confirmed the SI results. Barnacles were not affected by being in urbanised centres, while mussels were. Mussel FA signatures from city sites were enriched in polyunsaturated FA (PUFA), while the control sites had high percentages of SFA and MUFA. Enrichment in PUFA can indicate that specimens are exposed to high food quality (Dalsgaard et al. 2003) or to a high quantity of food (*i.e.* not under starvation; Wacker and von Elert 2001). As detailled previously, eutrophication due to an increase of anthropogenic-associated nutrients, can enhance primary production and an increase in food availability for primary consumers (Odum et al. 1962, Howarth et al. 1996, Downing 1997, Bouillon et al. 2002). It is hypothesised that the higher concentrations of PUFA found in mussels close to cities are linked to such a process.

However, this pattern was not observed for barnacles. Possible differences in metabolism as described previously could have contributed to the dissimilar pattern between mussels and barnacles. It is also possible that the enhancement of primary production affected mostly phytoplankton cells that are too large for the barnacles to feed on.

This study highlighted the profound lack of information on the effects of human activities on the food available for intertidal filter feeders in coastal areas. This is the first work to investigate potential anthropogenic effects on FA signatures of filter feeders. Further studies investigating anthropogenic effects in more controlled environments (*e.g.* mesocosm experiments) or comparing food sources of benthic populations, nutrient availability and the presence of pollution are required in order to clarify and identify the effects of human activities on food available for benthic communities and the resultant effects on coastal food webs. The present study indicated that anthropogenic effects can be detected through the diets of benthic consumers, and showed the importance of differentiating among species of filter feeders in ecological studies. Modification of the diet of these species can affect their survival and thus the species composition of the communities inhabiting coastal ecosystems. Although relatively few species are responsible for maintaining ecosystem functioning in natural systems (Mills et al. 1993, Bond 1994, Jones et al. 1996). Key species, such as mussels, can have far reaching impacts if disturbed (Paine 1969, Stachowicz et al. 1999). This is especially true because mussels are ecosystem engineers providing habitats for other organisms (Kelaher and Castilla 2005, Menge et al. 2008, Cole and McQuaid 2010). Consequently, changes in the quality and quantity of food available will not only affect intertidal filter feeders but could also affect the flora and fauna associated with them (Huston 1979, Hansen et al. 1995, Tilman 1999).

CHAPTER 4

The effect of freshwater input on the stable isotope and fatty acid signatures of marine benthic filter feeders along the South African east coast

Thukela River mouth- South Africa

4. The effect of freshwater input on the stable isotope and fatty acid signatures of marine filter feeders along the South African east coast

4.1. Introduction

Freshwater input may influences marine pelagic and benthic communities through the export of nutrients, sediments and detritus (Gillanders and Kingsford 2002, Robins et al. 2005). The increase of nutrients in coastal environments due to riverine input enhances phytoplankton and subsequently zooplankton production (D'Elia et al. 1986, Grimes and Kingsford 1996). Plankton being at the base of the food chain, can affect the survival of larval, juvenile and adult stages of higher predators that depend on this food source. Freshwater input may also promotes macroalgal and seagrass production in coastal areas (Bunt 1973, Eadie et al. 1994, Downing 1997, Cermeño et al. 2008). Fluvial sediment inputs may have a fundamental role in securing near shore habitats that are continuously subjected to erosion by oceanic currents and wave action, which is especially important because these habitats are essential refuges for many organisms such as fish and invertebrates (Halim et al. 1995, Dalrymple and Choi 2007). Detritus also may has a key role in aquatic ecosystems not only as a food source for detritivorous organisms, but because it can be remineralized by bacteria into nutrients available for primary producers (Nixon 1981, Whitfield 1998). Another very important aspect that needs to be considered when investigating the effect of freshwater input on marine systems is obviously the amount of freshwater flowing out of the estuary and the topography of the coast (Meybeck et al. 1996). Despite these well-established functions, published studies give different interpretations of the overall importance of rivers as potential nutrient sources for marine populations. De lecea et al. (2013), in a study conducted in the Natal Bight in South Africa demonstrated that the stable isotope signatures of demersal benthic populations were strongly dependent on fluvial input. Similarly, Darnaude et al. (2004) showed that the diet of deposit-feeding polychaetes in the Gulf of Lions originated from riverine organic matter. Drinkwater and Frank (1994) carried out a worldwide examination of riverine effects on the marine environment and highlighted a strong correlation between reductions in freshwater flow and a decline in coastal fisheries. Other studies have indicated that fluvial input becomes unimportant

in the presence of large-scale oceanographic processes such upwelling, or powerful currents, which overshadow or disperse the influence of freshwater input (Meyer et al. 2002, Hill et al. 2008).

Stable isotope (SI) signatures of $\delta^{13}C$ are a very powerful tool to discriminate between freshwater and marine sources of food (Kaehler et al. 2000, Darnaude et al. 2004, Bănaru et al. 2007) as the $\delta^{13}C$ of suspended organic matter (SPM) from rivers is depleted compared to marine SPM (Walsh et al. 1981, Coleman and Fry 1991). This is due to the presence of vegetation with depleted $\delta^{13}C$ in rivers, and carbon enrichment in marine systems (Fry and Sherr 1989, Vorwerk and Froneman 2009). For example Tallis (2009), in a study conducted at five river sites in the Olympic Peninsula (Washington, USA), used $\delta^{13}C$ values to show that benthic filter feeders consumed very small amounts of riverine SPM and that their primary resource was based on macroalgae and seagrass detritus.

Several studies have highlighted the benefits of using fatty acid techniques (FA) to differentiate sources of organic matter between marine and terrestrial (reviewed by Dalsgaard et al. 2003, Kelly and Scheibling 2012) and thus acquire information on the spatial and temporal variation in diet among individuals and within populations (Budge et al. 2006, Budge and Springer 2007, van den Meersche et al. 2009). Despite the relevance of FA analyses to investigate food sources, few studies have applied FA techniques to examine the effect of riverine organic input on marine organisms, however any effect of freshwater-associated organic input were identified on marine species located a few kilometres alongshore (either up- or downstream) of a river mouth (*e.g.* Richoux et al. 2014a).

South Africa has about 250 permanently open and temporarily closed estuaries along the south and east coasts (Whitfield and Bate 2007). Understanding the effect of freshwater input on the dietary regime of intertidal populations in the vicinity of estuaries along the South African coast is thus extremely important (Begg 1978). Especially in the context of climate change which predicts a reduction or an increase of freshwater input into the marine environment depending on the area in question (Harley et al. 2006), understanding or having a baseline to be able to predict the possible impact on costal environments is important. A few studies have examined the effects of freshwater dissolved and particulate material on the dietary regime of marine intertidal

populations using either SI or FA analyses (Tocher 2003, Tallis 2009, Vorwerk and Froneman 2009), but none have integrated results from both techniques. Using both FA and SI techniques, this study aims to increase our understanding of the effects of freshwater input on the dietary regimes of four rocky shore intertidal filter feeders over large spatial scales (100s km) on the South African east coast.

4.2. Materials and Methods

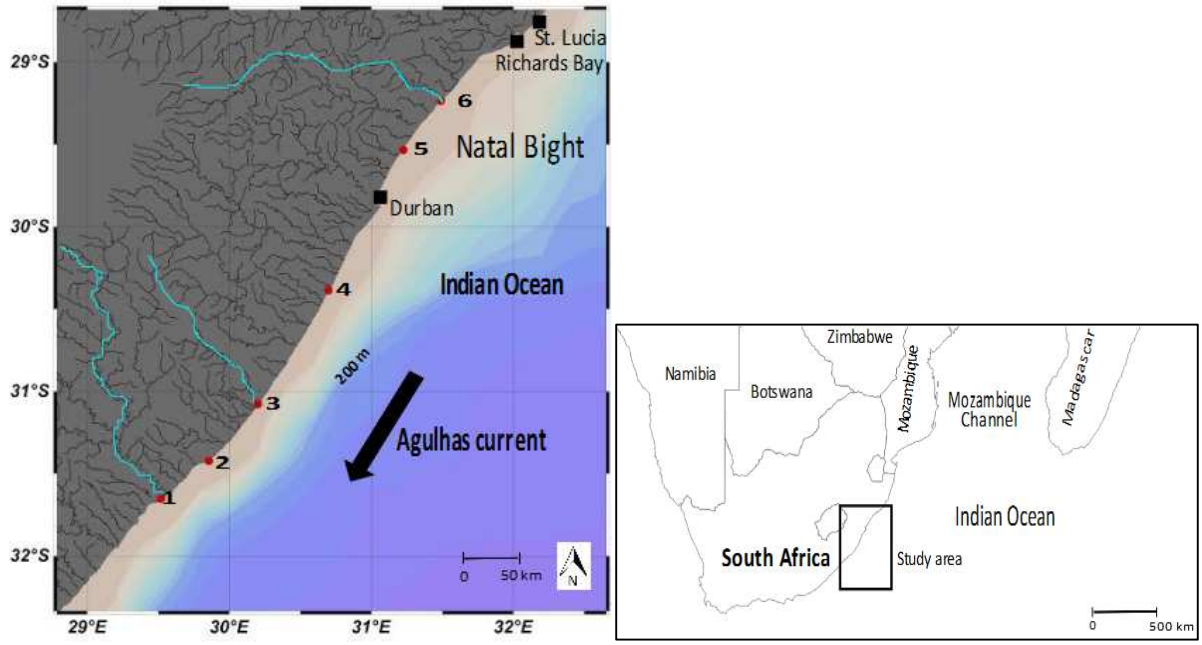


Fig 4.1 Map of the sampling sites on the east coast of South Africa with the bathymetry and the estuaries. Sites located at major estuaries (rivers highlighted in blue) were: Mzimvubu (site 1), Mtamvuna (site 3) and Thukela (site 6); sites used as controls were Mbotyi (site 2), Pennington (site 4) and Ballito (site 5).

4.2.1. Study area and sample collection

The study was conducted along the South African east coast, as most of the estuaries in South Africa are present along this coast (Fig 4.1, latitude 33.5-29.16 S° 27.13-31.26 E). This coast is characterized by a narrow continental shelf that widens in the northern part to form the Natal Bight between Durban and St. Lucia (Fig 4.1). The shelf break lies around the 200 m isobath and the Agulhas Current runs along the shelf break, enclosing well mixed water within the Natal Bight (Probyn et al. 1994, Lutjeharms et al. 2000). The Bight receives Indian tropical and subtropical water from the Mozambique Channel and from an upwelling cell which occurs off Cape St. Lucia, in the North of the Bight (Lutjeharms et al. 2000, Meyer et al. 2002).

In order to test the effect of freshwater input on the dietary composition of intertidal filter feeders, samples were collected at the mouths of three rivers that are large in the local context: Mzimvubu (site 1), Mtamvuna (site 3) and Thukela (site 6) and compared to samples from three control sites far (> 50 km) from large or permanently open river mouths: Mbotyi (site 2), Pennington (site 4) and Ballito (site 5; Fig 4.1). In addition, at each location measurements of instantaneous salinity were recorded. The Mzimvubu River (site 1) is one of the largest rivers of the region, and flows into the sea

at Port St. Johns. It is 250 km long with a catchment area of about 25 000 km^2 and mean annual runoff of over 2.8 x 10^9 m^3/ year (the fourth highest in South Africa, Table 4.1; Smakhtin et al. 1997, Madzena and Lasiak 1997). The Mtamvuna River (site 3) is 161 km long with a catchment area of 1570 km^2. Its mouth is situated in a deep gorge flanked by cliffs up to 200 m high at Port Edward (Table 4.1). The lower reach of this estuary is characterized by mangrove forests dominated by the genera *Bruguiera* and *Avicennia*, and it enters the Indian Ocean through a narrow inlet created by the cliffs (Cooper 1993). The Thukela River (site 6) is the longest river of the ones chosen for the comparison and has the largest catchment area. It follows a 502 km route through the province of KwaZulu-Natal, with a total catchment area of approximately 29 100 km^2. Annual freshwater input into the sea by the Thukela is higher than the other two rivers with 6.79 x 10^9 m^3/ year (Table 4.1), and its estuary is less influenced by marine waters, remaining freshwater dominated at all times (Schumann 2013), with abundant wet reed marshes and grasslands along the banks (Oliff 1960). All estuaries selected for this comparison remain the whole year permanently open to the ocean year around. However, it should be noted that there are other smaller estuaries along the whole coast that have very small catchment areas and are generally closed in the dry season, or even for several consecutive years (Fig 4.1; Froneman 2002, Perissinotto et al. 2004, Whitfield and Bate 2007). The control sites chosen for the comparison were Mbotyi (site 2), located in a rural area, far from high-density cities, while Pennington (site 4) and Ballito (site 5) are 70 km south and 50 km north of Durban respectively.

Table 4.1 Characteristics of the rivers chosen for the comparison.

River	Length (km)	Catchment area (km^2)	Run off m^3/year	Freshwater/Marine dominated
Mzimvubu	250	25 000	2.8 x 10^9	mixed
Mtamvuna	161	1570	3.03 x 10^8	mixed
Thukela	502	29 100	6.79 × 10^9	freshwater

The filter feeders investigated during this study were the indigenous mussel *Perna perna*, and three species of barnacle: *Chthamalus dentatus*, *Tetraclita serrata* and *Octomeris angulosa*. All are well represented along the east coast. Sampling was carried out in May 2012. Sites were separated by 40 - 100 km along a stretch of coast that

covered approximately 300 km. At each site, samples were collected from two locations (A and B), separated by 1 - 3 km. Specimens from sites close to rivers were taken at the closest rocky shore to the river mouth. Specimens were collected in corresponding of their natural microhabitats as described in Chapter 3 (paragraph 3.2.1). Three replicates of each species were used for the FA analyses and five for the SI analyses. Because the barnacle *C. dentatus* was relatively small (5 -10 mm diameter), each replicate was represented by a pool of animals (5 - 10 individuals), while replicates of *T. serrata* and *O. angulosa* comprised a single individual. For mussels, one replicate corresponded to the adductor muscle of one specimen. Muscle was chosen due to its low turnover rate, which makes it more representative of a time-integrated diet (Gorokhova and Hansson 1999). Samples for FA and SI analyses were processed using the methods described in Chapter 2 (Chapter 2; paragraph 2.2.1 and 2.2.2).

4.2.2. Data analysis

4.2.2.1. Stable isotopes

To examine spatial differences in $\delta^{13}C$ and $\delta^{15}N$ values between areas with and without freshwater input, a mixed model design was used, consisting of the factors: river (two levels, fixed), site (three levels, random and nested in river), location (two levels, random and nested in site) and species (four levels, fixed and crossed with all the other factors). Using this design, ANOVA tests were performed in order to assess differences among the different conditions. In the event of significant results, Tukey HSD *post hoc* comparisons were used to identify homogenous groups. Levene's test was used for testing the homogeneity of variances. The ANOVA and the *post hoc* tests were performed using STATISTICA v12 (StatSoft Inc. 2012).

4.2.2.2. Fatty acids

The differences in FA composition between large estuaries and control areas was conducted using a PERMANOVA based on a Bray-Curtis dissimilarities matrix with the same design as for the SI data analyses. Principal component analysis (PCA) and SIMPER were also performed as described in Chapter 2 (paragraph 2.2.3.2.).

4.3. Results

No differences were found between locations within the same sites for any of the species using either SI or FA analyses (ANOVA and PERMANOVA respectively, $p > 0.05$ in all cases). For this reason, the factor location was not considered in the present study and data for locations within sites were pooled. Consequently, n = 10 for SI analyses and n = 6 for FA analyses.

4.3.1. Species effect

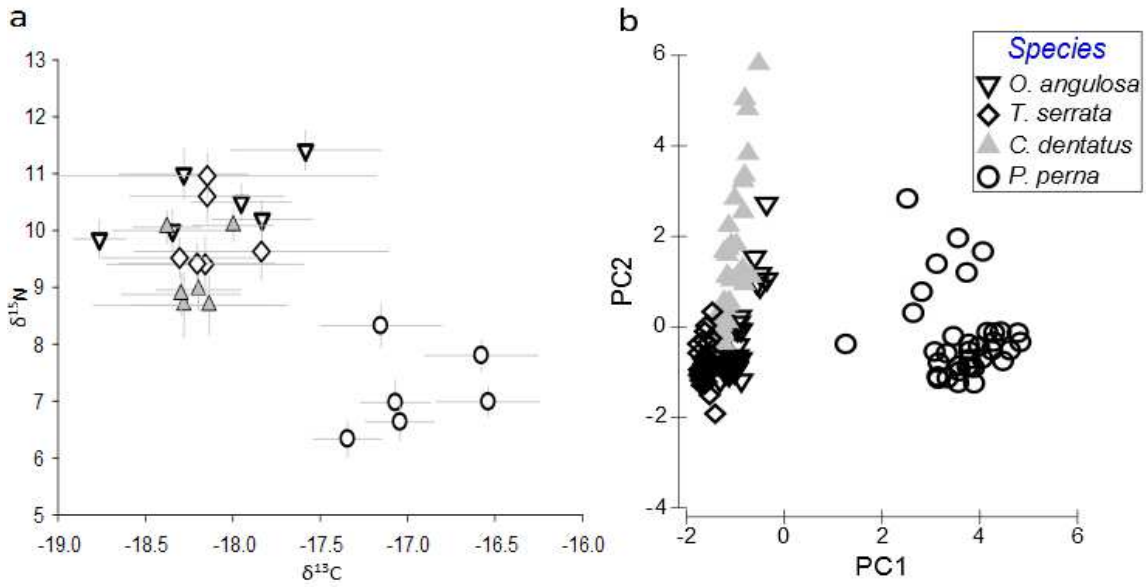


Fig 4.2 a) Stable isotope signatures $\delta^{13}C$ and $\delta^{15}N$ (mean ± SD; n = 10 per site), and b) PCA performed on the fatty acid composition of four species of filter feeders at six sites on the South African east coast. PC1 and PC2 explained 49.8 % and 20.8% of the total variance, respectively.

SI and FA signatures were different among the four species of filter feeders, as found in the previous chapter (Table 4.2 and 4.3; Fig 4.2; see Chapter 3), with the strongest differences being between mussels and the three barnacle species (both Tukey HSD and PERMANOVA, $p < 0.001$). Mussels had higher $\delta^{13}C$ and lower $\delta^{15}N$ values compared to barnacles across all sites, with values of $\delta^{13}C$ between -16.5 ‰ and 17.3 ‰ and $\delta^{15}N$ between 7 ‰ and 8.3 ‰, while barnacles were between -17.5 and -18.8 ‰ for $\delta^{13}C$ and from 8.6 to 11.4 ‰ for $\delta^{15}N$ (Fig 4.2, a). The FA profiles of mussels were characterized by higher proportions of non-methylene-interrupted FA (NMI), 20-monounsaturated FA (MUFA), 22:4w6 and 22:5w6, while barnacles showed higher values of saturated FA (SFA), 20:3-polyunsaturated FA (PUFA), 20:5w3 (EPA), 22:6w3

(DHA) and 22-MUFA explaining 65 % of the differences between mussels and barnacles (SIMPER). Surprisingly it was also noticed that *P. perna*, *O. angulosa* and *T. serrata* generally showed high levels of PUFA (50 – 60 %) at all sites followed by SFA (30 %) and MUFA (10 - 20 %), while *C. dentatus* were SFA enriched at all sites (45 - 70 %; for full list of FA see Appendix b).

Table 4.2 Results of ANOVA performed on $\delta^{15}N$ and $\delta^{13}C$ of four species of filter feeders at river and non-river sites. n = 10 per site for each species. Ri = River, Sp = Species, Si = Site, df = degrees of freedom, MS = mean square; * *p*, 0.05; ** *p*, 0.01; *** *p*, 0.001.

	$\delta^{15}N$				$\delta^{13}C$			
Source	**df**	**MS**	**F**		**df**	**MS**	**F**	
Ri	1	0.45	2.68		1	0.04	0.14	
Sp	3	110.03	649.50	***	3	22.33	69.27	***
Sp X Ri	3	1.90	11.22	***	3	0.30	0.93	
Si (Ri)	5	15.35	90.63	***	5	1.15	3.56	**
Si (Ri) X Sp	12	0.42	2.50	**	12	1.10	3.42	***
Error	215	0.17			215	0.32		

Strong dissimilarities in $\delta^{15}N$ and FA signatures were also recorded among barnacle species (Tukey HSD and PERMANOVA, both $p < 0.05$; Fig 4.2). *O. angulosa* had enriched values of $\delta^{15}N$ compared to the other two species (Tukey HSD, $p < 0.001$) and was characterized by higher levels of 14:0, 16:1w7, 16-PUFA, 20-MUFA, 20:4w3 and 20:4w6 FA (SIMPER). *C. dentatus* was enriched in SFA, bacterial FA (BAME) and 20:2w6 and *T. serrata* had higher values of 18:1w7, 20:1w11, 20:5w3 and 22:6w3 compared to the other two species (SIMPER). No difference was observed between $\delta^{15}N$ of *C. dentatus* and *T. serrata* (Tukey HSD, $p > 0.05$; Fig 4.2, a), nor among $\delta^{13}C$ signatures of the three barnacle species (Tukey HSD, $p > 0.05$).

Since the species showed strong dissimilarities among each other in both their SI and FA signatures, they were considered separately for the remaining analyses.

4.3.2. Riverine and site effects

None of the species of filter feeders showed a significant effect of the factor river using either SI or FA analyses (Table 4.2 and 4.3; Fig 4.3 and 4.4). Salinity records at the moment of the sample collection indicated sites had similar salinity value (Table 4.4).

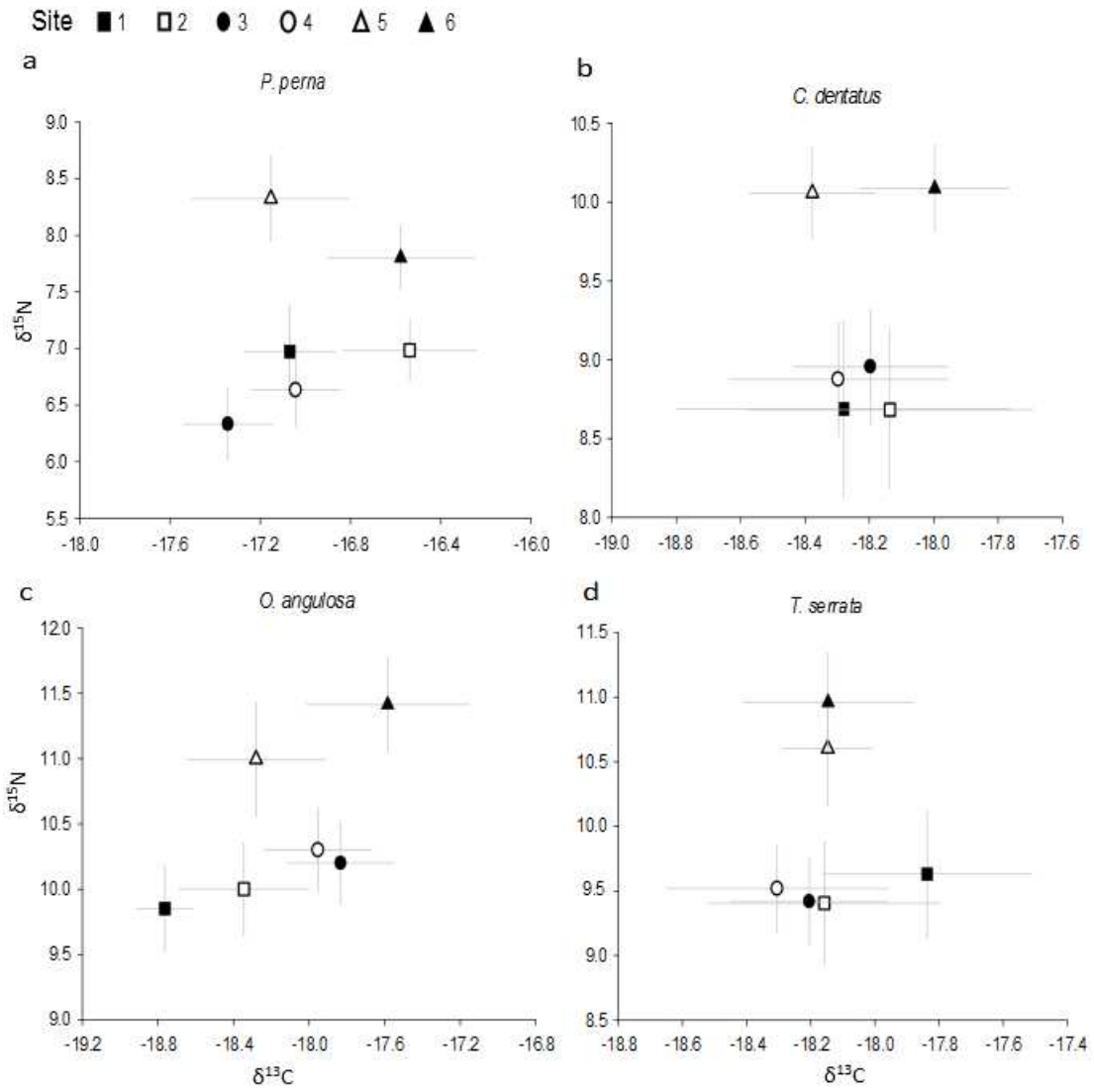


Fig 4.3 Stable isotope signatures (mean ± SD; n = 10) of a) *P. perna* b) *C. dentatus* c) *O. angulosa* d) *T. serrata* at sites close to a river mouth (black symbols) and sites far from freshwater input (open symbols) on the South African east coast.

For all species, dissimilarities were only found among sites (both ANOVA and PERMANOVA, $p < 0.001$). In particular, sites 5 and 6 had higher $\delta^{15}N$ values than the other sites, while $\delta^{13}C$ was significantly different among sites, but with no clear pattern (Tukey HSD, $p < 0.001$; Fig 4.3). FA analyses also showed strong dissimilarities among sites (PERMANOVA, $p < 0.05$), but again no clear pattern was found (Fig 4.4).

Table 4.3 PERMANOVA results on the fatty acid composition of filter feeders in relation to freshwater input on the South African east coasts. Ri = River, Sp = Species, Si = Site, df = degrees of freedom, MS = mean square; * *p*, 0.05; ** *p*, 0.01; *** *p*, 0.001.

Source	df	MS	F	p
Ri	1	145.71	0.32	
Sp	3	7512.90	40.18	***
Si (Ri)	4	452.54	9.54	***
Ri x Sp	3	64.44	0.34	
Si (Ri) x Sp	12	186.97	3.94	***
Residual	120	47.44		

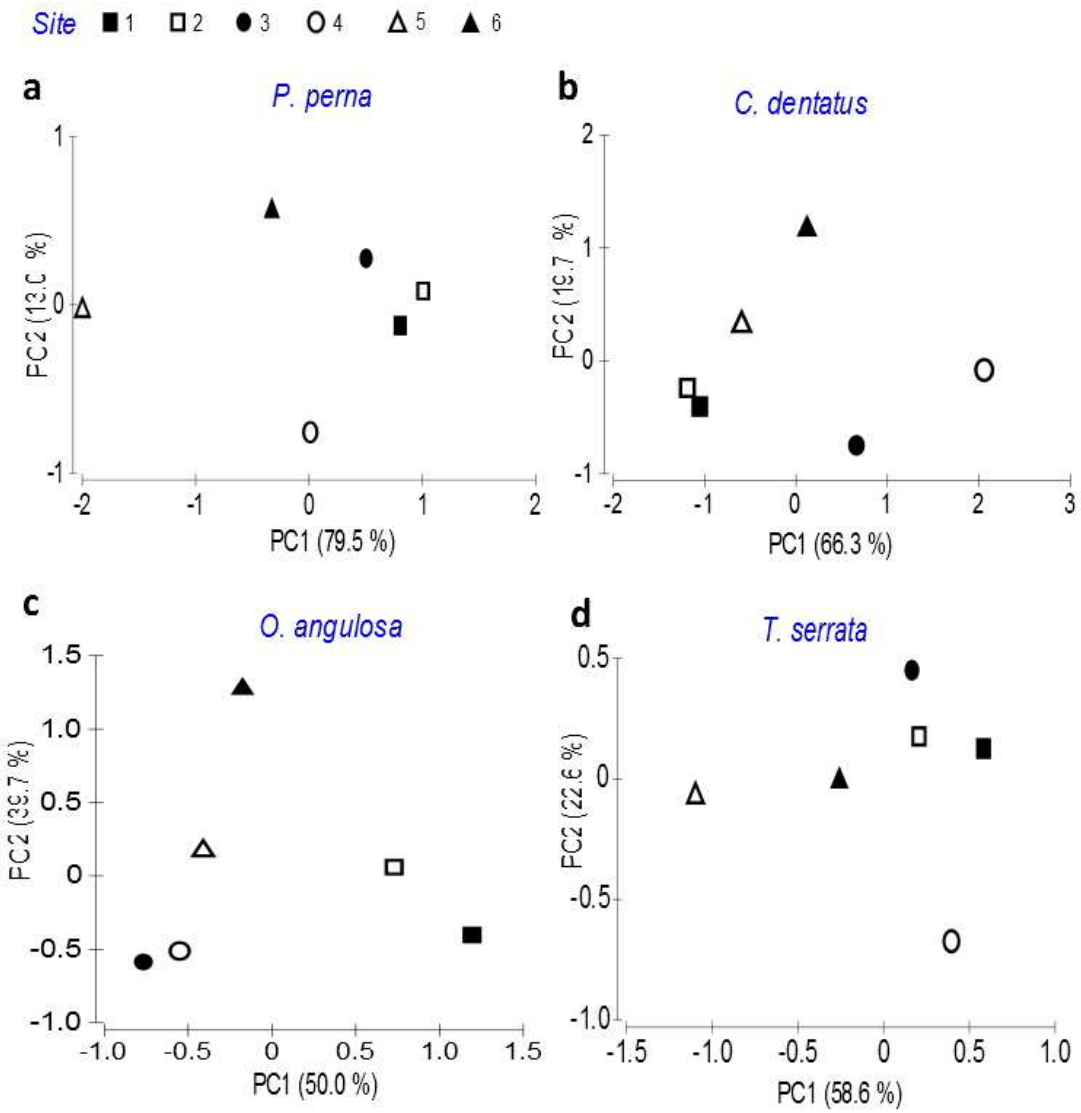


Fig 4.4 Principal component analyses (PCA) performed on the fatty acid composition of four species of filter feeders a) *P. perna* b) *C. dentatus* c) *O. angulosa* d) *T. serrata* at sites close to river mouths (black symbols) and sites far from freshwater input (open symbols) on the South African east coast (n = 6 per site).

Table 4.4 Salinity values recorded at each location at the moment of the sampling.

Site	Location	Salinity (‰)
1- Mzimvubu	A	33
	B	34
2- Mbotyi	A	33
	B	34
3- Mtamvuna	A	34
	B	36
4- Pennington	A	35
	B	34
5- Ballito	A	34.5
	B	36
6- Thukela	A	35
	B	36

4.4. Discussion

Strong differences among species across all sites were recorded with both SI and FA analyses. Mussels generally had higher $\delta^{13}C$ and lower $\delta^{15}N$ values than barnacles, and were characterized by different FA, including NMI FA which were completely absent from barnacles. In addition, differences among the $\delta^{15}N$ and FA signatures of the three barnacle species were also evident, in particular between *O. angulosa* and the other two species. These results are in agreement with the finding of Chapter 3, though mussels and barnacles from the present study had more depleted $\delta^{15}N$ than the conspecific of the south coast (see Chapter 3). These dissimilarities among species can be explained by different factors such as the microhabitats where the specimens were collected (Griffiths 1980, Wen-Xiong and Fisher 1996, Gardner 2002), different metabolic pathways or feeding mechanisms (Tenore and Dunstan 1973, Rubenstein and Koehl 1977, Narváez et al. 2008; Chapter 3) as discussed in Chapter 3.

The present study did not reveal any effect of freshwater input on the SI or FA signatures of filter feeders. These results are in agreement with a previous study conducted on the east coast of South Africa, which highlighted specimens of rocky shore filter feeders were exposed to a marine food source (Hill and McQuaid 2006). The South African east coast is characterized by a large number of rivers (Whitfield and Bate 2007), but, with the exception of the Natal Bight, the continental shelf is narrow, with the Agulhas Current remaining within few kilometers off the coast as it follows the 200 m isobath (Lutjeharms 2006). A few explanations can be given for the lack of freshwater effect. Firstly, coastal currents can change their directions in time depending on the hydrodynamic features of the area (*e.g.* presence of eddies) or wind direction (Roberts et al. 2010). Hence, freshwater input can reach areas either downstream or upstream of a river mouth and therefore lead to a well-mixed environment. As a consequence of this chaotic hydrodynamic on the coast, it is possible that specimens collected close to river mouths were as affected by freshwater input as the control sites, which would explain the absence of differences between the two conditions. Another explanation is simply dilution, with freshwater near the river mouth being thoroughly mixed with seawater, resulting in a riverine influence being too weak to be detectable in the FA or SI signatures of benthic populations. Very few studies have investigated the effects of freshwater input on the dietary regime of coastal benthic populations using FA techniques. Richoux

et al. (2014a) similarly found a minor contribution of (< 10 %) of estuarine carbon in the nearshore SPM in the vicinity region of a river mouth and concluded that freshwater input did not have a discernible effect on benthic populations located either upstream or downstream of a river mouth. The lack of effects of freshwater input on FA signatures suggests that this factor did not play a significant role in the food available for intertidal filter feeders in the present study. These results are supported by the instantaneous salinity data recorded at each location during the sampling collection, which did not show variation among sites close and far from a river mouth. However it is important to highlight that salinity changes very frequently on a hourly basis; consequently what was observed in the present study does not necessarily reflect what occurs in nature.

The present results contrast with another study conducted in the Natal Bight, in which the SI signatures of demersal organisms, sediment and SPM were analysed at several sites and depths during austral summer and winter (De Lecea et al. 2013). The authors found that the SPM available for demersal communities had mainly a freshwater origin. In the present study, the $\delta^{13}C$ values of filter feeders from sites within the Natal Bight (sites 5 and 6) were not statistically different from the other four sites with values comprised between -16.53 and -18.76 ‰, which reflected a marine origin signature according to Hill and McQuaid (2006). This suggests that intertidal filter feeders within and outside the Natal Bight, were reliant on the same food sources. In the present study the sampling was conducted in May 2012 (late autumn/winter), whereas De Lecea et al. (2013) sampled during the wet (summer) and dry (winter) seasons in 2010. Winter on the east coast of South Africa is the dry season where rainfall is low and freshwater inputs to the sea are reduced (Whitfield and Harrison 2003); in addition several estuaries of this coastline are temporarily closed during the dry season (Whitfield and Bate 2007). Therefore, it was expected that samples from the present study would be less strongly influenced by freshwater inputs compared with those of De Lecea et al. (2013). The different timing of sampling, however, cannot explain entirely the discrepancy between the two studies, as the SI turnover signatures of mussels is about 9 months (Hill and McQuaid 2009), which should reflect the diet observed averaged over the wet and dry seasons. A more likely explanation for this discrepancy can be attribute to the cycle of organic matter from rivers to marine systems. Several physical and biological processes are responsible for the transport of organic matter

from the continent to the ocean (Gagosian and Peltzer 1986, Hedges et al. 1997, Herfort 2006). Rivers carry large quantities of terrestrially derived dissolved and particulate organic matter into estuaries and then oceans, and have a fundamental role in the global carbon cycle (Schlesinger and Melack 1981, Hedges et al. 1997, Herfort 2006). Part of this organic material is remineralized in the water column (Williams and Druffel 1987), and another part is deposited in marine sediments (Hedges and Keil 1995, Mueller-Lupp et al. 2000, Hopmans et al. 2004). The intertidal area is generally considered as a transition zone between the terrestrial and marine environments where the organic matter of terrestrial origins does not remain, but simply passes through *en route* to the open sea (Levin et al. 2001). This is particularly true for rocky shores, which are areas of erosion (Ginsburg 1953). It might have been impossible to detect a riverine effect on the dietary regime of intertidal filter feeders in this study because the freshwater origin organic matter did not remain in the intertidal zone enough time. In contrast, De Lecea et al. (2013) observed an influences of freshwater SPM in the Natal Bight because organic matter is remineralized and deposited as sediment in the ocean as suggested by Ayers and Scharler (2011). It is important to notice that the conclusions of De Lecea et al. (2013) are based on the SI of the SPM collected and not on the consumers themselves. Indeed, in their investigation, the SI signature of the SPM had a clear freshwater signature, but the SI signatures of some demersal species, including filter feeders (*i.e.* bivalvia, decapoda) were within the same SI range as the species considered in the present study. This is surprising since species that rely on different food sources should exhibit different stable carbon signatures (Tieszen et al. 1983, Kaehler et al. 2000). Therefore, caution should be used in the comparison between these two studies.

The present study returned a higher $\delta^{15}N$ signature for all the species at sites within the Natal Bight (sites 5 and 6) compared to the other four sites further south along the South African coast. The Natal Bight is a relatively homogeneous environment in which water circulation involves a branch of water from the Agulhas Current (which has a southward direction), coming close to the shore and then taking a northerly direction in the area of Durban (Lutjeharms et al. 2000, Meyer et al. 2002). The Bight also receives nutrients from the upwelling cell located in the north of it (Meyer et al. 2002). A previous study conducted along the South African coast showed a biogeographic decrease from north-east to south-west in nitrogen isotope signatures of

filter feeders and suspended particulate matter (SPM; Hill and McQuaid 2006, see for further details Chapters 3 and 5), which seem to reflect an isotopic gradient from oligotrophic to more eutrophic water conditions occurring along the coast of South Africa, as described by Saino & Hattori (1980) and Minagawa & Wada (1984). The results of the present study seem to follow this same pattern. Another explanation of this pattern could also be related to the presence of cities inside the Bight (Richards Bay and Durban) with strong anthropogenic effects (Cloete and Oliff 1976, Vermeulen and Wepener 1999). As discussed in details in the previous chapter, cities such as these are likely to affect nutrient input into the sea, subsequently affecting the isotope signatures of benthic populations and may explain the enriched $\delta^{15}N$ (see Chapter 3). Both Durban and Richards Bay are cities with high levels of urbanization along the coast, and are characterized by the presence of harbours with extensive maritime traffic (Siko 1996). Considering the current circulation described here and the fact that the Natal Bight is an homogeneous environment (Meyer et al. 2002), it is possible that anthropogenic nutrients from the two cities affected the $\delta^{15}N$ of the specimens collected at the two sites 5 and 6. However, further studies to confirm or refute the existence of anthropogenic effects in this area are needed.

The present study clearly showed that freshwater input has little or no direct influence on the food sources for rocky intertidal populations of coastal areas, from few meters to a few km from the river mouth as observed in earlier studies (Vorwerk and Froneman 2009, Richoux et al. 2014a, 2014b). Since the hydrodynamics close to the river mouth, and consequently the contributions of terrestrially derived matter to coastal consumers, may diminish in the future with increased development of watersheds or human activities (*e.g.* harbour developments; Correll et al. 2001), even previously observed effects of freshwater input on marine populations are likely to be reduce.

Chapter 5

Effects of oceanographic processes operating at different spatial scales on the dietary regime of intertidal filter feeders

Bloubergstrand, Cape Town- *west coast*

Mbotyi- *east coast*

Brenton on sea- *south coast*

5. Effect of oceanographic processes operating at different spatial scales on the dietary regime of intertidal filter feeders

5.1. Introduction

The main factors driving marine large spatial scale (*i.e.* 100s km) patterns in primary production are temperature, solar radiation and nutrient availability (Field et al. 1998, Cramer et al. 1999, Elser et al. 2007). Temperature affects metabolic rates and as such acts on growth, reproduction and the productivity of primary producers (Grime 1977, Xiong et al. 2000). Solar radiation strongly affects the rate of photosynthesis (Bondeau et al. 1999, Nemani et al. 2003), while macro- and micro- nutrients frequently limit primary production in marine systems (Howarth 1988). All these factors control the intensiy of activity and the type of primary producers in the system, which subsequently affects the quantity and quality of the food available for primary consumers (McGuire et al. 1997, Blanchette et al. 2006, Lutjeharms 2006). Primary consumers, such as benthic filter feeders, play a critical role in natural ecosystems, as they act as an intermediate trophic pathway between autotrophic organisms and higher predators (Smith et al. 2009). Modifying the base of the food web can affect the physiology and distribution of higher consumers, and ultimately the functioning of the entire ecosystem (Connell 1985, Menge 2000, Dodson et al. 2000, Lavorel and Garnier 2002, Le Bauer and Treseder 2008).

Among biogeographic provinces (*i.e.* at large scales), primary production is strongly affected by large hydrogeographic regimes. In particular, currents play a fundamental role in coastal areas by mixing coastal and offshore production, potentially leading to changes in food availability for benthic populations. Along the South African coast, Bustamante et al. (1995) and Hill and McQuaid (2006) showed a biogeographic gradient of intertidal chlorophyll *a* concentration and a gradient in the stable isotope signatures of suspended particulate organic matter (SPM), respectively, that were mainly driven by the presence of the warm oligotrophic Agulhas Current on the south and east coasts and the eutrophic cold water Benguela Current on the west coast.

At a smaller spatial scales (*i.e.* mesoscales 10s-100s km), localized oceanographic events, such as upwelling or local currents, are amongst the main drivers of variability in primary production (Basterretxea and Arístegui 2000, Demarcq 2009). Coastal

upwelling events bring cold, nutrient-rich water into the euphotic zone, enhancing primary production locally. Consequently upwelling events affect the species composition of primary producers in the water column and thus the quantity and/or quality of food available for benthic populations (Figueiras et al. 2002, Blanchette et al. 2006). Changes in food availability in coastal areas can have profound consequences for benthic populations, influencing recruitment success, survival, growth, abundance and reproduction of organisms. For instance, Xavier et al. (2007) found that upwelling events along the South African west coast promote growth of intertidal mussels, and Wing et al. (1995) showed that upwelling enhances the settlement of marine invertebrates along the west North American coast, while Sanford and Menge (2001) found that barnacles living in upwelling areas show high growth rates during and after upwelling driven phytoplankton blooms, due to the combined benefits of an increase in phytoplankton and micro-zooplankton.

Finally, at local spatial scales (from one to a few km), other factors such as wave action or the presence of kelp forest, contribute to local variability in primary production. The intensity of wave action can influence the distribution of primary production in coastal areas and hence the food available for intertidal organisms (Eisma and Kalf 1987, Carter 1988, Bustamante et al. 1995). The presence of kelp forests is also another factor to take into consideration as kelp detritus represents an important source of primary production at local (one to few km) spatial scales. Duggins et al. (1989) showed that growth rates of benthic suspension feeders were two to five times higher on kelp dominated islands compared to islands without kelp, while Bustamante and Branch (1996a) highlighted the importance of kelp-derived detritus as the main source of organic carbon for benthic filter feeders on the South African west coast.

The aim of the present study is to evaluate the effect of large and mesoscale processes on the dietary regime of intertidal filter feeders. Specifically the effects of biogeography and upwelling were investigated. The biogeographic provinces of the South Africa coastline and the gradient of upwelling intensity around the South African coasts provide a unique opportunity to investigate how these oceanographic processes operating at different spatial scales affect the diet of benthic filter feeders. By using two complementary techniques (fatty acid and stable isotope analyses) the present work aims to establish: (1) the effect of biogeography on the dietary regimes of intertidal

primary consumers; (2) whether the diets of benthic filter-feeders are influenced by upwelling and (3) if the diet of these organisms differs at local spatial scales.

5.2. Materials and Methods

5.2.1. Study area and sample collection

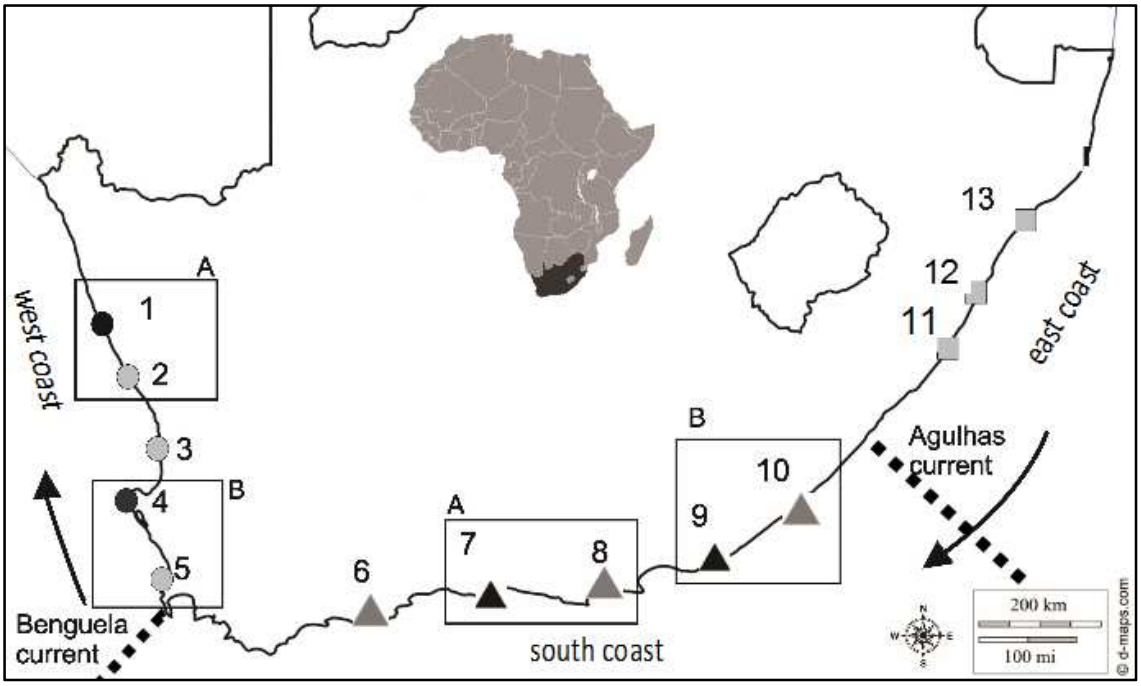


Fig 5.1 Map of South Africa showing the sampling sites in three biogeographic provinces: west coast (circles), south coast (triangles) and east coast (squares). Sites were in either upwelling or non-upwelling areas (black and grey, respectively). Site 1 (Groenrivier), site 2 (Doring Bay), site 3 (Lambert's Bay), site 4 (Cape Columbine), site 5 (Bloubergstrand), site 6 (Jongensfontein), site 7 (Brenton on Sea), site 8 (St. Francis Bay), site 9 (Port Alfred), site 10 (Kidd's beach), site 11 (Mbotyi), site 12 (Pennington) and site 13 (Ballito). All the sites in grey were used to test the effects of biogeography. To test the effects of upwelling within provinces, four sites each on the west and south coasts were allocated to regions (A or B) within each province. Tests of upwelling involved the 8 sites in these four regions.

The study was conducted along the South African coast (Fig 5.1, 34.4-29.1° S - 17.9-31.3° E). The South African coast can be divided into three biogeographic provinces roughly corresponding to the three coasts: west, south and east (Fig 5.1). Each coast is subjected to localized upwelling events occurring sporadically over the year for variable periods of time. On the west coast, the northwards flowing Benguela Current brings cold eutrophic waters and is characterised by strong, wind-driven upwelling events, occurring seasonally along the Cape Peninsula in the south, becoming more frequent northwards, with persistent upwelling around the region 60 km north of Groenrivier (site 1; Andrews and Hutchings 1980, Carr and Kearns 2003). On the south and east coasts, the Agulhas Current carries oligotrophic warm water flowing along the coast from the Mozambique Channel (Probyn et al. 1994, Lutjeharms et al. 2000). The south coast experiences wind driven upwelling, with a semi-permanent upwelling cell close to

Port Alfred (site 9). On this coast, upwelling events occur frequently over the year but with less intensity than on the west coast (Schumann et al. 1982, Walker 1986). The South African east coast experiences some upwelling events, but these are rare and weak (Lutjeharms 2006), and were not considered in the present study. The transition between the south and east coast biota corresponds to the offshore shift of the Agulhas Current. As a consequence, the two coasts have different water temperatures, with the south coast being slightly cooler that the east coast (Lutjeharms 2006).

The filter feeders investigated during this study were two species of mussels, the introduced *Mytilus galloprovincialis* and the indigenous *Perna perna*, and one species of barnacle *Chthamalus dentatus*. Neither species of mussel occurs around the whole coast of South Africa, *P. perna* is present on the south and east coasts, whereas *M. galloprovincialis* is present on the west and south coasts (Griffiths et al. 2009). In the present study, only one species of mussel was used on each coast, hence *M. galloprovincialis* was the species collected on the west coast and *P. perna* on the south and east coasts. The only barnacles used for this comparison was *C. dentatus* because it is present along all three coasts of South Africa.

To examine how biogeography and upwelling affect the dietary composition of these filter feeders, 13 sites were chosen (Fig 5.1). Sites were separated by hundreds of kilometres and within each site, samples were collected from two locations separated by 1-3 km. Sampling was conducted between April and June 2012. In order to test the effects of biogeography, three non-upwelling sites were chosen on each of the three coasts. These sites were: 2, 3, 5 on the west coast; 6, 8, 10 on the south coast and 11, 12 and 13 on the east coast (Fig 5.1). Two sites of upwelling and two sites of non-upwelling were sampled along the south and west coasts to test the effects of upwelling on filter-feeder diets. Sites 1 and 4 were considered as upwelling sites on the west coast and 7 and 9 on the south coast, while sites 2 and 5, 8 and 10 were the non-upwelling sites for the west and south coasts respectively (Fig 5.1). Sites 1 and 9 have continuous or very frequent upwelling events throughout the year, whereas site 4 has seasonal upwelling and site 7 experiences sporadic upwelling events (Andrews and Hutchings 1980, Schumann et al. 1982, Lutjeharms 2006). Further analyses were conducted to assess differences between upwelling cells within the same biogeographic province by comparing region A (sites 1 and 2) and B (sites 4 and 5) on the west coast, and region A

(sites 7 and 8) with region B (sites 9 and 10) on the south coast (Fig 5.1). At each location, haphazardly selected replicates of mussels and barnacles were collected. Three replicates of each species were used for the fatty acid (FA) analyses and five for the stable isotope (SI) analyses. Each barnacle replicate was represented by a pool of animals due to their small size, while for mussels the adductor muscle of a single individual was used due to the low turnover rate of this tissue, which renders it representative of a time integrated diet (Gorokhova and Hansson 1999). Samples for FA and SI analyses were processed with the same methods as described in Chapter 2 (Chapter 2; paragraph 2.2.1 and 2.2.2).

5.2.2. Data analysis

5.2.2.1. Stable isotopes

To examine spatial differences in $\delta^{13}C$ and $\delta^{15}N$ among the biogeographic provinces and between upwelling and non-upwelling areas, two mixed model ANOVA designs were used. To test the effects of biogeography, the design was composed of the factors: biogeographic province (three levels, fixed), site (three levels, random and nested in biogeographic province), location (two levels, random and nested in site) and species (two levels, fixed and crossed with biogeographic province, site and location). A second model used to test the effects of upwelling had the following four factors: upwelling (two levels, fixed), site (two levels, random and nested in upwelling), location (two levels, random and nested in site) and species (two levels, fixed and crossed with upwelling, site and location). In order to assess possible variability in the effects of upwelling within biogeographic provinces, a third mixed model design was used with the factors: upwelling (two levels, fixed), region (two levels, fixed and crossed with upwelling), location (two levels, random and nested in upwelling) and species (two levels, fixed and crossed with all the other factors). In the event of significant results, Tukey HSD *post hoc* tests was performed. The violation of homogeneity of variances was considered to be acceptable because ANOVA is relatively robust to heterogeneous variances for large designs such as the one in this study (Underwood 1997). Variance is reported as one standard deviation (SD) from the mean. Analyses were performed using STATISTICA v12 (StatSoft Inc. 2012).

5.2.2.2. Fatty acids

The study compared the FA composition of the species in the three biogeographic provinces of the South African coast, under upwelling and non-upwelling conditions and within provinces, using the same experimental designs as for the SI data analyses. A Multivariate Permutation Analysis (PERMOANVA; Anderson 2001) was used in order to assess differences among factors. Each term in the analysis was tested using > 9999 permutations as the relevant permutable units (Anderson and Braak 2003). Canonical Analysis of Principal coordinates (CAP, Anderson and Willis 2003, Anderson et al. 2008), a constrained ordination method that displays multivariate data with reference to *a priori* hypotheses, was used in order to investigate differences in FA signatures among species in relation to the different hypotheses (Anderson 2001, Anderson et al. 2008). The CAP analysis was based on Bray-Curtis dissimilarities calculated from percentage data after square root transformation. Vector overlays based on Pearson correlations (correlation between the variable (FA) and the CAP axes; correlation value > 0.3) were used in order to identify the FA influencing the axes. Only FA forming > 1 % of total FA (TFA) were considered in the analyses. All analyses were conducted using the PERMANOVA+ add-on package of PRIMER v6 (Clarke and Gorley 2006, Anderson et al. 2008).

5.3. Results

5.3.1. Stable isotopes

5.3.1.1. Differences among biogeographic provinces

Filter feeders clearly revealed different SI signatures depending on the biogeographic province considered (Table 5.1, Fig 5.2). West coast samples in particular were grouped significantly apart from samples from the south and east coasts (Tukey HSD, $p < 0.001$), which showed more dispersed $\delta^{13}C$ and $\delta^{15}N$ signatures. These patterns were observed for both mussels and barnacles (Tukey HSD, $p < 0.05$ in both cases, Fig 5.2). It is worth noticing that Chapters 3 and 4 revealed strong differences among species within the south and east coasts respectively. Here, however, it appears that biogeography has a stronger effect, with the different species tending to group together according to their biogeographic origin.

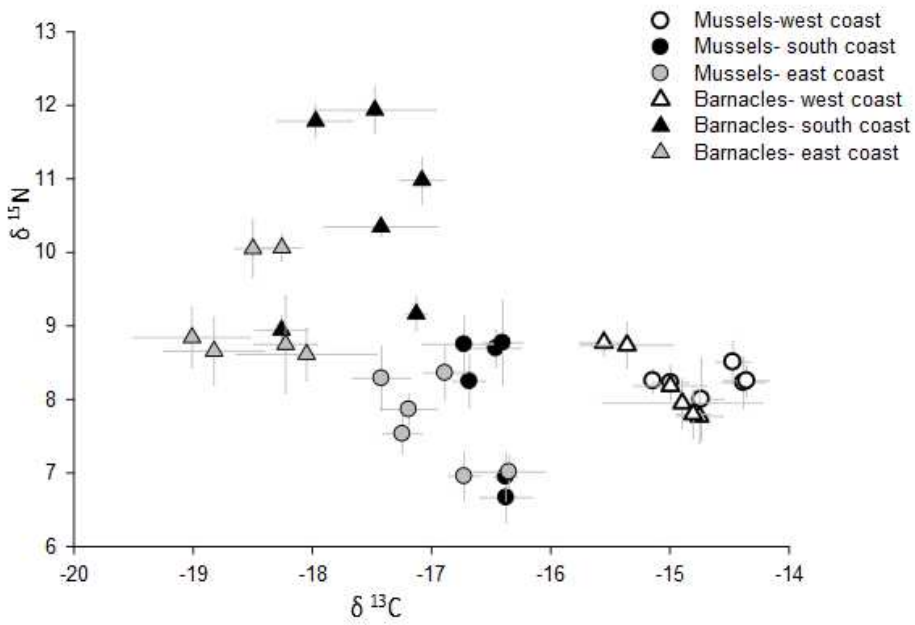


Fig 5.2 Stable isotope signatures (mean ± SD; n = 5) of mussels (circles) and barnacles (triangles), collected in the three South African biogeographic provinces: east (grey), south (black) and west (white) coasts.

Variations were also observed within each biogeographic province, with the $\delta^{15}N$ of mussels decreasing from 8.3 to 6.9 ‰ from north-east to south-west along the east coast and increasing from 6.9 to 8.7 ‰ from east to west on the south-west coasts (Fig 5.3). The $\delta^{13}C$ signatures of mussels varied widely among the three coasts. $\delta^{13}C$ became enriched from north-east to south-west along the east and south coast, and samples from the west coast were more enriched in carbon $\delta^{13}C$ than the other two coasts (-17/

-15.5 ‰ east, -16.5/ -15.5 ‰ south, -14.7/ -14.5 ‰ west). Barnacle signatures showed exactly the same pattern to mussels in terms of both carbon and nitrogen, but with generally higher average values ($\delta^{15}N$ values = 9.3, 10.5, 8.3 ‰, and $\delta^{13}C$ values = -18.6, -17.5, -15.2 ‰ respectively for the west, south and west coasts, Fig 5.3).

Table 5.1 ANOVA of stable isotope analyses performed on two groups of filter feeders (barnacles and mussels) in the three South African biogeographic provinces. Bp = Biogeographic province, Sp = Species, Si = Site, Loc = Location; df = degrees of freedom, MS = mean square; * p, 0.05; ** p, 0.01; *** p, 0.001.

	$\delta^{13}C$				$\delta^{15}N$			
Biogeography	**df**	**MS**	**F**		**df**	**MS**	**F**	
Bp	2	103.46	789.67	***	2	14.60	132.45	***
Sp	1	44.49	339.60	***	1	63.27	574.10	***
Sp X Bp	2	3.95	30.18	***	2	21.48	194.96	***
Si (Bp)	6	2.06	15.74	***	6	11.41	103.58	***
Si (Bp) X Sp	6	0.46	3.53	**	6	0.70	6.40	***
Loc (Si (Bp))	9	0.61	4.62	***	9	0.25	2.27	*
Loc (Si (Bp)) X Sp	9	0.36	2.76	**	9	0.09	0.78	
Error	109	0.13			109	0.11		

Significant differences among sites within the same province were found for both filter feeder types (Table 5.1 and 5.2). Tukey HSD tests highlighted dissimilarities among sites within the same biogeographic province for the $\delta^{13}C$ ($p < 0.01$), though not barnacles ($p > 0.05$), and for the $\delta^{15}N$ of both mussels and barnacles ($p < 0.01$); however no species showed a clear geographic pattern.

Differences between locations within the same sites were evident for $\delta^{15}N$ at site 8 for both species, while $\delta^{13}C$ showed more variation, differing between locations at almost all sites for mussels and only at site 10 for barnacles (Tukey HSD, $p < 0.01$).

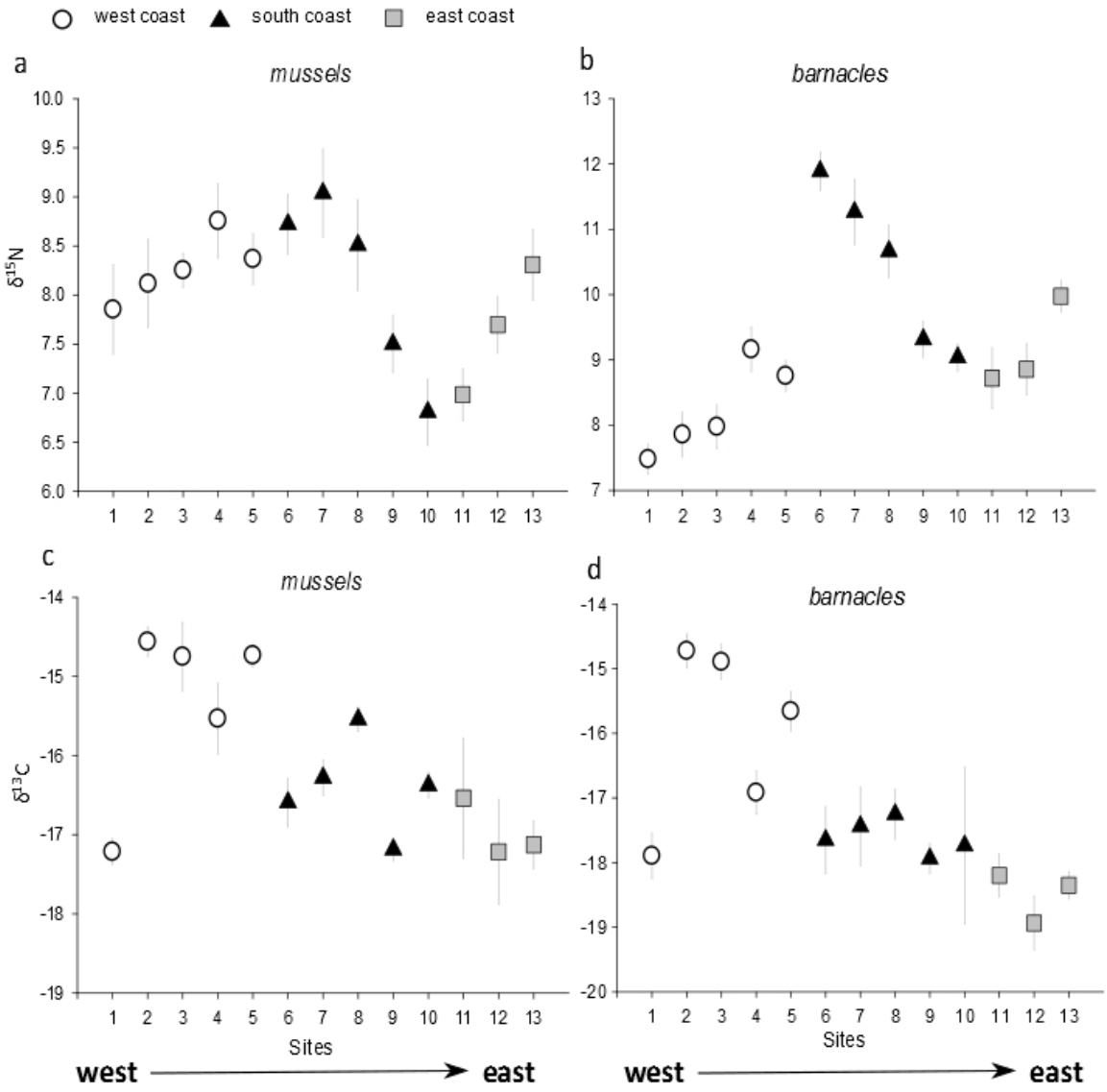


Fig 5.3 $\delta^{15}N$ and $\delta^{13}C$ (mean ± SD; n = 10) of mussels (a and c) and barnacles (b and d) at the 13 sites of the comparison within the 3 biogeographic provinces: east (grey square), south (black triangle) and west (white circle) coasts. Sites are numbered as in Fig 5.1, from the west to the east coast.

5.3.1.2. Upwelling effect

Potential upwelling effects were analysed separately for the west and south coasts due to the clear differences amongst biogeographic provinces highlighted above.

Table 5.2 Stable isotope ANOVA results preformed for filter feeders in relation to upwelling effect. Up = Upwelling, Sp = Species, Si = Site, Loc = Location; df = degrees of freedom, MS = mean square; * *p*, 0.05; ** *p*, 0.01; *** *p*, 0.001.

		$\delta^{13}C$				$\delta^{15}N$			
		df	**MS**	**F**		**df**	**MS**	**F**	
west coast	Up	1	75.89	730.32	***	1	0.03	0.27	
	Sp	1	13.30	128.00	***	1	0.04	0.29	
	Up X Sp	1	0.97	9.34	**	1	0.01	0.08	
	Si (Up)	2	10.14	97.59	***	2	9.94	80.38	***
	Si (Up) X Sp	2	1.16	11.12	***	2	1.26	10.20	***
	Loc (Si (Up))	4	0.38	3.68	**	4	0.10	0.79	
	Loc (Si (Up)) X Sp	4	0.19	1.82		4	0.12	0.94	
	Error	64	0.10			64	0.12		
south coast	Up	1	4.13	20.80	***	1	5.78	48.64	***
	Sp	1	28.54	143.63	***	1	90.54	762.32	***
	Up X Sp	1	2.04	10.28	**	1	0.11	0.91	
	Si (Up)	2	5.10	25.67	***	2	29.50	248.41	***
	Si (Up) X Sp	2	0.27	1.34		2	0.30	2.50	
	Loc (Si (Up))	4	0.76	3.80	**	4	0.81	6.79	***
	Loc (Si (Up)) X Sp	4	1.05	5.30	***	4	0.14	1.15	
	Error	64	0.20			64	0.12		

Significant upwelling effects were found for mussels and barnacles on both the south and the west coasts (Table 5.2). Mussels and barnacles from upwelling sites had depleted $\delta^{13}C$ signatures compared to the non-upwelling sites (Fig 5.3 and Fig 5.4, a and c). This effect was stronger on the west coast, in particular at site 1, which has continuous upwelling, and for mussels, for which a depletion of carbon at upwelling sites was more evident than for barnacles (Fig 5.3 and Fig 5.4, a and c). The two non-upwelling sites on the west coast (2 and 5) had similar $\delta^{13}C$ values (Tukey HSD, $p > 0.05$). In contrast, there were no effects of upwelling on the nitrogen signatures of mussels or barnacles on the west coast (Table 5.2, Fig 5.4 a and c). On the south coast, depletion of $\delta^{13}C$ ratios at upwelling sites was again evident for both types of filter feeders (Table 5.2) with a more intense effect for mussels. As for the west coast, the site with continuous upwelling, site 9, showed stronger effects than the other upwelling site on the south coast (Tukey HSD, $p < 0.01$; Fig 5.3 and Fig 5.4, b and d). There was also a significant effect of site on nitrogen signatures (Table 5.2). Tukey HSD tests highlighted sites 7 and 8 had similar $\delta^{15}N$ signatures in both species (Tukey HSD, $p > 0.05$), while site 9 had higher nitrogen signatures than site 10 only in mussels (Tukey HSD, $p < 0.05$). Tukey HSD also highlighted differences in $\delta^{15}N$ between locations at site 8 on the south

coast for both species ($p < 0.01$), and dissimilarity in $\delta^{13}C$ between locations at sites 2, 8, 9 for mussels and at site 10 for barnacles ($p < 0.01$). The location effect did not influence the results of the other factors.

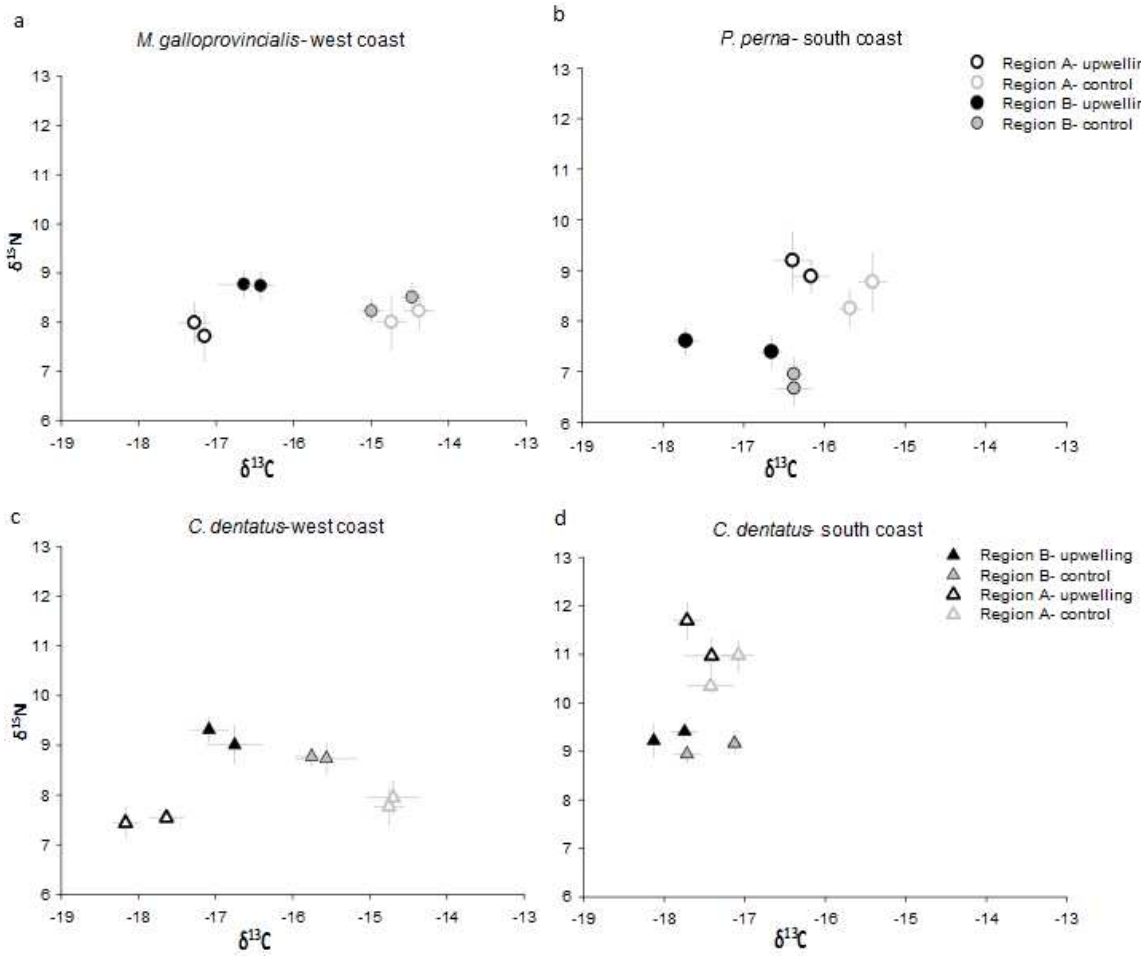


Fig 5.4 $\delta^{13}C$ and $\delta^{15}N$ signatures of intertidal mussels (circles) and barnacles (triangles) at locations within upwelling (black) and non-upwelling (grey) sites on the west and south coasts (mean ± SD; n = 5). The open symbols refer to samples from region A, filled symbols refer to region B.

5.3.1.3. Region effect

At the intra-province level, a clear separation between the two regions (A and B) was found on each coast for both filter feeder types (ANOVA, $p < 0.01$). $\delta^{13}C$ was depleted at upwelling sites on both coasts, with a stronger upwelling effect within each coast where upwelling was continuous or very frequent over the year (site 1, Groenrivier, and site 9, Port Alfred, for the west and south coasts respectively). When the factor region was considered on the west coast, upwelling still had no significant effect on $\delta^{15}N$ for either species (ANOVA, $p > 0.05$), while on the south coast in region A for barnacles and in

region B for mussels, nitrogen signatures were higher at upwelling sites compared to non-upwelling sites (ANOVA, both $p < 0.05$). In addition, Tukey HSD ($p < 0.01$) highlighted an increase of $\delta^{15}N$ from region A to region B for both species.

5.3.2. Fatty acid composition

PERMANOVA conducted on the FA composition of barnacles and mussels indicated significant differences in their signatures across all sites ($p < 0.001$). The presence of FA synthesized *de novo* (NMI, non-methylene interrupted fatty acids) by mussels prevented direct comparison with the barnacles (Table 5.3 andTable ***5.4***), hence barnacles and mussels were analysed separately.

5.3.2.1. Biogeography

Twenty-four FA were found for mussels and sixteen for barnacles that contributed > 1 % of the TFA (Table 5.3 and 5.4). The proportions of monounsaturated (MUFA), polyunsaturated (PUFA) and saturated FA (SFA) differed among the three coasts. Specifically, mussels generally showed high levels (50 - 60 %) of PUFA on all coasts, followed by SFA (30 %) and MUFA (10 - 20 %). Overall w-3 FA (*i.e.* 22:6w3, 20:5w3), 20:4w6 and 22 NMI were the predominant FA counting for 25 - 50 % of TFA (Table 5.3). For the barnacle *C. dentatus*, SFA were enriched on all coasts with high values of 16:0, 18:0 and bacterial FA (BAME, ~ 25 – 70 %), though 20:5w3 and 22:6w3 still formed a high (28 - 44%) proportion of the TFA (Table 5.4).

Table 5.3 Total fatty acid composition (% of TFA) of mussels (*P. perna* on the south and east coasts and *M. galloprovincialis* on the west coast) collected at 13 sites across three biogeographic provinces. The values are percentages expressed as mean ± standard deviation. Only FA contributing > 1 % to TFA are displayed.

Fatty acids	**west coast**					**south coast**					**east coast**		
	Site 1 Groenrivier	**Site 2** Doring Bay	**Site 3** Lambert's Bay	**Site 4** Cape Columbine	**Site 5** Bloubergstrand	**Site 6** Jongensfontein	**Site 7** Brenton on Sea	**Site 8** St. Francis Bay	**Site 9** Port Alfred	**Site 10** Kidd's beach	**Site 11** Mbotyi	**Site 12** Pennington	**Site 13** Ballito
14:0	0.73 ± 0.19	1.13 ± 0.31	0.85 ± 0.15	0.77 ± 0.20	1.50 ± 0.30	1.96 ± 0.32	4.09 ± 0.72	2.08 ± 0.41	2.33 ± 0.19	2.65 ± 0.47	2.30 ± 0.44	3.34 ± 0.99	3.33 ± 1.30
16:0	18.70 ± 1.41	24.07 ± 2.37	19.67 ± 1.60	18.70 ± 0.58	21.21 ± 1.63	20.32 ± 3.05	30.18 ± 6.89	17.45 ± 2.98	15.52 ± 2.69	18.64 ± 3.61	15.38 ± 1.14	17.71 ± 4.02	25.81 ± 8.49
16:1w7	1.82 ± 0.62	3.03 ± 0.90	2.41 ± 1.01	2.48 ± 0.91	4.12 ± 1.31	2.54 ± 0.33	3.07 ± 0.52	3.11 ± 0.52	4.02 ± 0.49	3.90 ± 0.27	4.31 ± 0.86	5.89 ± 1.78	5.58 ± 1.67
18:0	5.30 ± 0.77	6.53 ± 1.15	5.70 ± 0.48	5.30 ± 0.68	5.54 ± 0.99	8.41 ± 1.48	12.08 ± 2.75	8.57 ± 2.30	8.91 ± 0.89	10.10 ± 2.40	7.49 ± 0.76	7.23 ± 1.41	10.92 ± 2.08
18:1w9	1.36 ± 0.40	1.32 ± 0.28	1.06 ± 0.43	1.90 ± 0.61	1.50 ± 0.40	1.39 ± 0.16	1.98 ± 0.50	1.47 ± 0.31	1.45 ± 0.28	1.99 ± 0.45	1.62 ± 0.13	2.16 ± 0.68	2.97 ± 0.57
18:1w7	2.29 ± 0.38	1.85 ± 0.31	2.12 ± 0.44	2.29 ± 0.14	2.58 ± 0.28	2.11 ± 0.35	2.07 ± 0.24	2.51 ± 0.67	2.92 ± 0.56	2.57 ± 0.38	2.65 ± 0.73	2.87 ± 1.09	2.37 ± 0.61
18:2w6	1.52 ± 0.47	0.72 ± 0.12	0.77 ± 0.19	1.63 ± 0.26	1.04 ± 0.11	2.52 ± 0.46	1.33 ± 0.70	2.03 ± 0.57	2.30 ± 0.39	1.68 ± 0.68	3.37 ± 0.65	3.43 ± 0.54	1.97 ± 1.28
18:3w3	0.87 ± 0.38	0.23 ± 0.14	0.41 ± 0.14	0.92 ± 0.26	0.52 ± 0.14	0.84 ± 0.28	0.40 ± 0.47	0.73 ± 0.41	0.98 ± 0.52	0.47 ± 0.42	1.29 ± 0.25	1.02 ± 0.24	0.53 ± 0.46
18:4w3	1.09 ± 0.43	1.16 ± 0.47	1.50 ± 0.35	1.54 ± 0.49	1.14 ± 0.52	0.61 ± 0.20	0.34 ± 0.58	0.83 ± 0.61	0.69 ± 0.31	0.25 ± 0.30	1.00 ± 0.27	0.91 ± 0.25	0.21 ± 0.24
20:1w11	1.15 ± 0.26	1.24 ± 0.13	1.30 ± 0.26	0.90 ± 0.47	1.45 ± 0.12	1.53 ± 0.28	1.57 ± 0.31	1.50 ± 0.21	1.46 ± 0.18	1.48 ± 0.20	2.60 ± 0.57	2.24 ± 1.97	2.21 ± 0.89
20:1w9	4.66 ± 0.46	4.71 ± 0.87	4.30 ± 0.42	4.71 ± 1.74	3.75 ± 0.30	3.97 ± 1.06	6.70 ± 1.63	4.12 ± 0.95	3.58 ± 0.59	5.15 ± 1.14	2.80 ± 0.18	3.50 ± 0.95	7.07 ± 2.40
20:1w7	0.62 ± 0.22	0.79 ± 0.35	0.85 ± 0.13	1.45 ± 1.33	1.26 ± 0.19	0.70 ± 0.28	1.29 ± 0.39	1.41 ± 0.40	1.37 ± 0.27	1.66 ± 0.47	0.81 ± 0.19	1.62 ± 1.23	1.02 ± 0.44
20:2 NMI1	6.01 ± 1.18	4.73 ± 0.75	5.93 ± 0.96	5.55 ± 2.11	4.31 ± 0.91	4.28 ± 0.80	2.55 ± 0.84	4.68 ± 0.79	4.07 ± 0.64	3.87 ± 0.75	4.65 ± 1.64	5.12 ± 2.74	2.31 ± 0.79
20:2 NMI2	1.43 ± 0.56	1.26 ± 0.45	1.05 ± 0.16	1.89 ± 1.42	1.20 ± 0.73	0.58 ± 0.22	1.00 ± 0.67	0.99 ± 0.94	0.49 ± 0.28	0.84 ± 0.43	0.57 ± 0.26	1.08 ± 0.40	1.21 ± 0.59
20:4w6	4.84 ± 0.27	3.19 ± 0.67	2.97 ± 0.65	5.39 ± 1.75	3.31 ± 0.94	5.17 ± 0.86	2.31 ± 1.18	5.55 ± 1.38	5.27 ± 0.54	5.44 ± 1.27	7.91 ± 0.54	6.73 ± 1.67	4.36 ± 2.35
20:5w3	10.20 ± 1.79	8.25 ± 1.81	10.68 ± 1.41	13.98 ± 2.65	16.76 ± 3.78	5.76 ± 1.57	2.94 ± 2.51	7.28 ± 3.47	8.09 ± 1.66	5.09 ± 2.21	8.50 ± 2.04	6.12 ± 2.55	2.97 ± 1.93
22:2w6	0.85 ± 0.39	0.98 ± 0.37	1.08 ± 0.35	1.39 ± 0.37	0.69 ± 0.19	2.06 ± 0.49	1.18 ± 0.40	2.21 ± 0.44	2.17 ± 0.36	2.14 ± 0.53	1.98 ± 0.36	1.99 ± 0.74	1.58 ± 0.90
22:2 NMI1	4.97 ± 1.27	3.99 ± 0.80	4.99 ± 0.76	4.62 ± 0.93	5.22 ± 1.59	5.31 ± 0.78	2.94 ± 1.21	6.42 ± 1.28	7.21 ± 0.69	6.58 ± 0.94	6.17 ± 1.23	6.05 ± 2.39	3.76 ± 1.99
22:2 NMI2	0.91 ± 0.57	1.72 ± 0.39	1.31 ± 1.00	1.00 ± 0.18	0.79 ± 1.13	1.36 ± 0.55	2.01 ± 1.35	0.98 ± 0.68	1.99 ± 1.45	3.26 ± 2.17	0.35 ± 0.42	1.44 ± 0.41	2.46 ± 0.86
22:3 NMI	1.87 ± 0.30	1.36 ± 0.20	1.75 ± 0.34	1.71 ± 0.11	1.41 ± 0.14	1.52 ± 0.36	0.44 ± 0.71	1.46 ± 0.37	1.53 ± 0.16	1.33 ± 0.27	1.48 ± 0.19	1.11 ± 0.24	0.75 ± 0.49
22:4w6	0.72 ± 0.10	0.51 ± 0.11	0.36 ± 0.08	0.72 ± 0.27	0.40 ± 0.32	1.34 ± 0.43	0.31 ± 0.36	1.73 ± 0.39	1.51 ± 0.36	2.09 ± 0.33	2.54 ± 0.58	1.79 ± 0.54	1.29 ± 0.83
22:5w6	0.23 ± 0.18	0.08 ± 0.12	0.14 ± 0.16	0.24 ± 0.12	0.15 ± 0.12	1.26 ± 0.16	0.64 ± 0.41	1.06 ± 0.21	1.31 ± 0.21	1.11 ± 0.18	1.59 ± 0.32	1.52 ± 0.26	1.14 ± 0.62
22:5w3	2.00 ± 0.15	1.30 ± 0.17	1.60 ± 0.25	1.68 ± 0.15	1.95 ± 0.12	2.00 ± 0.31	1.06 ± 0.69	2.46 ± 0.63	3.21 ± 0.86	2.28 ± 0.64	2.43 ± 0.95	1.11 ± 0.05	1.22 ± 0.52
22:6w3	20.67 ± 2.04	20.42 ± 2.57	22.65 ± 2.42	15.16 ± 1.30	13.46 ± 1.56	16.89 ± 3.30	9.71 ± 5.32	14.58 ± 2.43	13.88 ± 2.15	10.21 ± 2.75	12.66 ± 1.92	9.72 ± 1.26	7.05 ± 4.17
BAME	5.20 ± 0.58	5.42 ± 0.49	4.56 ± 0.36	4.06 ± 0.25	4.70 ± 1.65	5.59 ± 0.78	7.81 ± 2.07	4.78 ± 1.14	3.75 ± 0.75	5.25 ± 1.29	3.53 ± 0.29	4.30 ± 0.75	5.94 ± 1.89
Σ20 NMI	7.44 ± 0.87	5.99 ± 0.60	6.98 ± 0.56	7.44 ± 1.76	5.51 ± 0.82	4.86 ± 0.51	3.55 ± 0.76	5.66 ± 0.86	4.55 ± 0.46	4.71 ± 0.59	5.22 ± 0.95	6.20 ± 1.57	3.51 ± 0.69
Σ22 NMI	7.75 ± 0.71	7.08 ± 0.46	8.04 ± 0.70	7.33 ± 0.41	7.42 ± 0.95	8.18 ± 0.57	5.39 ± 1.09	8.86 ± 0.78	10.73 ± 0.77	11.16 ± 1.12	8.00 ± 0.61	8.60 ± 1.01	6.97 ± 1.11
ΣSFA	24.73 ± 1.72	31.74 ± 2.70	26.22 ± 1.71	24.77 ± 0.95	28.26 ± 2.54	30.68 ± 3.50	46.35 ± 7.74	28.11 ± 3.95	26.75 ± 2.94	31.39 ± 4.55	25.17 ± 1.47	28.28 ± 4.43	40.05 ± 9.03
ΣMUFA	11.90 ± 0.94	12.94 ± 1.34	12.04 ± 1.21	13.75 ± 2.49	14.67 ± 1.41	12.24 ± 1.19	16.67 ± 1.85	14.11 ± 1.21	14.81 ± 0.88	16.74 ± 1.35	14.79 ± 1.07	18.28 ± 3.15	21.21 ± 3.14
ΣPUFA	58.17 ± 3.46	49.90 ± 3.52	57.17 ± 3.36	57.42 ± 4.45	52.37 ± 4.82	51.49 ± 4.08	29.17 ± 6.55	52.99 ± 5.03	54.69 ± 3.51	46.63 ± 4.69	56.51 ± 3.82	49.14 ± 5.08	32.80 ± 6.05

Table 5.4 Total fatty acid composition of the barnacle *C. dentatus* on the west, south and east coasts. The values are percentage expressed as mean standard ± deviation. Only FA contributing > 1 % are displayed.

Fatty acids	west coast					south coast					east coast		
	Site 1 Groenrivier	**Site 2** Doring Bay	**Site 3** Lambert's Bay	**Site 4** Cape Columbine	**Site 5** Bloubergstrand	**Site 6** Jongensfontein	**Site 7** Brenton on Sea	**Site 8** St. Francis Bay	**Site 9** Port Alfred	**Site 10** Kidd's beach	**Site 11** Mbotyi	**Site 12** Pennington	**Site 13** Ballito
14:0	1.45 ± 0.58	4.34 ± 1.32	3.45 ± 0.37	2.40 ± 0.41	5.95 ± 0.91	2.60 ± 0.85	3.10 ± 0.64	2.93 ± 0.53	4.48 ± 1.24	4.38 ± 0.60	3.86 ± 0.78	4.44 ± 0.84	3.25 ± 0.50
16:0	23.62 ± 4.73	28.91 ± 7.51	19.74 ± 2.00	16.38 ± 0.95	19.63 ± 2.30	26.13 ± 5.22	25.40 ± 4.24	35.87 ± 7.56	24.07 ± 6.33	21.05 ± 0.86	24.94 ± 3.33	41.92 ± 3.86	28.55 ± 2.52
16:1w7	4.03 ± 0.59	5.74 ± 1.01	5.94 ± 0.36	3.58 ± 0.40	6.21 ± 1.12	2.52 ± 0.62	2.43 ± 0.33	2.01 ± 0.56	4.45 ± 1.35	5.25 ± 0.55	4.86 ± 0.39	3.06 ± 1.63	3.80 ± 0.46
18:0	3.40 ± 0.94	2.98 ± 0.78	2.01 ± 0.38	3.48 ± 0.21	3.81 ± 0.64	15.84 ± 3.88	12.33 ± 3.57	23.94 ± 5.30	9.29 ± 3.28	9.12 ± 0.63	12.30 ± 2.54	21.01 ± 5.95	15.36 ± 2.33
18:1w9	6.98 ± 0.45	5.08 ± 0.58	4.93 ± 1.18	8.97 ± 1.22	8.60 ± 0.58	6.42 ± 0.40	5.14 ± 0.31	4.12 ± 1.78	5.12 ± 0.96	6.27 ± 0.28	6.51 ± 0.79	3.35 ± 1.60	5.63 ± 0.56
18:1w7	5.23 ± 0.84	4.82 ± 0.91	3.92 ± 0.57	5.44 ± 1.00	5.48 ± 1.10	3.33 ± 0.23	2.51 ± 0.22	2.74 ± 1.28	2.45 ± 0.96	3.02 ± 0.32	3.20 ± 0.44	3.04 ± 1.69	2.99 ± 0.72
18:2w6	3.97 ± 0.98	1.11 ± 0.21	1.55 ± 0.14	2.43 ± 0.25	0.95 ± 0.07	1.12 ± 0.33	1.26 ± 0.17	0.64 ± 0.30	0.76 ± 0.26	0.95 ± 0.04	1.16 ± 0.36	0.81 ± 0.52	1.30 ± 0.25
18:3w3	2.99 ± 0.92	0.39 ± 0.35	0.30 ± 0.03	1.89 ± 0.26	0.42 ± 0.08	0.47 ± 0.22	0.61 ± 0.13	0.26 ± 0.24	0.58 ± 0.18	0.60 ± 0.06	0.90 ± 0.48	0.44 ± 0.30	0.84 ± 0.21
18:4w3	7.23 ± 3.15	4.37 ± 2.07	6.93 ± 0.67	5.06 ± 1.19	1.77 ± 0.29	0.48 ± 0.30	0.93 ± 0.60	0.32 ± 0.35	1.29 ± 0.58	1.00 ± 0.17	1.22 ± 0.56	1.14 ± 0.89	0.80 ± 0.21
20:1w11	1.29 ± 0.15	1.40 ± 0.13	1.37 ± 0.53	3.03 ± 0.53	2.09 ± 0.56	0.52 ± 0.45	0.54 ± 0.36	0.86 ± 0.60	0.64 ± 0.19	0.87 ± 0.25	1.18 ± 0.26	0.72 ± 0.24	0.53 ± 0.26
20:1w9	0.74 ± 0.08	0.97 ± 0.22	0.83 ± 0.04	1.28 ± 0.05	1.04 ± 0.12	0.64 ± 0.17	0.76 ± 0.32	0.60 ± 0.23	0.50 ± 0.29	0.52 ± 0.05	0.46 ± 0.06	0.52 ± 0.20	0.54 ± 0.17
20:5w3	16.23 ± 2.75	11.31 ± 3.17	14.20 ± 0.92	22.23 ± 0.94	25.32 ± 2.12	12.99 ± 4.24	12.39 ± 2.49	6.69 ± 5.17	15.82 ± 6.55	20.73 ± 0.56	15.02 ± 4.10	4.58 ± 2.94	12.62 ± 1.50
22:0	0.29 ± 0.15	0.33 ± 0.12	0.18 ± 0.08	0.22 ± 0.05	0.22 ± 0.10	2.04 ± 0.41	1.72 ± 0.48	2.77 ± 0.36	1.25 ± 0.75	0.92 ± 0.05	1.25 ± 0.43	1.93 ± 0.76	1.75 ± 0.38
22:5w3	0.09 ± 0.10	0.13 ± 0.31	0.04 ± 0.09	0.73 ± 0.06	0.42 ± 0.07	0.79 ± 0.58	0.40 ± 0.21	0.30 ± 0.34	1.12 ± 0.63	1.75 ± 0.13	1.43 ± 0.38	0.43 ± 0.36	0.83 ± 0.13
22:6w3	20.29 ± 2.05	24.03 ± 4.75	30.63 ± 1.25	19.91 ± 1.04	13.80 ± 1.86	19.19 ± 5.86	21.59 ± 6.10	9.19 ± 5.59	17.63 ± 5.40	20.38 ± 1.81	16.93 ± 4.53	5.94 ± 2.09	16.31 ± 3.11
BAME	2.16 ± 0.49	3.92 ± 1.27	3.95 ± 0.21	2.13 ± 0.16	2.11 ± 0.24	4.88 ± 0.79	8.81 ± 1.13	6.75 ± 1.08	9.91 ± 4.25	2.93 ± 0.22	4.25 ± 1.55	6.58 ± 0.36	4.71 ± 0.70
ΣSFA	30.92 ± 4.88	40.47 ± 7.76	29.33 ± 2.08	24.61 ± 1.07	31.71 ± 2.56	51.48 ± 6.62	51.36 ± 5.71	72.27 ± 9.32	49.01 ± 8.43	38.40 ± 1.25	46.61 ± 4.55	75.89 ± 7.19	53.63 ± 3.56
ΣMUFA	18.27 ± 1.14	18.02 ± 1.50	16.98 ± 1.46	22.30 ± 1.71	23.42 ± 1.77	13.42 ± 0.92	11.38 ± 0.70	10.34 ± 2.35	13.17 ± 1.94	15.93 ± 0.75	16.21 ± 1.02	10.69 ± 2.86	13.49 ± 1.06
ΣPUFA	50.81 ± 4.85	41.33 ± 6.11	53.65 ± 1.70	52.25 ± 1.91	42.68 ± 2.86	35.03 ± 7.27	37.17 ± 6.62	17.39 ± 7.64	37.20 ± 8.57	45.41 ± 1.91	36.66 ± 6.19	13.33 ± 3.78	32.70 ± 3.48

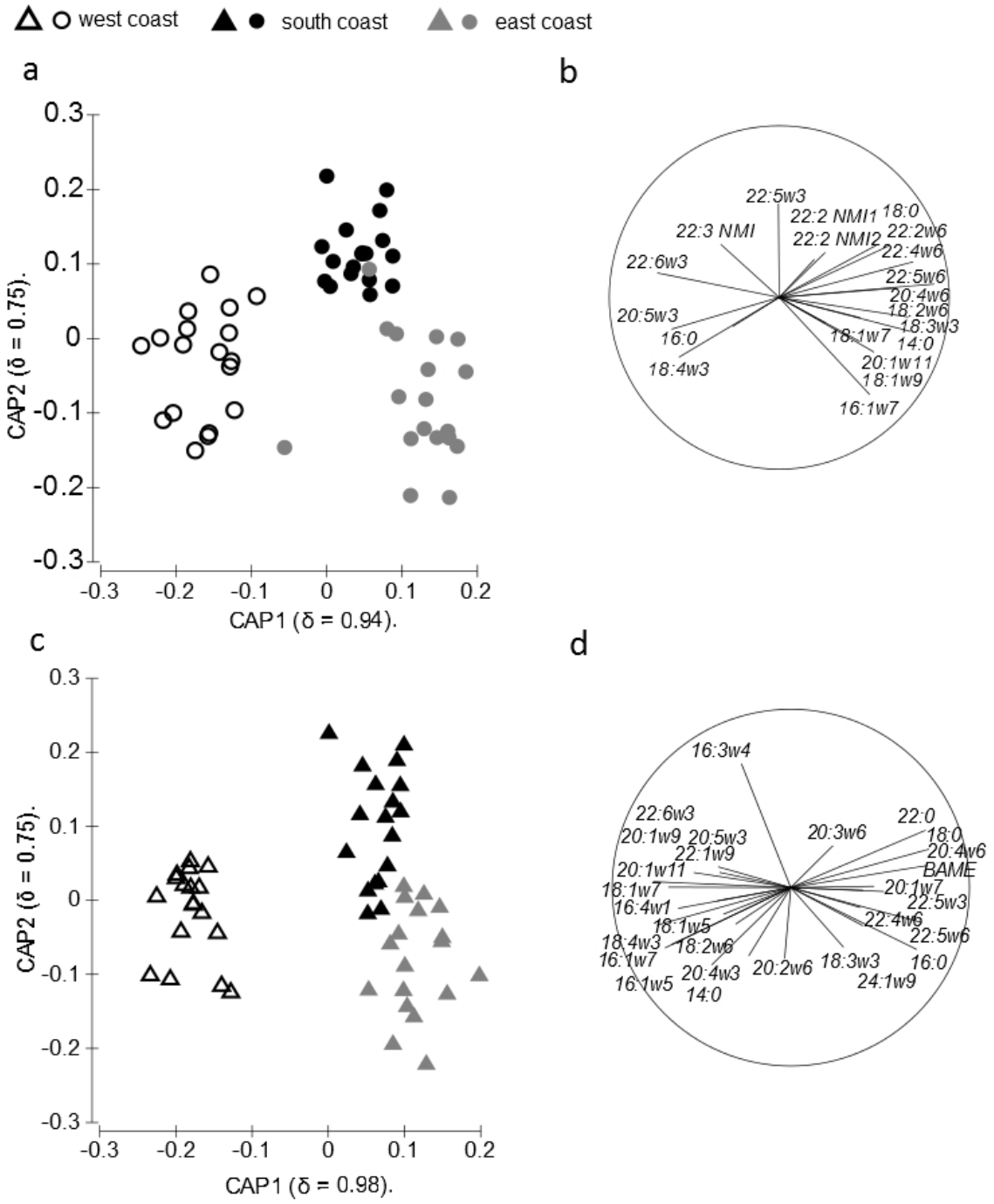


Fig 5.5 Canonical Analysis of Principal coordinates (CAP) of TFA composition of (a) mussels (circle) and (c) barnacles (triangle) in the three biogeographic provinces: west, east, south coast. δ values indicate the canonical correlation of each axis. (b) and (d), vectors for mussels and barnacles respectively, illustrating the Pearson correlations > 0.3 of the FA with the axes of the CAP, with the circle overlay scaled to the maximum correlation value and indicating the magnitude of effect.

PERMANOVA and CAP revealed significant differences in the signatures of both filter feeders among the biogeographic provinces (p < 0.01; Fig 5.5). For both taxa, the differences were mainly due to 16:1w7, 16:4w1, 18-MUFA, 18:4w3, 20:5w3 (EPA) and

22:6w3 (DHA), which were more abundant on the west coast; while 18:0, 18:3w3, 20:1w7, 20:4w6, 22:4w6 and 22:5w6 were predominant on the other two coasts (CAP; Fig 5.5, b and d; Table 5.3 and 5.4). In addition, mussels had higher levels of 22: NMI on the west coast (Table 5.3). Axis two of each of the two CAP highlighted dissimilarities between the south and east coasts. For mussels, the south coast was characterized by the FA 20 NMI, 22 NMI and 22:5w3, while the east coast had a higher proportion of 16:1w7, 18:1w9, 18:4w3 and 20:1w11 (CAP; Fig 5.5, b; Table 5.3). Barnacles showed a few dissimilarities from mussels. In particular, specimens from the east coast had a high proportion of 14:0, 18:3w3, 20:2w6 and 20:4w6; while on the south coast 16:3w4 and 20:3w6 were the predominant FA (CAP; Fig 5.5, d; Table 5.4).

Table 5.5 PERMANOVA results on the fatty acid composition of mussels and barnacles under upwelling and non-upwelling conditions on the South African west and south coasts. Up = Upwelling, Si = Site; df = degrees of freedom, MS = mean square, * *p*, 0.05; ** *p*, 0.01; *** *p*, 0.001

west coast		**df**	**MS**	**F**	
Mussels	Up	1	194.83	8.54	***
	Si (Up)	2	124.57	5.46	***
Barnacles	Up	1	549.24	23.36	***
	Site (Up)	2	375.54	15.97	***
south coast		**df**	**MS**	**F**	
Mussels	Up	1	160.05	2.91	
	Si (Up)	2	463.76	8.44	**
Barnacles	Up	1	166.52	2.97	
	Si (Up)	2	651.34	11.62	**

5.3.2.2. Upwelling effect

Replicates of locations from the same sites were grouped together (hence n = 6 for each site) in this analysis as no FA differences were found between locations at the same site (PERMANOVA, $p < 0.001$).

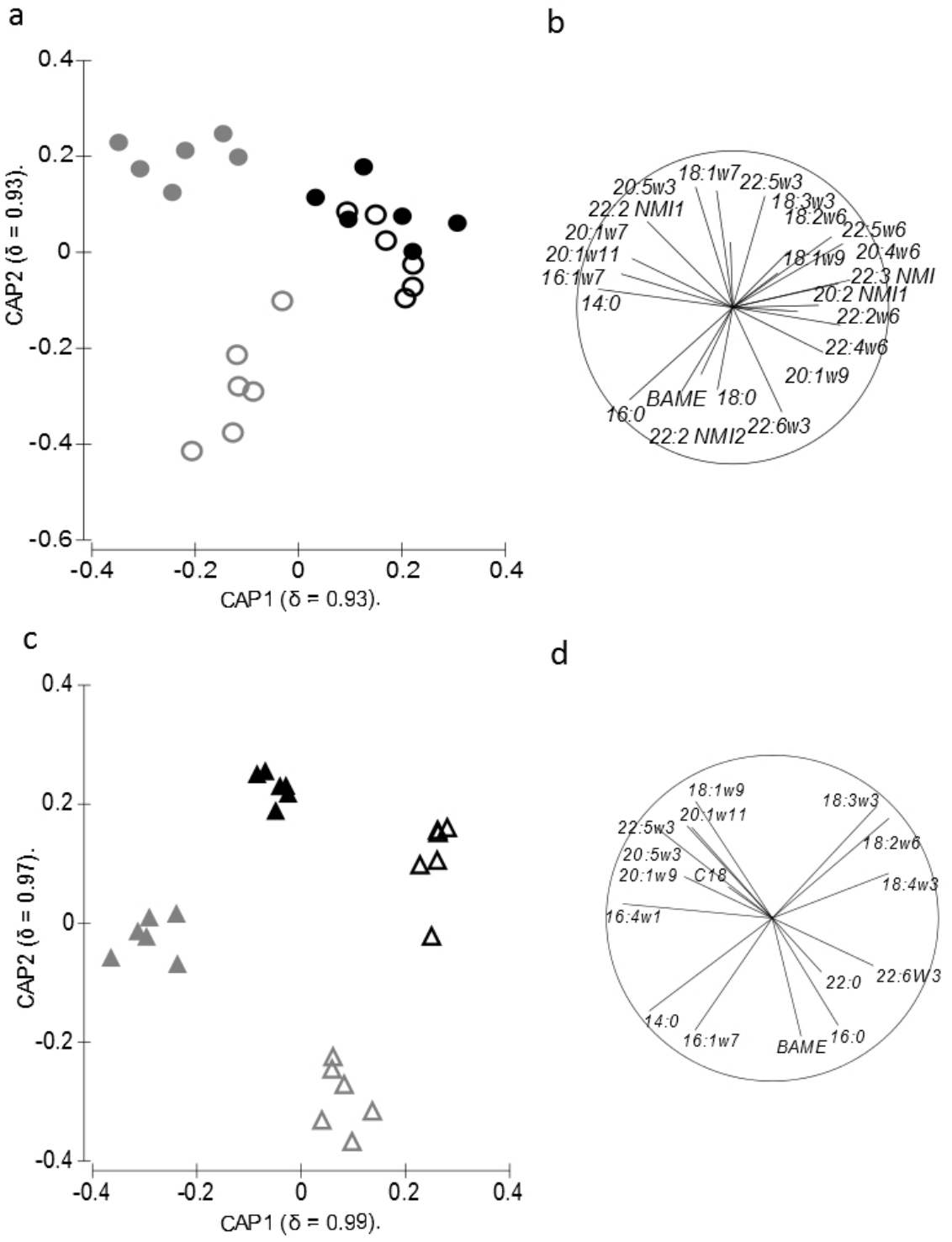


Fig 5.6 CAP of the TFA composition of mussels (a, circle) and barnacles (c, triangle) on the west coast at upwelling (black) and non-upwelling (grey) sites. The open symbols refer to samples from region A (sites 1 and 2), while the closed symbols refer to region B (sites 4 and 5). (b) and (d) vectors illustrating the Pearson correlations > 0.3 of the FA with the axes of the CAP, with the circle overlay scaled to the maximum correlation value and indicating the magnitude of effect.

Mussels and barnacles showed similar responses to upwelling on the west coast (Table 5.5), with organisms from upwelling areas being enriched in PUFA such as 18:2w6,

18:3w3, 20:2 NMI, 20:4w6 and 22:5w6, while those from non-upwelling areas having higher values of BAME, 14:0, 16:0, 16:1w7, 18:0 or 20:1w11 (Fig 5.6, b and d). In contrast, no effect of upwelling was detected on the FA composition of the south coast samples (Table 5.5; CAP not shown).

Significant differences among sites were found for both species (PERMANOVA, $p < 0.01$). CAP showed differences among samples from sites exposed to either upwelling or non-upwelling conditions on the west coast for both species (Fig 5.6). Specifically, sites 2 and 5 (non-upwelling) for mussels and barnacles and sites 1 and 4 (upwelling) for barnacles only, were statistically different from one another (PERMANOVA, $p < 0.001$). The FA responsible for the dissimilarities between upwelling areas for barnacles were 16:4w1, 20:5w3, 22:5w3, 20:1w11 and 18:1w9 that had high values at site 4, while at site 1, 18:2w6, 18:3w3, 18:4w3 and 22:6w3 were predominant (CAP; Fig 5.6 d; Table 5.4). For both species, site 2 had a higher proportion of BAME, 16:0, 18:0, 22:0 and 22:6w3 compared to site 5. In addition, mussels had high values of 22:2NMI2 at this site. Site 5 was characterized by 20:1w11, 20:5w3 and 22:5w3 (CAP; Fig 5.6 b and d; Table 5.3 and 5.4). On the south coast, *post-hoc* pair wise tests revealed differences among sites for both species, however no clear pattern was found (PERMANOVA $p < 0.01$; data not shown).

5.3.2.3. Region effect

At the local scale, no significant differences were found in the FA signatures of samples from different locations at the same site (PERMANOVA, $p > 0.05$). PERMANOVA, however, revealed intra-province differences on the west coast between regions A (Sites 1 and 2) and B (Sites 4 and 5) for both species ($p < 0.001$). Mussels were characterized by 16:0, 18:0, BAME, 20:1w9, 22:2NMI2 and 22:6w3 FA in region A, and by 18:1w7, 20:1w7, 20:5w3, 22:2NMI1 and 22:5w3 FA in region B. Barnacle samples from region A had high values of 16:0, 18:2w6, 18:3w3, 22:0 and 22:6w3; while in region B, 16:4w1, 18:0, 18:1w9, 20:1w9, 20:1w11, 20:5w3, and 22:5w3 were predominant (Fig 5.6 d). No intra-province effects were found for either species on the south coast (PERMANOVA, $p > 0.05$).

5.4. Discussion

SI and FA signatures of mussels and barnacles showed very distinct patterns in response to both biogeography and upwelling. Among the three South African biogeographic provinces, $\delta^{15}N$ of both species decreased along a geographical gradient from north to south along the east coast, it increased from east to west along the south coast and remained roughly constant along the west coast, where $\delta^{15}N$ signatures were more depleted than on the other two coasts. $\delta^{13}C$ values also differed among the biogeographic provinces, with mussels and barnacles showing the same pattern. $\delta^{13}C$ was enriched from north-east to south-west along the east and south coasts and samples from the west coast were more enriched in $\delta^{13}C$ than the other two coasts. This is in accord with results from a previous study which found a gradient of $\delta^{13}C$ and $\delta^{15}N$ signatures of the SPM and intertidal benthic organisms along the South African coast (Hill and McQuaid 2006). The strong spatial pattern in SI signatures of benthic primary consumers observed in the present study between the two coasts (*i.e.* south and east coast versus west coast) is likely to reflect the effects of the Benguela Current on the west coast and the Agulhas Current on the other two coasts. The presence of the cold, nutrient-rich Benguela Current on the west coast promotes high primary production dominated by phytoplankton, in particular diatoms and kelp (Andrews and Hutchings 1980, Emanuel et al. 1992, Pitcher et al. 1992, Shannon and Nelson 1996). While on the south and east coasts, the Agulhas Current brings oligotrophic warm water with low primary producer concentrations in coastal areas, and the coastal ecosystem has to mainly rely on remineralized nutrients (Miyake and Wada 1967). Thus, it is hypothesised that the enrichment of $\delta^{15}N$ in benthic filter feeders from north-east to south-west reflects an isotopic gradient from oligotrophic to more eutrophic conditions, as described by Saino & Hattori (1980) and Minagawa & Wada (1984). The differences in isotopic signatures of the species considered in this study are probably a result of the dependence of primary producers on recycled nitrogen in oligotrophic waters, which are depleted in $\delta^{15}N$ compared to eutrophic systems (Miyake and Wada 1967). FA signatures of filter feeders on the west coast were enriched in w-3 PUFA, while the south and east coasts had high values of w-6 PUFA. High levels of w-3 PUFA are indicative of microalgae, while macroalgae are w-6 PUFA enriched (Dalsgaard et al. 2003). This suggests a greater dependence on upwelling-supported phytoplankton blooms on the

west coast and a stronger dependence on macroalgal detritus on the south and east coasts, as previously showed by Hill and McQuaid (2006). The high levels of NMI FA in the mussels collected on the west coast also support this hypothesis as these FA are synthesized *de novo* by bivalves via elongation and Δ5 desaturation of 18:1n-9 and 16:1n-7, which are both very abundant in phytoplankton (prymnesiophyta and diatoms) and in heterotroph organisms such as ciliates (Zhukova 1991, Dalsgaard et al. 2003, Peters et al. 2006). The Benguela Current is a highly productive system with nitrate concentrations between 5 - 8 mg L^{-1} (Payne and Crawford 1989, Basterretxea and Arístegui 2000) and an annual mean chlorophyll *a* concentration of 2.15 mg m^{-3} (Brown et al. 1991). In contrast, the south and east coasts are characterized by the nutrient-depleted Agulhas Current (nitrate concentration < 0.62 mg L^{-1} and an annual mean chlorophyll *a* concentration of 1.48 mg m^{-3}; Probyn et al. 1994, Machu et al. 2005), resulting in lower rates of primary production offshore and in the intertidal zone compared to the west coast (Schleyer 1981, Shannon 1989, Bustamante et al. 1995). In addition, the two coasts are dominated by different macroalgae compositions. For examples the west coast is characterized by extensive forests of the kelp *Ecklonia maxima* and *Laminaria pallida* (Velimirov 1980, Bustamante et al. 1995), while the south and east coasts are typified by seaweeds, such as rhodophytes and coralline algae (Bustamante et al. 1995, Griffiths and Branch 1997, Bolton et al. 2004). As a result, the three biogeographic provinces are characterized by different primary producer' composition, which appears to be mirrored in the FA composition of the mussels and barnacles studied. Indeed, a high proportion of phytoplankton trophic markers were found in the samples from the west coast, and high macroalgal detritus markers in the south and east coasts samples. However, surprisingly the result of the present study did not indicate an influence of kelp detritus on specimens' diet on the west coast despite the presence of extensive kelp forests.

According to chapter 2, *P. perna* and *M. galloprovincialis* have different FA composition. Therefore, the differences in mussel FA signatures between the west and south-east coasts can also partly be due to a difference between species, and not solely due to the effects of biogeography. However, *C. dentatus* also showed strong separation between the two coasts, exhibiting a similar pattern to mussels. This suggests that

probably all filter feeders are strongly influenced by the biogeography of the coast of South Africa, which seemed to be the main driver of variability in this study.

Upwelling had a profound effect on the SI and FA signatures of the studied species. Carbon signatures were significantly depleted at upwelling sites for all three species on both the west and the south coasts, with values lying between those for filter feeders at non-upwelling sites (from -15.5 to -18 ‰) and offshore phytoplankton (from -20 to -22 ‰; Hill and McQuaid 2006). $\delta^{13}C$ is an indicator of food source and as such, the results of the present study indicate that these benthic filter feeders fed on a food source depleted in the heavy carbon isotope. Previous SI studies indicated the dependence of intertidal organisms on macroalgal detritus for food (Bustamante and Branch 1996a, Hill and McQuaid 2006), and showed that the $\delta^{13}C$ of SPM displays significant depletion when moving from the near to the offshore environments (Hill et al. 2008). In addition, other studies showed that upwelling enhances nutrient levels, and thus stimulates phytoplankton and onshore macrophyte growth (Nielsen and Navarrete 2004, Wieters 2005). These considerations and the depleted $\delta^{13}C$ signatures of specimens at upwelling sites compared to non-upwelling sites suggest that benthic filter feeders at upwelling areas consumed a mix of coastal macroalgal detritus and phytoplankton brought onshore during downwelling events.

On the west coast, the $\delta^{15}N$ signatures of mussels and barnacles were not significantly different between upwelling and non-upwelling sites, indicating that the trophic level of these two filter feeders was not affected by the influence of upwelling. On the south coast, specimens from the upwelling sites in region A for barnacles and region B for mussels had enriched $\delta^{15}N$ compared to the non-upwelling sites; in addition, there was an enrichment of $\delta^{15}N$ from region B (sites 9 and 10) to region A (sites 7 and 8). This enrichment of $\delta^{15}N$ could be due to the geographic location of the sites. The results for biogeographic effects indicated a gradient of increasing $\delta^{15}N$ from south-east to south-west, probably due to the proximity of the Agulhas Current to the coastline, as described. The same explanation could be applied in the present case, in which the apparent increase in $\delta^{15}N$ at the upwelling influenced site 7 for barnacles and site 9 for mussels, and from region A to region B on the south coast could be an artefact of this geographic gradient.

FA analyses confirmed the SI results for the west coast, but did not show any effect of upwelling on the south coast for either mussels or barnacles. Upwelling intensity and frequency differ markedly between the west and south coasts. On the west coast, the Benguela Current is a highly productive system with strong upwelling events along the coast (Shannon et al. 1983, Shannon and Nelson 1996), while the south coast generally tends to experience more ephemeral, localised upwelling events (Lutjeharms et al. 2000). Samples from upwelling sites on the west coast showed higher percentages of PUFA such as 18:3w3, 18:2w6, 20:5w3, 22:5w3 or 22:5w6 compared to non-upwelling sites, which had high values of MUFA and SFA, for example 14:0, 16:0, 20:1w11 and 16:1w7. High levels of PUFA are usually associated with highly productive systems and are an indication of good food quality (Brett and Müller-Navarra 1997, Müller-Navarra et al. 2000, Dalsgaard et al. 2003). PUFA are also required during growth due to their essential role in membrane structure and activity (Wacker and von Elert 2001, Alkanani et al. 2007). Hence, the present results suggest that filter feeders experience better quality food at upwelling sites compared to non-upwelling sites. A few studies have highlighted the positive effects of upwelling on food for benthic populations. For instance Corbisier et al. (2014) in an investigation of demersal populations off Cabo Frio in Brazil showed that consumers were heavily dependent on the organic matter produced and deposited during the downwelling following strong upwelling events. It is not possible to completely exclude the potential impact of water temperature on the PUFA composition of these organisms as many studies have highlighted an increase of PUFA associated with the maintenance of membrane fluidity in cold environments (Tooke and Holland 1985, Hall et al. 2002).

Both SI and FA analyses highlighted differences between sites of upwelling within the same coast. Site 1 (Groenrivier, region A) was characterized by more depletion of $\delta^{13}C$ and dinoflagellate trophic markers (*i.e.* 18:4w3, 22:6w3) than site 4 (Cape Columbine, region B), which had high levels of diatom FA such as 16:1w7, 20:5w3. Along the south coast, differences between upwelling sites were identified only in the SI analysis, with $\delta^{13}C$ being more depleted at site 9 (Port Alfred, region A) than site 7 (Brenton on Sea, region B). Site 1 (Groenrivier) and site 9 (Port Alfred) are centres of either continuous or frequent upwelling, whereas site 4 (Cape Columbine) experiences seasonal events of upwelling, mostly during summer season and finally upwelling is

sporadic and ephemeral at site 7 (Velimirov 1980, Newell 1982, Schumann et al. 1982, Lutjeharms et al. 2000). The different frequencies and intensities of upwelling events will result in different inputs of nutrients, with consequences for the food available to benthic organisms. Cole and McQuaid (2010) also found variability in the abundances and species composition of organisms associated with mussel beds in different areas within the same biogeographic province of South Africa. Smith et al. (2009) in a study conducted in two biogeographic provinces of California, showed how recruitment and growth can differ considerably within the same province. These studies and the present findings highlight the concept that sites of upwelling are not equal to each other in term of intensity and frequency of the event and that this is reflected in the diets of benthic populations. Consequently, the different FA and SI signatures of specimens in the present study could also be due to dissimilar characteristics of upwelling depending on the biogeographic province considered.

Apart from the obvious effects of upwelling and biogeography described, there were differences among sites on the south and east coasts and between non-upwelling sites on the west coast. The south and east coasts are broadly characterized by the same oceanographic regimes, although the influence of the Agulhas Current weakens towards the south-west. However others factors (*e.g.* urbanization, bays) can also influence the food available for benthic organisms which would explain the differences between sites within a same oceanographic regime (Probyn et al. 1994, Hooper et al. 2005, Bode et al. 2014).

The present results also illustrated differences in primary consumer trophic markers, which probably mirror differences of food at local smaller scales. Dissimilarities were observed in $\delta^{13}C$ and $\delta^{15}N$ signatures between locations at the same sites, particularly for mussels. Such scales of variability can be attributed to a wide range of processes. McQuaid and Mostert (2010) experimentally manipulated water flow at centimetre scales around mussel beds, which altered growth rates, while Kon et al. (2007) observed that the diet of bivalves in an Australian mangrove forest changed according to the microhabitat (tidal creek, mangrove forest, mangrove forest gap) inhabited. Schall et al. (2011a) also suggested that processes happening at the microhabitat level, such as bacterial degradation of macroalgae, can change the food available for intertidal consumers that are living only centimetres apart. These

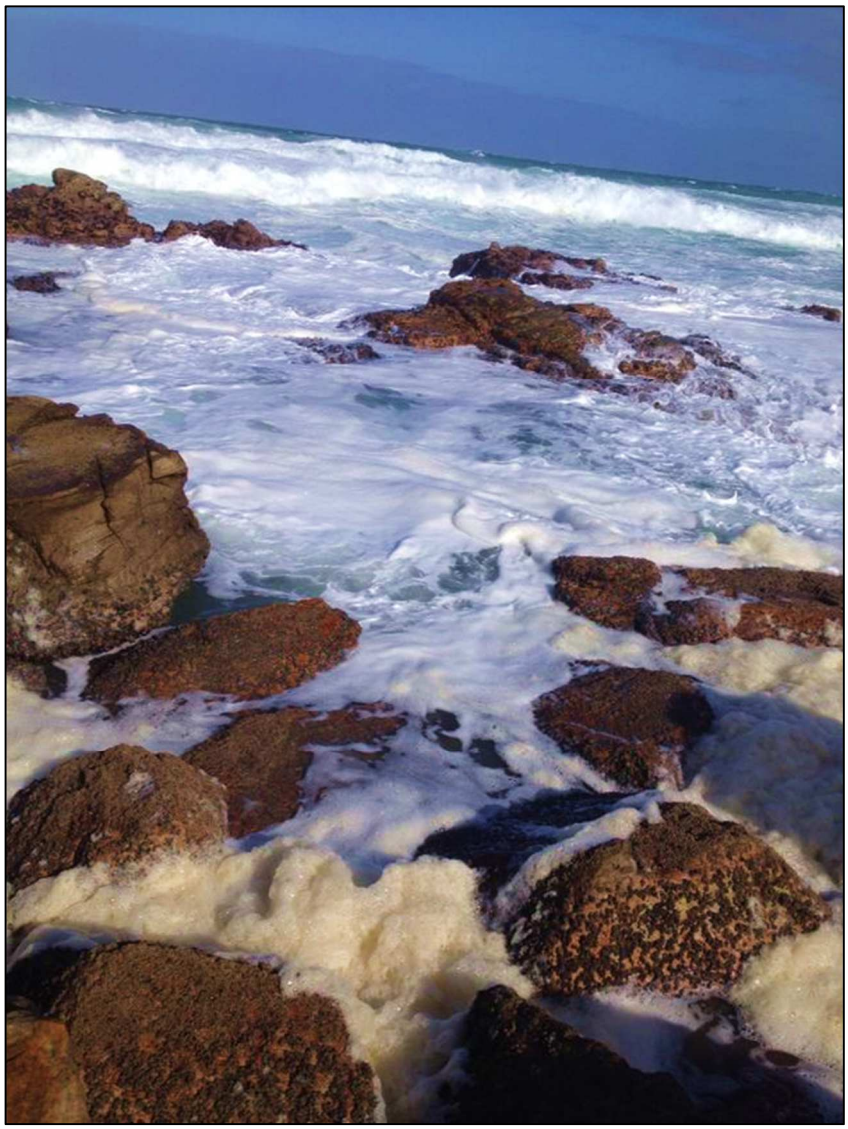
observations, including the results from this study, are consistent with Levin (1992) who highlighted the importance of planning experiments and studies to take into account ecological processes that occur at different spatial scales.

Mussels and barnacles had different SI and FA signatures, but showed the same patterns in relation to biogeography and upwelling. In the present study, all samples were collected from shores with similar wave action. Furthermore, both mussels and barnacles are non-selective filter feeders, so that the differences in their trophic signatures are driven by other mechanisms such as their size or feeding mechanisms (Griffiths and King 1979). The size of filter feeders often determines the size of prey they can ingest and in the present study the specimens of mussels ranged from 4 to 8 cm, while barnacles reached a maximum size of 2 cm which can lead to different prey being ingested (Rubenstein and Koehl 1977). In addition, *C. dentatus* is a passive feeder, whereas mussels are active feeders (Branch et al. 2007). The last factor that can explain some of the differences amongst species is their FA pathways which are controlled genetically (Napolitano 1999) and as such would be different from one taxa to the other. The combined effect of these aspects can hence contribute to explain the differences observed in their SI FA signatures.

The present study highlights the importance of the linkage between oceanographic processes acting at different spatial scales on the diet of primary consumers. Biogeographic regions and upwelling have an impact of the diet of the benthic filter feeders studied. The biogeography factor is the most important while upwelling events are nested within the biogeographic effect and have a different impact depending on their frequency and intensity. This highlights that, for both filter feeders, the importance of the processes influencing diet depends on the spatial scale considered.

CHAPTER 6

Spatio-temporal effects of upwelling on the fatty acid composition of benthic filter feeders in the Benguela Current ecosystem



Upwelling event- South Africa

6. Spatio-temporal effects on the fatty acid composition of benthic filter feeders in the Benguela Current ecosystem

6.1. Introduction

Temporal and spatial variations in mesoscale nearshore oceanographic conditions play an important role in the distribution of primary production (Wieters 2005, Blanchette et al. 2006), resulting in differences in the availability of resources for intertidal consumers (Menge et al. 1997, McQuaid and Payne 1998). One such feature is represented by upwelling events (Bosman et al. 1987, Smith et al. 2009). Changes in food availability can have powerful effects on the distribution and metabolism of intertidal organisms (Connell 1985, Raimondi 1990, Figueiras et al. 2002). For example, Ventura et al. (1997) showed that the reproductive peak of *Astropecten brasiliensis* coincided with the upwelling season, which enabled their larvae to benefit from the phytoplankton rich water; while Wieters et al. (2005) in a study conducted along the coast of Chile showed that turf algae grew faster at the upwelling centre compared to downstream of the upwelling.

Upwelling events can change strongly in intensity and frequency over time (*e.g.* Adamec and O'Brien 1978, Lewis 1981, Picaut 1983, Field and Shillington 2006). These changes have far-reaching effects on coastal systems and intertidal organisms. For instance, Smith et al. (2009) on the California coast found that strong upwelling reduced mussel recruitment in coastal areas due to the export of larvae offshore, whereas when upwelling weakened, it was facilitated onshore larval transport and high recruitment to rocky intertidal habitats. Likewise, changing characteristics of upwelling can also have strong repercussions on coastal primary production (Pitcher et al. 1992, 1993). For example, Corbisier et al. (2014), in an investigation of the subtidal benthic trophic structure in Cabo du Frio (Brazil), showed temporal variability in the stable isotope (SI) signatures of consumers that reflected changes in the pattern of food distribution due to temporal variation in upwelling events. During an upwelling event, the phytoplankton composition in coastal areas changes. The advection of nutrient rich waters allows the proliferation of diatoms, sometimes reaching extremely high concentrations (Kiørboe et al. 1998). After an upwelling event, diatoms are usually replaced by dinoflagellates mainly due to silicon becoming limiting for diatom growth (silicon being the main

constituent of diatom frustules; Humborg et al. 2000, Martin-Jézéquel et al. 2000, Tilstone et al. 2000).

The present study aims to examine the spatio-temporal effect of upwelling on the diet of benthic intertidal filter feeders. The natural seasonality of upwelling events on the South African west coast provides a unique opportunity to investigate the relationship between temporal variability in nearshore oceanographic conditions and food availability for benthic populations. The main hypothesis of this chapter is that the fatty acid composition of mussels would be characterized by diatom fatty acid trophic markers (FATM) at upwelling centres while this signature should decrease downstream of the upwelling centre. Similarly it is expected that the proportion of dinoflagellate biomarkers in the mussels will be higher downstream of upwelling sites (as observed by Allan et al. 2010) or to increase soon after an upwelling event (*e.g.* during the downwelling period). In addition, the specimens of upwelling centre are expected to be in better condition than in non-upwelling areas. Within this broad context, this study also aims to identify if mussels adopt a specific life-strategy in response to this high food seasonality. Indeed when food is highly variable in time, organisms usually tend to advantage reproduction or survival (Ricklefs 1977, Erikstad et al. 1998, Post and Parkinson 2001). By investigating the fatty acid (FA) composition of the adductor muscles and the gonads, one can infer whether the organisms are investing more energy in reproduction or growth, respectively.

Small scale (from cm to a few m) differences in food availability can also be important for benthic populations, especially for sessile or sedentary species. For example, McQuaid and Mostert (2010) showed that very small (cm) scale changes in hydrodynamics around mussel clumps can affect their growth rates. Similarly, other studies showed isotopic variation among organisms only few cm apart due to microscale variation in food availability (Guest et al. 2004, Kon et al. 2007, Schaal et al. 2011). Although several studies proved that many factors affect populations from different heights on the shore (*e.g.* thermal stress. desiccation or wave action), no study has investigated if food availability changes over a vertical intertidal gradient, for example between low shore and high shore populations.

To summarise, this work investigates the spatio-temporal variability of upwelling events on the FA composition of mussels in the Benguela upwe*lling system. Special

focus was directed towards: (1) the investigation of gonads and adductor muscles to determine a preferred life-strategy in response to food pulses, (2) determine if upwelling is favourable for mussels (*i.e.* condition indices), (3) how different heights habitats can affect mussels in upwelling conditions. FA analysis was used as represents an appropriate tool to investigate organisms' diet and it can also provide information on food quality and life-strategy metabolism (*i.e.* energetic storage for growth or reproduction).

6.2. Materials and Methods

6.2.1. Study area

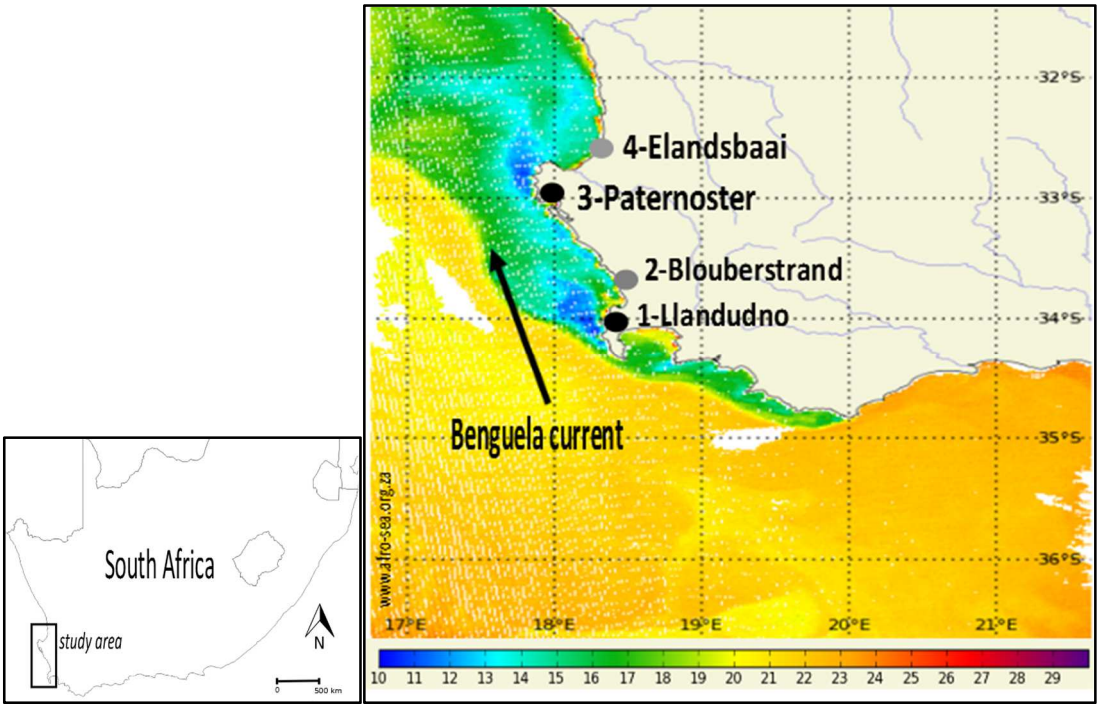


Fig 6.1 Map of the study area on the west coast of South Africa showing the sampling sites in upwelling (black) and non-upwelling (grey) areas. The right-hand side map illustrates an upwelling event (13/01/2014) with the colours bar indicating sea surface temperatures (SST).

The study was conducted along the South African west coast (Fig. 1, 34.4-29.1 S° 17.9-31.3 E). This coast is characterized by the Benguela Current, cold eutrophic waters with an Antarctic origin, which flow northerly. This current is associated with wind driven upwelling events, occurring seasonally along the study area (Andrews and Hutchings 1980, Verheye Dua and Lucas 1988).

6.2.2. Sampling sites

Sites were identified as upwelling or non-upwelling centres following Xavier et al. (2007) based on *in situ* sea surface temperatures using temperature data logger and Advanced Very High Resolution Radar (AVHRR) satellite thermal imagery. Sites 1 (Llandudno) and 3 (Paternoster) were upwelling sites and 2 (Bloubergstrand) and 4 (Elandsbaai) were non-upwelling sites (Fig 6.1). Both upwelling sites experience seasonal upwelling events which are more intense during the summer months (Andrews and Hutchings 1980, Shannon et al. 1984, Field and Shillington 2006). Four samplings were conducted: two in the austral summer (10 - 11th December 2012 and 8 - 9th February

2013) when the upwelling events are usually predominant, and two in winter (12 - 13th June and 8 - 9th July 2013) during the non-upwelling season.

6.2.3. Temperature data

To characterize upwelling frequency, sea temperature was recorded *in situ a*t each site during the course of this study, from December 2012 to July 2013. Four temperature loggers were deployed on the intertidal rocky shore at each of the four sampling sites. The loggers were composed of an iButton model DS 1922L Dallas Maxim, CA, USA (Thermochron high resolution (-40 °C to +85 °C), accuracy 0.0625°C) covered with Teflon and glued with two-component epoxy (Alcolin rapid-epoxy) onto grey 8.0 X 5.5 cm, perspex plates that resembled a natural rock surface as closely as possible (Fig 6.2). They were programmed to measure temperature every 30 min and they were replaced every three months due to memory limitation. The iButtons were programmed using the software ColdChain Thermo Dynamics. The location of each logger varied slightly between sites but remained within the same 20 cm tidal range. Hourly data of predicted tidal height at the sampling sites were provided from the website XTide: harmonic tide clock and tide predictor (http://www.flaterco.com/xtide/xtide.html). Following Harley and Helmuth (2003) the time of the tide's return was assumed, based on temperature decreases/increases of 3.0 °C over 20 min (or 2.25 °C per 15 min) during rising and falling tides. Consequently, this study assumed a change of ± 4.5°C over 30 min separated temperature data for when the data loggers were in or out of the water.

In order to assess how the glue and the plate affected the values recorded by the iButton a laboratory experiment was run. In particular, five loggers attached to plates and five "free" (without Teflon, glue and plate) iButtons were placed in a water bath for 24 h at a constant temperature of 20 °C. The difference between the mean values of the two groups was 0.25 °C (ANOVA, $p < 0.01$). Consequently, this value was subtracted from the temperature values recorded by the data loggers.

The temperature data were used to compare temperature at sites in upwelling centres with their adjacent non-upwelling sites, and temperature changes within sites, including the calculation of the frequency of upwelling events. These were defined as a decline in mean daily temperature (ΔT) of ≥ 5°C (Shannon et al. 1984, Lutjeharms et al.

2000) and they were categorized as strong rapid cooling events. The number of successive days after each cooling event in which the temperature remained constant ($\Delta T \geq 5$ °C) wad also recorded. When the mean daily temperature dropped 1 - 4 °C, these events were defined as weaker cooling events and are also described in the results. An ANOVA was performed on the mean daily temperature among sites and over time in order to assess when upwelling events occurred. The design was composed of the factors: upwelling (two levels, fixed) and site (two levels, random and nested in upwelling).

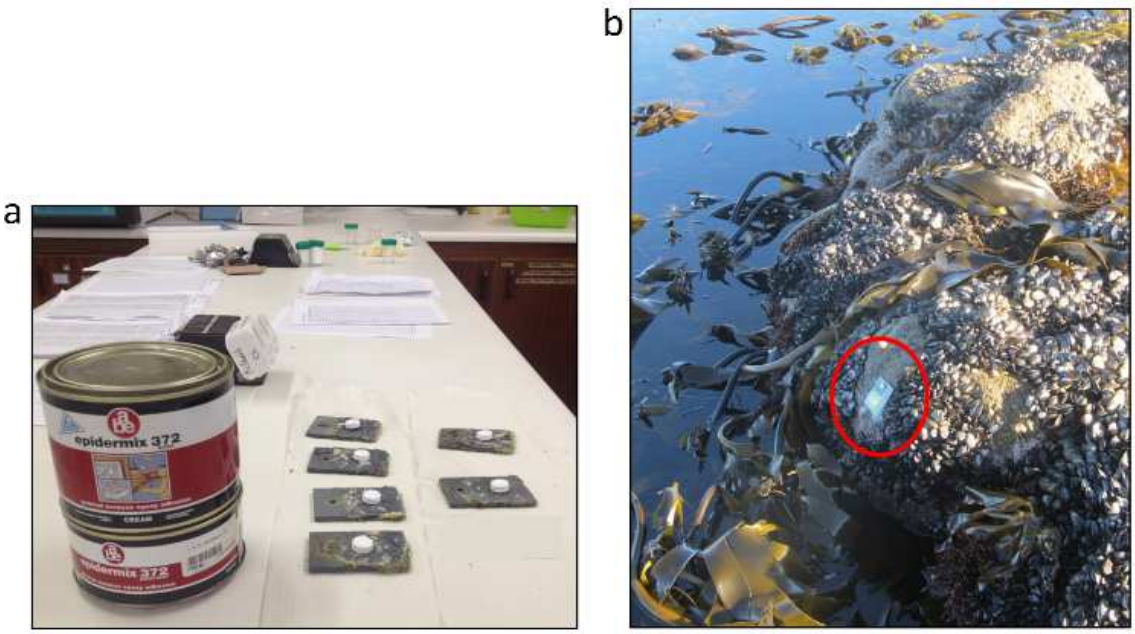


Fig 6.2 (a) Data loggers in preparation in the laboratory and (b) logger deployed on the shore.

6.2.4. Sampling and sample processing

The filter feeders chosen for this study was the mussel *Mytilus galloprovincialis*. The tissues used for the comparison were the adductor muscles and gonads. The adductor muscle was used due to its low turnover rates and is therefore more representative of a time-integrated diet (Gorokhova and Hansson 1999), while gonads will give insights on the reproduction strategy of the animals. The mussels were collected at the four sites described previously - two upwelling and two non-upwelling sites. In order to assess if food quality changed with height on the shore, samples were taken at two different heights, one in the high mussel zone and one in the low mussel

zone at each site. Replicates collected at each site and at each height were used to evaluate small-scale variability (*i.e.* few metres apart).

At each height, three haphazardly selected individual mussels were collected, dissected and processed for further FA analyses. In addition, to assess how food quality varied between sites in upwelling and non-upwelling conditions and among months, three replicates of 5L samples of seawater were collected from the shore at each site to measure the suspended particulate organic matter (SPM). All water samples were filtered gently (< 5 cm Hg vacuum) onto pre-combusted (450°C) GF/F filters (0.7 µm pore size and 47 mm diameter). The specimen and SPM samples were then flash frozen in liquid nitrogen and transferred to a –80 °C freezer until processing. Samples were processed as described in Chapter 2 (Chapter 2; paragraph 2.2.1 and 2.2.2).

6.2.5. Condition and gonad indices

To understand the possible effect of upwelling on the condition of mussels, the condition index (CI) and the gonad index (GI) of *M. galloprovincialis* were measured from specimens at each site on each of the four sampling events. For the CI, 20 random replicates of mussels were collected and kept frozen at – 20 °C until processing. The soft tissue of mussel was dissected and dried on a piece of pre-weighed aluminium foil at 60 °C for 48 h. The shells were dried separately. In addition, the CI was also calculated for the samples used for the FA analyses (an additional 3 individuals per tidal height). For these FA samples, the soft parts were freeze dried for 24 h. No differences in the CI were recorded between specimens dried with the two different methods (oven at 60°C for 24 h vs freeze-dry; ANOVA, $p > 0.05$). Consequently all the replicates were pooled together for the CI analyses (hence n = 26 per site). The CI was calculated as a percentage of the dry weight of the soft tissue over the dry shell weight, following Davenport and Chen (1987):

$$CI = \text{dry soft tissue weight} / \text{dry shell weight} \times 100$$

The GI was measured only for the samples used for the FA analyses (n = 3 per height). The gonads were freeze dried for 24 h. The GI was calculated as a percentage of

the dry weight of the gonad over the dry total body weight according to Williams and Babcock (2005):

$$GI = \text{dry gonads weight} / \text{dry total body weight} \times 100$$

Gonads and adductor muscles used for the FA analyses were weighed after being freeze-dried and frozen (– 80 °C) until processing for FA analyses. The whole body (without adductor muscle and gonads) was freeze-dried separately and weighed in order to calculate the total body weight.

6.2.6. Data analysis

6.2.6.1. Fatty acids

To test the influence of the different factors investigated during this study, a mixed model design was used consisting of the factors: month (four levels, random), upwelling (two levels, fixed), site (two levels, random and nested in upwelling), height (two levels, random and nested in site) and tissue (two levels, fixed and crossed with all the other factors). The experimental design to analyse the FA differences of the SPM was composed of the factors: month (four levels, random), upwelling (two levels, fixed), and site (two levels, random and nested in upwelling). The FA composition of species under the different conditions was compared using a PERMANOVA based on a Bray-Curtis dissimilarities matrix. Principal component analysis (PCA) and SIMPER were also performed as described in Chapter 2 (paragraph 2.2.3.2.).

6.2.6.2. Condition Index and Gonad Index

To test for an effect of upwelling on the CI of *M. galloprovincialis*, a two way ANOVA was performed. It comprised the factors: month (four levels, random), upwelling (two levels, fixed) and site (two levels, random and nested in upwelling). To assess the effect of season, upwelling and height on the GI of the specimens used for the FA analyses (n = 3 per height) a second ANOVA was performed. The experiment design was composed of the factors: month (four levels, random), upwelling (two levels, fixed), site (two levels, random and nested in upwelling) and height (two levels, random and nested

in site). The violation of homogeneity of variances was considered to be acceptable because ANOVA is relatively robust to heterogeneous variances for large designs such as the one in this study (Underwood 1997).

6.3. Results

6.3.1. Seawater temperature

Onshore water temperature showed pronounced variability over the study period and among sites (Fig 6.3). From December to February the upwelling sites 1 and 3 (Llandudno and Paternoster) exhibited consistently colder conditions by an average of 4.3 and 2.4 °C respectively, less than at their corresponding proximate downstream non-upwelling sites 2 and 4 (Bloubergstrand and Elandsbaai; ANOVA, $p < 0.01$). Site 1 experienced three events of strong upwelling. The first began on the 31 of December and involved low temperature (10 - 12 °C) that lasted until the 13 of February (ΔT 8 °C); the second was recorded on the 3 of March and lasted a week (ΔT 5 °C); and the third occurred on the 26 and stayed until the 31 of March (ΔT 5 °C; Fig 6.3). Even though site 2 was a non-upwelling site, rapid decreases in temperature were recorded on several occasions, suggesting that cold upwelled waters also influenced this site. Cooling events occurred: from the 31 of December to the 3 of January (ΔT 5°C) as observed at site 1; on the 16 for three days (ΔT 4 °C) and on the 27 of February for 10 days (ΔT 4 °C; Fig 6.3). Site 3 experienced six upwelling events. The first started on the 17 of December lasting for eight days (ΔT 6°C); the second began on the 31 of December and it finished on the 30 of January, similar to the other upwelling site 1 (ΔT 5 °C); on the 4 - 13 and on the 16 - 18 of February (both ΔT 5°C); the last two events started on the 28 of February and on the 23 of March and they stayed for nine and 10 days respectively (both ΔT 5 °C; Fig 6.3). Similar to the other non-upwelling site (site 2), site 4 showed also strong cooling events over the summer months. In particular they were recorded on the 31 of December (ΔT 7 °C), and 16 of February (ΔT 6 °C). The first cooling event lasted for all of January, while the second event lasted for four days (Fig 6.3). The average temperature values at upwelling sites during upwelling events was 11 °C, while in the absence of upwelling the temperature was between 16 - 17 °C. At non-upwelling sites the temperature recorded during cooling events was between 11 - 15 °C for site 2 and between 12 - 15°C for site 4, while through non-cooling events it was 15 - 20 °C and 19 - 22 °C for sites 2 and 4 respectively (Fig 6.3). Between March and April all sites showed average temperature values between 11.5 - 17 °C, while during the winter months, May to July, temperatures were between 13 - 15 °C at all sites.

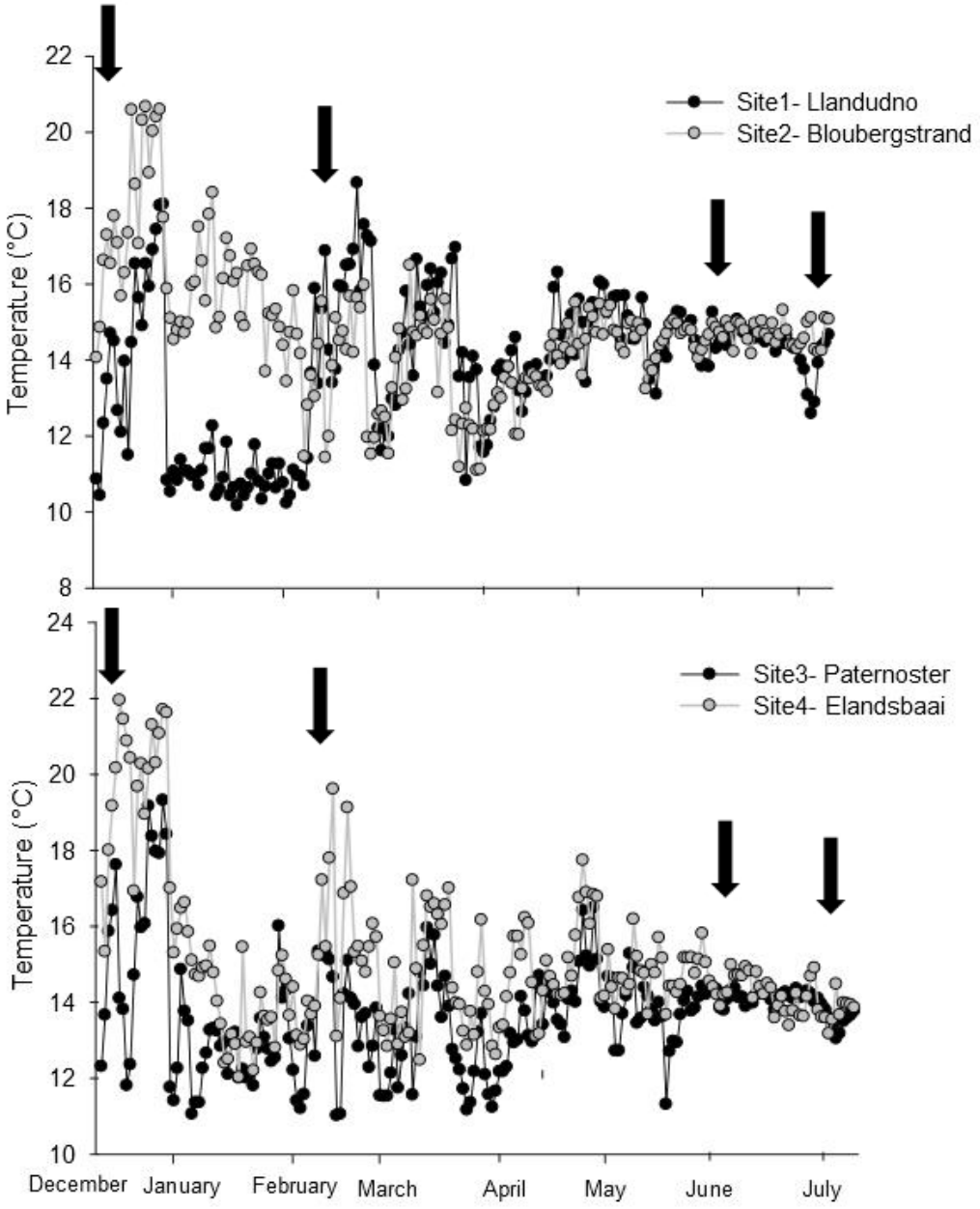


Fig 6.3 Mean daily in situ sea temperatures at 2 upwelling (black symbols) and 2 non-upwelling (grey symbols) sites on the South African west coast derived from onshore loggers for December 2012 to July 2013. The arrows indicate the four sampling events.

6.3.2. Fatty acid composition

6.3.2.1. Differences between tissues

A total of 28 and 29 FA contributing to > 1 % to TFA were found in the adductor muscles and the gonads respectively (Table 6.1 and 6.2). The major FA found in the adductor muscles were 20:1w9 (3 - 6 %), 22:2NMI (4 - 5 %), 18:0 (5 - 7 %), 20:2NMI1 (5 - 7 %), 20:5w3 (10 -13 %), 22:6w3 (12 - 20 %) and 16:0 (18-23 %; Table 6.1 and 6.2) while

those with highest proportion of gonad tissue were 16:1w7 (3 - 7 %), 18:0 (3 - 7 %), 22:6w3 (10 - 26 %), 20:5w3 (12 - 27 %) and 16:0 (20 - 23 %; Table 6.2).

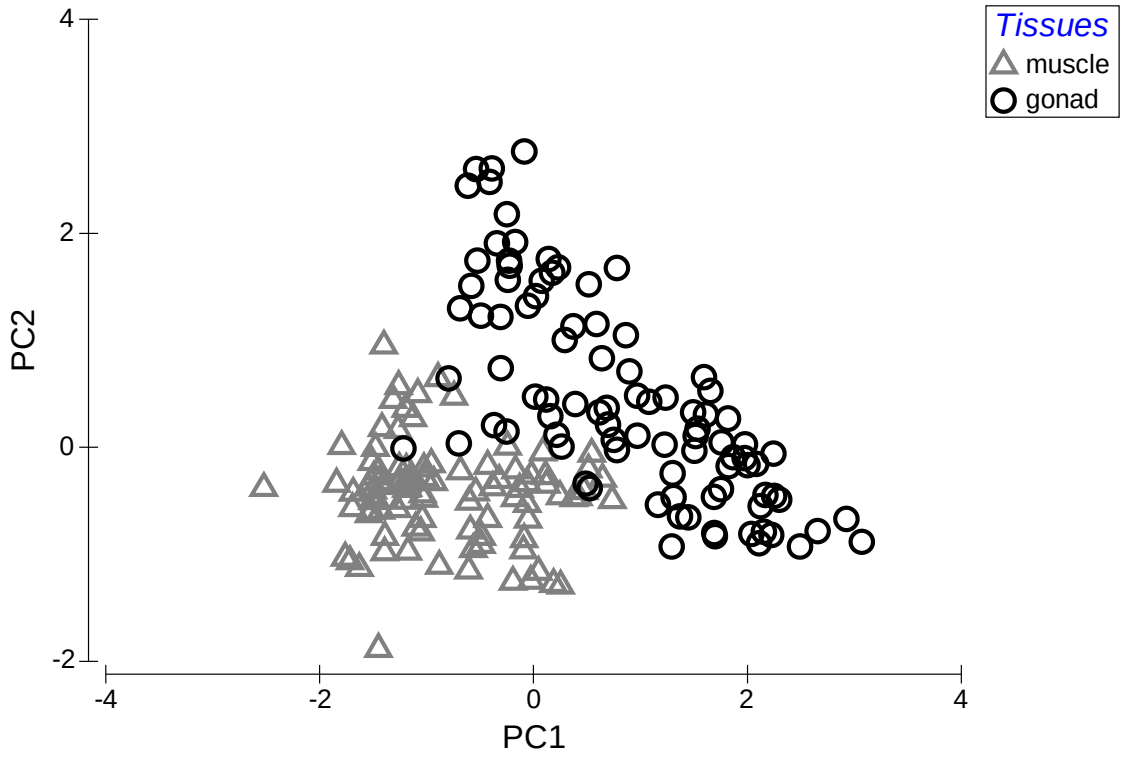


Fig 6.4 PCA on the FA composition of gonads and adductor muscles of *M. galloprovincialis* collected at four sites on the South Africa west coast over summer (December 2012 and February 2013) and winter months (June and July 2013). PC1 explained 38.5 % of the total variance and PC2 18.9%.

The main difference amongst all the samples was between the two types of tissues (PCA, Fig 6.4), however it was difficult to explain both axes due to some overlapping samples; in addition it is worth noting that the "best fitting" factorial plan explained only 57.4% of the total variance. SIMPER analysis highlighted that the main differences between gonads and muscles were due to higher proportions of 16:1w7, 18:1w9, 18:4w3, 20:5w3 and 22:6w3 in gonads; and higher proportions of 18:0, 20:2NMI1, 20:4w6 and 22:2NMI1 in the adductor muscles; explaining 55 % of the FA differences between the two tissues.

Table 6.1 Total fatty acid composition of adductor muscles of adult *M. galloprovincialis* collected during four sampling events and across four sites along the South African west coast. The values are percentages expressed as mean ± standard deviation (n = 6 per site). Only FA > 1 % were displayed below. PUFA = Polyunsaturated Fatty Acids, MUFA= Monounsaturated Fatty Acids, SFA= Saturated Fatty Acids, EFA = Essential Fatty Acids (20:4w6, 20:5w3 and 22:6w3), BAME = Bacterial Fatty acids.

	December				February				June				July			
	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4
14:0	0.49 + 0.14	0.60 + 0.13	0.89 + 0.63	0.56 + 0.35	1.02 + 0.40	0.91 + 0.21	0.88 + 0.37	0.44 + 0.11	0.51 + 0.12	1.22 + 0.27	0.95 + 0.49	0.57 + 0.31	1.25 + 1.91	1.04 + 0.31	0.72 + 0.58	0.45 + 0.17
16:0	19.69 + 0.79	19.95 + 1.02	21.10 + 1.36	20.48 + 0.89	19.13 + 1.29	18.28 + 1.34	18.70 + 1.71	17.02 + 0.68	21.62 + 0.90	21.34 + 0.90	22.25 + 1.56	22.83 + 2.11	23.36 + 2.65	20.98 + 0.70	22.98 + 0.50	22.01 + 1.69
16:1w7	1.52 + 0.65	1.90 + 0.44	2.90 + 1.89	1.96 + 1.28	5.08 + 2.07	3.98 + 0.88	3.69 + 1.66	1.57 + 0.72	1.69 + 0.80	3.67 + 1.06	3.39 + 1.88	1.68 + 0.66	1.36 + 0.34	3.28 + 1.34	2.64 + 1.95	1.48 + 0.33
18:0	7.39 + 0.70	7.80 + 0.69	6.24 + 1.38	6.31 + 0.78	5.24 + 0.60	6.14 + 0.67	5.12 + 0.81	5.17 + 0.21	6.71 + 0.96	6.15 + 0.71	5.63 + 1.41	6.47 + 1.10	7.84 + 1.20	6.46 + 0.68	6.38 + 1.88	6.92 + 1.30
18:1w9	0.98 + 0.18	0.57 + 0.11	1.44 + 0.59	1.19 + 0.39	2.13 + 0.59	1.42 + 1.31	1.62 + 0.48	1.14 + 0.16	1.85 + 0.55	0.89 + 0.07	1.67 + 0.70	1.36 + 0.19	1.30 + 0.29	0.81 + 0.21	1.61 + 0.88	1.24 + 0.20
18:1w7	2.02 + 0.30	2.02 + 0.21	1.80 + 0.45	2.13 + 0.16	3.53 + 0.85	4.24 + 1.52	2.83 + 0.55	2.87 + 0.75	2.23 + 0.19	2.38 + 0.16	1.96 + 0.22	2.06 + 0.14	2.09 + 0.24	2.59 + 0.25	1.98 + 0.15	1.97 + 0.22
18:2w6	0.90 + 0.15	0.84 + 0.15	1.07 + 0.26	0.93 + 0.07	1.17 + 0.07	1.17 + 0.22	1.24 + 0.22	1.03 + 0.17	1.09 + 0.14	1.29 + 0.18	1.08 + 0.18	1.08 + 0.22	1.00 + 0.30	1.26 + 0.18	1.11 + 0.30	1.03 + 0.26
18:4w3	0.84 + 0.31	1.17 + 0.25	0.53 + 0.29	0.50 + 0.21	1.00 + 0.40	0.89 + 0.24	1.47 + 0.80	0.58 + 0.29	0.48 + 0.22	1.21 + 0.48	1.26 + 0.63	1.06 + 0.62	0.67 + 0.39	0.84 + 0.50	1.04 + 0.75	0.82 + 0.39
20:00	0.43 + 0.33	0.17 + 0.16	0.35 + 0.36	0.51 + 0.49	0.51 + 0.25	0.40 + 0.51	0.43 + 0.20	0.37 + 0.31	0.22 + 0.18	0.33 + 0.31	0.51 + 0.31	0.29 + 0.28	0.82 + 0.75	0.69 + 0.56	0.42 + 0.20	0.80 + 1.31
20:1w11	1.04 + 0.44	1.23 + 0.24	0.91 + 0.38	1.76 + 1.30	1.02 + 0.33	1.63 + 1.28	1.63 + 0.24	1.13 + 0.67	1.18 + 0.98	2.46 + 0.68	1.76 + 0.66	1.50 + 0.36	1.84 + 1.12	1.54 + 0.91	1.56 + 1.06	1.15 + 1.12
20:1w9	5.32 + 0.44	4.43 + 0.35	4.80 + 0.96	4.72 + 1.17	4.58 + 0.77	3.89 + 0.87	2.98 + 0.39	4.10 + 0.98	5.03 + 0.81	2.38 + 0.30	3.46 + 1.30	4.03 + 0.44	4.01 + 0.71	3.43 + 1.18	3.74 + 1.20	6.00 + 1.33
20:2 NMI1	7.87 + 0.54	6.84 + 0.68	6.15 + 1.03	7.15 + 0.91	7.11 + 0.63	6.72 + 1.45	6.68 + 0.80	9.30 + 0.81	7.30 + 0.98	5.26 + 0.82	5.57 + 1.02	6.26 + 0.61	6.69 + 1.04	4.43 + 1.49	5.04 + 0.96	6.69 + 1.11
20:2 NMI2	1.17 + 0.44	1.14 + 0.18	0.92 + 0.25	1.14 + 0.44	1.06 + 0.24	1.05 + 0.36	1.62 + 1.06	2.28 + 1.11	1.27 + 0.34	1.07 + 0.14	0.85 + 0.23	1.04 + 0.19	1.33 + 0.42	1.65 + 1.63	1.42 + 1.38	0.95 + 0.23
20:4w6	5.31 + 0.38	3.40 + 0.39	3.73 + 0.90	5.15 + 0.37	4.52 + 0.59	2.31 + 0.27	2.84 + 0.56	4.74 + 0.44	7.21 + 0.96	3.42 + 0.53	3.57 + 1.11	5.56 + 1.40	5.78 + 1.27	2.93 + 1.28	3.65 + 0.88	5.52 + 0.57
20:4w3	0.01 + 0.03	0.00 + 0.00	0.15 + 0.18	0.15 + 0.18	1.45 + 0.89	2.48 + 0.78	2.26 + 1.81	4.70 + 1.01	0.02 + 0.06	0.17 + 0.07	0.15 + 0.08	0.03 + 0.09	0.06 + 0.06	0.60 + 1.22	0.09 + 0.11	0.16 + 0.27
20:5w3	11.68 + 0.93	13.84 + 1.51	12.29 + 0.53	11.35 + 1.66	12.88 + 1.40	14.77 + 2.39	11.88 + 1.61	9.23 + 0.69	11.63 + 1.74	18.09 + 2.22	12.22 + 1.75	9.33 + 1.44	9.73 + 3.27	17.65 + 2.97	12.17 + 1.62	8.88 + 1.71
22:0	0.11 + 0.09	0.16 + 0.13	0.04 + 0.11	0.15 + 0.14	1.12 + 0.51	1.92 + 0.70	0.83 + 0.30	1.55 + 1.01	0.04 + 0.04	0.03 + 0.04	0.12 + 0.09	0.10 + 0.13	0.48 + 0.60	0.13 + 0.24	0.04 + 0.09	0.00 + 0.00
22:2w6	1.20 + 0.18	0.61 + 0.07	1.20 + 0.40	1.02 + 0.22	0.92 + 0.22	0.52 + 0.35	1.85 + 1.15	2.97 + 1.79	0.85 + 0.12	0.63 + 0.34	0.76 + 0.16	1.01 + 0.17	1.91 + 1.86	0.73 + 0.44	0.88 + 0.35	1.04 + 0.09
22:2 NMI1	5.82 + 0.37	6.89 + 0.53	4.28 + 1.00	5.02 + 0.82	4.12 + 0.37	4.81 + 0.89	3.31 + 0.56	4.33 + 0.62	4.59 + 0.76	4.61 + 1.40	3.65 + 0.69	4.49 + 0.78	4.55 + 0.32	4.22 + 1.17	3.16 + 1.00	4.52 + 0.98
22:3 NMI	1.32 + 0.04	1.16 + 0.22	1.16 + 0.28	1.31 + 0.16	1.06 + 0.09	0.97 + 0.13	1.02 + 0.23	1.25 + 0.22	1.16 + 0.20	0.81 + 0.21	1.00 + 0.12	1.19 + 0.16	1.17 + 0.24	0.79 + 0.17	0.96 + 0.22	1.14 + 0.18
22:5w6	0.53 + 0.05	0.42 + 0.06	0.29 + 0.05	0.24 + 0.05	1.03 + 0.53	1.70 + 0.57	1.33 + 0.48	2.75 + 0.70	0.39 + 0.04	0.38 + 0.03	0.27 + 0.04	0.17 + 0.14	0.47 + 0.11	0.34 + 0.15	0.31 + 0.02	0.43 + 0.41
22:5w3	2.02 + 0.13	2.29 + 0.26	1.57 + 0.18	2.22 + 0.35	1.67 + 0.17	2.03 + 0.17	1.35 + 0.10	1.72 + 0.34	1.78 + 0.15	2.08 + 0.12	1.55 + 0.23	1.87 + 0.43	1.78 + 0.42	2.36 + 0.59	1.36 + 0.09	1.77 + 0.37
22:6w3	15.92 + 0.55	16.22 + 0.71	20.74 + 2.08	17.29 + 2.05	12.67 + 1.81	12.12 + 1.23	18.95 + 1.38	12.65 + 1.28	14.46 + 1.63	14.38 + 1.34	20.11 + 2.27	18.87 + 1.10	12.83 + 3.60	15.36 + 3.59	20.07 + 2.22	17.20 + 3.26
BAME	6.42 + 0.41	6.34 + 0.55	5.45 + 0.85	6.77 + 0.41	5.97 + 0.45	5.64 + 0.46	5.48 + 0.40	7.11 + 0.31	6.70 + 0.65	5.76 + 0.50	6.26 + 1.74	7.15 + 0.75	7.66 + 1.53	5.86 + 0.84	6.68 + 2.57	7.82 + 1.98
ΣSFA	34.53 + 1.06	35.02 + 0.67	34.07 + 1.54	34.78 + 0.97	33.00 + 1.20	33.29 + 0.32	31.44 + 2.19	31.67 + 1.29	35.80 + 2.26	34.82 + 1.21	35.72 + 2.49	37.40 + 3.00	41.42 + 6.98	35.16 + 1.48	37.22 + 4.28	38.00 + 4.46
ΣMUFA	10.88 + 0.97	10.15 + 0.72	11.85 + 2.31	11.76 + 1.14	16.33 + 2.59	15.17 + 2.19	12.76 + 2.42	10.82 + 2.34	11.98 + 1.58	11.77 + 1.29	12.24 + 2.31	10.63 + 0.93	10.60 + 0.52	11.66 + 2.18	11.52 + 2.44	11.84 + 1.97
ΣPUFA	54.59 + 1.24	54.83 + 0.86	54.07 + 2.63	53.46 + 1.18	50.67 + 2.22	51.54 + 2.11	55.81 + 3.84	57.52 + 3.51	52.23 + 1.74	53.41 + 0.76	52.04 + 1.26	51.97 + 3.59	47.98 + 6.85	53.18 + 2.49	51.26 + 2.88	50.16 + 3.63
ΣEFA	32.9 + 0.66	33.5 + 1.25	36.8 + 2.60	33.8 + 2.69	30.1 + 2.16	29.2 + 3.03	33.7 + 2.34	26.6 + 1.53	33.3 + 3.01	35.9 + 2.37	35.9 + 1.39	33.8 + 2.84	28.34 + 7.87	35.94 + 4.13	35.89 + 3.99	31.6 + 3.43

Table 6.2 Total fatty acid composition of gonads of *M. galloprovincialis* collected in December, February, June and July and at the four sampling sites along the South African west coast. The values are percentages expressed as mean ± standard deviation (n = 6 per each site). Only FA > 1 % were displayed below. PUFA = Polyunsaturated Fatty Acids, MUFA= Monounsaturated Fatty Acids, SFA= Saturated Fatty Acids, EFA = Essential Fatty Acids (20:4w6, 20:5w3 and 22:6w3).

	December				February				June				July			
	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4
14:0	1.43 + 1.33	1.45 + 1.30	1.15 + 1.49	1.78 + 0.94	2.09 + 1.03	2.51 + 1.66	0.88 + 0.89	1.67 + 0.89	0.88 + 0.60	2.24 + 0.75	2.13 + 1.06	1.13 + 0.76	0.66 + 0.59	1.55 + 1.06	0.80 + 0.72	0.95 + 0.60
16:0	21.37 + 2.65	18.21 + 2.78	23.20 + 2.45	20.89 + 0.95	20.97 + 0.81	22.17 + 1.81	20.80 + 1.67	20.69 + 1.36	18.97 + 0.98	20.49 + 0.90	23.62 + 2.19	22.13 + 1.61	17.38 + 5.12	21.77 + 1.89	21.96 + 0.81	21.08 + 1.43
16:1w7	4.52 + 4.03	5.18 + 3.29	2.95 + 3.46	6.76 + 3.45	7.83 + 3.74	7.01 + 4.01	3.70 + 3.18	6.19 + 2.72	3.90 + 2.45	6.55 + 2.49	5.99 + 2.67	3.45 + 2.40	1.95 + 1.06	5.02 + 3.12	2.65 + 2.44	3.83 + 2.65
16:1w5	0.08 + 0.10	0.10 + 0.05	0.19 + 0.19	0.20 + 0.07	0.13 + 0.04	0.13 + 0.06	0.15 + 0.14	0.19 + 0.04	0.15 + 0.07	0.26 + 0.13	0.29 + 0.09	0.28 + 0.09	0.63 + 0.86	0.14 + 0.08	0.24 + 0.10	0.33 + 0.19
16:2w4	0.37 + 0.21	0.12 + 0.08	0.21 + 0.04	0.26 + 0.12	0.35 + 0.12	0.49 + 0.30	0.24 + 0.03	0.32 + 0.18	0.08 + 0.06	0.16 + 0.22	0.21 + 0.06	0.27 + 0.12	0.29 + 0.33	0.12 + 0.09	0.20 + 0.09	0.18 + 0.12
17:1w7	0.35 + 0.55	0.13 + 0.04	0.20 + 0.34	0.17 + 0.05	0.33 + 0.36	0.13 + 0.04	0.42 + 0.64	0.16 + 0.05	0.10 + 0.05	0.13 + 0.02	0.09 + 0.04	0.10 + 0.07	0.21 + 0.24	0.10 + 0.07	0.06 + 0.05	0.10 + 0.05
16:4w1	0.14 + 0.10	0.13 + 0.09	0.15 + 0.08	0.11 + 0.04	0.11 + 0.03	0.05 + 0.03	0.14 + 0.02	0.09 + 0.04	0.16 + 0.04	0.06 + 0.03	0.12 + 0.02	0.18 + 0.07	0.22 + 0.05	0.08 + 0.04	0.12 + 0.02	0.19 + 0.12
18:0	5.93 + 2.37	6.16 + 1.81	5.98 + 4.44	3.70 + 1.94	3.64 + 1.66	5.22 + 3.40	7.32 + 4.69	3.99 + 1.36	5.57 + 2.03	4.13 + 0.92	3.81 + 1.50	4.44 + 1.59	6.36 + 1.31	6.02 + 3.08	6.65 + 2.37	4.79 + 2.32
18:1w9	1.86 + 1.33	1.19 + 0.40	2.93 + 3.45	3.00 + 1.17	3.15 + 0.84	1.37 + 0.72	2.00 + 1.52	3.67 + 0.69	2.94 + 0.95	0.97 + 0.28	2.75 + 1.00	2.01 + 0.90	2.57 + 1.44	0.93 + 0.44	1.56 + 0.69	2.22 + 1.03
18:1w7	2.12 + 0.56	3.34 + 0.29	1.26 + 0.47	2.81 + 0.29	2.45 + 0.37	2.90 + 0.35	1.75 + 0.43	2.86 + 0.28	2.37 + 0.40	2.51 + 0.43	1.91 + 0.32	2.27 + 0.24	2.38 + 0.72	2.75 + 0.26	1.95 + 0.21	2.40 + 0.24
18:2w6	0.80 + 0.16	0.85 + 0.22	0.94 + 0.45	1.28 + 0.25	1.16 + 0.12	0.99 + 0.09	1.24 + 0.65	1.50 + 0.14	1.25 + 0.24	1.35 + 0.27	1.30 + 0.21	1.35 + 0.34	1.17 + 0.44	1.42 + 0.38	1.00 + 0.27	1.35 + 0.30
18:3w6	0.07 + 0.07	0.12 + 0.02	0.14 + 0.24	0.11 + 0.07	0.09 + 0.05	0.11 + 0.05	0.06 + 0.08	0.15 + 0.01	0.07 + 0.04	0.30 + 0.24	0.08 + 0.09	0.06 + 0.03	0.10 + 0.11	0.19 + 0.15	0.08 + 0.08	0.51 + 0.99
18:4w3	0.94 + 0.76	0.78 + 0.50	2.24 + 2.60	1.37 + 0.55	1.38 + 0.48	1.59 + 0.81	1.35 + 1.54	1.66 + 0.48	1.10 + 0.26	2.11 + 0.78	2.31 + 0.88	2.32 + 1.37	1.14 + 1.10	1.71 + 1.21	1.26 + 0.94	2.08 + 1.60
20:1w11	0.88 + 0.58	1.82 + 1.89	0.97 + 0.71	1.34 + 0.50	1.67 + 0.79	1.07 + 0.85	1.57 + 0.83	1.64 + 0.80	0.95 + 0.45	1.75 + 0.99	1.50 + 0.38	1.13 + 0.50	1.40 + 0.63	0.61 + 0.24	0.98 + 0.96	1.29 + 1.39
20:1w9	3.85 + 0.65	3.39 + 1.13	2.53 + 1.19	3.44 + 0.60	2.84 + 0.93	2.84 + 1.17	2.52 + 0.66	3.25 + 0.75	3.98 + 0.47	2.47 + 0.93	3.84 + 1.09	3.42 + 0.84	4.38 + 0.99	2.68 + 0.38	3.50 + 0.91	2.92 + 1.12
20:2 NMI1	3.11 + 1.13	4.69 + 1.56	2.53 + 1.12	4.20 + 0.49	3.67 + 0.74	2.88 + 0.47	2.94 + 1.63	4.13 + 0.67	4.08 + 1.23	3.14 + 1.43	2.53 + 0.90	3.61 + 1.03	5.10 + 1.54	2.89 + 0.44	2.34 + 0.44	3.62 + 0.66
20:2 NMI2	0.85 + 0.59	0.76 + 0.25	0.58 + 0.65	0.53 + 0.15	0.39 + 0.18	0.50 + 0.16	0.36 + 0.26	0.61 + 0.16	0.71 + 0.33	0.63 + 0.39	0.56 + 0.38	0.69 + 0.35	1.07 + 0.55	0.59 + 0.19	0.48 + 0.33	1.58 + 1.65
20:4w6	3.25 + 2.00	1.82 + 0.99	1.44 + 0.93	3.77 + 0.95	3.56 + 1.13	1.89 + 0.62	2.79 + 0.83	4.35 + 0.93	6.48 + 1.30	2.57 + 0.85	2.05 + 0.66	3.98 + 0.94	6.07 + 3.35	2.63 + 0.53	3.30 + 0.79	3.42 + 1.82
20:5w3	23.56 + 1.90	24.83 + 4.25	16.04 + 2.56	18.27 + 2.15	21.61 + 1.75	27.85 + 0.81	16.36 + 1.81	19.17 + 1.58	20.10 + 2.64	23.54 + 3.15	14.13 + 1.88	12.37 + 1.39	18.13 + 4.37	23.30 + 2.60	17.49 + 1.69	13.39 + 1.66
22:2w6	0.98 + 0.74	0.35 + 0.12	0.47 + 0.46	0.98 + 0.23	0.83 + 0.36	0.41 + 0.24	0.45 + 0.33	1.04 + 0.25	0.58 + 0.30	0.34 + 0.19	1.00 + 0.74	0.65 + 0.34	1.63 + 1.71	0.44 + 0.22	0.31 + 0.11	0.42 + 0.32
22:2 NMI1	1.99 + 0.73	2.81 + 0.78	0.91 + 0.65	2.87 + 0.43	2.43 + 0.72	2.12 + 0.81	1.81 + 1.29	2.41 + 0.71	2.30 + 1.11	2.93 + 1.47	1.68 + 0.75	2.17 + 0.88	2.24 + 1.88	2.10 + 0.57	1.22 + 0.66	2.20 + 0.65
22:5w3	2.16 + 0.53	2.29 + 0.36	1.20 + 0.35	1.84 + 0.21	1.93 + 0.17	2.29 + 0.37	1.43 + 0.09	1.96 + 0.22	1.90 + 0.20	2.00 + 0.31	1.29 + 0.22	1.39 + 0.12	1.96 + 0.23	2.16 + 0.49	1.38 + 0.08	1.61 + 0.44
22:6w3	13.31 + 3.99	14.31 + 3.88	27.59 + 5.35	14.91 + 4.68	12.48 + 3.13	9.79 + 3.71	24.94 + 2.93	12.88 + 3.48	15.84 + 2.71	14.04 + 2.74	23.29 + 4.19	25.29 + 4.23	16.21 + 1.41	15.73 + 3.64	25.77 + 3.08	23.69 + 2.81
BAME	6.08 + 2.95	5.17 + 1.12	4.21 + 1.65	5.41 + 1.04	4.91 + 1.71	3.67 + 0.80	4.76 + 1.18	5.42 + 0.76	5.53 + 0.56	5.32 + 1.31	3.48 + 1.04	5.31 + 1.00	6.76 + 1.59	5.05 + 0.66	4.71 + 0.72	5.85 + 0.89
ΣSFA	34.82 + 3.15	31.00 + 2.48	34.54 + 2.76	31.78 + 2.00	31.61 + 2.54	33.58 + 1.67	33.76 + 5.40	31.76 + 1.24	30.96 + 1.90	32.18 + 1.18	33.04 + 2.12	33.02 + 0.65	31.16 + 7.09	34.40 + 2.70	34.11 + 1.67	32.67 + 1.38
ΣMUFA	13.66 + 4.63	15.14 + 2.76	11.02 + 4.96	17.72 + 4.82	18.40 + 4.69	15.46 + 4.18	12.13 + 5.02	17.97 + 3.74	14.39 + 3.69	14.64 + 2.64	16.37 + 3.84	12.66 + 3.14	13.51 + 2.68	12.24 + 3.82	10.94 + 3.24	13.09 + 3.39
ΣPUFA	51.52 + 3.15	53.86 + 2.36	54.44 + 4.93	50.51 + 4.14	49.99 + 3.19	50.96 + 3.74	54.11 + 1.85	50.27 + 3.70	54.65 + 2.94	53.18 + 2.28	50.58 + 4.18	54.32 + 3.19	55.33 + 4.55	53.37 + 1.48	54.95 + 1.67	54.23 + 2.75
ΣEFA	40.11 + 5.12	40.96 + 2.91	45.07 + 8.36	36.95 + 4.52	37.65 + 4.06	39.54 + 4.95	41.30 + 4.74	36.39 + 3.38	42.43 + 4.32	40.16 + 1.95	39.48 + 4.78	41.63 + 4.33	40.41 + 2.60	41.66 + 3.13	46.56 + 4.36	40.49 + 4.79

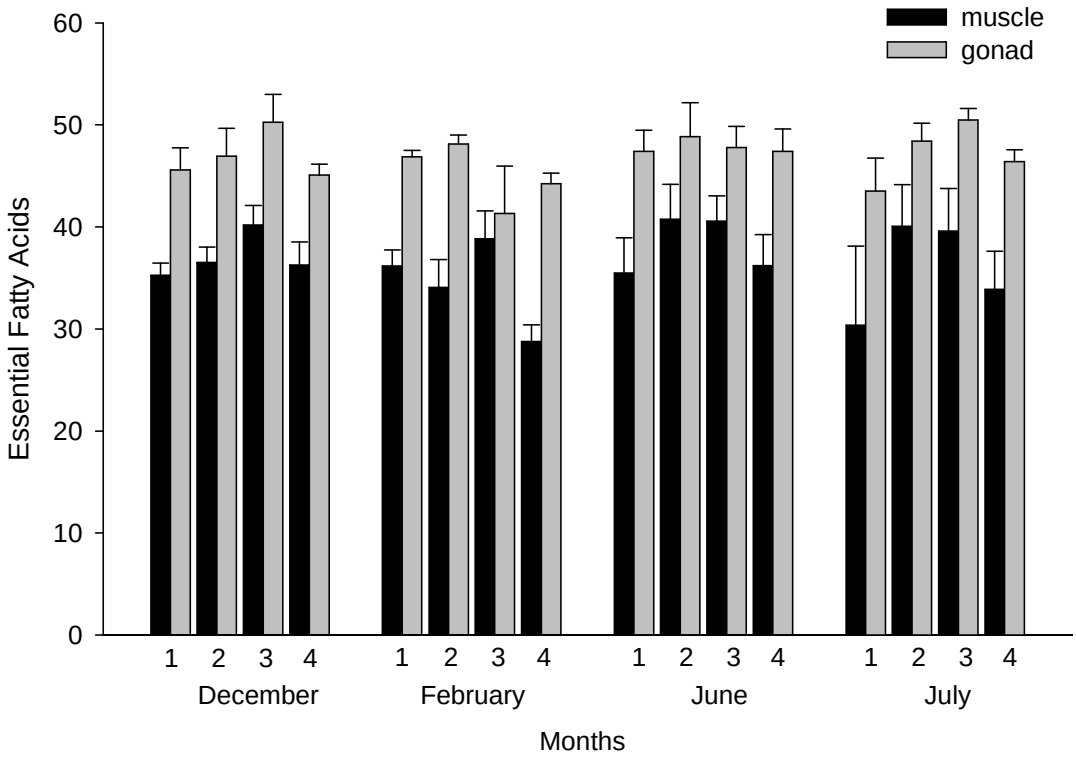


Fig 6.5 Percentages of Essential fatty acids (EFA; mean ± SD) for adductor muscle and gonad tissue of *M. galloprovinci*alis at four sites (1, 2, 3 and 4) on the South African west coast and during December, February, June and July. EFA= 20:4w6, 20:5w3 and 22:6w3.

Gonads showed a higher proportion of essential FA (EFA, *i.e.* 20:4w6, 20:5w3, 22:6w3, 36 – 46 % of TFA; Alkanani et al. 2007) than muscles (26 – 35 % of TFA) at all sites and across all months, Fig 6.5). In particular, gonads had double the proportion of 20:5w3 relative to adductor muscles (Table 6.1 and 6.2). Considering the strong dissimilarities between tissues, FA composition of gonads and adductor muscles were investigated separately from the other analyses.

6.3.2.2. Temporal variation

6.3.2.2.1. Adductor muscle

FA analyses conducted on adductor muscles showed strong dissimilarities among months (Table 6.3). Axis one of the PCA, which explains 34 % of the total variance of the data set, separated the samples collected in February from all other months (Table 6.3, Fig 6.6). The FA responsible for this pattern were 16:1w7, 20:4w3, 20:5w3, 22:0 and

22:5w6, that were more abundant in February, and 16:0, 20:1w9, 20:4w6 and 22:6w3 that characterized the other three months (PCA and SIMPER; Fig 6.6). PC2, which explained 20.5 % of the total variance, did not seem to highlight any clear pattern amongst the samples.

Table 6.3 PERMANOVA results on the fatty acid composition of the adductor muscles of *M. galloprovincialis* at four sites and across four month on the South African west coast. M = Month, Up = Upwelling, Si = Site, He = Height; df = degrees of freedom, MS = mean square; * p*, 0.05; ** p, 0.01; *** p, 0.001.*

	df	**MS**	**Pseudo-F**	**P**	
M	3	488.66	9.57	0.003	**
Up	1	157.98	0.37	0.971	
Si (Up)	2	502.22	4.76	0.000	**
He (Si (Up))	4	60.69	2.04	0.233	
M x Up	3	58.28	1.14	0.379	
M x Si (Up)	6	51.04	1.71	0.045	**
M x He (Si (Up))	12	29.71	1.24	0.096	
Res	64	23.9			

No effect of the factors upwelling, height or any interactions were found (Table 6.3). However, PERMANOVA highlighted a significant effect of the factor site and the interaction between site and month (Table 6.3), indicating that the FA characterizing each sites changed with month.

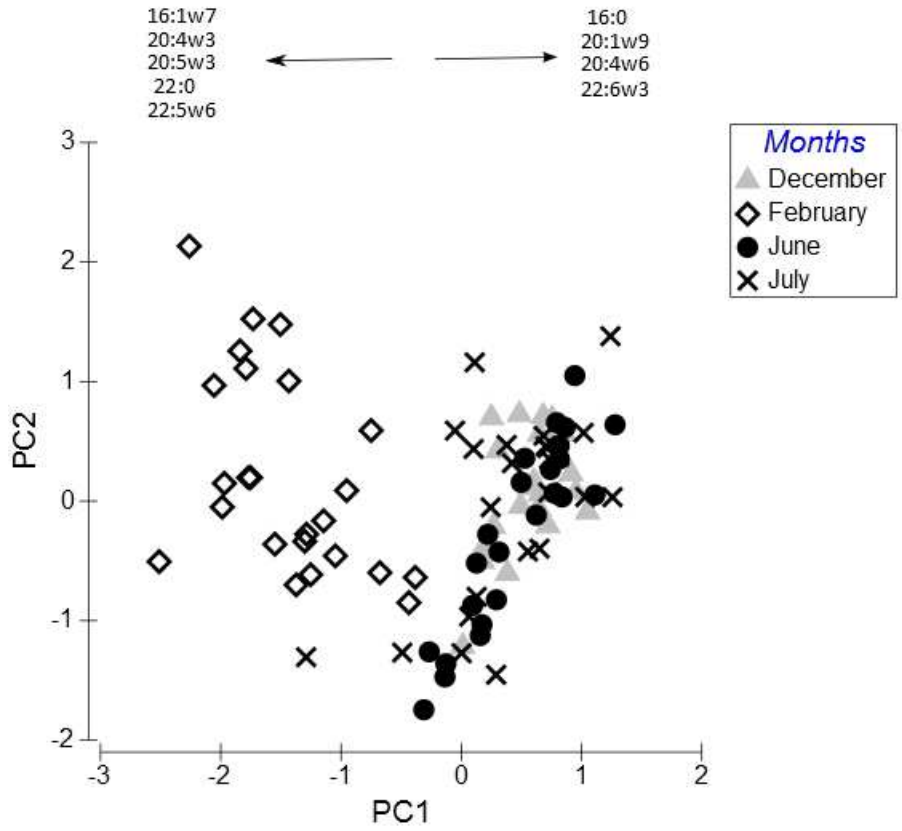


Fig 6.6 PCA conducted on the adductor muscles of *M. galloprovincialis* collected at the intertidal rocky shore at all sites and at both heights, on the South African west coast in December, January, June and July. PC1 explain 34.0 % of the total variance and PC2 a further 20.5 %.

For the months of December, June and July, the 2 sites of upwelling were different from each other (site 1 ≠ site 3) as well as the 2 sites of non-upwelling (site 2 ≠ site 4; PCA and PERMANOVA *post hoc* pair-wise test, $p < 0.05$). Indeed PCA and SIMPER underlined that site 3 exhibited a high proportion of 16:1w7 and dinoflagellate trophic markers (TM; 18:4w3 and 22:6w3; FA contributing to 40 % of TFA, Table 6.1) compared to site 1 which was characterized by bacterial FA (BAME), 20:2NMI1, 20:4w6 and 22:2NMI1 (26 % of TFA, Table 6.1). These FA explained 55 % of the dissimilarities between groups (SIMPER). Site 2 was different from site 4 in having high percentages of 20:1w11 and diatom TM (16:1w7 and 20:5w3; 32 % of TFA), while site 4 was typified by 20:1w9, 20:4w6, 20:2NMI1 and 22:6w3 (35 % of TFA, Table 6.1; SIMPER, FA explaining 50 % of the total variance). In February, only sites 2 and 4, the two non-upwelling sites, were different from each other (PERMANOVA *post hoc* pair-wise test and PCA). During this month, PCA and SIMPER showed the two sites of upwelling and site 2 had higher proportion of 14:0 and diatom trophic markers compared to site 4 (20 % of TFA, Table

6.1), which was enriched in 20:4w3, 20:2NMI1 and 22:2w6 (17 % of TFA, Table 6.1). These FA contributed to explain 53 % of the dissimilarities between groups.

6.3.2.2.2. Gonad

Gonad FA compositions had a high proportion of PUFA (50 - 55 % of TFA) at all sites and months, followed by SFA (30 - 35 %) and MUFA (10 - 15 %; Table 6.3). PERMANOVA showed the factors upwelling, month, height and their interactions were not significant; however, an effect of the factor site and its interaction with month was recorded, indicating that the FA composition of gonads across sites changed in the different months (Table 6.4).

Table 6.4 PERMANOVA results on the fatty acid compositions of gonads of *M. galloprovincialis* at four sites and across for month on the South African west coast in relation to the different factors. M = Month, Up = Upwelling, Si = Site, He = Height; df = degrees of freedom, MS = mean square; * *p*, 0.05; ** *p*, 0.01; *** *p*, 0.001.

	df	**MS**	**F**	**P**	
M	3	158.86	1.67	0.169	
Up	1	396.91	0.63	0.769	
Si (Up)	2	673.62	4.87	0.000	***
He (Si (Up))	4	55.45	1.06	0.399	
M x Up	3	99.58	1.06	0.432	
M x Si (Up)	6	95.03	1.83	0.043	*
M x He (Si (Up))	12	51.58	0.78	0.792	
Res	60	65.91			

The dissimilarities among sites depended on the month considered, however no clear pattern was recorded for any of them. PCA confirmed the PERMANOVA (Fig 6.7). PC1 that explained the majority of the variance (45.4 %) could not separate specimens based on site or any other factors (Fig 6.7). PC2 separated site 1 and 2 from sites 3, while site 4 is spread on both side of axis 2; however it only explains 17.7 % of the total variance.

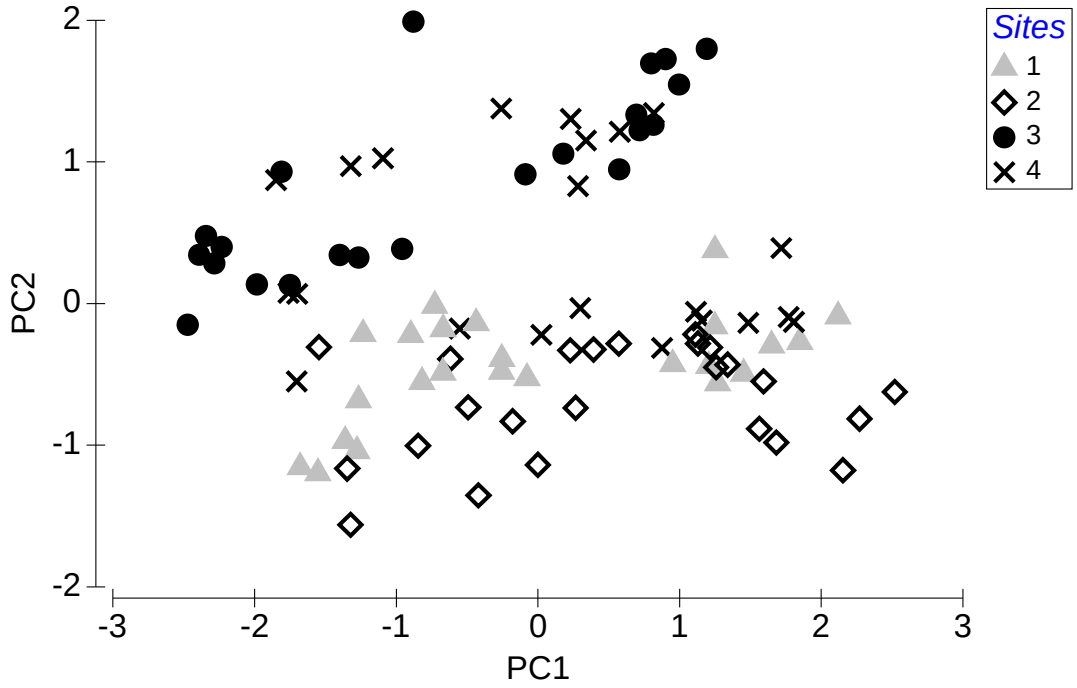


Fig 6.7 PCA conducted on the FA composition of the gonads of *M. galloprovincialis* collected from intertidal rocky shore at four sites during the four sampling events. PC1 explain 45.4 % of the total variance and PC2 17.7 %.

6.3.2.2.3. Food source

PERMANOVA analyses showed a significant effect of the factor month and the interaction between month and site on the SPM signatures, with no effects of neither upwelling nor site (Table 6.5). Generally, SPM had higher proportions of SFA (40 - 60 %) than MUFA (20 - 45 %) and PUFA (10 - 25 %) in all months (Table 6.6). Despite the differences highlighted by PERMANOVA, the FA signatures of SPM changed over the months and across sites without a clear pattern and no similarity with the pattern seen for the adductor muscles.

Table 6.5 PERMANOVA results on the fatty acid compositions of SPM at four sites and across for month on the South African west coast in relation to the different factors. M = Month, Up = Upwelling, Si = Site; df = degrees of freedom, MS = mean square; * *p*, 0.05; ** *p*, 0.01; *** *p*, 0.001.

	df	MS	F	p	
M	3	829.38	3.06	0.001	**
Up	1	193.57	0.62	0.864	
Si (Up)	2	197.21	0.72	0.753	
M x Up	3	543.57	2.00	0.055	
M x Si (Up)	6	270.65	1.69	0.002	**
Res	32	159.92			

Table 6.6 Total fatty acid composition of suspended particulate matter (SPM) collected during the four sampling events and across four sites along the South African west coast. The values are percentages expressed as mean ± standard deviation (n = 3 per site). Only FA > 1 % are displayed. PUFA = Polyunsaturated Fatty Acids, MUFA= Monounsaturated Fatty Acids, SFA= Saturated Fatty Acids.

	December				February				June				July			
	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4
14:0	5.28 + 1.22	5.93 + 0.54	3.90 + 0.34	3.89 + 1.04	3.06 + 0.13	3.28 + 1.09	3.04 + 0.07	2.75 + 1.00	3.87 + 0.89	10.50 + 0.48	7.96 + 2.50	6.84 + 0.40	4.75 + 0.89	3.34 + 0.48	2.72 + 1.14	2.89 + 0.55
14:1w5	0.99 + 0.95	1.04 + 1.12	0.29 + 0.26	0.44 + 0.36	0.21 + 0.26	0.74 + 0.82	1.53 + 0.27	0.61 + 0.23	0.18 + 0.27	0.67 + 0.20	0.53 + 0.31	0.72 + 0.33	0.45 + 0.27	0.49 + 0.20	0.21 + 0.14	0.50 + 0.25
16:0	20.82 + 5.28	26.49 + 2.33	33.01 + 1.32	33.09 + 0.86	20.01 + 7.17	19.21 + 1.41	22.41 + 0.53	17.34 + 5.80	29.17 + 5.55	31.73 + 3.47	28.95 + 5.26	31.35 + 2.35	28.40 + 5.55	14.12 + 3.47	20.02 + 17.55	17.17 + 2.96
16:1w7	6.36 + 4.87	11.58 + 1.66	2.65 + 0.68	3.22 + 1.68	4.01 + 0.89	2.68 + 0.27	6.19 + 0.07	1.31 + 0.53	4.23 + 2.00	1.43 + 1.15	2.44 + 1.95	6.44 + 6.65	3.99 + 2.00	3.09 + 1.15	8.25 + 9.43	1.56 + 0.39
16:1w5	3.55 + 2.61	0.33 + 0.21	1.84 + 1.02	2.91 + 0.83	0.76 + 0.61	3.04 + 2.07	1.96 + 0.42	6.19 + 1.81	1.51 + 2.58	1.93 + 0.47	2.03 + 2.20	1.35 + 1.17	5.45 + 2.58	5.36 + 0.47	4.05 + 0.87	4.19 + 0.65
17:1w7	0.77 + 0.59	1.22 + 0.35	0.38 + 0.02	0.49 + 0.21	0.46 + 0.14	0.30 + 0.18	0.62 + 0.14	0.82 + 0.36	0.28 + 0.04	0.31 + 0.09	0.44 + 0.39	0.96 + 1.17	0.15 + 0.04	0.25 + 0.09	0.29 + 0.16	0.31 + 0.32
C18:0	11.36 + 3.80	8.92 + 1.26	11.22 + 1.54	9.06 + 1.46	14.43 + 7.45	9.77 + 4.85	7.08 + 0.73	7.74 + 2.24	5.98 + 3.15	12.42 + 1.51	10.68 + 3.96	14.76 + 7.56	12.50 + 3.15	8.19 + 1.51	15.01 + 5.78	7.30 + 4.11
18:1w9	9.55 + 3.35	18.78 + 8.83	20.07 + 8.26	26.32 + 4.84	18.44 + 16.74	16.87 + 4.26	22.23 + 1.57	11.11 + 4.30	18.13 + 2.50	12.26 + 1.97	10.28 + 10.11	2.82 + 2.67	12.95 + 2.50	9.66 + 1.97	10.20 + 3.03	12.24 + 4.18
18:1w7	3.31 + 2.19	1.69 + 2.07	4.28 + 4.56	0.91 + 0.22	10.44 + 10.28	2.30 + 0.69	2.27 + 2.02	3.94 + 0.61	3.08 + 1.47	3.15 + 0.22	5.22 + 2.63	2.52 + 1.68	5.29 + 1.47	4.31 + 0.22	4.36 + 0.12	9.56 + 8.02
18:1w5	0.99 + 0.58	0.76 + 0.13	0.74 + 0.34	0.57 + 0.17	0.42 + 0.21	0.24 + 0.07	0.62 + 0.02	1.99 + 1.75	1.78 + 1.75	0.07 + 1.65	1.66 + 1.01	0.57 + 0.75	3.61 + 1.75	3.79 + 1.65	3.42 + 1.12	2.78 + 1.90
18:2w6	1.34 + 0.51	2.12 + 1.28	2.35 + 1.07	2.89 + 0.77	8.12 + 4.91	5.69 + 3.34	9.60 + 0.42	2.02 + 0.54	3.26 + 1.01	2.25 + 2.76	1.73 + 1.47	0.84 + 0.69	3.74 + 1.01	9.63 + 2.76	4.40 + 3.62	22.06 + 2.83
18:4w3	1.22 + 1.05	0.91 + 1.57	0.76 + 0.48	0.82 + 0.51	0.04 + 0.08	2.87 + 1.06	2.24 + 0.71	4.99 + 2.15	3.61 + 2.75	0.77 + 0.67	0.53 + 0.68	2.22 + 0.75	2.17 + 2.75	1.99 + 0.67	2.41 + 1.84	1.08 + 0.64
20:0	1.46 + 1.32	0.69 + 0.21	1.18 + 0.04	1.16 + 0.26	1.76 + 2.53	0.96 + 0.79	0.60 + 0.23	2.03 + 2.58	7.25 + 1.55	1.32 + 3.18	1.82 + 1.26	1.75 + 0.38	1.87 + 1.55	4.43 + 3.18	1.25 + 0.16	2.98 + 2.10
20:1w11	4.50 + 7.62	1.03 + 0.68	0.52 + 0.09	0.94 + 0.61	6.02 + 9.47	4.37 + 4.02	2.88 + 0.37	3.13 + 2.35	3.21 + 0.04	0.69 + 1.40	1.87 + 0.44	0.79 + 0.23	0.52 + 0.04	3.08 + 1.40	3.01 + 2.32	1.23 + 0.54
20:1w9	3.60 + 2.03	1.97 + 1.08	1.35 + 0.82	0.95 + 0.19	2.43 + 3.30	3.99 + 1.29	1.03 + 1.66	5.62 + 3.90	1.55 + 0.24	0.85 + 2.27	1.30 + 0.85	1.20 + 1.29	0.37 + 0.24	4.14 + 2.27	1.77 + 0.53	0.79 + 0.09
20:1w7	1.18 + 1.22	0.80 + 0.60	0.34 + 0.33	0.64 + 0.33	1.85 + 2.10	0.17 + 0.23	0.17 + 0.29	0.54 + 0.48	0.38 + 0.43	0.22 + 1.36	0.60 + 0.22	1.53 + 1.86	0.39 + 0.43	3.07 + 1.36	0.63 + 0.62	0.69 + 0.67
20:3w3	1.97 + 2.20	1.20 + 0.67	1.65 + 0.94	0.74 + 0.38	0.63 + 0.26	3.54 + 2.76	2.36 + 0.66	5.84 + 1.99	0.57 + 0.49	0.95 + 0.42	0.87 + 0.78	0.95 + 1.09	0.66 + 0.49	0.67 + 0.42	0.75 + 0.32	0.33 + 0.13
20:5w3	4.84 + 4.31	0.25 + 0.27	1.04 + 0.74	1.89 + 1.31	0.33 + 0.54	4.25 + 3.94	1.24 + 0.14	2.10 + 0.93	1.54 + 0.56	1.40 + 2.49	1.60 + 0.57	1.33 + 1.01	1.03 + 0.56	3.48 + 2.49	1.66 + 0.77	1.49 + 0.45
22:0	0.91 + 1.18	0.50 + 0.07	0.76 + 0.40	0.42 + 0.03	0.45 + 0.26	3.41 + 2.48	0.54 + 0.06	6.00 + 1.83	1.38 + 0.10	2.12 + 0.01	3.00 + 1.96	1.00 + 0.14	0.67 + 0.10	0.48 + 0.01	0.42 + 0.15	1.25 + 1.22
22:1w9	3.80 + 2.37	2.68 + 0.52	3.14 + 1.36	1.69 + 1.19	1.35 + 0.58	4.15 + 2.43	2.51 + 0.57	1.60 + 1.38	1.96 + 2.04	4.00 + 2.56	2.86 + 1.63	2.27 + 3.28	2.40 + 2.04	4.54 + 2.56	2.92 + 1.75	2.04 + 0.73
22:2w6	2.23 + 2.98	2.27 + 3.50	0.38 + 0.66	0.66 + 0.75	0.66 + 0.48	0.61 + 0.49	0.78 + 0.14	1.17 + 0.38	0.26 + 0.11	1.14 + 0.27	1.21 + 0.67	1.86 + 2.19	0.37 + 0.11	1.02 + 0.27	0.41 + 0.11	0.29 + 0.30
22:5w3	2.26 + 2.74	1.39 + 0.81	2.03 + 1.14	1.51 + 0.83	0.85 + 0.42	3.72 + 1.25	1.91 + 0.54	4.53 + 1.27	0.95 + 0.47	1.25 + 1.30	2.92 + 1.42	2.14 + 1.83	1.30 + 0.47	1.52 + 1.30	0.71 + 0.29	0.49 + 0.20
22:6w3	2.60 + 1.67	2.14 + 2.31	2.99 + 1.04	1.29 + 0.39	0.97 + 0.81	1.70 + 1.03	1.29 + 0.23	2.89 + 1.10	1.96 + 0.57	2.48 + 1.66	3.34 + 2.30	2.64 + 2.24	1.19 + 0.57	2.62 + 1.66	2.03 + 0.74	1.19 + 0.24
BAME	5.12 + 2.83	5.31 + 0.83	3.11 + 0.70	3.51 + 1.87	2.30 + 0.69	2.15 + 1.05	4.88 + 0.24	3.76 + 1.84	3.90 + 0.68	6.10 + 2.29	6.16 + 2.56	11.14 + 7.16	5.78 + 0.68	6.72 + 2.29	9.09 + 5.60	5.60 + 1.54
ΣSFA	44.95 + 3.03	47.84 + 3.11	53.19 + 0.40	51.13 + 2.25	42.01 + 7.93	38.77 + 6.64	38.55 + 0.44	39.61 + 7.58	51.56 + 6.17	64.20 + 6.90	58.56 + 8.55	66.85 + 15.84	53.98 + 6.17	37.29 + 6.90	48.52 + 16.53	37.19 + 7.68
ΣMUFA	38.60 + 6.58	41.88 + 2.05	35.60 + 1.37	39.07 + 2.15	46.40 + 5.56	38.85 + 3.44	42.02 + 1.55	36.85 + 2.33	36.29 + 9.30	25.56 + 6.11	29.25 + 13.38	21.16 + 10.48	35.56 + 9.30	41.79 + 6.11	39.11 + 13.89	35.89 + 5.05
ΣPUFA	16.45 + 3.59	10.28 + 4.95	11.21 + 1.23	9.80 + 2.03	11.60 + 4.85	22.38 + 3.53	19.43 + 1.52	23.53 + 5.37	12.15 + 3.51	10.24 + 8.31	12.19 + 4.93	11.99 + 9.07	10.46 + 3.51	20.92 + 8.31	12.37 + 3.10	26.92 + 2.77

6.3.3. Condition index

Neither upwelling nor month affected the condition index of *M. galloprovincialis* (ANOVA, $p > 0.05$); however, there were significant differences among sites (ANOVA, $p < 0.001$). The Tukey HSD tests showed site 1 had the lowest CI over time and compared to the other sites ($p < 0.001$, Fig 6.8). Site 2 had CI values significantly intermediate between site 1 and sites 3-4 ($p < 0.001$; Fig 6.8). Sites 3 and 4 were not statistically different from each other and they showed the highest CI values among sites.

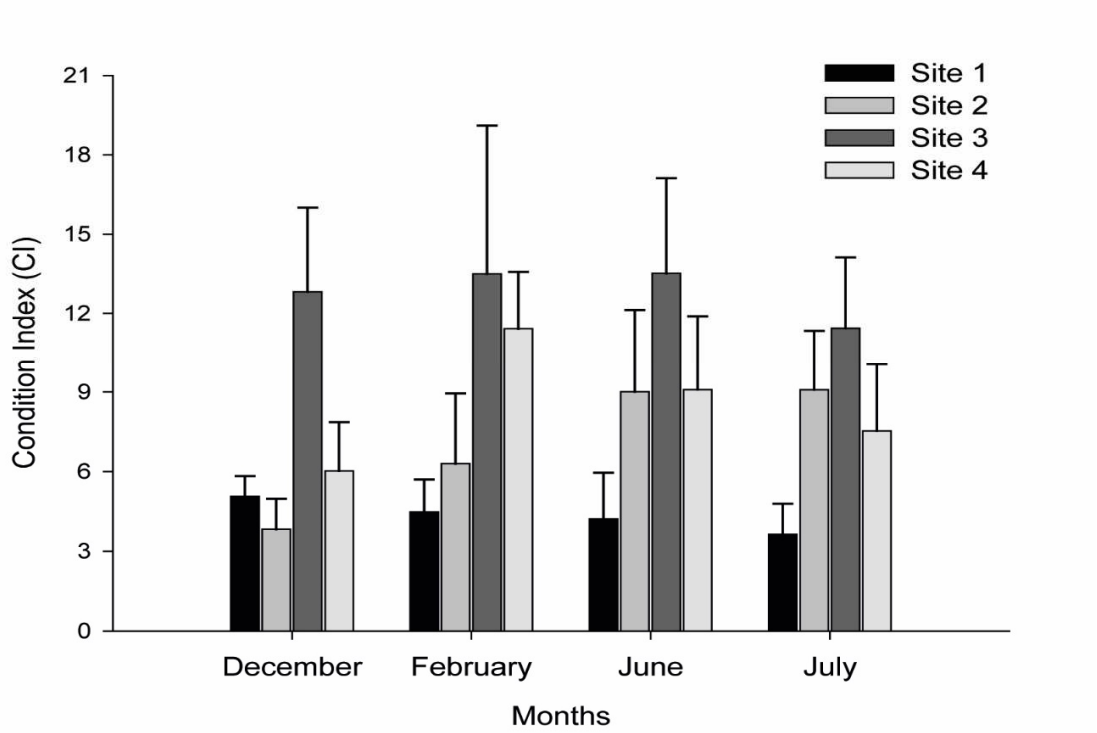


Fig 6.8 Condition Index of *M. galloprovincialis* at the four sites and across the four months sampled. Sites are arranged from south to north (left to right) and the error bars indicate standard deviation. Site 1 and 3 were sites of upwelling, while site 2 and 4 of non-upwelling.

6.3.4. Gonad index

Upwelling and month did not have an effect on the GI of mussels on the South African west coast, however there were significant effects of the factor site and the interactions between month and site (Table 6.7).

Table 6.7 ANOVA to test the temporal effect of upwelling on the Gonad Index of *M. galloprovincialis* on the South African west coast. M = Month, Up = Upwelling, Si = Site, He = Height; df = degrees of freedom, MS = mean square, F = f-ratio, P = p-value. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

	df	MS	F	P	
T	3	184.99	2.52	0.061	
Up	1	12.41	0.17	0.682	
Si (Up)	2	2679.15	36.49	0.000	***
He (Si (Up))	4	8.11	0.11	0.979	
T X Up	3	99.56	1.36	0.259	
T X Si (Up)	6	225.18	3.07	0.008	**
T x He (Si (Up))	12	197.29	2.69	0.06	
Error	123	73.41			

As the CI, site 3 had the highest GI across all months (Fig 6.9). Sites 1 and 2 had similar GI and they were constant over the four months, while site 4 showed an increase of GI from the summer to the winter months (Tukey HSD, $p < 0.05$; Fig 6.9).

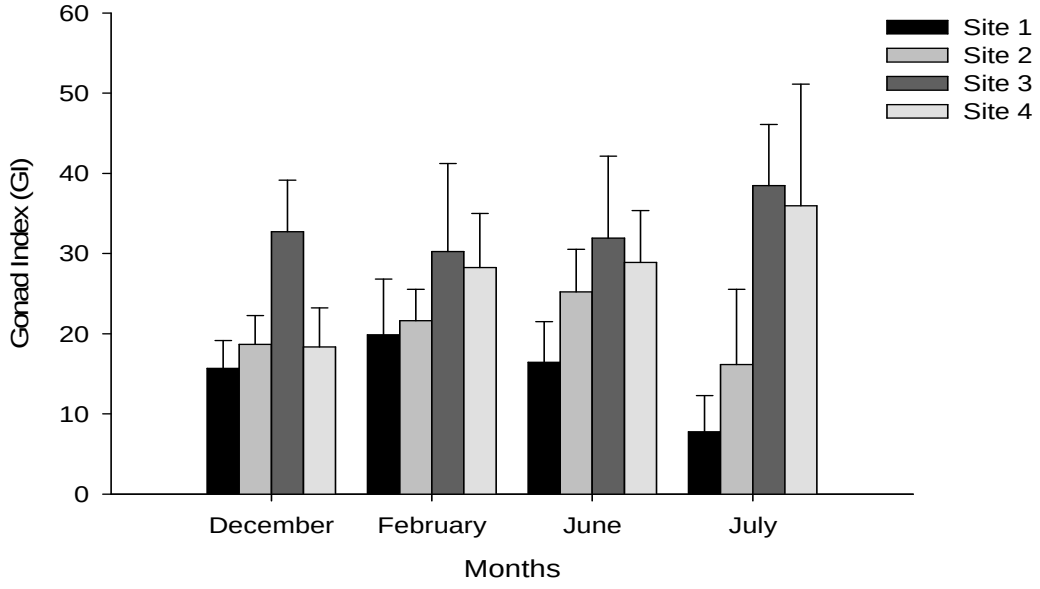


Fig 6.9 Gonad Index of the specimens of *M. galloprovincialis* processed with fatty acid analyses at four sites and across the four moths sampled. Sites are arranged from south to north (left to right) and the error bars indicate standard deviation. Sites 1 and 3 were sites of upwelling, while site 2 and 4 of non-upwelling.

6.4. Discussion

The present study highlights marked differences in the FA compositions of gonad and adductor muscle tissue of *M. galloprovincialis*, with gonads showing a higher proportion of PUFA and more specifically EFA across all months and sites. EFA are FA essential for the maintenance of cell membrane structure and function, and they are precursors of bioactive compound in invertebrates, essential for survival, growth and reproduction (Parrish et al. 2000, Sushchik 2008). However organisms cannot synthetize them in sufficient quantity (missing the desaturase enzyme Δ6; Pirini et al. 2007), and therefore, they have to be acquired through their diet (Arts et al. 2001, Kelly and Scheibling 2012). Studies have suggested preferential retention of specific FA in gonads, in order to ensure reproduction and to increase the chances of survival of the offspring (Pollero et al. 1983, Soudant et al. 1996a, 1996b, Palacios et al. 2005, Kattner and Hagen 2009). For instance Estefanell et al. (2014) found *Octopus vulgaris* retains 20:4w6 in its gonads; Blanchard et al. (2005) showed the concentration of 18:2w6, 18:3w3 and 22:6w3 increased in the gonads of the fish *Perca fluviatilis* during the reproduction season; while Besnard et al. (1989) highlighted that peaks of 20:5w3 in the bivalve *Pecten maximus* gonad coincide with sexual maturity, whereas 22:6w3 is essential for membranes before oocyte maturity. As expected, the gonads of mussels presented a higher proportion of EFA and in particular double the proportion of 20:5w3 compared to adductor muscles. This suggests a preferential retention of EFA and specifically of 20:5w3 in gonads, most likely for reproduction purposes. The results of the GI and CI provide support to this hypothesis. Although the overall patterns of CI and GI among sites were similar throughout the year, CI at site 1 remained consistently low over time, while GI was variable, often having similar values to the other sites within each month. This may suggest that despite specimens from site 1 exhibiting poorer condition than those at the other sites, they still invested substantial amounts of energy in reproduction.

Although gonads did not show a clear pattern over the sampling period, they were significantly different to the muscles during all months. At all sites considered, adductor muscle samples from February were separated from specimens of the other three months for having a higher proportion of diatom trophic markers compared to December, June and July. The temperature data from the present study showed most

upwelling events occurred between December and February. Upwelling enhances nutrient levels in coastal areas, stimulating phytoplankton growth mainly in the form of diatoms (Napolitano 1999) and would thus modify the food quality for intertidal consumers (see Chapter 5). Therefore, the FA signatures of adductor muscle in February seems to reflect the influence of upwelling events, which occurred during the previous months. This matches the one month FA turn-over period found for mussels (Pirini et al. 2007). However, this effect was not detectable in gonads, which showed differences in FA signatures among months, but without a clear pattern. A possible explanation for the different pattern between tissues could be related to the different turnover rates of gonads and muscles with gonads changing FA signatures faster than adductor muscle (Napolitano et al. 1993, Ezgeta-Balić et al. 2014). Tissues, such as gonads or the digestive gland, which have a higher metabolic activity and a faster turnover rate, are more appropriate for revealing recent diet, while tissues with lower metabolic activity, such as muscles, have a slower turnover rate and thus provide an integration of dietary sources over a longer period of time (Ezgeta-Balić et al. 2014). Some studies have investigated the FA turnover rate in other organisms such as fish or zooplankton (*e.g.* Graeve et al. 1994, Robin et al. 2003), however, no studies have investigated the FA turnover rates of gonads or muscles in filter feeders. Further investigations in this regard are needed in order to clarify this pattern. Another hypothesis, which corroborates with the previous paragraph, is that there is a preferential retention of some FA in the gonads for reproduction purposes. A preferential FA pathway mechanism may thus explain the miss-match between the food quality and the FA signature of the gonads and therefore clarify the lack of resemblance between the gonad and the muscle pattern (Caers et al. 1999, Blanchard et al. 2005, Uysal et al. 2006, Kebir et al. 2007).

The factor upwelling was not significant for either gonads or muscles for any of the months, however strong dissimilarities among sites were recorded. In particular, muscles showed that diatom trophic markers characterized the non-upwelling site 2 at all months and site 3 was enriched in dinoflagellate trophic markers, while site 1 and 4 had generally high proportions of PUFA, but no specific FATM were identified. Gonads showed some minor variations among sites and months but without a clear pattern. As discussed above, the faster turnover rate and different metabolic pathway of gonads compared to adductor muscle are probably the drivers of the dissimilarities between

tissues. Therefore, only the results of the more conservative tissue, the adductor muscle, are discussed in relation to the factor month. Adductor muscles results contradict with the prior expectations of the study. It was expected stronger diatom TM or dinoflagellate TM at upwelling sites (sites 1 and 3), which are the two main taxa present during upwelling events (Chavez and Messié 2009). Site 3 confirmed this pattern, being typified by dinoflagellate markers, but site 1 was not characterised by any distinct phytoplankton TM. Similar disparities were found with the condition indexes. Site 3 had the highest CI and GI among sites in all months, while site 1 showed the lowest CI and GI at all time, which indicate a lower quality and may quantity of food at site 1 compared to the other sites. Temperature showed both upwelling sites were exposed to long periods of upwelled water; however the temperature data showed even the two non-upwelling sites were influenced by a few upwelling events. A theory associated with upwelling proposed that at upwelling sites, nutrients and particles can be carried offshore during upwelling due to the hydrogeography of the area or to wind intensity and direction, resulting in phytoplankton-poor waters inshore (Brown and Field 1986, Wieters et al. 2003, Roughan et al. 2005). This offshore advection during upwelling is followed by onshore advection (either to the same point on the shore or farther downstream) during upwelling relaxation or downwelling. This suggests that the section of the coast downstream of the upwelling centres can exhibit higher phytoplankton concentrations than sites located at the upwelling centres, generating a source-sink type of geographic pattern of nearshore nutrients and phytoplankton along the coast. A few other studies support instead the idea that upwelling enhances nutrient levels, and thus stimulates phytoplankton and macrophyte growth at the site of upwelling (Nielsen and Navarrete 2004, Wieters 2005). The first hypothesis can be applied to sites 1 and 2, while the second hypothesis seems to apply to sites 3 and 4. Indeed, at site 2 upwelling occurred (*e.g. in situ* temperature data loggers) and the FA composition of the adductor muscles of specimens from this site were more enriched in diatom trophic markers compared to site 1. In addition, site 1 had the lowest CI values during all sampling events. Site 3 was characterised by dinoflagellate TM, and this suggests that the FA composition may reflect a later stage of upwelling (post diatom blooms) and site 4 did not reveal any clear FATM. The two perspectives can depend on the hydrogeography of the specific sites and perhaps the intensity of the event. For instance, the hydrogeographics at site

1 may result in offshore advection of water, whereas at site 3 the hydrogeography may retain phytoplankton onshore after upwelling blooms. The result of the present study partially contradict the finding of Xavier et al. (2007), which compared growth rate, recruitment and population structure of *M. galloprovincialis* at upwelling centres and downstream sites on the west coast of South Africa. They found strong variability among sites as here; however, they did not highlight dissimilarities between site 1 and 3 as found in this study. The dissimilarities between the two investigations are difficult to explain and further sampling would be required to fully explain these patterns. Certainly, the present study was conducted over a long period of time, across both upwelling and non-upwelling seasons, with several sampling events, while Xavier et al. (2007) evaluated the effects of upwelling on the CI of mussels abundances on the basis of a single sampling campaign. Inter-annual variability in upwelling intensity and frequency needs also to be considered as intensity of upwelling might change the characteristics of an event as observed by Smith et al. (2009) for recruitment of mussels.

In the present investigation, no differences in FA signatures were found among samples from two heights of the shore (low and high intertidal). This suggests that specimens from the low and high intertidal mussel zones were exposed to the same food quality and they assimilated FA into their tissues in a similar way. It can also illustrate similar metabolic stress and survival strategies at both tidal heights. This is in contradiction of a previous study showing that SI signatures of individuals of the same species located only a few cm apart where subjected to different food sources due to different processes acting at microscales (Schaal et al. 2011). The present study showed at the opposite no differences between the FA composition of mussels suggesting a certain homogeneity of the food quality between the two heights. An important aspect to be considered is that the two studies were conducted using two different techniques, and this may explain the dissimilarities. SI are a more conservative tool compared to FA, integrating over a longer period of time (Pirini et al. 2007, Hill and McQuaid 2009), and for this reason can underline effects not detectable with FA analyses. However, further studies are needed to try to explain this discrepancy.

Surprisingly in this study, no differences were found in the FA composition of the SPM at the different sites and sampling events suggesting a certain homogeneity of the food for intertidal populations. SPM reflects the FA composition of the water at the

moment of the sampling and for this reason it does not necessarily represent what the organisms where feeding on a few days/weeks before. It was therefore not expected to link the POM FA composition with the FA composition of the consumers but it was expected to detect some differences, in relation to upwelling. For example, more phytoplankton TM were expected at upwelling sites during the upwelling season. The spatial and temporal homogeneity of the SPM was even more unexpected as differences were found in the FA signature of the consumers. A possible explanation for this pattern is that every time SPM was sampled, the conditions among sites were similar, which could indicate that the conditions are changing rapidly after an upwelling event and go back to a common "baseline" status.

Previous studies showed the importance of upwelling on the South African west coast to enhance primary production, as well as for growth and food quality for primary consumers (Bustamante et al. 1995, Xavier et al. 2007). In this study, adductor muscles had a strong phytoplankton FA signature in February, during the upwelling season and at all sites, suggesting that a very strong upwelling event occurred during the previous weeks, as was shown by the temperature results. In addition, even if upwelled waters were present only during the summer months, tissues of mussels showed similar proportions of PUFA-MUFA-SFA over time, indicating that the specimens were exposed to similar quality and quantity of food both in austral summer and winter and in both upwelling and non-upwelling conditions. Although the factor upwelling was not significant in any of the analyses, the present results suggest that upwelling played a role at all sites of the investigation and that it equally influences specimens at upwelling and non-upwelling sites. This indicates that the influence of upwelling on the west coast of South Africa is pervasive, rather than discrete, and that it necessary to categorize upwelling influence by referring to upwelling centres and downstream areas and by measuring their occurrence and intensity as they seem to be highly variable in time and space.

CHAPTER 7

SYNTHESIS

7. Synthesis

7.1. Spatial variation in dietary regimes

The diet of benthic filter feeders is affected by a range of factors operating simultaneously and at different spatial scales. Despite interspecific differences among the five species considered in this thesis, all of them responded to factors operating at large (100s km), meso (10s - 100s km) and local (from one to few km) scales.

Along the South African coast, the main factor that was found to influence the dietary regime of filter feeders was biogeography. The fatty acid (FA) and stable isotope (SI) signatures of conspecific specimens from the three coasts were very different from each other, with the strongest differences being between specimens from the west coast and the other two coasts, where specimens of the west coast showed signs of better food quality than the south and east coasts (see Chapter 5). I attributed these dissimilarities to the influence of the cold eutrophic Benguela Current on the west coast and to the warm oligotrophic Agulhas Current on the south and east coasts. The Benguela is a highly productive system that is strongly characterised by upwelling events that enhance primary and secondary production (Andrews and Hutchings 1980, Shannon et al. 1984). In contrast, the Agulhas Current is oligotrophic and the south and east coasts exhibit less frequent and weaker upwelling events. These oceanographic systems would have an impact on the distribution and composition of the suspended particulate matter (SPM) as well as the production on coastal areas. Therefore, I hypothesised that the different hydrogeographic regimes along the coast of South Africa affected the dietary regime of primary consumers. In a broader scale, dissimilarities in food availability can also influence species richness, growth rates and patterns of distribution of benthic invertebrates (Huntley and Brooks 1982, Jones 1986, Rosenberg 1995, Sogard and Olla 1996). While climate, water and air temperature are generally the main drivers of differences among bioregions in coastal areas (Rohde 1992, 1999, Willig et al. 2003, Ruttenberg et al. 2005, Dunn et al. 2009), it seems that the other critical factor along this coast is the gradient of nutrient concentrations, that increases from east to west reflecting a shift from oligotrophic to more eutrophic conditions as described in Chapters 3 and 5 (Fig 7.1). These strong dissimilarities among bioregions can have

profound consequences for the coastal environment. For example a few studies showed recruitment, growth and abundance of mussel populations differ among biogeographic provinces due to the effects of oceanographic patterns (*e.g.* currents and upwelling) on larval transport and seawater temperature (Smith et al. 2009). Another example is *Mytilus galloprovincialis*. This invasive species in South Africa is present only on the west and south coasts. Previous studies indicated the pattern of distribution of this species is driven by a wide range of factors, such as larval dispersal and the effects of temperature on adult performance (Zardi et al. 2007b, 2011). On the south coast, its abundance and biomass broadly increased from east to west, in parallel with the pattern of nutrients described above. This may suggest that the abundance and distribution of this species is also related to food availability. Similarly, Archambault at al. (1999) showed that mussels within a bay grow faster than specimens on the open coast due to a higher food availability within bays and Thompson and Nichols (1988) indicated that the clam *Macoma balthica* had a fast growth rate in San Francisco Bay due to the high availability of chlorophyll *a* and the warmer temperature.

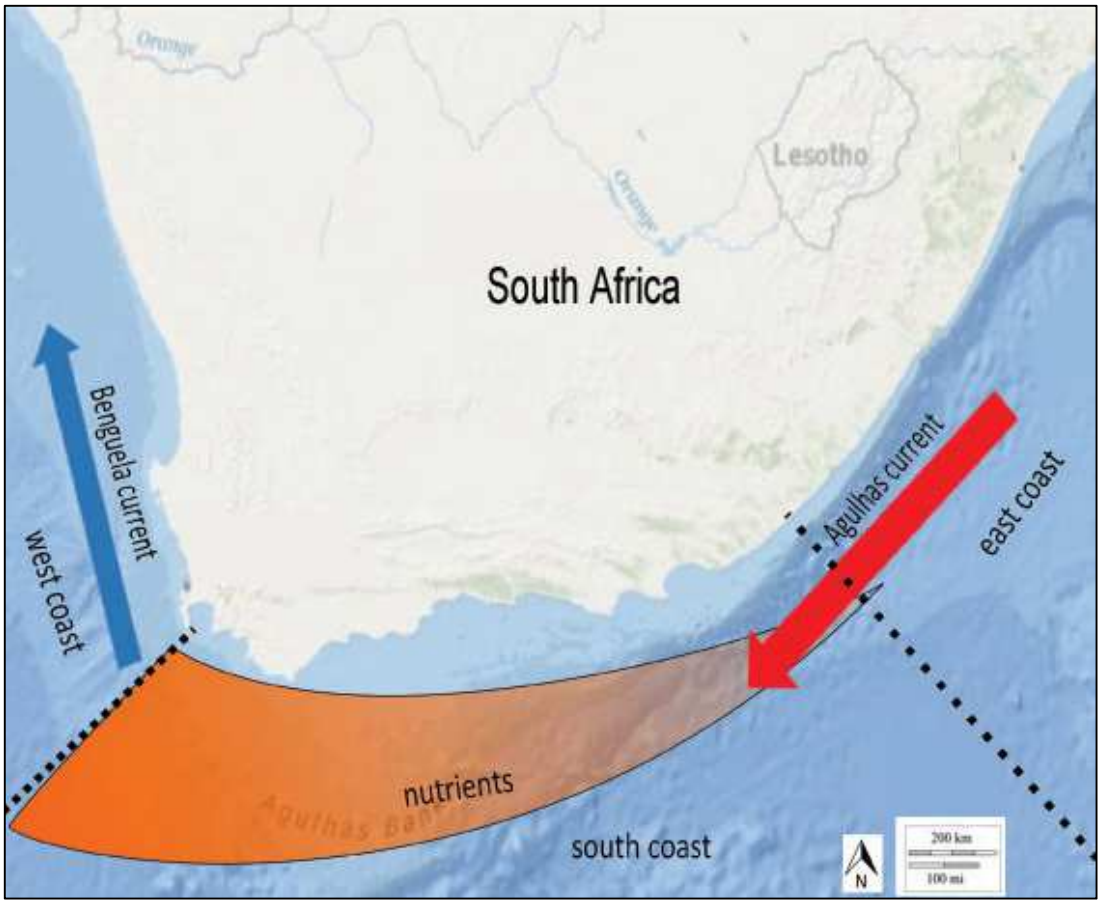


Fig 7.1 Schematic of the increase of nutrients concentration from east to west passing from an oligotrophic to more eutrophic system. Map from http://www.sanbi.org/.

Within biogeographic provinces, other factors that operate at meso or local scales also influenced the diet of filter feeders, particularly upwelling. Upwelling events strongly influenced the SI and FA signatures of benthic filter feeders on the west coast, and the SI of specimens on the south coast. The upwelling effect on the diet of filter feeders was stronger in the highly productive Benguela system, a pattern that reflected the greater intensity of upwelling of this area, compared to the more oligotrophic system of the south coast. Generally, upwelling sites were influenced by upwelling, however within both coasts, the sites characterized by very frequent and/or strong upwelling events (Groenrivier and Port Alfred on the west and south coast, respectively), showed the strongest effect within each coast respectively (Chapter 5). Consequently, the factor upwelling is effectively nested within the factor biogeography, with generally weak upwelling in the more oligotrophic system (*i.e.* south coast) and more intense events in the eutrophic system (*i.e.* west coast). An alternative view to interpret the results is that biogeography is driven by upwelling. The large Benguela Current system is recognised as one of the main western boundary upwelling systems in the world oceans (Carr and Kearns 2003, Chavez and Messié 2009) and it strongly influences the ecosystems of the west coast of Southern Africa (Andrews and Hutchings 1980). Upwelling could represent the primary factor responsible for the biotype of the west coast, and therefore in this case the relationship between upwelling and bioregion would be hierarchical, with biogeography nested in upwelling. This hypothesis is supported by the findings of Chapter 6, which showed clearly that upwelling along the region between the Cape Peninsula and Elandsbaai (200 km) is relatively homogeneous and influences specimens at both upwelling centres and at downstream sites. In contrast, Chapter 5, in a study conducted over 400 km of coastline, indicated that the effect of upwelling on the west coast was discrete, with mussels and barnacles at upwelling sites having different FA and SI signatures from specimens at non-upwelling sites from the same coast and biogeographic province. The patterns found at Chapter 5 and 6 are very surprising since they showed different responses in relation to upwelling within the same bioregion. A possible explanation for this discrepancy could be related to the different spatial resolution of the two studies. One study was conducted over 400 km of coastline, which includes areas with frequent upwelling events (Chapter 5), whereas the other was at a

smaller scale and upwelling occurs only seasonally (200 km, Chapter 6). In addition, in Chapter 5, one of the sites chosen for the comparison experiences very frequent and intense upwelling events, which are stronger than the events on the southern west coast that occur only over the summer months (Andrews and Hutchings 1980, Shannon et al. 1983, Field and Shillington 2006). These considerations suggest that, depending on the spatial scale of resolution used on the west coast, an upwelling effect is either discrete (at about 400 km) or diffuse (within 200 km).

Other factors affecting the diet of primary producers at meso or local scales have also been investigated in this thesis. It was shown that anthropogenic factors can affect mussels and barnacles on the south coast of South Africa. Specifically, Chapter 3 highlighted the effect of proximity to urbanized centres on the $\delta^{15}N$ and FA signatures of benthic populations. Indeed this coast hosts few cities that can increase the amount of nutrients in the nearby coastal environment and thus modify the food source for benthic filter feeders. In this case, the consumers seemed to be positively influenced by the proximity of urbanized centres, showing an increase of polyunsaturated fatty acids (PUFA). A few studies have highlighted a negative correlation between increased urbanization and species diversity. For instance, Mangialajo et al. (2008) found a decrease in the abundance of a habitat forming species toward urbanized centres, with a complete loss of this species in urbanized area. In contrast, this study suggests that along the South African coast, human activities can positively influence natural benthic populations by improving food availability. Most studies of the effects of human activities on natural populations were conducted in highly urbanized centres (*i.e.* Europe and North America), and have focused on pollution or eutrophication (Phillips 1977, López Gappa et al. 1990, Mallin et al. 2000, Karlson et al. 2002, Verdelhos et al. 2005, Yang et al. 2008). Maybe the status of the cities in a developing country like South Africa offers an intermediate level of urbanisation, which is less detrimental to benthic populations compared to over-urbanised cities especially in developing countries. At the same time, this ecosystem offers an opportunity to evaluate anthropogenic effects in a developing countries. An anomalous finding here was *M. galloprovincialis* specimens collected in Mossel Bay, which seemed to be suffering from starvation or poor food quality, unlike *Perna perna* from the same mussels bed (Chapter 2). There is no obvious

explanation for this. Another anomaly was related to different responses of specimens depending on the city considered. Indeed, the specimens from Mossel Bay and Port Elizabeth contrasted from the specimens collected in East London, which showed no effect of urbanization on their FA or SI signatures. It was suggested that this discrepancy might originate from the location of East London on the open coastline while the former are located in bays, where water retention will be greater.

Other variability was observed during this work among regions and site. On the south coast, specimens from the eastern region B (sites 1 and 2) were depleted in $\delta^{13}C$ compared to the more western region A (site 3, 4, 5 and 6; Chapter 3). This pattern was probably due to the presence of a cell of continuous upwelling located in region B (Chapter 3 and 5). Benthic populations in upwelling areas showed lower $\delta^{13}C$ signatures compared to specimens from non-upwelling areas, which reveal a different food source origin of specimens at upwelling centres compared to non-upwelling sites (Chapter 5). Multiple stressors can generate additive effects that may be synergistic, which amplify their effects, or antagonistic, when stressors mitigate each other (Crain et al. 2008). It is possible that the lack of an effect of urbanisation on specimens from East London was due to the combined effects of urbanisation and upwelling resulting in an antagonist effect. This would thus explain why no urbanization effect was observed on any of the specimens collected in East London. It is crucial to investigate the interactions of multiple stressors on ecosystems, as their combined action can determine the occurrence of other effects in marine assemblages. For example, a negative effect of a stressor may precondition a species or a community to be more or less sensitive to a second stressor, subsequently affecting the ecosystem (Crain et al. 2008). Importantly, the effect of urbanization was evaluated at cities along the south coast and should be considered within the larger context of biogeography. Possibly, in another biogeographic province that is a more productive, such as the west coast, the effect of urbanization would not be detectable because the productivity of the natural system would buffer the effect of urbanisation.

One factor that was not found to influence the diet of filter feeders was freshwater inputs (Chapter 4). No freshwater effects were found on either the FA or SI signatures

of any of the study species along the east coast of South Africa, at either meso or local scales. As discussed in Chapter 4 there are a few possible explanations for this, but importantly, all species of filter feeders showed the same lack of response. SI and FA are time-integrated techniques that reflect diet signatures over time. Thus supports the generalization that the dietary regimes of intertidal marine filter feeders are not influenced by freshwater inputs along this coast.

Other factors that operate at small scales (*i.e.* few metres) did not play a role on the diet of filter feeders. Particularly no differences were recorded in either the condition index or the FA signatures between specimens from two heights of the shores (Chapter 6). A few factors have an important role in determining food availability for benthic populations at small scales. Wave action is one of the primary factors determines food distribution in coastal area, and it can strongly influence benthic populations (Paine and Levin 1981, Sousa 1984, Eisma and Kalf 1987, Bustamante et al. 1995). For instance, McQuaid and Branch (1984) showed that populations from exposed shores had higher biomass compared to specimens from sheltered areas due to higher wave exposure, which is one of the primary mechanisms of food supply for the intertidal benthos. High wave action can ensure more food availability for benthic organisms, compared to specimens in sheltered areas, and underpins the shift between filter-feeder and macroalgal domination of intertidal biomass (McQuaid and Branch 1985). The sites chosen for this comparison had the same wave exposure. In addition, the condition index of specimens from the two heights was not significantly different. Consequently, it was indicated that the populations at the two heights were subject to the same food availability.

7.2. Critique of the techniques

The present study showed the novelty of looking at the diet of marine organisms using a combination of SI and FA analyses. Separately, these techniques have been widely used in the past to look at trophic relationships in marine environments (McConnaughey and McRoy 1979, DeNiro and Epstein 1981, Napolitano 1999, Zhukova 2000, Hanson et al. 2010). Here, they provided exhaustive information on the diet of filter feeders in relation to the different hypotheses. However, a few criticisms can be made of these

techniques. Both provide information on the food sources assimilated by the organisms (Budge et al. 2006, Pirini et al. 2007). Fatty acid trophic markers (FATM) represent a particularly useful tool to trace which taxon of species was ingested and/ or assimilated by the consumers (Dalsgaard et al. 2003, Budge et al. 2006). However, with consumers, which often have a mixed diet, such as filter feeders, the results can be very difficult to interpret due to a mixture of trophic markers that cannot be assigned to a single prey, but only to a group of prey. For example, at upwelling sites on the west coast, filter feeders presented a mixed SI signature between those of phytoplankton from offshore and macroalgae from onshore and a FA composition enriched in PUFA compared to specimens from non-upwelling sites (Chapter 5). It was not possible, however, to identify which taxon (e.g. diatom, dinoflagellate, brown algae) was predominant in their diet.

Little knowledge is available on the FA metabolic pathways of filter feeders. Specifically, there is a limited information on how and which FA are assimilated by the different filter feeder species, how they are stored or used by the organisms, and whether they are apportioned differently to the consumer's tissues and, if so, in which proportions. Particular attention should be also given to non-methylene-interrupted FA (NMI). These FA are synthesised *de novo* in bivalves by elongation and Δ5 desaturation of 18:1w9 and 16:1w7, with the latter being very abundant in several taxa of phytoplankton and the first being common in several marine organisms such as ciliate and copepods, as well as fish (Farkas et al. 1977, Sargent and Falk-Petersen 1988, Pirini et al. 2007, Barnathan 2009). For instance, when the proportion of 16:1w7 is low in a consumers' FA composition, but a high proportion of 16: PUFA, 20:5w3 and NMI is found, it is possible to suggest that the bivalves transformed 16:1w7 into NMI, and therefore, were feeding on diatoms. Having more information on the metabolism of these animals would increase our understanding of what is actually assimilated by the consumers, and how they invest in reproduction or growth. An integration with other techniques, for example laboratory experiments where the specimens are subject to a controlled diet (*e.g.* Pirini et al. 2007), or combining FA analyses and compound-specific carbon isotopic analyses of FA in order to trace changes in dietary sources (Koussoroplis et al. 2010), could provide information on the dietary assimilation and apportionment.

The biological role and function of NMI FA is an important issue to consider. In the present study, mussel species were characterized by a relatively high proportion of 20:2 NMI and 22:2NMI relative to the other FAs. In addition, specimens from the west coast had higher proportions of these FA compared to those from the other two coasts (Chapter 5). Previous studies found high concentrations of NMI FA in phospholipids and their amounts were reversely related to the sum of 20:5w3 and 22:6w3 FAs. Their preferential incorporation into polar lipids suggests a structural and functional role of NMI FA in biological membranes (Barnathan 2009). Irazu et al. (1984) highlighted how the unsaturated structure of NMI FA confers on cell membranes a higher resistance to oxidative stress and microbial lipases than the common PUFA, and thus can represent a biochemical acclimation feature of benthic organisms to their specific habitat. Kraffe et al. (2004) suggested that the functional role of NMI FA could be related to the control and repair of structural and functional inadequacies due to a decrease of long-chain PUFA in structural lipids, particularly in the inner layer of the membrane where NMI FA are preferentially distributed. Other studies, showed that NMI have a melting point that is lower than that of homologes with double bonds interrupted by only one methylenic group ($-CH_3$) and thus they may be more suitable for maintaining homeoviscosity at low temperatures (Barton and Gunstone 1975, Zakhartsev et al. 1998, Pirini et al. 2007). This implies that in cold environments, or during the transition from summer to winter, the proportion of NMI would increase. This is in accordance with the differences among biogeographic provinces found in this study. The temperature of the water on the west coast is substantially lower than on the other two coasts (Andrews and Hutchings 1980, Probyn et al. 1994). This suggests specimens on the west coast have higher values of NMI FA in order to ensure membrane fluidity.

An aspect of FA techniques that was not evaluated in the present study, but that could be useful for future investigations, involves separating the neutral lipids (NL) from the polar lipids (PL). PL have a functional role controlling metabolic activities (*e.g.* membranes; Nevenzel 1970, Volkman et al. 1989). They are genetically controlled and as such, their FA composition remains relatively constant in an organism. Neutral lipids, in contrast, constitute the FA that are used for short or longer energy term storage and

are used by organisms as the need arises (Harrington et al. 1970, Sinanoglou and Miniadis-Meimaroglou 1998, Lee et al. 2006). In the present work, the total FA were analysed without prior separation of the lipid classes. An interesting approach would be to investigate if stronger levels of storage lipid change in relation to the different hypotheses investigated in this study. For instance, assess whether specimens under upwelling conditions built more lipid storage compared to specimens at non-upwelling sites in response to food pulses. This would provide more information on the metabolism of filter feeders. However, a negative aspect of separating FA into classes is that it requires substantially more time and higher costs than the extraction of TFA (Dalsgaard et al. 2003).

Another limitation of this study is that different tissues were used for mussels and barnacles. For mussels, the adductor muscle was used due to its low turnover rate (Gorokhova and Hansson 1999); while the whole body of the barnacles was analysed as it was difficult to separate tissues from one and another due to small size, particularly in *Chthamalus dentatus*. Different mussel' tissues can show dissimilar SI and FA signatures (Chapter 6; Hill and McQuaid 2009); therefore when the entire body is investigated the resultant signatures are a mixture from the various tissues. Possibly the dissimilarities in FA and SI signatures observed between barnacles and mussels (Chapter 3 and 4) could be partially due to differences in the tissues analysed.

Differences in the turnover rate among tissue types and among species also requires further investigation. Mussel tissues have different SI turnover times, for example for adductor muscle it is about nine months while for gills it is around three months (Hill and McQuaid 2009), while no information is available on the turnover times of barnacle tissues. In addition, it is known that the FA turnover rate of the whole mussel body is about one month (Pirini et al. 2007), but no information is available on the FA turnover of different mussel tissues or of barnacles. These kinds of information are particularly useful when an experiment is planned, for instance in order to decide how often the sampling should be conducted. If a study aims to investigate the temporal effect of a specific factor (*e.g.* upwelling), then it is essential to understand the rates of tissue turnover so that sampling can be conducted at the appropriate temporal scale. This is particularly important if different tissues have dissimilar turnover rates. In this case, a

study needs to also focus on the appropriate tissue to use in relation to the specific hypothesis.

7.3. Conclusion and perspectives

This study constitutes a baseline for understanding the diet of filter feeders in relation to several factors operating at different spatial scales. This is fundamentally important because filter feeders play a key functional role in coastal areas as bioengineers and as habitat forming species (Jones et al. 1996, Gutiérrez et al. 2003, Cole et al. 2011). In this study, the species considered differed, and showed different diets, however they all showed similar responses to the factors investigated. With changing environmental conditions and anthropogenic influences, it is increasingly important to understand how these factors interact with coastal systems. As a fundamental ecological feature, food availability exerts bottom-up control on coastal ecosystems (Menge 2000). The fact that these effects influence habitat-forming intertidal species raises the potential for knock-on effects within the broader system. The effect of changing food availability therefore has the potential to alter species richness and population dynamics, ultimately with consequences for ecosystem functioning.

Given a scenario of climate change, ocean currents and upwelling are likely to change in response to alterations in temperature, precipitation and wind intensities (Timmermann et al. 1999). An increase of freshwater flux will potentially increase stratification of the water column, increasing the input of terrestrial nutrients, while decreasing vertical nutrient flux. Bakun (1990) predicted that the intensity and frequency of upwelling will increase due to an intensification of alongshore wind stress on the ocean surface; while Timmermann et al. (1999) predicted an increase of El Niño due to climate change. All these phenomena could affect nutrient supply and the transport of primary production, with important consequences for the population dynamics of consumers (Harley et al. 2006). Although these changes are likely to influence biological productivity, changes are likely to be region-specific and at this stage they are not yet predictable (Harley et al. 2006). This thesis indicates that such oceanographic processes influence the diet of benthic organisms in different ways, at different scales and sometimes in an interactive

way. As a result making predictions about the consequences of large-scale and long-term environmental changes is likely to be much more difficult than anticipated.

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9. Appendices

Appendix a Total fatty acid (TFA) composition of filter feeders in relation to anthropogenic effects. The values are percentages expressed as mean ± standard deviation. Only FA >1 % were displayed below. a) Ts- *T. serrata*. b) Oa- *O. angulosa*. c) Cd- *C. dentatus*. d) Pp- *P. perna*. BAME = bacterial fatty acids, SFA = saturated fatty acids, MUFA = monounsaturated fatty acids, PUFA = polyunsaturated fatty acids.

a)

Ts	1- East London		2- Kidd's beach		3- Port Elizabeth		4- St. Francis Bay		5- Mossel Bay		6- Jongensfontein	
TFA	A	B	A	B	A	B	A	B	A	B	A	B
14:0	3.17 ± 0.73	2.71 ± 0.79	4.05 ± 1.27	3.88 ± 0.46	3.14 ± 1.49	2.50 ± 1.39	2.57 ± 0.42	2.98 ± 0.16	3.42 ± 0.41	1.37 ± 0.84	3.81 ± 1.09	3.48 ± 0.70
16:0	18.79 ± 1.61	16.47 ± 0.68	18.31 ± 1.09	17.79 ± 0.96	17.09 ± 1.18	16.54 ± 1.67	15.99 ± 1.09	20.01 ± 1.01	18.73 ± 3.06	14.47 ± 2.66	16.33 ± 1.48	16.11 ± 0.22
16:1w7	3.60 ± 0.49	3.47 ± 0.71	4.85 ± 1.19	5.02 ± 0.19	3.59 ± 1.29	2.74 ± 1.31	3.60 ± 0.54	3.15 ± 0.35	3.74 ± 0.37	2.16 ± 0.67	3.72 ± 0.68	3.39 ± 0.33
18:0	8.18 ± 0.75	9.45 ± 0.32	8.05 ± 1.08	7.92 ± 0.49	8.15 ± 1.41	9.12 ± 1.52	8.53 ± 0.78	11.43 ± 1.01	9.20 ± 1.40	10.56 ± 0.85	8.38 ± 0.94	8.10 ± 0.46
18:1w9	5.03 ± 0.12	5.24 ± 0.54	5.39 ± 0.23	5.48 ± 0.11	5.39 ± 0.60	5.46 ± 1.01	5.72 ± 0.50	5.24 ± 0.58	7.00 ± 0.57	5.26 ± 0.86	5.83 ± 0.26	5.03 ± 0.09
18:1w7	3.73 ± 0.12	4.09 ± 0.33	3.79 ± 0.84	4.21 ± 0.17	3.20 ± 1.18	3.65 ± 1.15	5.14 ± 0.11	6.18 ± 0.45	7.27 ± 0.75	6.11 ± 0.38	2.87 ± 2.49	4.11 ± 0.12
18:2w6	1.06 ± 0.13	0.87 ± 0.06	0.79 ± 0.10	0.87 ± 0.02	0.93 ± 0.21	0.92 ± 0.19	0.99 ± 0.12	0.74 ± 0.26	0.98 ± 0.27	0.96 ± 0.27	1.23 ± 0.06	1.26 ± 0.06
18:3w3	3.37 ± 0.43	4.13 ± 1.00	3.04 ± 1.42	3.97 ± 0.52	4.45 ± 3.10	4.25 ± 3.10	3.93 ± 0.68	9.52 ± 2.33	5.51 ± 1.77	13.08 ± 7.18	5.54 ± 1.58	6.83 ± 1.87
18:4w3	1.47 ± 0.29	0.97 ± 0.24	1.30 ± 0.46	1.31 ± 0.06	1.70 ± 0.80	1.48 ± 0.82	1.23 ± 0.33	0.70 ± 0.19	0.70 ± 0.15	0.60 ± 0.34	1.39 ± 0.23	1.40 ± 0.17
20:1w11	0.70 ± 0.12	0.93 ± 0.22	0.76 ± 0.03	1.02 ± 0.24	0.97 ± 0.75	1.77 ± 0.09	1.54 ± 0.23	2.11 ± 0.12	1.02 ± 0.28	1.78 ± 0.72	1.06 ± 0.22	1.01 ± 0.09
20:1w9	1.21 ± 0.16	1.50 ± 0.06	1.19 ± 0.20	1.18 ± 0.06	1.18 ± 0.25	1.24 ± 0.45	1.27 ± 0.19	1.29 ± 0.15	1.27 ± 0.20	1.31 ± 0.29	1.02 ± 0.15	1.04 ± 0.04
20:1w7	0.57 ± 0.08	0.73 ± 0.06	0.64 ± 0.09	0.71 ± 0.10	0.61 ± 0.17	0.67 ± 0.16	0.89 ± 0.04	1.20 ± 0.11	1.46 ± 0.29	0.96 ± 0.08	0.80 ± 0.18	0.59 ± 0.01
20:4w6	1.83 ± 0.27	2.35 ± 0.16	1.97 ± 0.10	2.09 ± 0.29	2.04 ± 0.05	1.94 ± 0.27	2.80 ± 0.18	1.75 ± 0.41	3.95 ± 1.29	2.87 ± 0.31	2.96 ± 0.68	2.24 ± 0.37
20:5w3	20.73 ± 1.35	21.17 ± 1.37	21.54 ± 1.71	19.25 ± 1.06	19.98 ± 1.07	18.85 ± 2.18	17.37 ± 1.09	11.06 ± 1.57	12.65 ± 3.27	13.75 ± 4.38	16.68 ± 2.62	16.47 ± 0.37
22:0	0.74 ± 0.05	0.87 ± 0.02	0.66 ± 0.14	0.77 ± 0.09	0.83 ± 0.27	0.95 ± 0.32	0.86 ± 0.15	1.30 ± 0.13	1.19 ± 0.16	1.87 ± 0.62	0.89 ± 0.09	0.97 ± 0.06
22:5w3	1.29 ± 0.14	1.29 ± 0.15	1.85 ± 0.41	1.75 ± 0.24	1.06 ± 0.22	0.87 ± 0.29	1.22 ± 0.20	0.96 ± 0.09	1.99 ± 0.11	1.25 ± 0.47	1.17 ± 0.02	0.96 ± 0.10
22:6w3	21.67 ± 0.49	21.15 ± 0.64	19.42 ± 0.62	19.93 ± 1.33	22.33 ± 2.05	23.56 ± 2.03	21.89 ± 0.78	15.15 ± 1.32	14.09 ± 2.25	17.88 ± 3.09	22.18 ± 0.76	22.90 ± 1.00
BAME	2.86 ± 0.33	2.61 ± 0.37	2.39 ± 0.06	2.84 ± 0.19	3.34 ± 0.14	3.49 ± 0.42	4.48 ± 0.12	5.25 ± 0.15	5.82 ± 0.66	3.77 ± 0.50	4.13 ± 0.57	4.09 ± 0.07
SFA	33.74 ± 1.71	32.11 ± 1.42	33.47 ± 1.32	33.21 ± 2.02	32.56 ± 1.24	32.61 ± 1.64	32.42 ± 1.08	40.96 ± 1.96	38.37 ± 5.40	32.04 ± 2.44	33.55 ± 2.13	32.75 ± 0.56
MUFA	14.83 ± 0.40	15.96 ± 1.21	16.62 ± 0.27	17.62 ± 0.28	14.94 ± 0.85	15.53 ± 0.91	18.16 ± 0.80	19.17 ± 0.75	21.76 ± 0.78	17.58 ± 0.74	15.30 ± 3.15	15.18 ± 0.16
PUFA	51.43 ± 1.65	51.93 ± 2.60	49.91 ± 1.59	49.17 ± 2.29	52.50 ± 0.74	51.86 ± 2.24	49.42 ± 1.74	39.87 ± 1.61	39.87 ± 6.15	50.38 ± 3.07	51.15 ± 5.26	52.07 ± 0.66

b)

Oa	1- East London		2- Kidd's beach		3- Port Elizabeth		4- St. Francis Bay		5- Mossel Bay		6- Jongensfontein	
TFA	A	B	A	B	A	B	A	B	A	B	A	B
14:0	5.82 ± 0.67	6.47 ± 0.40	7.11 ± 0.48	6.76 ± 0.97	5.83 ± 0.25	5.66 ± 0.30	2.37 ± 0.65	3.84 ± 1.05	5.40 ± 1.17	5.54 ± 0.28	5.21 ± 4.35	6.17 ± 0.38
16:0	20.44 ± 0.77	21.07 ± 0.73	21.42 ± 0.19	21.49 ± 1.04	20.99 ± 1.48	20.96 ± 0.42	17.10 ± 1.65	17.64 ± 0.32	17.89 ± 1.39	18.99 ± 0.46	18.25 ± 1.85	19.79 ± 0.36
16:1w7	6.90 ± 0.57	7.23 ± 0.39	7.91 ± 0.27	7.56 ± 0.43	6.04 ± 0.34	5.77 ± 0.09	3.66 ± 1.09	4.94 ± 0.66	5.79 ± 0.49	5.61 ± 0.14	4.77 ± 1.72	5.57 ± 0.42
18:0	8.43 ± 0.60	8.38 ± 0.52	7.85 ± 0.59	8.41 ± 0.46	8.73 ± 1.13	8.43 ± 0.42	9.92 ± 0.46	9.08 ± 0.98	8.19 ± 0.93	8.17 ± 0.40	8.57 ± 2.95	7.43 ± 0.49
18:1w9	5.44 ± 0.43	5.76 ± 0.16	5.21 ± 0.21	5.27 ± 0.04	5.70 ± 0.29	6.05 ± 0.26	5.31 ± 0.56	5.87 ± 0.22	6.84 ± 0.67	6.51 ± 0.20	5.96 ± 0.48	5.59 ± 0.09
18:1w7	3.57 ± 0.47	2.99 ± 0.39	3.15 ± 0.11	3.37 ± 0.28	3.11 ± 0.11	3.08 ± 0.15	4.46 ± 0.31	5.02 ± 0.25	4.89 ± 0.95	4.95 ± 0.28	3.70 ± 1.47	3.09 ± 0.34
18:2w6	1.24 ± 0.08	1.20 ± 0.06	1.05 ± 0.02	1.03 ± 0.06	1.26 ± 0.08	1.36 ± 0.16	1.26 ± 0.17	1.45 ± 0.12	1.67 ± 0.16	1.76 ± 0.06	1.34 ± 0.15	1.50 ± 0.04
18:3w3	1.06 ± 0.10	0.86 ± 0.05	0.85 ± 0.05	0.82 ± 0.03	1.11 ± 0.06	1.20 ± 0.18	1.01 ± 0.04	1.19 ± 0.15	1.17 ± 0.13	1.30 ± 0.08	0.95 ± 0.18	1.15 ± 0.03
18:4w3	1.99 ± 0.13	1.67 ± 0.11	1.83 ± 0.16	1.69 ± 0.11	2.18 ± 0.22	2.35 ± 0.14	1.33 ± 0.04	1.90 ± 0.39	1.45 ± 0.43	1.98 ± 0.05	1.78 ± 0.83	2.07 ± 0.03
20:1w9	0.89 ± 0.09	0.83 ± 0.12	0.83 ± 0.05	0.88 ± 0.09	0.74 ± 0.12	0.79 ± 0.14	1.72 ± 0.31	1.15 ± 0.17	1.08 ± 0.03	0.80 ± 0.04	1.15 ± 0.71	0.86 ± 0.02
20:4w6	1.70 ± 0.06	1.74 ± 0.05	1.66 ± 0.04	1.62 ± 0.12	1.42 ± 0.36	1.56 ± 0.17	1.60 ± 0.31	1.69 ± 0.30	2.76 ± 0.46	1.89 ± 0.19	1.83 ± 0.58	1.34 ± 0.20
20:5w3	17.35 ± 0.73	17.70 ± 0.52	18.01 ± 0.05	17.69 ± 0.87	15.76 ± 0.87	15.67 ± 0.64	18.62 ± 1.51	17.44 ± 0.12	15.74 ± 1.77	15.98 ± 0.17	17.21 ± 3.09	15.62 ± 0.42
22:5w3	1.87 ± 0.16	1.64 ± 0.13	1.91 ± 0.44	1.73 ± 0.17	1.13 ± 0.13	1.06 ± 0.09	1.05 ± 0.13	1.18 ± 0.04	1.27 ± 0.07	1.08 ± 0.11	0.96 ± 0.09	0.89 ± 0.01
22:6w3	18.90 ± 1.55	18.44 ± 0.81	17.20 ± 0.08	17.48 ± 0.71	20.38 ± 1.29	20.91 ± 0.63	25.11 ± 2.80	21.41 ± 1.08	18.13 ± 0.27	19.27 ± 1.00	22.86 ± 0.72	22.81 ± 0.75
BAME	3.69 ± 0.44	3.41 ± 0.33	3.30 ± 0.43	3.54 ± 0.57	4.98 ± 0.50	4.50 ± 0.24	4.72 ± 0.96	5.54 ± 0.41	7.24 ± 0.71	5.57 ± 0.24	4.67 ± 0.18	5.16 ± 0.23
SFA	38.38 ± 0.93	39.33 ± 1.31	39.69 ± 0.32	40.19 ± 1.41	40.53 ± 2.65	39.56 ± 0.97	34.11 ± 2.73	36.11 ± 0.56	38.72 ± 0.99	38.27 ± 0.58	36.71 ± 3.43	38.55 ± 0.70
MUFA	16.79 ± 1.01	16.81 ± 0.21	17.11 ± 0.90	17.08 ± 0.39	15.59 ± 0.50	15.69 ± 0.48	15.15 ± 1.39	16.99 ± 0.24	18.60 ± 0.32	17.86 ± 0.41	15.58 ± 0.19	15.11 ± 0.64
PUFA	44.84 ± 1.87	43.86 ± 1.44	43.21 ± 0.23	42.72 ± 1.79	43.88 ± 2.74	44.75 ± 1.39	50.74 ± 4.08	46.91 ± 0.80	42.68 ± 1.22	43.87 ± 0.81	47.71 ± 3.27	46.33 ± 1.22

c)

C.d	1- East London		2- Kidd's beach		3- Port Elizabeth		4- St. Francis Bay		5- Mossel Bay		6- Jongensfontein	
TFA	A	B	A	B	A	B	A	B	A	B	A	B
14:0	2.32 ± 0.88	3.47 ± 0.56	4.30 ± 0.74	4.20 ± 0.52	4.58 ± 0.86	3.74 ± 0.81	2.48 ± 0.48	3.16 ± 0.35	4.32 ± 0.52	1.64 ± 0.47	1.83 ± 0.29	3.18 ± 0.53
16:0	20.76 ± 2.67	24.25 ± 0.92	20.96 ± 0.54	19.86 ± 0.17	32.29 ± 4.67	33.73 ± 8.96	28.00 ± 2.49	41.05 ± 2.43	33.50 ± 2.69	32.53 ± 3.73	24.01 ± 5.40	26.32 ± 5.76
16:1w7	3.47 ± 0.94	4.73 ± 0.52	5.13 ± 0.71	5.05 ± 0.43	2.93 ± 0.27	2.32 ± 0.44	2.36 ± 0.23	1.50 ± 0.30	2.64 ± 0.22	1.77 ± 0.29	1.88 ± 0.07	2.97 ± 0.27
18:0	10.95 ± 1.23	12.37 ± 0.94	9.18 ± 0.62	8.51 ± 0.35	19.16 ± 2.52	21.29 ± 4.92	18.47 ± 1.86	27.62 ± 1.49	14.42 ± 1.60	25.15 ± 3.43	15.94 ± 4.13	14.55 ± 4.12
18:1w9	5.82 ± 0.70	6.72 ± 0.37	6.21 ± 0.28	5.96 ± 0.12	6.00 ± 0.77	5.43 ± 1.19	5.40 ± 0.36	2.51 ± 0.86	5.91 ± 0.17	5.13 ± 1.17	5.94 ± 0.30	6.42 ± 0.45
18:1w7	3.13 ± 0.39	3.64 ± 0.17	3.14 ± 0.04	2.71 ± 0.28	2.46 ± 0.25	3.73 ± 0.78	3.63 ± 0.52	1.63 ± 0.65	3.63 ± 0.15	2.75 ± 0.65	3.36 ± 0.14	3.04 ± 0.07
18:2w6	1.23 ± 0.15	0.93 ± 0.09	0.90 ± 0.06	0.94 ± 0.03	0.50 ± 0.28	0.40 ± 0.36	0.82 ± 0.16	0.41 ± 0.23	1.34 ± 0.22	0.61 ± 0.20	1.05 ± 0.30	1.11 ± 0.40
18:3w3	0.84 ± 0.17	0.58 ± 0.04	0.54 ± 0.05	0.62 ± 0.05	0.43 ± 0.12	0.59 ± 0.22	0.45 ± 0.05	0.04 ± 0.07	0.54 ± 0.06	0.52 ± 0.11	0.43 ± 0.22	0.48 ± 0.25
18:4w3	0.96 ± 0.16	0.67 ± 0.17	0.87 ± 0.17	1.07 ± 0.09	0.42 ± 0.19	0.46 ± 0.14	0.32 ± 0.16	0.29 ± 0.51	0.79 ± 0.36	0.33 ± 0.19	0.36 ± 0.22	0.56 ± 0.36
20:1w9	0.82 ± 0.07	0.94 ± 0.17	0.54 ± 0.02	0.46 ± 0.01	0.83 ± 0.04	0.86 ± 0.28	0.63 ± 0.18	0.53 ± 0.29	0.74 ± 0.03	1.00 ± 0.26	0.56 ± 0.17	0.68 ± 0.17
20:1w7	0.48 ± 0.04	0.56 ± 0.04	0.35 ± 0.30	0.00 ± 0.00	0.81 ± 0.19	0.55 ± 0.23	0.55 ± 0.09	0.16 ± 0.14	0.45 ± 0.14	0.82 ± 0.28	0.16 ± 0.23	0.40 ± 0.15
20:4w6	2.61 ± 0.17	2.24 ± 0.10	3.35 ± 0.12	2.61 ± 0.02	1.14 ± 0.44	1.13 ± 0.89	2.70 ± 0.35	0.54 ± 0.50	3.32 ± 0.52	2.16 ± 0.86	3.74 ± 0.78	2.21 ± 0.53
20:5w3	17.78 ± 2.68	14.35 ± 1.14	19.82 ± 0.65	20.40 ± 0.51	6.01 ± 3.35	5.41 ± 5.06	10.62 ± 2.36	2.19 ± 1.44	9.15 ± 2.41	6.74 ± 3.32	13.05 ± 4.52	11.92 ± 4.36
22:0	0.97 ± 0.25	1.23 ± 0.16	0.87 ± 0.07	0.90 ± 0.04	2.51 ± 0.28	2.46 ± 0.28	2.37 ± 0.16	2.97 ± 0.15	1.86 ± 0.37	2.76 ± 0.36	1.92 ± 0.42	2.00 ± 0.47
22:5w3	1.23 ± 0.15	1.08 ± 0.05	1.79 ± 0.05	1.60 ± 0.04	0.70 ± 0.11	0.71 ± 0.32	0.57 ± 0.11	0.00 ± 0.00	0.54 ± 0.16	0.14 ± 0.21	0.50 ± 0.16	1.01 ± 0.77
22:6w3	23.01 ± 3.95	18.23 ± 1.67	18.41 ± 1.31	21.14 ± 1.18	10.78 ± 5.41	8.32 ± 8.18	13.29 ± 2.70	4.33 ± 1.81	9.79 ± 1.26	7.95 ± 4.59	19.16 ± 5.42	17.72 ± 6.84
BAME	3.35 ± 0.59	3.79 ± 0.50	2.91 ± 0.23	2.98 ± 0.25	8.17 ± 1.65	8.70 ± 1.87	6.71 ± 0.68	10.04 ± 0.70	6.24 ± 0.64	7.40 ± 1.17	5.31 ± 1.20	5.23 ± 1.28
SFA	38.35 ± 5.38	45.11 ± 2.28	38.23 ± 0.88	36.46 ± 0.87	66.70 ± 9.78	69.92 ± 16.17	58.03 ± 5.05	84.84 ± 4.69	60.35 ± 5.12	69.49 ± 9.14	49.01 ± 11.41	51.28 ± 11.86
MUFA	13.98 ± 1.96	16.81 ± 0.64	16.10 ± 0.93	15.16 ± 0.36	13.31 ± 1.12	13.06 ± 2.29	13.20 ± 0.75	7.36 ± 1.13	14.18 ± 0.34	12.07 ± 1.33	12.69 ± 0.38	13.70 ± 0.72
PUFA	47.66 ± 7.34	38.08 ± 2.66	45.68 ± 1.70	48.38 ± 1.23	19.98 ± 9.52	17.01 ± 14.19	28.77 ± 5.49	7.81 ± 3.84	25.47 ± 4.79	18.44 ± 8.90	38.30 ± 11.53	35.01 ± 11.88

d)

Pp	1- East London		2- Kidd's beach		3- Port Elizabeth		4- St. Francis Bay		5- Mossel Bay		6- Jongensfontein	
TFA	**A**	**B**	**A**	**B**	**A**	**B**	**A**	**B**	**A**	**B**	**A**	**B**
14:0	1.95 ± 0.41	2.73 ± 0.13	2.04 ± 0.14	1.82 ± 0.37	1.54 ± 0.30	2.11 ± 0.17	3.43 ± 0.78	3.96 ± 0.64	1.17 ± 0.18	1.83 ± 0.15	1.80 ± 0.22	1.64 ± 0.39
16:0	14.61 ± 1.90	19.99 ± 2.59	15.25 ± 0.36	14.95 ± 0.73	13.91 ± 1.06	18.24 ± 2.40	26.61 ± 8.51	30.71 ± 5.87	15.94 ± 0.12	15.01 ± 1.22	18.80 ± 2.72	18.68 ± 3.80
16:1w7	3.66 ± 0.29	3.68 ± 0.25	2.68 ± 0.30	2.42 ± 0.26	2.93 ± 0.42	2.87 ± 0.63	3.24 ± 0.55	2.66 ± 0.12	2.10 ± 0.25	2.84 ± 0.26	2.64 ± 0.19	2.12 ± 0.06
18:0	8.05 ± 2.03	11.45 ± 1.91	7.02 ± 0.55	7.04 ± 0.38	6.49 ± 0.45	9.93 ± 1.98	12.20 ± 3.91	11.64 ± 2.24	6.33 ± 1.12	6.53 ± 0.83	7.82 ± 1.01	8.30 ± 2.03
18:1w9	2.09 ± 0.53	1.79 ± 0.25	0.78 ± 0.09	0.94 ± 0.15	1.19 ± 0.24	1.65 ± 0.15	1.95 ± 0.72	2.02 ± 0.39	1.25 ± 0.28	1.22 ± 0.35	1.46 ± 0.05	1.24 ± 0.16
18:1w7	2.73 ± 0.33	2.29 ± 0.27	1.19 ± 1.03	2.17 ± 0.19	2.85 ± 0.66	2.02 ± 0.24	2.06 ± 0.10	2.05 ± 0.32	1.91 ± 0.42	1.56 ± 0.33	1.97 ± 0.42	2.12 ± 0.30
18:2w6	2.16 ± 0.48	1.16 ± 0.41	2.42 ± 0.21	2.55 ± 0.36	2.28 ± 0.33	1.70 ± 0.63	1.48 ± 0.97	1.19 ± 0.38	2.77 ± 0.65	3.09 ± 0.73	2.41 ± 0.42	2.55 ± 0.53
18:3w3	0.79 ± 0.31	0.14 ± 0.24	1.13 ± 0.04	1.14 ± 0.20	1.02 ± 0.12	0.43 ± 0.38	0.39 ± 0.68	0.41 ± 0.28	1.12 ± 0.35	1.15 ± 0.15	0.77 ± 0.22	0.89 ± 0.36
18:4w3	0.50 ± 0.34	0.00 ± 0.00	1.00 ± 0.25	0.88 ± 0.04	1.36 ± 0.19	0.32 ± 0.27	0.47 ± 0.82	0.22 ± 0.38	1.05 ± 0.33	1.12 ± 0.17	0.54 ± 0.18	0.69 ± 0.23
20:1w11	1.37 ± 0.22	1.60 ± 0.19	1.93 ± 0.28	1.61 ± 0.20	1.40 ± 0.18	1.59 ± 0.24	1.60 ± 0.43	1.61 ± 0.19	1.95 ± 0.29	1.90 ± 0.41	1.69 ± 0.21	1.38 ± 0.29
20:1w9	4.38 ± 1.21	5.99 ± 0.63	2.68 ± 0.18	2.80 ± 0.18	3.25 ± 0.25	4.97 ± 0.37	6.65 ± 2.60	7.15 ± 0.88	3.05 ± 0.33	2.83 ± 0.09	4.30 ± 1.37	3.65 ± 0.90
20:1w7	1.40 ± 0.53	1.94 ± 0.35	0.46 ± 0.80	0.24 ± 0.21	1.29 ± 0.38	1.53 ± 0.49	1.20 ± 0.51	1.46 ± 0.32	0.64 ± 0.23	0.68 ± 0.27	0.84 ± 0.35	0.55 ± 0.11
20:2 NMI1	4.49 ± 0.23	3.38 ± 0.63	6.52 ± 0.41	5.61 ± 1.10	4.34 ± 0.92	5.11 ± 0.58	2.93 ± 0.80	2.35 ± 0.90	5.48 ± 0.99	5.95 ± 0.78	4.91 ± 0.55	3.75 ± 0.53
20:2 NMI2	0.61 ± 0.39	1.11 ± 0.47	1.04 ± 0.73	0.55 ± 0.21	0.37 ± 0.01	1.64 ± 1.06	0.90 ± 0.94	1.21 ± 0.55	0.51 ± 0.28	0.38 ± 0.09	0.71 ± 0.26	0.47 ± 0.15
20:4w6	6.52 ± 1.19	4.82 ± 0.73	5.41 ± 0.83	5.69 ± 0.30	6.68 ± 0.89	4.80 ± 1.16	2.88 ± 1.64	2.02 ± 0.59	8.32 ± 1.66	6.18 ± 0.36	5.52 ± 0.97	5.20 ± 0.92
20:5w3	7.13 ± 2.52	3.80 ± 1.09	7.82 ± 0.86	8.43 ± 0.62	11.01 ± 1.26	4.52 ± 0.47	3.83 ± 3.66	2.57 ± 1.81	6.80 ± 0.74	7.01 ± 0.37	5.43 ± 1.57	6.89 ± 1.62
22:2w6	2.64 ± 0.64	1.82 ± 0.25	2.39 ± 0.63	2.16 ± 0.28	1.95 ± 0.36	2.63 ± 0.29	1.35 ± 0.47	1.15 ± 0.38	2.39 ± 0.31	3.10 ± 0.73	2.37 ± 0.21	1.90 ± 0.64
22:2 NMI1	7.34 ± 0.20	6.37 ± 0.28	6.61 ± 0.78	5.76 ± 0.29	6.24 ± 1.25	7.05 ± 1.53	3.38 ± 1.28	2.85 ± 1.38	6.13 ± 0.99	6.44 ± 0.88	5.92 ± 0.54	5.10 ± 0.95
22:2 NMI2	2.62 ± 1.19	4.20 ± 3.02	0.97 ± 0.37	1.19 ± 0.28	0.65 ± 0.16	1.38 ± 0.92	1.78 ± 1.54	2.53 ± 1.58	1.61 ± 0.96	0.91 ± 0.31	1.38 ± 0.47	1.44 ± 0.79
22:3 NMI	1.57 ± 0.20	1.23 ± 0.10	1.01 ± 0.35	1.28 ± 0.45	1.64 ± 0.11	1.42 ± 0.56	0.57 ± 0.99	0.36 ± 0.62	1.92 ± 0.14	1.99 ± 0.02	1.59 ± 0.33	1.59 ± 0.47
22:4w6	2.40 ± 0.05	2.07 ± 0.42	1.79 ± 0.10	1.77 ± 0.24	1.94 ± 0.50	1.71 ± 0.33	0.30 ± 0.52	0.36 ± 0.32	2.69 ± 0.78	1.50 ± 0.11	1.78 ± 0.36	1.07 ± 0.17
22:5w6	1.25 ± 0.12	1.14 ± 0.28	1.47 ± 0.38	1.37 ± 0.12	1.14 ± 0.24	1.13 ± 0.25	0.68 ± 0.69	0.73 ± 0.11	1.24 ± 0.08	1.37 ± 0.05	1.38 ± 0.26	1.33 ± 0.04
22:5w3	2.90 ± 0.71	2.01 ± 0.31	2.67 ± 0.55	3.05 ± 0.29	3.17 ± 0.27	2.08 ± 0.35	1.18 ± 1.12	1.15 ± 0.37	2.23 ± 0.33	2.22 ± 0.34	2.30 ± 0.15	2.00 ± 0.42
22:6w3	12.93 ± 2.17	9.34 ± 2.71	18.14 ± 2.43	19.38 ± 0.76	17.85 ± 1.30	13.71 ± 1.03	11.97 ± 7.55	9.55 ± 4.45	16.47 ± 1.01	18.92 ± 0.96	16.42 ± 3.54	20.23 ± 2.55
BAME	3.91 ± 0.91	5.97 ± 0.55	5.57 ± 0.63	5.17 ± 0.61	3.49 ± 0.31	5.46 ± 0.19	6.96 ± 2.12	8.11 ± 2.35	4.93 ± 0.22	4.29 ± 0.08	5.28 ± 0.61	5.20 ± 1.04
SFA	28.52 ± 5.21	40.13 ± 3.22	29.87 ± 0.84	28.99 ± 1.15	25.43 ± 1.15	35.73 ± 4.66	49.21 ± 15.22	54.42 ± 10.95	28.37 ± 1.18	27.67 ± 0.64	33.70 ± 4.47	33.82 ± 6.92
MUFA	15.62 ± 2.46	17.30 ± 0.19	9.71 ± 1.97	10.18 ± 0.18	12.91 ± 1.97	14.64 ± 0.28	16.71 ± 3.38	16.94 ± 0.88	10.91 ± 0.92	11.02 ± 0.33	12.89 ± 2.19	11.06 ± 0.37
PUFA	55.86 ± 7.43	42.57 ± 3.41	60.41 ± 2.75	60.83 ± 1.10	61.66 ± 3.09	49.63 ± 4.45	34.08 ± 18.35	28.64 ± 10.73	60.73 ± 1.40	61.32 ± 0.96	53.41 ± 6.66	55.12 ± 7.27

Appendix b Total fatty acids (TFA) composition of filter feeders in relation to effect of riverine input. The values are percentages expressed as mean ± standard deviation. Only FA >1 % were displayed below. A) Ts- *T. serrata*. B) Oa- *O. angulosa*. C) Cd- *C. dentatus*. D) Pp- *P. perna*. BAME = bacterial fatty acids. SFA = saturated fatty acids. MUFA = monounsaturated fatty acids. PUFA = polyunsaturated fatty acids. 16-PUFA = 16:2w4, 16:3w4 and 16:4w1; 20-MUFA= 20:1ww1. 20:1w9 and 20:1w7; 20:3 PUFA= 20:3w6 and 20:3w3; 22-MUFA= 22:1w11, 20:1w9 and 20:1w7.

a)

Ts TFA	**Site 1- Mzimvubu**	**Site 2- Mbotyi**	**Site 3- Mtamvuna**	**Site 4- Pennington**	**Site 5- Ballito**	**Site 6- Thukela**
14:0	3.51 ± 0.88	3.44 ± 0.90	3.42 ± 0.92	3.07 ± 0.83	3.03 ± 0.80	3.16 ± 0.70
16:0	19.00 ± 0.98	18.72 ± 0.88	18.97 ± 0.54	18.93 ± 0.53	19.38 ± 1.30	19.41 ± 1.28
16:1w7	4.44 ± 1.07	4.36 ± 1.10	4.36 ± 1.10	3.96 ± 1.11	3.90 ± 1.08	4.06 ± 1.00
18:0	8.91 ± 1.04	9.12 ± 0.95	9.01 ± 0.83	9.42 ± 1.02	9.82 ± 1.11	9.75 ± 1.09
18:1w9	5.10 ± 0.24	5.14 ± 0.30	5.13 ± 0.30	5.13 ± 0.30	5.40 ± 0.78	5.56 ± 0.75
18:1w7	4.33 ± 0.45	4.31 ± 0.46	4.23 ± 0.39	4.38 ± 0.38	4.58 ± 0.52	4.35 ± 0.62
18:4w3	0.93 ± 0.19	0.93 ± 0.20	1.01 ± 0.24	0.98 ± 0.23	0.89 ± 0.33	0.93 ± 0.30
20:1w9	1.31 ± 0.21	1.35 ± 0.21	1.31 ± 0.17	1.41 ± 0.25	1.54 ± 0.38	1.58 ± 0.38
20:4w6	2.86 ± 0.15	2.93 ± 0.28	2.95 ± 0.27	2.92 ± 0.29	2.93 ± 0.29	3.01 ± 0.37
20:5w3	21.40 ± 0.91	21.20 ± 1.07	20.93 ± 0.75	20.77 ± 0.83	19.47 ± 2.67	19.00 ± 2.50
22:5w3	1.96 ± 0.28	1.93 ± 0.28	1.93 ± 0.27	1.86 ± 0.32	1.65 ± 0.37	1.65 ± 0.37
22:6w3	18.81 ± 1.21	19.06 ± 1.38	18.91 ± 1.35	19.03 ± 1.25	18.83 ± 1.47	18.83 ± 1.48
24:1w9	0.76 ± 0.07	0.78 ± 0.05	0.81 ± 0.07	0.98 ± 0.40	1.07 ± 0.40	1.11 ± 0.38
BAME	2.54 ± 0.32	2.57 ± 0.32	2.72 ± 0.37	2.77 ± 0.37	2.99 ± 0.28	2.96 ± 0.30
SFA	34.63 ± 0.87	34.53 ± 0.92	34.83 ± 0.79	34.97 ± 0.94	36.11 ± 2.44	36.18 ± 2.41
MUFA	16.90 ± 0.76	16.86 ± 0.77	16.83 ± 0.80	16.78 ± 0.76	17.44 ± 1.93	17.61 ± 1.80
PUFA	48.46 ± 1.45	48.61 ± 1.53	48.34 ± 1.33	48.25 ± 1.43	46.45 ± 4.32	46.21 ± 4.16

b)

Oa TFA	Site 1- Mzimvubu	Site 2- Mbotyi	Site 3- Mtamvuna	Site 4- Pennington	Site 5- Ballito	Site 6- Thukela
14:0	6.71 ± 0.92	5.62 ± 0.94	5.12 ± 1.25	4.76 ± 1.87	3.43 ± 1.10	1.83 ± 1.23
16:0	20.25 ± 0.88	19.82 ± 1.03	24.78 ± 5.06	23.65 ± 2.85	22.27 ± 8.08	17.54 ± 1.89
16:1w7	8.05 ± 0.62	5.57 ± 2.75	4.36 ± 2.16	6.01 ± 1.78	4.91 ± 1.15	3.52 ± 1.45
16-PUFA	1.24 ± 0.31	0.93 ± 0.22	0.76 ± 0.10	0.93 ± 0.50	0.76 ± 0.29	0.91 ± 0.22
18:0	7.67 ± 0.33	8.51 ± 0.52	13.19 ± 3.02	11.63 ± 2.97	11.19 ± 3.86	11.63 ± 1.42
18:1w9	4.59 ± 0.20	5.22 ± 0.31	4.66 ± 0.77	4.80 ± 0.46	5.68 ± 1.43	5.64 ± 0.60
18:1w7	3.39 ± 0.28	3.26 ± 0.12	3.18 ± 0.17	4.16 ± 0.70	3.84 ± 0.18	4.55 ± 0.85
18:2w6	1.00 ± 0.04	1.12 ± 0.07	0.89 ± 0.14	1.06 ± 0.25	1.25 ± 0.11	1.16 ± 0.24
18:4w3	1.29 ± 0.10	1.24 ± 0.20	0.79 ± 0.28	0.81 ± 0.19	0.90 ± 0.25	0.97 ± 0.23
20-MUFA	1.34 ± 0.21	1.60 ± 0.15	2.49 ± 0.74	2.07 ± 0.79	2.11 ± 0.24	3.01 ± 0.73
20:4w6	2.09 ± 0.07	2.13 ± 0.11	1.87 ± 0.50	1.82 ± 0.32	1.81 ± 0.28	2.22 ± 0.17
20:5w3	18.00 ± 0.52	16.80 ± 0.44	12.88 ± 4.34	12.66 ± 2.06	12.68 ± 4.70	15.38 ± 0.97
22-MUFA	0.51 ± 0.36	0.81 ± 0.56	1.05 ± 0.48	0.91 ± 0.21	1.14 ± 0.27	1.40 ± 0.47
22:5w3	1.88 ± 0.08	1.75 ± 0.13	1.65 ± 0.37	1.23 ± 0.18	1.09 ± 0.38	1.31 ± 0.27
22:6w3	16.62 ± 0.94	18.96 ± 1.35	13.07 ± 5.43	13.40 ± 3.27	18.48 ± 7.20	20.88 ± 0.61
24:1w9	0.81 ± 0.08	0.96 ± 0.14	0.66 ± 0.73	1.13 ± 0.29	1.06 ± 0.21	1.45 ± 0.60
BAME	2.67 ± 0.11	3.53 ± 0.54	4.93 ± 0.62	4.89 ± 0.93	4.45 ± 1.79	3.85 ± 0.54
SFA	37.30 ± 0.99	37.48 ± 1.59	48.02 ± 8.75	44.94 ± 5.44	41.34 ± 14.36	34.85 ± 2.36
MUFA	17.35 ± 0.50	15.83 ± 2.66	13.91 ± 1.46	17.01 ± 1.15	16.63 ± 2.54	16.56 ± 1.15
PUFA	44.01 ± 0.85	45.10 ± 1.38	35.57 ± 9.46	35.98 ± 5.39	39.91 ± 12.44	45.58 ± 1.28

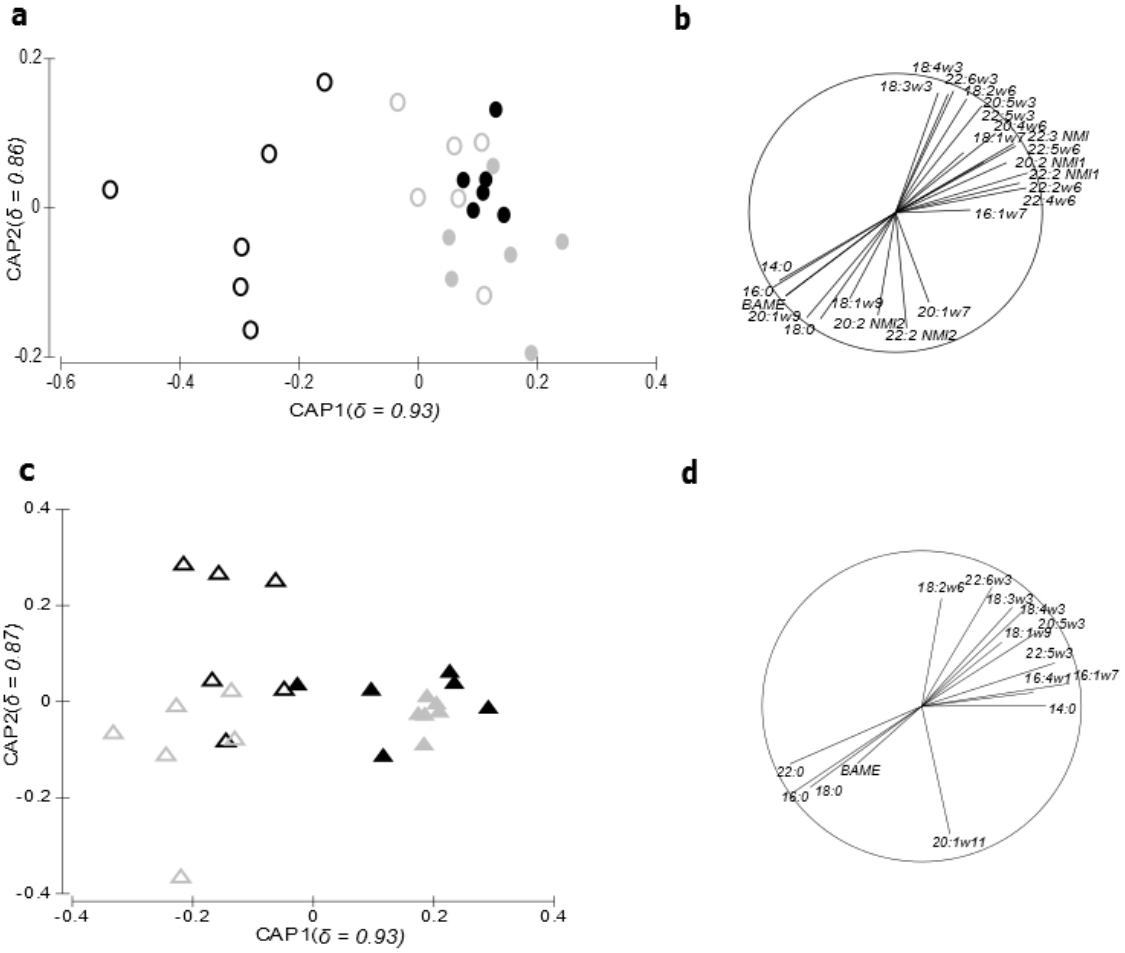
c)

Cd TFA	Site 1- Mzimvubu	Site 2- Mbotyi	Site 3- Mtamvuna	Site 4- Pennington	Site 5- Ballito	Site 6- Thukela
14:0	4.51 ± 0.85	3.62 ± 0.73	3.79 ± 0.51	4.20 ± 0.83	3.06 ± 0.47	1.68 ± 0.39
16:0	23.91 ± 1.99	23.40 ± 3.20	30.19 ± 6.78	39.54 ± 3.20	26.83 ± 2.40	29.28 ± 6.58
16:1w7	5.54 ± 0.84	4.56 ± 0.37	3.69 ± 0.98	2.91 ± 1.56	3.57 ± 0.42	1.74 ± 0.57
18:0	12.37 ± 1.44	11.54 ± 2.42	15.47 ± 5.61	19.79 ± 5.38	14.45 ± 2.25	20.33 ± 5.84
18:1w9	6.13 ± 0.60	6.10 ± 0.77	4.71 ± 0.80	3.18 ± 1.53	5.29 ± 0.51	4.22 ± 1.48
18:1w7	3.48 ± 0.29	3.00 ± 0.42	2.84 ± 1.47	2.89 ± 1.61	2.81 ± 0.67	1.81 ± 0.64
18:2w6	0.89 ± 0.07	1.08 ± 0.34	0.27 ± 0.09	0.77 ± 0.49	1.22 ± 0.23	0.84 ± 0.37
18:3w3	0.56 ± 0.09	0.85 ± 0.45	0.65 ± 0.34	0.41 ± 0.29	0.79 ± 0.20	0.59 ± 0.30
18:4w3	0.72 ± 0.15	1.15 ± 0.53	1.07 ± 0.23	1.06 ± 0.81	0.75 ± 0.20	0.54 ± 0.14
20-MUFA	1.00 ± 0.07	1.55 ± 0.24	2.83 ± 0.61	1.17 ± 0.22	1.20 ± 0.24	1.12 ± 0.33
20:1w9	0.70 ± 0.10	0.44 ± 0.06	2.16 ± 1.39	0.49 ± 0.18	0.70 ± 0.61	0.54 ± 0.28
20:4w6	2.70 ± 0.41	2.95 ± 0.82	3.62 ± 2.49	1.26 ± 0.70	3.44 ± 0.39	3.21 ± 1.47
20:5w3	13.97 ± 2.50	14.08 ± 3.82	8.51 ± 4.67	4.35 ± 2.81	11.86 ± 1.39	9.39 ± 4.65
22:0	1.31 ± 0.27	1.17 ± 0.41	1.67 ± 0.83	1.82 ± 0.69	1.65 ± 0.36	2.11 ± 0.59
20-MUFA	0.84 ± 0.38	0.81 ± 0.48	1.90 ± 0.87	0.83 ± 0.32	0.47 ± 0.17	1.03 ± 1.10
22:5w3	1.27 ± 0.22	1.34 ± 0.35	1.67 ± 0.75	0.60 ± 0.37	0.78 ± 0.12	0.83 ± 0.17
22:6w3	14.70 ± 3.18	15.87 ± 4.22	7.82 ± 2.38	5.62 ± 2.03	15.33 ± 2.92	15.18 ± 6.61
BAME	4.23 ± 0.90	4.46 ± 1.75	6.79 ± 1.45	7.27 ± 0.53	4.69 ± 0.68	4.35 ± 1.27
SFA	46.32 ± 4.33	44.20 ± 7.56	57.91 ± 13.87	72.62 ± 8.70	50.67 ± 4.31	57.76 ± 14.38
MUFA	17.11 ± 1.64	16.20 ± 2.06	16.12 ± 4.62	10.99 ± 4.21	13.40 ± 1.46	9.97 ± 1.78
PUFA	36.56 ± 5.84	39.60 ± 9.56	25.98 ± 9.44	16.39 ± 4.77	35.93 ± 4.26	32.27 ± 12.78

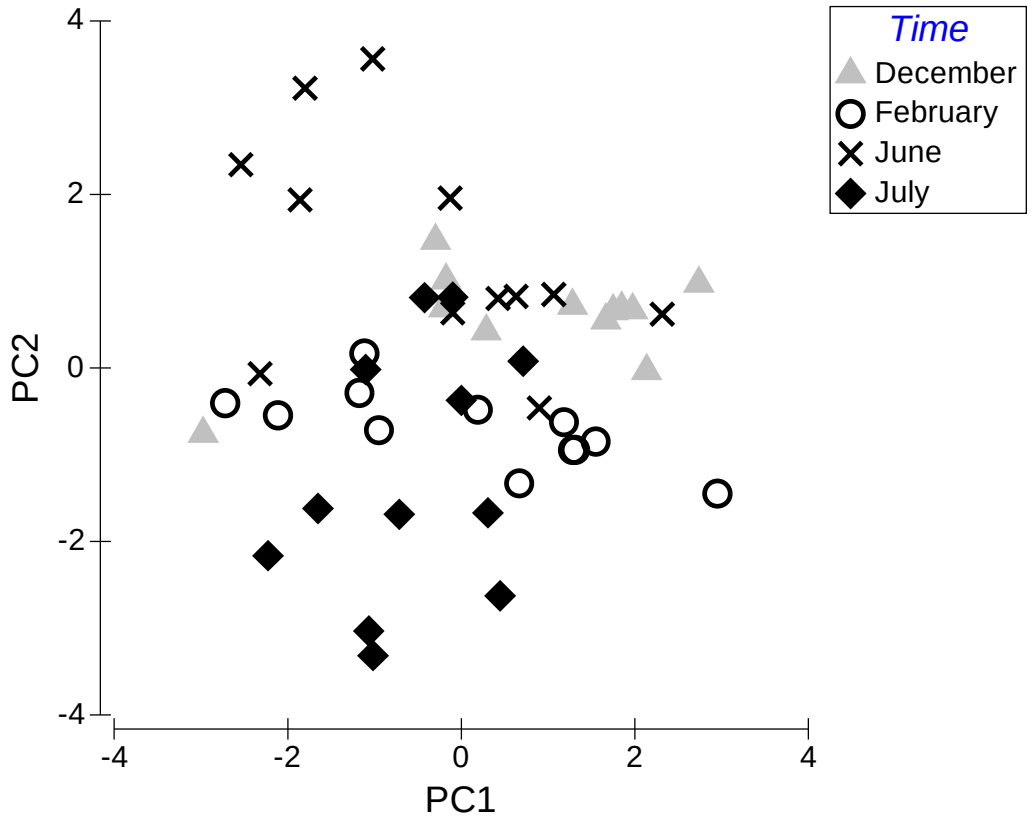
d)

Pp TFA	Site 1- Mzimvubu	Site 2- Mbotyi	Site 3- Mtamvuna	Site 4- Pennington	Site 5- Ballito	Site 6- Thukela
14:0	2.78 ± 0.17	2.74 ± 0.22	2.61 ± 0.34	2.62 ± 0.35	2.53 ± 0.44	2.35 ± 0.47
16:0	14.93 ± 1.66	15.68 ± 0.47	15.70 ± 0.46	15.82 ± 0.51	15.55 ± 1.06	15.39 ± 1.15
16:1w7	5.40 ± 0.36	5.29 ± 0.50	5.11 ± 0.76	5.15 ± 0.80	4.77 ± 0.91	4.44 ± 0.92
18:0	7.08 ± 0.60	7.07 ± 0.57	7.28 ± 0.71	7.18 ± 0.79	7.39 ± 0.69	7.31 ± 0.75
18:1w9	1.69 ± 0.28	1.67 ± 0.30	1.65 ± 0.30	1.58 ± 0.15	1.58 ± 0.15	1.56 ± 0.13
18:1w7	2.52 ± 0.21	2.45 ± 0.31	2.52 ± 0.39	2.65 ± 0.57	2.55 ± 0.69	2.67 ± 0.73
18:2w6	2.82 ± 0.14	2.89 ± 0.24	2.90 ± 0.24	2.89 ± 0.24	3.18 ± 0.74	3.31 ± 0.70
18:3w3	1.22 ± 0.11	1.19 ± 0.15	1.20 ± 0.16	1.21 ± 0.18	1.20 ± 0.17	1.29 ± 0.24
18:4w3	1.16 ± 0.26	1.10 ± 0.27	1.02 ± 0.23	1.04 ± 0.28	1.06 ± 0.25	1.07 ± 0.25
20-MUFA	6.39 ± 0.64	6.38 ± 0.61	6.46 ± 0.56	6.50 ± 0.51	6.41 ± 0.45	6.19 ± 0.58
20:2 NMI1	4.36 ± 0.75	4.39 ± 0.81	4.34 ± 0.85	4.24 ± 0.99	4.73 ± 1.66	4.56 ± 1.68
20:4w6	6.64 ± 0.54	6.86 ± 0.87	7.11 ± 0.77	7.28 ± 0.71	7.51 ± 0.95	7.75 ± 0.70
20:5w3	9.40 ± 1.66	9.00 ± 1.75	8.63 ± 1.33	8.79 ± 1.63	8.47 ± 2.12	8.65 ± 2.07
22:2w6	1.60 ± 0.40	1.67 ± 0.50	1.75 ± 0.48	1.84 ± 0.35	1.98 ± 0.34	2.00 ± 0.34
22:2 NMI1	5.61 ± 0.97	5.60 ± 0.96	5.63 ± 0.96	5.74 ± 0.72	6.22 ± 1.22	6.16 ± 1.23
22:2 NMI2	1.32 ± 0.47	1.35 ± 0.41	1.30 ± 0.44	1.16 ± 0.58	0.92 ± 0.72	0.59 ± 0.58
22:3 NMI	1.40 ± 0.13	1.38 ± 0.12	1.38 ± 0.12	1.40 ± 0.08	1.47 ± 0.17	1.48 ± 0.18
22:4w6	2.00 ± 0.52	2.15 ± 0.69	2.30 ± 0.51	2.32 ± 0.48	2.47 ± 0.62	2.56 ± 0.58
22:5w6	1.43 ± 0.17	1.45 ± 0.21	1.50 ± 0.16	1.50 ± 0.16	1.58 ± 0.29	1.54 ± 0.32
22:5w3	2.22 ± 0.32	2.11 ± 0.49	2.22 ± 0.53	2.20 ± 0.51	2.13 ± 0.52	2.50 ± 0.95
22:6w3	13.32 ± 1.41	12.74 ± 2.11	12.65 ± 2.03	12.31 ± 1.83	11.86 ± 1.48	12.43 ± 1.92
BAME	3.95 ± 0.30	4.03 ± 0.20	3.93 ± 0.39	3.85 ± 0.41	3.73 ± 0.31	3.62 ± 0.31
SFA	28.74 ± 1.43	29.52 ± 0.78	29.53 ± 0.77	29.46 ± 0.78	29.20 ± 1.30	28.67 ± 1.71
MUFA	15.99 ± 0.65	15.78 ± 0.87	15.75 ± 0.90	15.88 ± 1.02	15.31 ± 1.46	14.87 ± 1.28
PUFA	55.27 ± 1.58	54.70 ± 0.74	54.73 ± 0.79	54.66 ± 0.86	55.48 ± 2.39	56.46 ± 2.64

Appendix c CAP of the TFA composition of mussels (a) and barnacles (c) on the south coast at upwelling (black) and non-upwelling (grey) sites. The open symbols refer to samples from region A (sites 7 and 8), while the closed symbols refer to region B (sites 9 and 10). (b) and (d) vectors illustrating the Pearson correlations > 0.3 of the FA with the axes of the CAP, with the circle overlay scaled to the maximum correlation value and indicating the magnitude of effect..



Appendix d PCA of SPM collected at four sites on the South Africa west coast over the four months of the sampling events. PC1 explained 20.2 % of the total variability and PC2 18.0 %.



Appendix e Table reporting the locations sampled for the entire study. The table also reports the geographical coordinates in decimal degrees, the bio-provinces (west, south or east coast) of interest and the number of the chapters where the location is mentioned.

Site	Coordinate	Bio-province	Chapter
Groenrivier	S30.4949 E17.3353	west coast	5
Doring bay	S31.4817 E18.1352	west coast	5
Lambert's bay	S32.0629 E18.1804	west coast	5
Elandsbaai	S32.1903 E18.1922	west coast	6
Cape columbine	S32.4850 E17.5212	west coast	5,6
Bloubergstrand	S33.4819 E18.2746	west coast	5,6
Llandudno	S34.0018 E18.2028	west coast	6
Jongensfontein	S34.2530 E21.2034	south coast	2,3,5
Mossel Bay	S34.1048 E22.0920	south coast	2,3
Brenton	S34.0321 E23.1208	south coast	2,5
St. Francis Bay	S34.1028 E24.5023	south coast	3,5
Port Elizabeth	S33.5856 E25.4007	south coast	2,3
Port Alfred	S33.3647 E26.5323	south coast	5
Kidd's beach	S33.0854 E27.4213	south coast	3,5
East London	S33.0118 E27.5521	south coast	3
Mzimvubu River	S31.3856 E29.3129	east coast	4
Mbotyi	S31.2501 E29.4758	east coast	4,5
Mtamvuna River	S31.0408 E30.1130	east coast	4
Pennington	S30.2348 E30.4205	east coast	4,5
Ballito	S29.3132 E31.1301	east coast	4,5
Thukela River	S29.1400 E31.2923	east coast	4