A STABLE ISOTOPE APPROACH TO TROPHIC ECOLOGY: RESOLVING FOOD WEBS IN INTERTIDAL ECOSYSTEMS.

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ABSTRACT

There are broad differences in regional oceanography and primary production around the South African coast, which we might expect to give rise to major differences in trophic pathways. $\delta^{13}C$ and $\delta^{15}N$ isotopic ratios of suspended particulate matter (SPM), mussels, various intertidal consumers and common macroalgae along the South African coastline were explored using stable isotope analysis to investigate biogeographic and temporal variability of isotopic signatures of marine intertidal consumers and their food sources around the coast of South Africa, with a focus on evaluating the dependence of intertidal mussels on phytoplankton and macroalgal-derived organic carbon.

Isotopic equilibration rates of four mussel tissues were determined through laboratory feeding experiments, which established that adductor tissue had the slowest isotopic turnover rate, and was subsequently used as an indication of overall mussel diet. Biogeographic, temporal and nearshore/offshore trends of isotopic ratios of SPM were investigated along 10km transects perpendicular to the coast and SPM exhibited overall trends of carbon depletion when moving from west to east along the coastline and from nearshore to offshore water, in both cases suggesting a shift from macrophyte detritus to a phytoplankton signature. $\delta^{13}C$ signatures of SPM also revealed temporal and biogeographic variation that had strong ties to local oceanography, being closely correlated to regional hydrographic features and tidal influences. Mixing models indicated filter feeders demonstrated over 50% dependence on nearshore SPM for organic carbon and it was possible to categorize them into geographic groups based on their carbon and nitrogen signatures, suggesting biogeographic shifts in resources. Biogeographic shifts in diet were also seen in some grazers. Difficulties in relating macroalgae to mussel diet led to investigations into the isotopic changes associated with macroalgal decomposition. Variation in photosynthetic fractionation, leaching and microbial mineralization are believed to have resulted from species-specific patterns of degradation. Although the strong links between carbon signatures and local oceanography indicate that stable isotope analysis is a powerful tool for the study of water mixing and coastal hydrography in relation to food-web analyses, substantial variation in fractionation of primary consumers, along with different periods of time integration between consumers and their food sources must be considered in future studies, to resolve trophic links in marine food webs successfully.

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TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

‘THERE’S A UNIVERSE INSIDE THESE WATERS’

‘PATY MARSHALL-STACE, ‘MEZA GEBEL’ (1995)

CHAPTER 1: INTRODUCTION AND OVERVIEW

1.1 Food webs

Food web investigations typically focus on the flow of energy and nutrients up through trophic levels, with the ultimate goal of understanding transfer patterns among communities (e.g. Odum 1968, 1971; Pimm & Lawton 1977; Menge *et al*. 1986). At each transfer, a proportion of potential energy is lost as heat (frequently upwards of 80%) and consequently the amount of energy available to each community determines the length of the food chain (Odum 1971; Pimm & Lawton 1977). Food webs can be roughly broken down into two categories for the purposes of biological investigation; (1) grazing food chains, referring to primary production that is successively utilized by primary, secondary and tertiary consumers and (2) detrital food chains, that incorporate the microbial colonization of non-living organic matter and subsequent assimilation of both microbes and non-living organic matter by detritivores (Barnes & Hughes 1982; Valiela 1995). Food web resolution requires an understanding of community dynamics and the factors which regulate community structure. Research suggests that community dynamics of aquatic and terrestrial ecosystems are regulated by two general types of processes: top-down and bottom-up interactions, which have qualitative or quantitative effects on community structure (e.g. Dayton 1971; Dungan 1987; Menge 1992; Menge *et al*. 1999). Top-down controls (e.g. abundance and distribution of predators and/or prey) refer to situations where the structure of lower trophic levels depends, in some fashion, on higher trophic level activities (e.g. Paine 1974; Power 1992; Menge *et al*. 1999), while bottom-up controls (e.g. nutrients and productivity) are those that influence community structure directly or indirectly through variation in lower trophic levels or resource availability (Neill & Peacock 1980; Menge 1992; Hunter & Price 1992; Menge *et al*. 1999).

1.2 Rocky shores

Rocky shore ecosystems are ideal for food web investigations because they provide large populations of small and easily manipulated organisms that are accessible on a daily basis and incorporate extremely steep environmental gradients across very small physical scales. Rocky shores represent an interface between land and sea that sustains communities within a transitional environment subject to dynamic daily variability in

physical conditions. Rocky shore communities are found throughout the world, from environments that endure regular to intermittent freezing and ice abrasion to those subjected to temperatures high enough to denature proteins (Menge & Branch 2001), and the intensity of this variability stems primarily from exposure to terrestrial conditions for part of the tidal cycle and marine conditions for the remainder (Denny & Wethey 2001). This semi-marine, semi-terrestrial environment is characterized by dramatic fluctuations of many abiotic factors, particularly heat transport, temperature and desiccation. During high tide, intertidal organisms rapidly achieve thermal equilibrium due to the high coefficient of heat transfer in water, whereas during low tide, air and ground temperature, wind speed and humidity result in continual changes in internal body temperatures (Barnes & Hughes 1982). The thermal severity of intertidal environments is such that during some outgoing tides organisms may experience internal temperatures hotter than that of ambient air (Etter 1988; Hayworth & Quinn 1990; Helmuth 1999), and during incoming tides may undergo up to 20°C decreases in body temperature in less than a minute (Denny & Wethey 2001).

The harshness of these physical conditions manifests itself in zonation patterns within all rocky shore communities (e.g. Lewis 1964; Connell 1972; Stephenson & Stephenson 1972). Organisms have developed a variety of adaptations to withstand desiccation and high temperatures, as well as changes in salinity, wave exposure and sand inundation, allowing for differential exploitation of habitat. On South African shores, for example, *Choromytilus meridionalis* (Kr.) and *Perna perna* (L.) demonstrate intrashore habitat segregation. *C. meridionalis* can withstand prolonged exposure to hypoxia and is characteristically found living on the lower shore and on rocks associated with sand, while *P. perna* demonstrates much higher hypoxia-related mortality and consequently lives further up on the midshore (Marshall & McQuaid 1993). Another comparison by Zardi *et al*. (2006) between the exotic blue Mediterranean mussel, *Mytilus galloprovincialis* (characteristically found on the higher shore) and *P. perna* showed that *P. perna* secretes more and thicker byssal threads than *M. galloprovincialis* resulting in a greater capability to withstand hydrodynamic stress. Other adaptations of intertidal organisms include the expression of heat shock proteins during periods of physiological stress (Roberts *et al*. 1997; Buckley *et al*. 2001; Halpin *et al*. 2002) and aggregational

behaviours (e.g. mussels and barnacles) that reduce hydrodynamic and thermal stress (Bertness 1989; Helmuth 1998). These adaptations to physical stress however, may involve tradeoffs in terms of biotic interactions, as large aggregates of mussels may experience less physical stress but more competition for food (Menge *et al*. 1986). These evolutionary adaptations combined with biotic pressures not only determine zonation but also affect community structure and consequently influence trophic relationships in intertidal communities.

1.3 The southern African coastline

The coastline of southern Africa is exposed to two separate current systems which are key components in global ocean circulation. The Benguela Current moves towards the equator on the west coast, bringing up cold (11-16 ºC; Andrews & Hutchings 1980; Demarcq *et al*. 2003), eutrophic water of Antarctic origins, with nitrate concentrations between 5-8µg-at N/l^{-1} (Verheye-Dua & Lucas 1988), and resulting in semi-permanent upwelling cells that support extensive beds of kelp on and north of the Cape of Good Hope (Andrews & Hutchings 1980; Barnes & Hughes 1982). The oligotrophic Agulhas Current however, draws warmer water (between 22-26ºC; Gordon *et al*. 1987; Lutjeharms 2006) from the Moçambique channel, with nutrient concentrations of < 0.62µg-at N/l^{-1} (Machu *et al*. 2005), down along the east and south coasts, passing the southern limit of land and then flowing south towards Antarctica before executing a sharp turn known as the Agulhas Retroflection Current (ARC), which continues east with significant meandering (Quartly & Srowkosz 2003; Lutjeharms 2005). Early descriptions of rocky shore assemblages with respect to basic biogeography and the influence of coastal currents (Stephenson 1936, 1943; Stephenson *et al*. 1940; Branch & Branch 1985; Branch & Griffiths 1988) suggested that rocky shore communities exposed to these two different systems (Benguela and Agulhas) experience significant differences in available nutrients and hydrographic regimes that potentially influence dietary and community structures.

Rocky shores along the coastline of southern Africa exposed to strong wave action are often dominated by sessile filter feeders such as bivalves, barnacles and polychaetes

(Bustamante *et al*. 1995). These intertidal consumers obtain nutrients from suspended particulate matter (SPM) available in the water column, which consists of a variable mixture of pelagic phytoplankton, detritus and resuspended organic and inorganic debris (Stuart *et al*. 1982; Grant & Cranford 1991; Bustamante & Branch 1996; Levinton *et al*. 2002). It is generally accepted that pelagic phytoplankton is the most important food source for bivalves in both estuarine and coastal environments (Widdows *et al*. 1979; Asmus & Asmus 1991; Yelenik *et al*. 1996; Dame & Prins 1998), and empirical studies have shown strong correlations between mussel populations and phytoplankton biomass (Cloern 1982; Asmus & Asmus 1991). Some studies however, have indicated that macroalgal detritus is an important component in the diet of rocky shore suspension feeders (Mann 1988; Duggins *et al*. 1989), and that, where available, kelp-derived carbon in particular may play a significant part in bivalve nutrition (Stuart 1982; Bustamante & Branch 1996; Levinton *et al*. 2002). The kelp forests seen around the Cape of Good Hope comprise two species: *Ecklonia maxima* and *Laminaria pallida* and kelp-derived detritus often represents up to 65% of intertidal SPM (Bustamante & Branch 1996). Therefore, bottom-up controls such as nutrient variability and the uptake of detrital macroalgal carbon by filter feeders may have been substantially underestimated in both the benthic and the pelagic environments (e.g. Dunton & Schell 1987; Duggins *et al*. 1989; Bustamante & Branch 1996). As a result, the importance of phytoplankton versus macroalgal detritus as primary food sources for intertidal communities is increasingly being called into question (Bustamante & Branch 1996; Vizzini *et al*. 2002).

1.4 Food web analysis

Traditional approaches to food web analysis include direct observations in the laboratory and field, radio-labeled tracer techniques, gut-content analysis and the application of immunological methods (Rounick & Winterbourn 1986; Michener & Schell 1994). Few of these techniques however, have provided adequate resolution of the food webs studied and each method has its disadvantages. Direct observations in the laboratory, for example, involve the use of artificial environments that may introduce artifacts into the results (Morton & Yuen 2000) while field observations of feeding behaviour are logistically impractical in most aquatic ecosystems. Radio-labeled tracer

techniques (e.g. Smith *et al*. 1979; Beviss-Challinor & Field 1982; Sherwood *et al*. 2002) normally require specialized licenses and large quantities of radioactive isotope to overcome dilution effects (Michener & Schell 1994). Furthermore, successful application of the radio-tracer method requires the recovery of statistically significant numbers of labeled species. Gut-content analysis (e.g. Taylor 1986; Fukami *et al*. 1999; Gudmundsson *et al*. 2000) allows the determination of food sources at only one point in time, and it infers the dietary composition of animals based on ingestion rather than assimilation (Gearing 1991; Créach *et al*. 1997). Components that are digested relatively easily are frequently impossible to identify, therefore data derived from this technique may show significant deviations from the actual dietary composition of an organism (Dahl 1979). Serological methods using immunoassays of gut-contents are an alternative or complimentary way of resolving food web structures (e.g. Boreham & Ohiagu 1978; Feller *et al*. 1979; Feller & Gallagher 1982), and this process involves the development of antisera from whole organism extracts and immunodiffusion tests of antigen specificity. These antigens are usually taxon specific and help to identify partially digested stomach contents (Feller *et al*. 1979; Heusser *et al*. 1981). Unfortunately, this method is limited by the specificities of the antigens developed and is consequently time consuming, expensive and yields only qualitative results (Michener & Schell 1994).

1.5 Stable isotope analysis

The drawbacks of the traditional methods in studying food webs have led biologists to techniques that utilize naturally occurring biomarkers, such as the determination of fatty acid profiles and stable isotopes analyses. Fatty acid profiling is a technique which shows considerable promise for associating individuals to a particular biome (Hobson 1999), as unusual fatty acids, not synthesized by most marine animals, occur in marine lipids as unique and characteristic food web markers (Ratnayake *et al*. 1989; Castell *et al*. 1994; Smith *et al*. 1996). The second technique, stable isotope analysis, is one that has been used in the archaeological and geological disciplines for many years (e.g. Craig 1954; Nagy 1970; van der Merwe 1982; DeNiro 1987). Elements having more than one stable isotope that are most commonly utilized are hydrogen (H), oxygen (O), carbon (C),

nitrogen (N) and sulphur (S). Studies of trophic ecology within marine aquatic systems, however, depend primarily on C and N, as both these elements demonstrate predictable changes in isotope ratios when moving from food source to consumer. Both H and S show little change between trophic levels, and O is used most often to investigate changes in water temperature (Archambeau *et al*. 1998; Mannino *et al*. 2003; Hoie *et al*. 2004).

The investigation of food-web and ecosystem dynamics with isotopic analysis utilizes the abundance of stable isotopes in nature to follow pathways of organic matter through successive trophic levels (DeNiro & Epstein 1978; DeNiro & Epstein 1981; Fry & Sherr 1984). By measuring the ratios of the two stable isotopes of C (^{12}C and ^{13}C) and N (^{14}N and ^{15}N), the source of organic C and N can often be determined. The isotopic compositions of these elements change in predictable ways as they cycle through the biosphere. These changes are typically mass dependant, however the addition of neutral mass (neutrons) does not alter most aspects of chemical reactivity, and consequently different isotopes of the same element are functionally equivalent in the majority of chemical/biochemical reactions (Peterson & Fry 1987). The minute changes that do occur are referred to as isotopic fractionation. While assimilating C and N from their diets, consumers preferentially respire the light C isotope (^{12}C) and preferentially excrete the light N isotope (^{14}N). As a result, consumers are generally enriched with heavier isotopes in relation to their food. A variety of studies have demonstrated that C fractionates minimally by an average of 1‰ per trophic level (DeNiro & Epstein 1978; Fry & Sherr 1984; Peterson & Fry 1987; France & Peters 1997), while N has a larger fractionation factor of 3-4‰ per trophic level (DeNiro & Epstein 1981; Fry & Sherr 1984; Vander Zanden & Rasmussen 2001). However, this estimate of nitrogen fractionation is an average value over a wide variety of taxa and some of the literature suggests that bivalves, for example, have smaller fractionation values ranging between 1.26 – 2.5‰ (Fry 1988; Raikow & Hamilton 2001; Moore & Suthers 2005). Stable isotope tracing offers two potential advantages in terms of food-web analyses; firstly, the $\delta^{13}C$ and $\delta^{15}N$ ratios of animal tissue represent the integration of carbon and nitrogen over a prolonged period, and secondly, they are based on assimilation rather than ingestion (Peterson & Fry 1987). This approach to food-web resolution has been successfully applied in both

benthic and pelagic ecosystems on many occasions, for example: to determine the position of horseshoe crabs in estuarine food webs (Carmichael *et al*. 2004), to track migratory feeding behaviour in pinnepeds (Burton & Koch 1999; Hirons *et al*. 2001), to describe the importance of zooplankton in anchovy diet (Monterio *et al*. 1991) and to resolve food web structure in Mediterranean rocky littoral fishes (Pinnegar & Polunin 2000). Stable isotope techniques have also established the importance of kelp-derived organic carbon in both intertidal communities on the west coast of South Africa (Bustamante & Branch 1996) and coastal ecosystems of the sub-Antarctic Prince Edward Islands (Kaehler *et al*. 2000).

1.6 Thesis Overview

Numerous studies have used stable isotope analysis to explore the flow of carbon through trophic levels (e.g. Fry & Sherr 1984; Rounick & Winterbourn 1986; Grant & Cranford 1991; Gu *et al*. 1993; Persic *et al*. 2004), and a few have considered isotopic variability due to seasonal dietary changes (Goering *et al*. 1990; Kreeger & Newell 2001; Vizzini & Mazzola 2003). However, there has been limited research on spatial variation in isotope signatures such as biogeographic variation or on temporal variation in the isotopic composition of marine consumers and their food, particularly within intertidal benthic communities. The primary aim of this dissertation was to investigate both the biogeographic and temporal variability of isotopic signatures of marine intertidal consumers and their food sources around the coast of South Africa with a focus on assessing the trophic dependence of intertidal mussels on macroalgal and phytoplankton-derived organic carbon.

To identify links between mussel tissue and diet on both long and short term scales, it is important to establish the natural turnover rates of isotopes within mussel tissue. Data on the time integration period of mussel adductor tissue, and comparisons of this period with the rate of change in isotopic values of different mussel tissue types, are investigated in chapter 2.

Chapter 3 explores temporal variation in the isotopic signatures of mussel adductor muscle tissue and macroalgae in an effort to assess the trophic relationships between

mussels and their food sources at a coastal site in southeastern South Africa. Furthermore, data on the spatial and temporal isotopic gradients in nearshore and offshore SPM are described with respect to coastal hydrography.

The nearshore hydrography of southern Africa is profoundly influenced by two very different current systems as described above; Chapter 4 includes descriptions of the biogeographic and temporal isotope patterns of nearshore and offshore SPM gradients, mussel tissue and macroalgae along the coastline from Namibia to the Moçambican border.

Chapter 5 explores this biogeographic isotopic pattern in intertidal communities at four trophic levels from multiple sites along the South African coastline to clarify the effect of biogeography on the importance of macroalgal versus phytoplankton-derived organic carbon as a base for intertidal food webs.

Throughout this dissertation, numerous difficulties were encountered in relating living macroalgal signatures to those of mussels or SPM. However, linear mixing models indicated that macroalgal detritus contributed the majority of organic carbon to the diet of mussels. Chapter 6 outlines the effects of decomposition on macrophyte stable isotope integrity in an attempt to link living macroalgae with macroalgal detritus, and to gain further understanding of the role of algal decomposition in the enrichment or depletion of $\delta^{13}C$ and $\delta^{15}N$ signatures.

An integration of all segments of the dissertation is presented in Chapter 7 to summarize the isotopic patterns in producers and consumers with respect to temporal and biogeographic variation. The general discussion focuses on the importance of macroalgal derived organic carbon in mussel diet, attempts to provide an overview of energy flow in South African rocky intertidal communities and suggests future approaches to food web analyses.

CHAPTER 2: INVESTIGATION OF TISSUE-DEPENDANT ISOTOPIC EQUILIBRATION RATES IN THE BROWN MUSSEL *PERNA PERNA*.

2.1 Introduction

In ecological systems, stable isotope analysis is increasingly being used to explore behavioural patterns in consumer feeding and/or habitat utilization in both time and space (Sweeting *et al*. 2005). Isotopic signatures have been used to identify sources of organic matter for marine consumers, (e.g. Dunton & Schell 1987; Cranford & Hill 1999; Bode *et al*. 2006) infer patterns of migration (Hobson 1999; Augley *et al*. 2007; Bodin *et al*. 2007), elucidate trophic positioning in food webs (Sholto-Douglas *et al*. 1991; Froneman 2002; Hobson *et al*. 2002; Fredriksen 2003) and to describe seasonal oscillations of prey/food abundance (Kreeger & Newell 2001; Vizzini & Mazzola 2003). Recently, stable isotopes have also been used to investigate ontogenetic and body size based dietary shifts (Fry *et al*. 1999; Vander Zanden & Rasmussen 2001; Badalamenti *et al*. 2002 Sweeting *et al*. 2007) and to quantify consumer tissue turnover rates within marine ecosystems (Fry & Arnold 1982; Frazer *et al*. 1997; Gorokhova & Hansson 1999; Herzka *et al*. 2002; Sweeting *et al*. 2005, 2007).

Typical consumers are opportunistic feeders with diets that often vary over time; consequently higher variation is expected in generalist feeders than in monospecific feeders or generalist feeders exposed to a uniform food supply (Bearhop *et al*. 2004). All animal tissues and/or biochemical fractions possess an isotopic memory (Tieszen *et al*. 1983) and shifts in diet are not immediately evident in consumer tissue signatures, but are lagged over a period of time required for isotopic equilibration (Fry & Arnold 1982; Hobson & Clark 1992; Sweeting *et al*. 2005). Isotopic memory is a function of the turnover rate of the tissue/fraction in question, the isotopic ratio of the food at the time of tissue/fraction synthesis and the isotopic ratios of all assimilated food thereafter (Tieszen *et al*. 1983). Accordingly, equilibration of consumer isotopic signatures is driven by two mechanisms; the formation of new tissue with a new dietary signature (termed ‘dilution’ or ‘turnover’) and the replacement of old tissue signatures with new ones during tissue repair (Fry & Arnold 1982; Sweeting *et al*. 2005). Although these two mechanisms are influenced by relative body size and growth rate, tissue turnover is agreed to be the dominant process (Hesslein *et al*. 1993; Herzka & Holt 2000; Sweeting *et al*. 2005). The

isotopic incorporation of multiple dietary and metabolic changes over time suggests that consumer signatures will never solely reflect current or previous diets, but instead represent a little of both (Sweeting *et al*. 2005). Consequently, equilibration time (and thus isotopic signatures) may vary at tissue, individual, population and species levels, exhibiting isotopic signatures that can be additionally influenced by food stress such as starvation (Hobson *et al*. 1993; Gannes *et al*. 1997) and seasonal metabolic changes unrelated to diet (Lorrain *et al*. 2002).

Multiple studies have shown that different tissues demonstrate unique equilibration times, with more metabolically active tissues such as the liver, pancreas and fat, possessing faster dilution rates than less metabolically active ones such as connective tissue and bone (Thompson & Ballou 1956; Libby *et al*. 1964; Tieszen *et al*. 1983; Hobson & Clark 1992; Sweeting *et al*. 2005). Using variance in mussel isotopic signatures as a descriptor of tissue turnover, the aims of this chapter were: (1) to compare isotopic signatures of four separate tissues and (2) to establish dilution rates of each tissue, to help clarify the link between macroalgae, SPM and mussel diet. I hypothesize that tissue-biased equilibration (Tieszen *et al*. 1983; Gorokhova & Hansson 1999; Sweeting *et al*. 2005) will result in different mussel tissues possessing different isotopic ratios and equilibration rates and that, although mussel tissues may not reach 100% dilution, they will demonstrate patterns consistent with respective dietary switches to more enriched or more depleted food sources. Quantifying the signatures and dilution rates of multiple tissues within *Perna perna* will help to relate mussel signatures to those of potential food sources such as SPM and macroalgae in later chapters.

2.2 Methods

2.2.1 Experimental set-up

Two aquaria with under-gravel and submersible carbon-less corner filters were filled with 20L of filtered (5µm) seawater. Each tank was equipped with a Via Aqua submersible pump (VA 300A) to produce a water current and maintain food in suspension. 100% of the water was replaced every three days, each tank was cleaned every three weeks and the corner filters were cleaned as necessary. 130 *Perna perna* (shell length 30-40mm) were collected from Kenton-on-Sea (see chapter 3 for site

description) and 65 mussels were placed in each tank. Tank one was fed a diet of powdered baby fish food (AquaPlus; Appendix 1) and tank two was fed a diet of powdered *Ecklonia maxima* (Bettaway health food products; Appendix 1), mussels were fed 0.8 – 1.0g of food every day. Water temperature (16-20°C) and day/night light cycle were ambient throughout the experiment and salinity was maintained at 34 ± 2 ppt.

2.2.2 Sample Collection

Three mussels from each tank were removed once a week for three months. To investigate variation in tissue turnover rates, four tissues from each mussel were sampled: gill, gonad, mantle and adductor muscle tissue. Initial $\delta^{13}C$ and $\delta^{15}N$ signatures for each mussel tissue (x 3) and both diets (kelp; fish food, x 3) were obtained for day 0.

2.2.3 Sample Preparation

All tissue samples were rinsed in distilled water (dH_20) and oven dried (60°C, 48hrs).

2.2.4 Isotopic Analysis

$\delta^{13}C$ and $\delta^{15}N$ signatures of all samples were determined using a continuous flow Isotope Ratio Mass Spectrometer (IRMS), after sample combustion in on-line Carlo-Erba preparation units at the University of Cape Town, South Africa and the Environmental Engineering Research Centre, Queens University Belfast, Northern Ireland. Beet sugar and Merck gelatine were used as standards, calibrated against International Atomic Energy reference materials (PeeDee Belemnite and air for $\delta^{13}C$ and $\delta^{15}N$ respectively). Results are expressed in standard delta notation, $\delta X = ([R_{sample}/R_{standard}] - 1) \times 1000$, where X is the element in question and R is the ratio of the heavy over the light isotope. Precision of replicate determinations for both carbon and nitrogen was ± 0.05‰.

2.2.5 Data Analysis

Non-parametric, permutational multivariate analyses of variance (PERMANOVA; McArdle & Anderson 2001; Anderson 2001), using Bray-Curtis dissimilarities, were performed to assess the effect of tissue type and time (days) on $\delta^{13}C$, $\delta^{15}N$ and C: N ratios of *Perna perna*, separately for both fish food and kelp food sources. If significant differences were found, pair-wise *a posteriori* t-test comparisons were made using permutational analyses based on distance. Where sufficient numbers of unique

permutation values were possible, the permutational probability (p_1) is reported, where few unique permutation values were possible, the more conservative Monte-Carlo probability (p_2) is reported.

2.3 Results

Different tissues behaved differently over time and responses differed between diets in several cases (Figs 2.1 - 2.3), though C: N ratios were more similar between diets than $\delta^{13}C$ or $\delta^{15}N$. There was a clear interaction between tissue type and time, which will be discussed presently, but the significant differences between tissue types seen throughout the experiment must first be highlighted.

2.3.2 Effect of tissue

PERMANOVA analyses (9999 permutations) showed that tissue had significant effects on $\delta^{13}C$, $\delta^{15}N$ and C: N ratios with five exceptions: no significant differences were seen between $\delta^{13}C$ signatures of mantle and gill tissue for mussels fed on a kelp diet and between $\delta^{15}N$ signatures of adductor and mantle tissue and mantle and gill tissue for both kelp and fish food diets (see Tables 2.1 & 2.2).

Table 2.1: Permutational probability values (p_1) of PERMANOVA results for mussels fed on fish food, comparing significant differences in $\delta^{13}C$, $\delta^{15}N$ and C: N ratios among tissues. * $p_1 < 0.05$

Tissue Comparison	**$\delta^{13}C$**	**$\delta^{15}N$**	**C: N Ratio**
Adductor VS Mantle	0.0002*	0.8126	0.0001*
Adductor VS Gill	0.0162*	0.0219*	0.0051*
Adductor VS Gonad	0.0001*	0.0009*	0.0001*
Mantle VS Gill	0.0152*	0.1063	0.0149*
Mantle VS Gonad	0.0015*	0.0019*	0.0082*
Gill VS Gonad	0.0001*	0.0003*	0.0001*
$F_{3, 47}$	86.50	23.09	37.80
SS	162.64	451.68	2563.25
MS	54.21	150.56	854.42

Table 2.2: Permutational probability values (p_1) of PERMANOVA results for mussels fed on kelp, comparing significant differences in $\delta^{13}C$, $\delta^{15}N$ and C: N ratios among tissues. * $p_1 < 0.05$

Tissue Comparison	$\delta^{13}C$	$\delta^{15}N$	C: N Ratio
Adductor VS Mantle	0.0016*	0.2663	0.0001*
Adductor VS Gill	0.0011*	0.0346*	0.0129*
Adductor VS Gonad	0.0001*	0.0003*	0.0001*
Mantle VS Gill	0.9961	0.1050	0.0025*
Mantle VS Gonad	0.0001*	0.0001*	0.0001*
Gill VS Gonad	0.0001*	0.0001*	0.0001*
$F_{3, 47}$	125.41	26.46	91.45
SS	375.54	383.32	3894.42
MS	125.18	127.77	1298.14

Overall $\delta^{13}C$ and $\delta^{15}N$ signatures of gonad tissue were lower than those from any other tissue at all times, with the exception of carbon in mantle tissue at day 81 (0.5‰ lower), and C: N ratios were consistently higher than any other tissue at all times with the exception of mantle tissue on day 56 (0.3‰ higher). Differences among tissues in $\delta^{13}C$ and $\delta^{15}N$ ranged between 0 – 2.5‰ and 0 - 1.5‰ respectively and C: N ratios differed by between 0 and 2.5.

2.3.3 Interaction of time x tissue

Mean (± SD) isotopic signatures ($\delta^{13}C$ and $\delta^{15}N$) and C: N ratios of the kelp diet were -16.22 ± 0.11, 5.67 ± 0.54, 22.96 ± 0.64 respectively. Mean (± SD) signatures of the fish food diet were -21.88 ± 0.15, 6.21 ± 0.29, 5.87 ± 0.50 respectively (Figs 2.1 - 2.3).

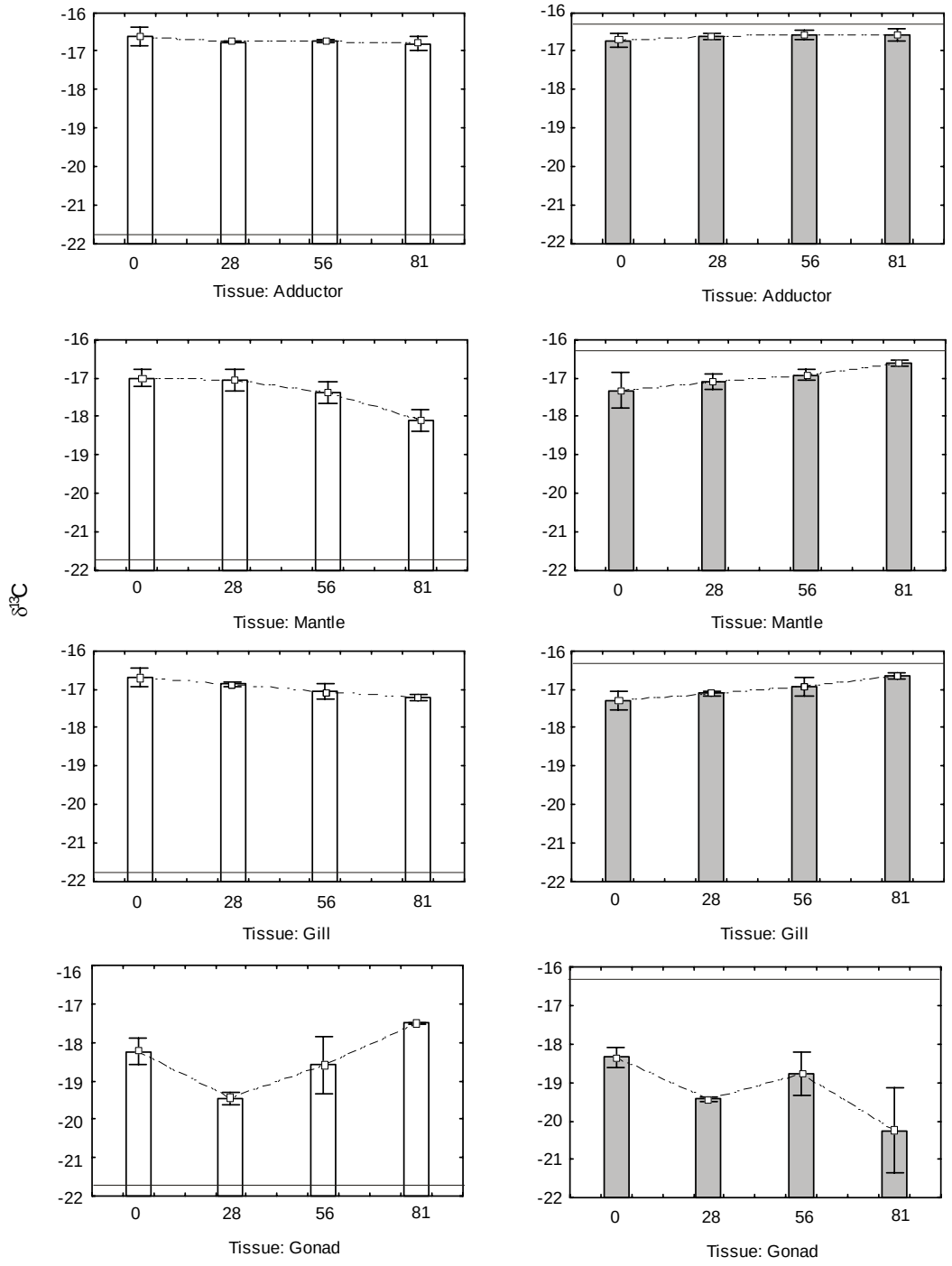

Figure 2.1: Tissue specific changes in $\delta^{13}C$ (‰) over time for *Perna perna* fed on fish food and kelp diets. Values are ± SD. Letters indicate homogenous groups within tissues and solid lines indicate food source signatures.

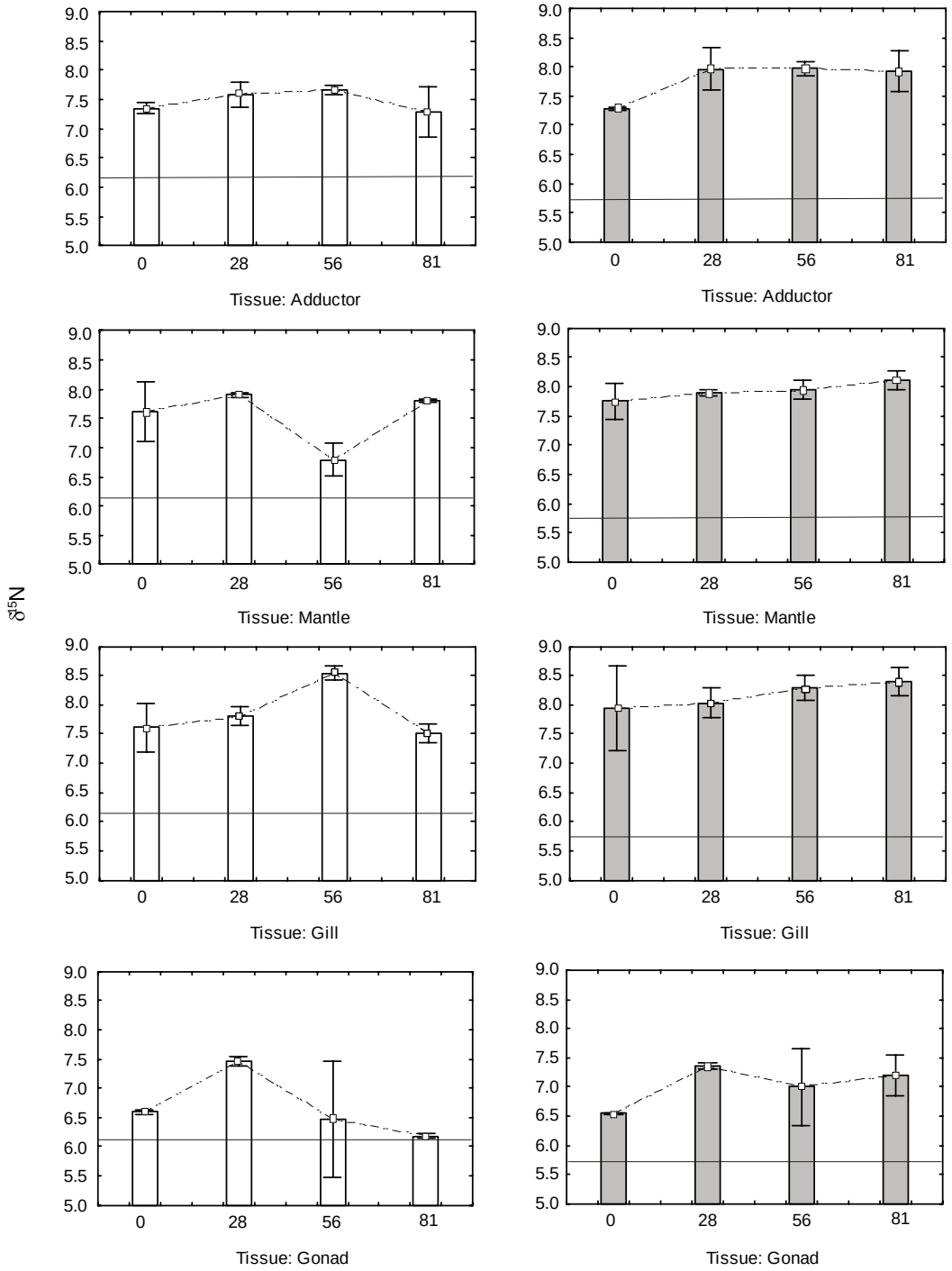

Figure 2.2: Tissue specific changes in $\delta^{15}N$ (‰) over time for *Perna perna* fed on fish food and kelp diets. Values are ± SD. Letters indicate homogenous groups within tissues and solid lines indicate food source signatures.

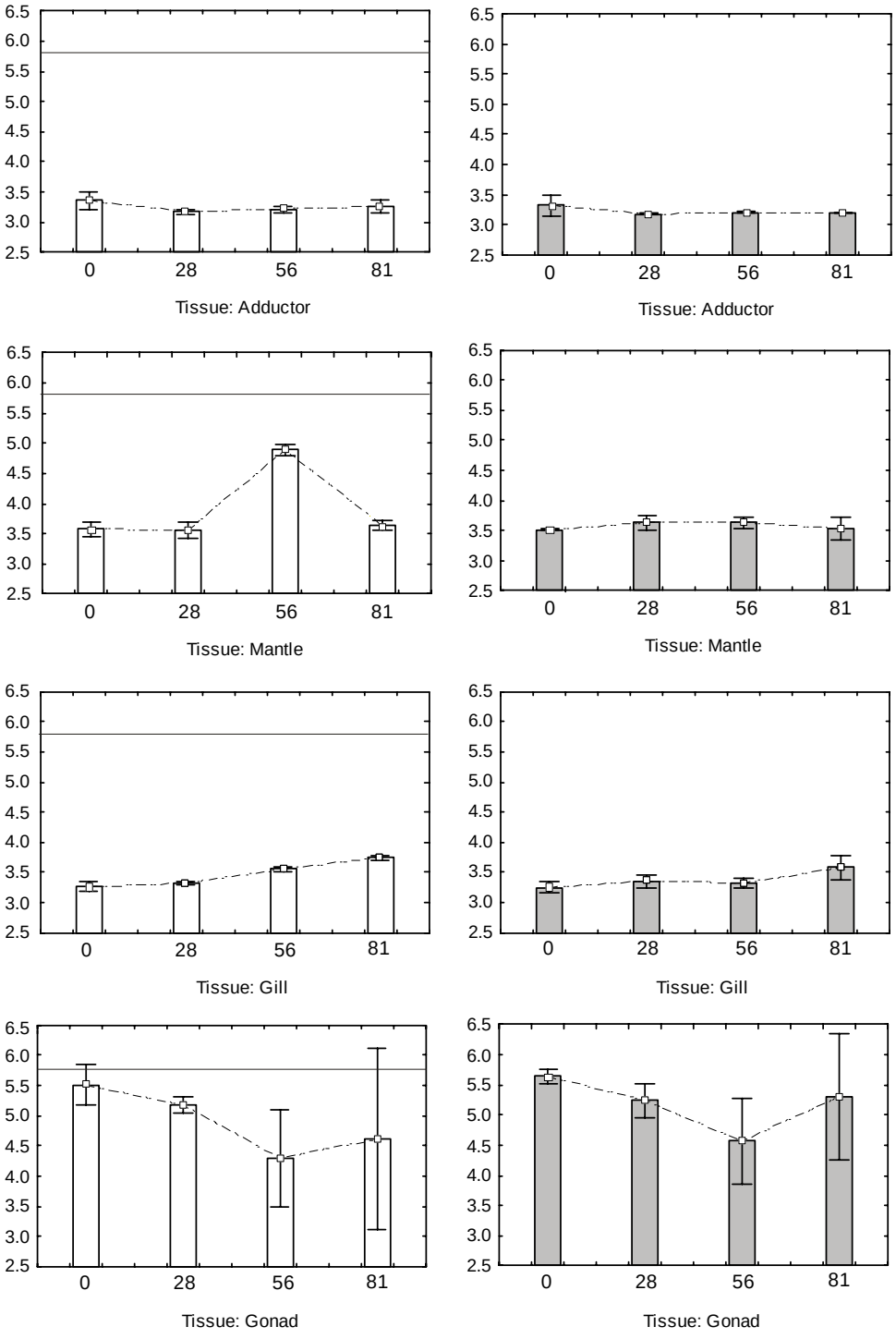

Figure 2.3: Tissue specific changes in C: N ratios over time for *Perna perna* fed on fish food and kelp diets. Values are ± SD. Letters indicate homogenous groups within tissues and solid lines indicate fish food C: N ratio (kelp C: N ratio was 22.96).

The PERMANOVA analyses (9999 permutations) revealed significant changes in tissue $\delta^{13}C$ with both diets (Fig 2.1), in $\delta^{15}N$ for the fish food diet only (Fig 2.2) and in C: N ratios for the fish food diet only (Fig 2.3) over time (see Table 2.3).

Table 2.3: Monte Carlo probability values (p_2) of PERMANOVA results for mussels fed on kelp (K) and on fish food (F), comparing significant changes in tissue $\delta^{13}C$, $\delta^{15}N$ and C: N ratios over time. The time x tissue interaction was insignificant for kelp $\delta^{15}N$ and C: N ratios. * $p_2 < 0.05$.

Time Comparison	$\delta^{13}C_K$	$\delta^{13}C_F$	$\delta^{15}N_F$	C: N Ratio$_F$
Adductor tissue				
Day 0 VS Day 28	0.4292	0.3881	0.1609	0.1293
Day 0 VS Day 56	0.3363	0.4181	0.0162*	0.2194
Day 0 VS Day 81	0.4323	0.3740	0.7828	0.4770
Day 28 VS Day 56	0.6888	0.7188	0.6346	0.3718
Day 28 VS Day 81	0.8502	0.7207	0.3378	0.2848
Day 56 VS Day 81	0.8853	0.6524	0.2201	0.5536
Mantle tissue				
Day 0 VS Day 28	0.4817	0.7988	0.3992	0.9312
Day 0 VS Day 56	0.2363	0.1539	0.0770*	0.0005*
Day 0 VS Day 81	0.0662*	0.0082*	0.5781	0.5572
Day 28 VS Day 56	0.2969	0.2329	0.0055*	0.0002*
Day 28 VS Day 81	0.0216*	0.0103*	0.0252*	0.5346
Day 56 VS Day 81	0.0353*	0.0378*	0.0045*	0.0001*
Gill tissue				
Day 0 VS Day 28	0.3045	0.3278	0.5100	0.2530
Day 0 VS Day 56	0.1479	0.1341	0.0297*	0.0049*
Day 0 VS Day 81	0.0149*	0.0366*	0.7905	0.0006*
Day 28 VS Day 56	0.2953	0.2101	0.0038*	0.0006*
Day 28 VS Day 81	0.0030*	0.0061*	0.1100	0.0002*
Day 56 VS Day 81	0.1567	0.3263	0.0011*	0.0027*
Gonad tissue				
Day 0 VS Day 28	0.0026*	0.0052*	0.0002*	0.2077
Day 0 VS Day 56	0.3114	0.5239	0.7752	0.0786
Day 0 VS Day 81	0.0461*	0.0194*	0.0003*	0.3115
Day 28 VS Day 56	0.1271	0.1427	0.1766	0.1372
Day 28 VS Day 81	0.3037	0.0001*	0.0001*	0.4385
Day 56 VS Day 81	0.1184	0.0697	0.7097	0.8301
$F_{9, 47}$	125.41	10.29	4.46	37.80
SS	375.54	58.04	261.66	2563.25
MS	125.18	6.45	29.07	854.42

Although adductor muscle tissue demonstrated very little change over time, overall patterns of $\delta^{13}C$ in mantle and gill tissue showed isotopic changes that gradually approached the new food source signatures. Tissues from mussels on the kelp diet became gradually more enriched and mussels on the fish food diet became gradually more depleted over time. Contrastingly, carbon patterns in gonad tissue showed a sharp, significant depletion by day 28 for both diets, after which $\delta^{13}C$ moved in opposite directions to dietary signatures (see Fig 2.1).

Patterns in $\delta^{15}N$ were less clear. There were no significant effects of time on tissue nitrogen for mussels fed on kelp, but significant differences occurred in all tissue types for mussels fed on fish food. Adductor muscle tissue showed significant increases between day 1 and 56, mantle and gill tissue showed insignificant enrichments from day 0 – 28 and then demonstrated opposite changes. Mantle $\delta^{15}N$ signatures became sharply depleted by day 56 and then increased by day 81. Gill signatures became enriched by day 56 and depleted by day 81. Overlapping homogenous groups however, suggest there was little overall change in $\delta^{15}N$ and comparisons of dietary signatures (fish food: 6.21 ± 0.29 and kelp: 5.67 ± 0.54) with average tissue signatures over time showed no more than 1.26 – 2.5 ‰ fractionation between diet and mussel tissue. Interestingly, changes in gonad nitrogen for both diets demonstrated the inverse of their respective $\delta^{13}C$ patterns (although kelp changes were insignificant), with initial enrichment from day 0 – 28, subsequent depletion from day 28 - 56, followed by further depletion for mussels on the fish food diet and enrichment in those eating kelp by day 81 (see Fig 2.2).

Overall C: N ratios were fairly conservative; there were significant changes over time only in mantle and gill tissues from mussels fed on fish food. Mantle tissue ratios spiked dramatically at day 56 and decreased towards initial values by day 81, whereas gill tissue appeared to increase slowly over time (Fig 2.3).

2.3.4 Equilibration rates

Percentage tissue turnover in adductor, mantle and gill tissue of both diets over the 81 day period are reported for $\delta^{13}C$ only as there was very little overall change in $\delta^{15}N$ signatures and C: N ratios. Based on these percentages, equilibrations rates (time) were calculated. Percentage turnover (and consequently equilibration times) for the 81 day period differed among tissues and between diets (see table 2.4).

Table 2.4: % tissue turnover in $\delta^{13}C$ over 81 days and calculated $\delta^{13}C$ equilibration rates for adductor, mantle and gill tissues in both diets.

Tissue	% Turnover	Calculated whole tissue turnover
Fish Food		
Adductor	4.14	5.35 years
Mantle	28.00	289 days
Gill	12.45	1.78 years
Kelp		
Adductor	8.85	2.5 years
Mantle	33.89	239 days
Gill	30.90	262 days

2.4 Discussion

2.4.1 Differences between diets

Even though animals in this experiment were fed isotopically invariable and homogeneous food for approximately 12 weeks, no carbon isotopic composition of any tissue reached the $\delta^{13}C$ signature of the new diet. Nevertheless, although no significant changes were seen over time in adductor tissue, the general isotopic $\delta^{13}C$ pattern in all tissues from both diets (with the exception of gonad) conformed to the stated hypothesis, with tissue signatures gradually approaching those of the new food source. However, tissue turnover rates and calculated equilibration times not only varied between tissues but also between diets (see Table 2.4). These dietary differences may be a function of metabolic adaptations to food stress which have been well documented. Modification of clearance rates occurs at the gill level, with negative correlations to food quantity (Wong

& Cheung 1999) and positive correlations to organic content (Widdows *et al*. 1979; Wong & Cheung 1999; Wong & Cheung 2001; Gardner 2002). Pre-ingestive particle selection by bivalves based on size (Vahl 1973; Newell *et al*. 1989; Defossez & Hawkins 1997; Ward & Shumway 2004) and organic content (Newell & Jordan 1983; Shumway *et al*. 1985; Prins *et al*. 1991) can be carried out by the gills and labial palps and Shumway *et al*. (1985) and Rouillon & Navarro (2003) demonstrated differential utilization by bivalves of ingested particles within the gut. Although both food sources were powdered in this study in an attempt to standardize particle size, their organic contents were clearly different. As absorption efficiencies range from about -60% to 60% (Bayne & Hawkins 1990) depending on net energy gain, with positive correlations with decreasing food quality (Navarro *et al*. 1991; Gardner & Thompson 2001; Gardner 2002), the ease of assimilation will vary with food source and result in different isotopic equilibration times. Russel-Hunter (1970) estimated that consumers require a diet with a C: N ratio of 17 or less. The C: N ratio of powdered kelp (approx. 23) was much higher than that of fish food (approx. 5.8), suggesting that initially fish food was the better food source. However, tissue equilibration times in kelp-fed mussels were faster, indicating better absorption efficiencies and assimilation rates, which may be the result of higher/more readily accessible carbon relative to fish food.

Although no differences were seen in tissue $\delta^{15}N$ signatures between diets, the identification of homogenous groups in tissues fed on fish food revealed that day-to-day variation in nitrogen was more pronounced with the fish food diet than with kelp. This disparity is likely to be due in part to the chemical composition of each food source. Powdered baby fish food is formulated specifically to encourage growth of young fish and contains high amounts of protein that can be readily assimilated by mussels. Many macroalgae (including kelp) are fibrous in nature (Stuart *et al*. 1981; Mann 1988) and are comparatively poor in available nitrogen. Dried and powdered kelp is presumably just as nitrogen poor and probably contributes comparatively little to the animal's nitrogen requirements; this role is often fulfilled in nature by bacteria with low C: N ratios that colonize fresh kelp detritus (Newell & Field 1983). The lack of significant change among tissues with respect to C: N ratios however, suggest that very little microbial mineralization of either food source took place. As no significant differences in any tissue were seen in $\delta^{15}N$ from the kelp diet and little overall change occurred in tissues from the

fish food diet (with the exception of gonad), no equilibration rates were calculated for $\delta^{15}N$ turnover.

Although the fractional turnover rates of nitrogen are often assumed to match those of carbon (e.g. Hobson & Wassenaar 1997; Gorokhova & Hansson 1999; Cherel *et al*. 2000), there is evidence that suggests the two elements might not be assimilated at the same rate. Firstly, isotopic tissue composition is affected by isotopic routing, where the differential assimilation of carbon from amino acids, lipids, carbohydrates, etc. (Gorokhova & Hansson 1999) results in not just a simple reflection of the bulk diet, but rather the particular nutrient components from which they are synthesized (Schwarcz 1991; Gannes *et al*. 1997; Bearhop *et al*. 2002). The isotopic composition of body protein in omnivores for example, generally reflects that of assimilated dietary protein and not the bulk diet (Ambrose & Norr 1993). Secondly, lipids and carbohydrates contain little or no nitrogen and fractionation discrepancies could occur between assimilation of carbon and nitrogen in tissues where lipid and/or carbohydrate content is high (Bearhop *et al*. 2002). This is an important consideration as, in order for tissue signatures to be comparable to those in chapters 2-5, no lipid extractions were performed on any tissues in this study. Consequently it is impractical to assume $\delta^{15}N$ tissue turnover rates match those of carbon in this study and they clearly require further investigation.

2.4.2 Differences between tissues

Although there was a large difference in equilibration times between diets, adductor tissue demonstrated the slowest carbon turnover of between 2.5 - 5.35 years. Contrastingly, mantle tissue showed the fastest equilibration rates, with 100% turnover in less than a year for both diets (ranging from 239 - 289 days), while gill turnover rates ranged between 262 days – 1.78 years. According to McQuaid & Lindsay (2000) the maximum lifetime of a brown mussel (*Perna perna*) living on sheltered rocky shores is 6.8 years, suggesting that adductor tissue in particular represents very long-term isotope integration that may span the entire lifetime. Such slow rates of tissue turnover have also been documented by Thompson & Ballou (1956) who reported that most of the collagen (a large component of connective tissue) in mature rats is apparently not replaced in their lifetime and by McCutchan *et al*. (2003) who observed that it can take longer for consumer tissues to reach isotopic equilibrium than to quadruple in mass.

The slow and variable rates of turnover seen in this study are most likely a reflection of the suitability of the food sources, with the supplied diet failing to fulfill the mussels´ nutritional requirements and so retarding tissue turnover. Alternatively, respiration rates, metabolic activity and growth rates have been shown to have inverse relationships with mussel size and age (Hawkins *et al*. 1986; Sukhotin & Pörtner 2001; Sukhotin *et al*. 2006), with more investment in reproductive output than somatic growth in older animals (Aldridge *et al*. 1983; Peterson 1983; Iglesias & Navarro 1991). Individuals in the 30-40mm size class may then demonstrate slower turnover rates than smaller and younger mussels, which may also explain the extreme length of the calculated period required for 100% tissue turnover. Nevertheless, calculated equilibration times indicate that isotopic turnover rates can be ranked as adductor muscle > gill > mantle.

The variation in isotopic signatures and turnover rates among tissues within each diet may be explained through isotopic routing, and/or by differential fractionation through metabolism and the allocation of assimilated carbon among tissues (Thompson & Ballou 1956; Libby *et al*. 1964; Tieszen *et al*. 1983; Gorokhova & Hansson 1999; Sweeting *et al*. 2005). Moreover, in tissues where rare or limiting nutrients are required for synthesis, they may be mobilized from long-term stores rather than from the present diet (Bearhop *et al*. 2002). It must also be noted that during times of nutritional stress the metabolism of body lipids (which are more depleted in ^{13}C; DeNiro & Epstein 1977) would presumably result in ^{13}C enrichment in the remaining tissue pool (Tieszen *et al*. 1983; Doucett *et al*. 1999). The faster equilibration rate of mantle versus gill tissue is somewhat surprising as there is some evidence that gill tissue is considerably more active metabolically (Livingstone & Farrar 1984). However, mantle tissue in mussels has two interrelated physiological functions which may be responsible for this discrepancy. Firstly, the mantle accumulates reserve tissue composed mostly of glycogen, lipids and proteins and secondly, it serves as a development site for gonad synthesis, which is done at the expense of the reserve tissue (Lubet *et al*. 1978; Pipe 1987; Crespo & Espinosa 1990; Freites *et al*. 2002; Lombraña *et al*. 2005). In *Mytilus* spp., mantle glycogen is the main energy source for sustaining gonadal development and a clear relationship exists between its degradation and gonad synthesis (Gabbott & Peek 1991; Suárez *et al*. 2005; Lombraña *et al*. 2005). There is some evidence that sexually mature mussels demonstrate stress induced spawning (Young 1945; Breese *et al*. 1963; Ram *et al*. 1993) and the majority of

Perna perna individuals collected in this study spawned within two hours of being placed in experimental tanks. The patterns seen in gonad $\delta^{13}C$ in this study may therefore be indicative of a shift in fractionation after a spawning event (sharp depletion between days 0 – 28), during which mussels were adjusting their metabolism to acclimate to laboratory conditions (i.e. temperature, flow rates, $[O_2]$, food and light conditions etc.) while re-attaching to the substratum. After day 28, fractionation shifted again in what may have been a metabolic response to increased reproductive effort and the faster turnover rate in mantle tissue and the change in gonadal carbon signature (i.e. away from the dietary $\delta^{13}C$ signature) may therefore be a result of the consumption of mantle reserve tissue as more energy was invested in gonad production.

No significant time-tissue interactions in $\delta^{15}N$ were seen in any tissue from mussels eating kelp and, although significant interactions were demonstrated by all four mussel tissues on the powdered fish food diet, no general patterns could be seen over time. The temporal variations seen in adductor, mantle and gill tissue were due to considerable changes occurring at day 56, followed by a return to values similar to initial signatures, suggesting a lack of overall change in $\delta^{15}N$ over time. It is interesting to note that the $\delta^{15}N$ signatures of gonad from both diets demonstrate a general inverse pattern of their respective $\delta^{13}C$ patterns, again suggesting a shift in fractionation associated with a change in energy investment from gonad production to acclimation to laboratory conditions and back again.

The overall lack of isotopic change in nitrogen (with the exception of gonad), suggests that nitrogen signatures of original food sources were similar to those of fish food and kelp diets and supports the findings of Fry (1988), Raikow & Hamilton (2001), Moore & Suthers (2005), Hansson *et al*. (1997) who suggest that $\delta^{15}N$ fractionation in mussel tissues is considerably smaller (1.26 – 2.5‰) than previously assumed (e.g. DeNiro & Epstein 1981; Minagawa & Wada 1984; Post 2002). This corroborates the trophic correction of 1.7‰ (Fry 1988; Hansson *et al*. 1997; Raikow & Hamilton 2001; Moore & Suthers 2005) applied in chapters 2 – 4 and indicates a need to validate isotopic fractionation at the species level.

Hawkins (1985) found that laboratory-measured whole body protein turnover rates in *Mytilus edulis* varied with absorption rates, reaching maximum values of 0.29-0.4% per day, suggesting 100% whole body turnover between 244-350 days, which is a great deal

faster than turnover rates calculated in this study for both adductor and gill tissue. However, due to the high nitrogen content of protein, Hawkins' turnover rates would represent a generalized measure of $\delta^{15}N$ equilibration rates, and consequently cannot be compared with the carbon turnover rates found in this study.

2.4.3 Summary and conclusions

Overall, $\delta^{13}C$ ratios in adductor, mantle and gill tissues gradually approached food source signatures in both diets. Overall, adductor tissue had the slowest carbon turnover rate (indicating long-term isotope integration) followed by gill and then mantle tissue, although calculated equilibration times were excessively long, suggesting that the nutritional demands of the experimental mussels were not met by the supplied diet. Tissue turnover rates have the potential to differ between elements, and as no significant changes in tissue nitrogen were apparent over time, no equilibration rates could be calculated for $\delta^{15}N$. Changes in gonad signatures are likely to be representative of fractionation shifts due to changes in energy investment and the results from this study indicate that whole body nitrogen fractionation ranges between 1.26 – 2.5 ‰.

Hobson & Clark (1992, 1993) and Nagy (1987) noted that metabolic rates in captive animals can be substantially different from free-living individuals and so relationships derived from laboratory studies may not accurately represent those in the natural environment. This complicates the application of laboratory measured equilibration rates to isotopic patterns of wild animals, yet the natural environment often presents too many variables at ecological, biological and biochemical levels to obtain reliable measurements. Consequently linking laboratory experiments with field studies will help to clarify overall isotope patterns, as understanding variation on one level, requires an awareness of processes at others (Gannes *et al*. 1997). Interpretations of isotopic variation necessitate a more thorough understanding of mechanisms behind metabolic fractionation at the tissue and species levels.

CHAPTER 3: TEMPORAL ISOTOPIC VARIATION IN SUSPENDED PARTICULATE MATTER SUGGESTS STRONG LINKS TO NEARSHORE HYDROGRAPHY AND HEAVY DEPENDENCE OF MUSSELS ON VERY NEARSHORE PRODUCTION.

3.1 Introduction

Coastal ecosystems constitute complex and dynamic environments in terms of both nutrient regimes and hydrography (Ostrom *et al*. 1997). Benthic and intertidal communities are profoundly influenced by their environments through the physical processes that drive food and nutrient delivery (Blanchette *et al*. 2006). Consequently changes in these processes can affect intertidal community structure at a variety of spatial and temporal scales (Roughgarden *et al*. 1988; Bustamante *et al*. 1995; Nielsen & Navarrete 2004). Intertidal mussel beds are generally thought to obtain their organic carbon through the ingestion of pelagic phytoplankton (Widdows *et al*. 1979; Asmus & Asmus 1991; Dame & Prins 1998), although several studies have shown that inshore production, including macroalgal carbon from detrital food webs, may be of equal importance (e.g. Stuart *et al*. 1982; Dunton & Schell 1987; Duggins *et al*. 1989; Bustamante & Branch 1996).

In many cases, the biological application of stable isotope analysis has successfully linked primary producers to higher trophic levels in marine and estuarine environments (DeNiro & Epstein 1978; Deegan & Garritt 1997; Kaehler *et al*. 2000; Vizzini *et al*. 2002), but only recently has temporal variation in the isotopic signatures of marine producers and consumers become a focus of trophic investigations (e.g. Goering *et al*. 1990; Reira & Richard 1997; Kang *et al*. 1999). Frazer (1996) discovered that winter $\delta^{13}C$ ratios of Antarctic larval krill were enriched relative to summer, while Megens *et al*. (2001) reported temporally enriched carbon values of particulate organic matter (POM) in the North Sea that were related to season. Rolff (2000) described interannual variation in both $\delta^{13}C$ and $\delta^{15}N$ in the plankton of temperate fresh and marine waters and Kreeger & Newell (2001) described monthly changes in hydrography, temperature and runoff that can affect SPM composition and distribution in coastal systems. This suggests that a more detailed understanding of intertidal food webs may require attention to temporal variation in the isotopic signature of food sources and consumers.

Establishing isotopic links between producers and consumers in dynamic marine environments is a complex undertaking, as the turnover of consumer tissues tends to be slower than that of their diet (O'Reilly *et al*. 2002). Consumer isotope signatures are time-integrated (reflecting average diet over a period of time) and may not reflect short-term dietary variations in $\delta^{13}C$ or $\delta^{15}N$. Consequently, consumers are considered to experience far less temporal variation than primary producers.

My study investigates temporal variation in the $\delta^{13}C$ and $\delta^{15}N$ signatures of macroalgae, suspended particulate matter (SPM) and intertidal mussels. This was done in order to (1) investigate nearshore to offshore gradients in SPM and evaluate their temporal consistency (2) investigate the short-term temporal variability of nearshore SPM, measured over 24hr periods, (3) investigate longer term (seasonal) temporal variability of mussel diet and its relation to distinct sources of production and (4) determine the relative importance of nearshore detritus versus pelagic primary production (i.e. phytoplankton) in the diet of the intertidal mussel *Perna perna*.

3.2 Methods

3.2.1 Site

Kenton-on-Sea (S33° 41' 0, E26° 40' 60; Fig 4.1) is on the south coast of South Africa, possessing rocky shores comprised of Aeolian dune rock, with three permanently open estuaries in close proximity. Both the Kariega Estuary, which enters the sea directly at Kenton, and the Kowie River, approximately 25km east along the coastline, have heavily impounded catchment areas with very limited outflow to the sea, while the Great Fish River, approximately 55km east along the coastline, has a strong flow regime making considerable outputs to the surrounding seawater (Allanson & Read 1995).

3.2.2 Sample collection

Two individual SPM samples were obtained from five-liter surface water samples on a monthly basis, along a transect perpendicular to the coast at distances of 0, 0.25, 0.5, 1, 2, 4 and 10km from the shoreline at Kenton-on-Sea. The sampling period lasted from April 2004 to May 2005, between GPS coordinates S33º 46.276, E26º 43.130 (0km) and S33º 41.640, E26º 41.041 (10km). Each month five intertidal mussels (*Perna perna*) and

duplicate samples of three of the most abundant macroalgae species (*Gelidium pristoides*, *Hypnea spicifera* and *Ulva* sp.) were collected from the same site.

Chlorophyll a (chl-a) samples (3 x 250 ml) were collected at each station along the same transect from September 2004 to May 2005 to investigate changes in primary producer standing stocks. 200ml surface water samples (x 3) were collected in October and November 2005 at each station along the same transect for a preliminary exploration of offshore variation in the species composition of phytoplankton communities.

Five-liter estuarine surface water samples (x 2) were collected from the Kariega Estuary mouth on five separate outgoing (April, July, November 2004, February, April 2005) and incoming tides (May, August, October 2004, January, March 2005).

SPM samples (x 2) were also collected once, along a 50km transect with samples from 0 - 7.5km collected in June 2003 and then from 3.5 – 50km offshore in August 2003 (total of 14 stations) to investigate isotopic patterns in offshore water.

On two occasions (winter, June 21-22nd and summer, November 18-19th 2005) nearshore (0km) SPM samples were collected at Kenton-on-Sea at hourly intervals for 24 hours to investigate short-term variation in $\delta^{13}C$ and $\delta^{15}N$ signatures.

3.2.3 Sample Preparation

Water samples were filtered through pre-combusted (500°C, 6hrs) Whatman® GF/F filters (0.45 µm pore size), using a vacuum pump (≤ 4 cm Hg) and oven dried at 60°C for 24hrs. Zooplankton and other large particle contaminants were manually removed under a dissecting microscope (16 x magnification). Mussel adductor muscle tissue was dissected from fresh samples, rinsed in distilled water (dH_20) and oven dried (60°C, 48hrs). Adductor tissue was used in this study as muscle tissue has low turnover rates (chapter 2; Gorokhova & Hansson 1999) and is therefore representative of a time-integrated diet. Additionally, as muscle has minimal lipid content (Soudant *et al*. 1999; Chu *et al*. 2000), no lipid extractions were performed on any tissue. All macroalgae were rinsed in dH_20, and visible epiphytes were removed before drying (60°C, 48hrs). Size fractionated chl-a was determined through serial filtration of 250 ml samples, using a vacuum pump (≤ 4cm Hg), through 20.0 (Nitex), 2.0 and 0.20 µm (plastic Nucleopore™) filters and extracted in 90% acetone for 24hrs in the dark. Chl-a concentrations (mg chl-a m^{-3}) were determined fluorometrically (Turner designs 10AU fluorometer) before and after acidification (Holm-Hansen & Riemann 1978). 200ml surface water samples for

phytoplankton identification were fixed in 4% buffered formalin, samples were then reduced to 100 ml and phytoplankton taxa were identified in one-tenth samples using the works of Boden & Reid (1989) and Tomas (1996) and reported as cells/litre.

3.2.4 Isotope Analysis

All isotopic analyses were completed as described in Chapter 2.

3.2.5 Data Analysis

Carbon and nitrogen contour plots were created using Ocean Data View 3 (Schlitzer 2005; available at www.awi-bremerhaven.de/GEO/ODV). Monthly positional patterns of the Agulhas Current were investigated via sea surface temperatures (SST) through MODIS (Moderate Resolution Imaging Spectroradiometer) real time weighted daily images and PATHFINDER climatology (http://www.rsmarinesa.org.za/), to determine the distance offshore at which the current was found (measured in UTHSCSA Image Tool v3 (2002)), with the Agulhas Front designated as water with an SST of 22°C or higher. Monthly climatological chl-a concentrations were also explored through satellite imagery using the ocean colour SeaWiFS sensor (Sea-viewing of Wide Field Sensor) to compare temporal changes in primary production using chl-a as an indicator of phytoplankton biomass. Correlation analyses were performed in Statistica v7 (StatSoft Inc. 2004) between $\delta^{13}C$ signatures at 0, 0.25, 0.5, 1, 2, 4 or 10km and wind direction, wind speed, rainfall, water temperature or the distance offshore of the Agulhas Current.

One-way ANOVA were performed using Statistica v7 (StatSoft Inc. 2004) on $\delta^{13}C$ and $\delta^{15}N$ ratios of mussels to assess seasonal variability in isotope signatures. The IsoSource model described by Phillips & Gregg (2003) was applied as a two isotope system ($\delta^{13}C$ and $\delta^{15}N$) with three sources: average nearshore (0km; -15.91‰, 6.46‰), average offshore (10-50km; -19.72‰, 5.89‰) and average estuarine SPM (-18.37 ‰, 6.86 ‰) to determine the percentage contribution of organic carbon and nitrogen to mussel diet of nearshore SPM during each month, using an average fractionation of 1‰ for $\delta^{13}C$ (as reported by DeNiro & Epstein 1978; Fry & Sherr 1984; Peterson & Howarth 1987) and 1.7‰ (Hansson *et al*. 1997; Raikow & Hamilton 2001; Moore & Suthers 2005) for $\delta^{15}N$. Isotopic signatures for estuarine SPM were used from low tide values only, as this is the only time (except in extreme floods) that the Kariega may flow out to the sea.

IsoSource modeling (Phillips & Gregg 2003) requires a minimum of three sources for a 2 element analysis, consequently a two-source linear mixing model (Bustamante & Branch 1996) was used to determine the isotopic percentage contribution of organic carbon to nearshore SPM by macroalgae and phytoplankton:

$$\% \text{ Isotope} = [(d_{\text{nearshore}} - d_{\text{offshore}}) / (d_{\text{macroalgae}} - d_{\text{offshore}})] \times 100$$

where d is the isotopic ratio of the sample, using an overall average of all macroalgal species over 14 months (-14.66 ‰) and monthly offshore (10km) SPM as a measure of phytoplankton (see Fig 3.1A).

Non-parametric, permutational multivariate analyses of variance (PERMANOVA; McArdle & Anderson 2001; Anderson 2001), using Bray-Curtis dissimilarities was performed on phytoplankton abundance data (transformed to $\log_{x+1}$) to investigate the effect of time and distance on phytoplankton species composition. Subsequent multidimensional scaling (MDS) in Primer v5.2.6 (2001) using a Bray-Curtis distance matrix, was performed on phytoplankton abundance data (transformed to $\log_{x+1}$) over both months at each station along the sampled transect and then separately for stations within October and November 2005. Where sufficient numbers of unique permutation values were possible, the permutational probability (p_1) is reported, where few unique permutation values were possible, the more conservative Monte-Carlo probability (p_2) is reported.

3.3 Results

3.3.1 Large-scale patterns in SPM

While $\delta^{15}N$ for SPM lacked clear spatial or temporal trends, distinct spatial patterns existed in $\delta^{13}C$, with consistent nearshore to offshore depletions seen during all months (Fig 3.1), with an average carbon signature of -15.83 ± 1.48‰ nearshore (0km) and -19.88 ± 1.30‰ offshore (10km). The extended offshore transect reaching 50km from the coast of Kenton-on-Sea, also showed clear, strong carbon depletion from directly inshore (0km; -14.26 ± 0.75‰) to 50km from the coast (-18.53 ± 0.61‰; Fig 3.2). Initial $\delta^{13}C$ values decreased rapidly with distance, leveling out around 20km and remained within 2.0‰ of the offshore average (-19.88‰). Contour plots of $\delta^{13}C$ monthly data (Fig 3.1) revealed projections of nearshore SPM with enriched $\delta^{13}C$ values extending up to 2km

offshore between the months of June – August 2004 and again in May 2005. During May and September 2004 nearshore production remained close inshore and highly depleted offshore water incursions (approx. –20.00‰) reached as far inshore as 3km from the beach. Interestingly, May 2005 showed both projections of nearshore water and incursions of offshore water (Fig 3.1). Except for January 2005 there was little congruence with the results for $\delta^{15}N$ and I focus here on the carbon data.

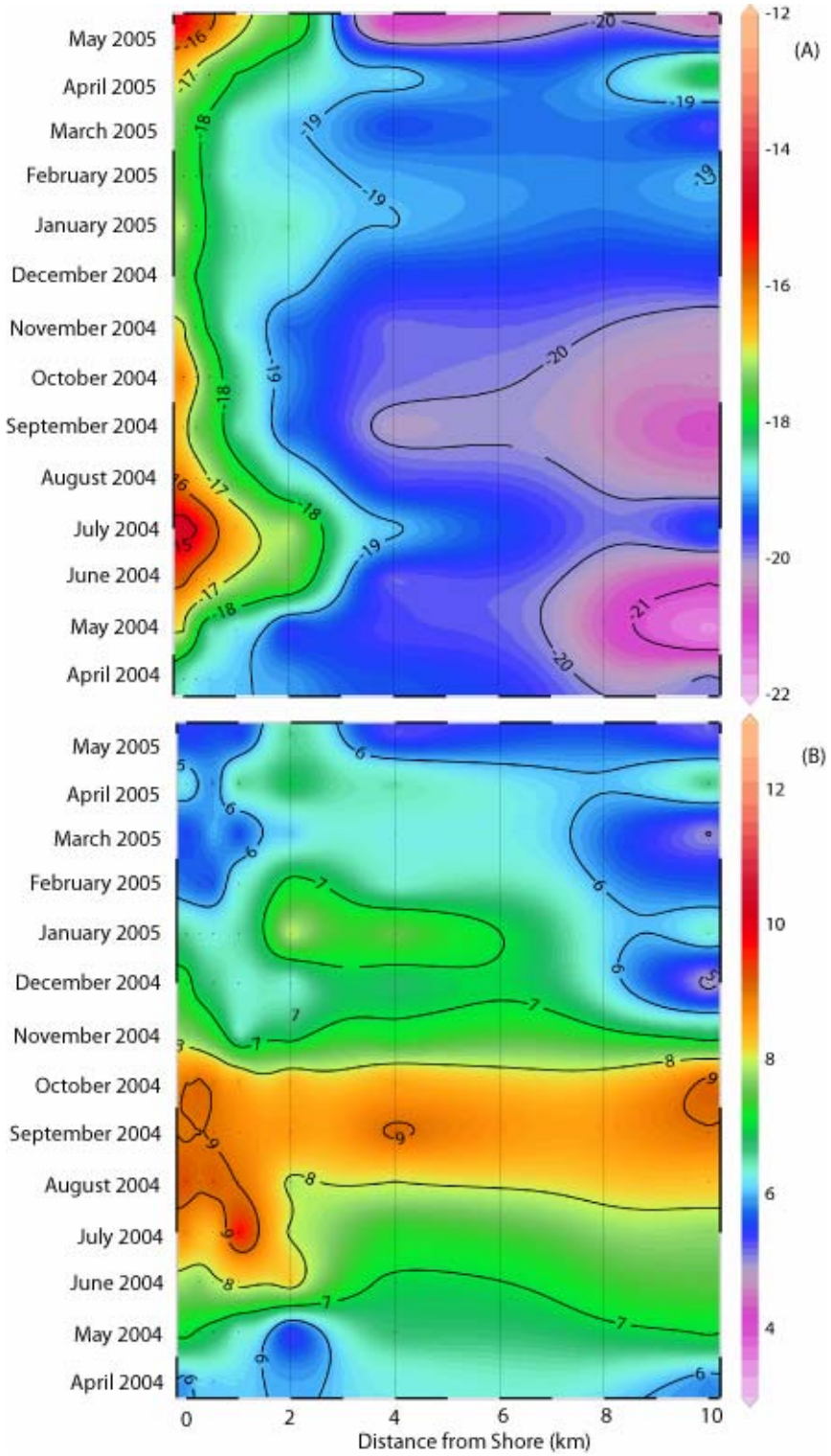

Figure 3.1: Contour plot of $\delta^{13}C$ (A) and $\delta^{15}N$ (B) signatures (‰) from monthly SPM transects at Kenton-on-Sea

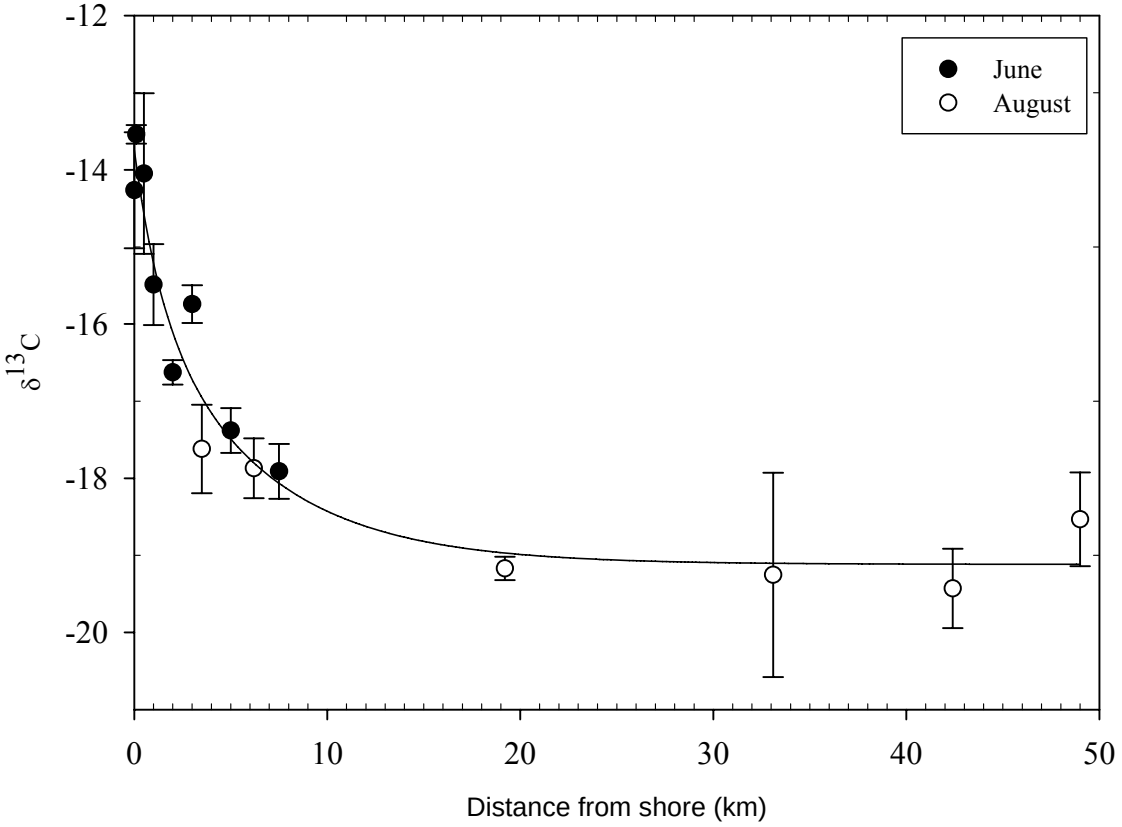

Figure 3.2: Nearshore to offshore gradient in $\delta^{13}C$ (‰) from 0 to 50km offshore. Regression: $y = -19.1176 + 1.71^{-0.7539x} + 1.84^{0.1672x} + 1.83^{-0.1673}$, $r^2 = 0.0431$. Values are means ± SD.

Correlation analyses showed no significant relationships between carbon signatures at any distance and wind direction, wind speed, rainfall, water temperature or the distance of the Agulhas Current on any sampling occasion ($p > 0.05$ in all analyses).

3.3.2 Small-scale patterns in SPM

Average estuarine $\delta^{13}C$ and $\delta^{15}N$ values from the Kariega mouth for incoming and outgoing tides were -18.37 ± 0.14 ‰; 6.85 ± 0.77 ‰ and -15.80 ± 0.50 ‰; 6.27 ± 0.54 ‰ respectively, with incoming tides being isotopically more representative of marine water.

$\delta^{13}C$ ratios of SPM showed cyclical enrichment patterns over a 24 hour period. Inshore carbon signatures at high tides were enriched to a maximum of -14.84 ± 0.36 and at low tides depleted to a minimum of -17.39 ± 1.49, while variation in the SPM ratios of $\delta^{15}N$ was less clear, lacking any overall pattern (Fig 3.3).

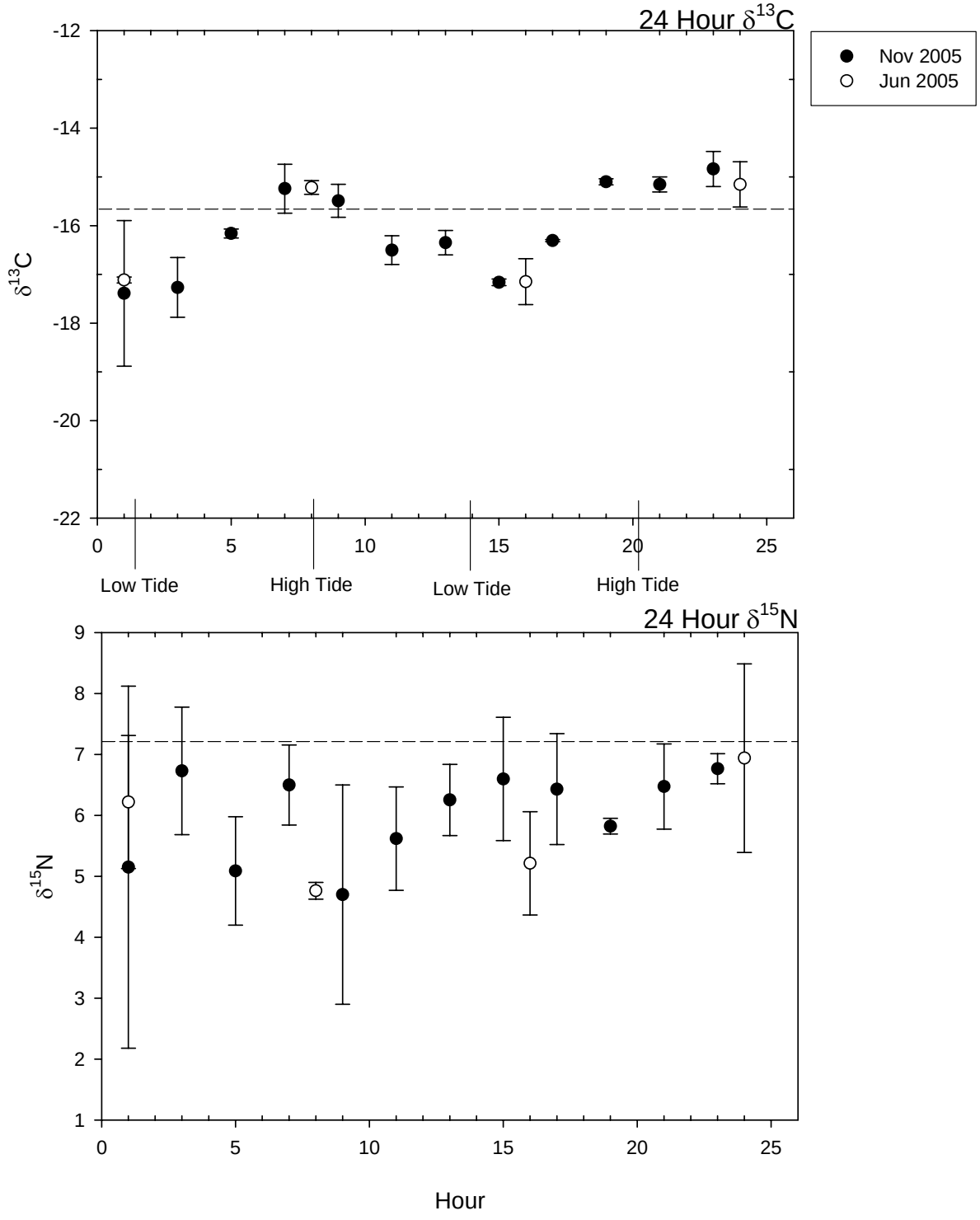

Figure 3.3: $\delta^{13}C$ (A) and $\delta^{15}N$ (B) signatures (‰ ± 1SD) of nearshore SPM over 24 hours. LT and HT indicate low tide and high tide respectively. Dotted lines represent average mussel signature (‰) over 14 months.

3.3.3 Mussels and diet

Adductor muscle isotope signatures showed little temporal variation over the 14 month period, with no significant differences in nitrogen ($p > 0.05$), and with carbon showing small but significant temporal differences (homogenous groupings, Newman-Keuls $p < 0.05$) within 1.0‰ that could not be related to season (Fig 3.4). In 11 out of 14 months $\delta^{13}C$ ratios of mussel adductor tissue were similar or identical to carbon values of nearshore SPM, while three out of 14 months showed mussel $\delta^{13}C$ ratios that fell between those for nearshore and offshore SPM (Fig 3.5). However, no consistent 1‰ fractionation was seen between nearshore or offshore SPM and mussel signatures.

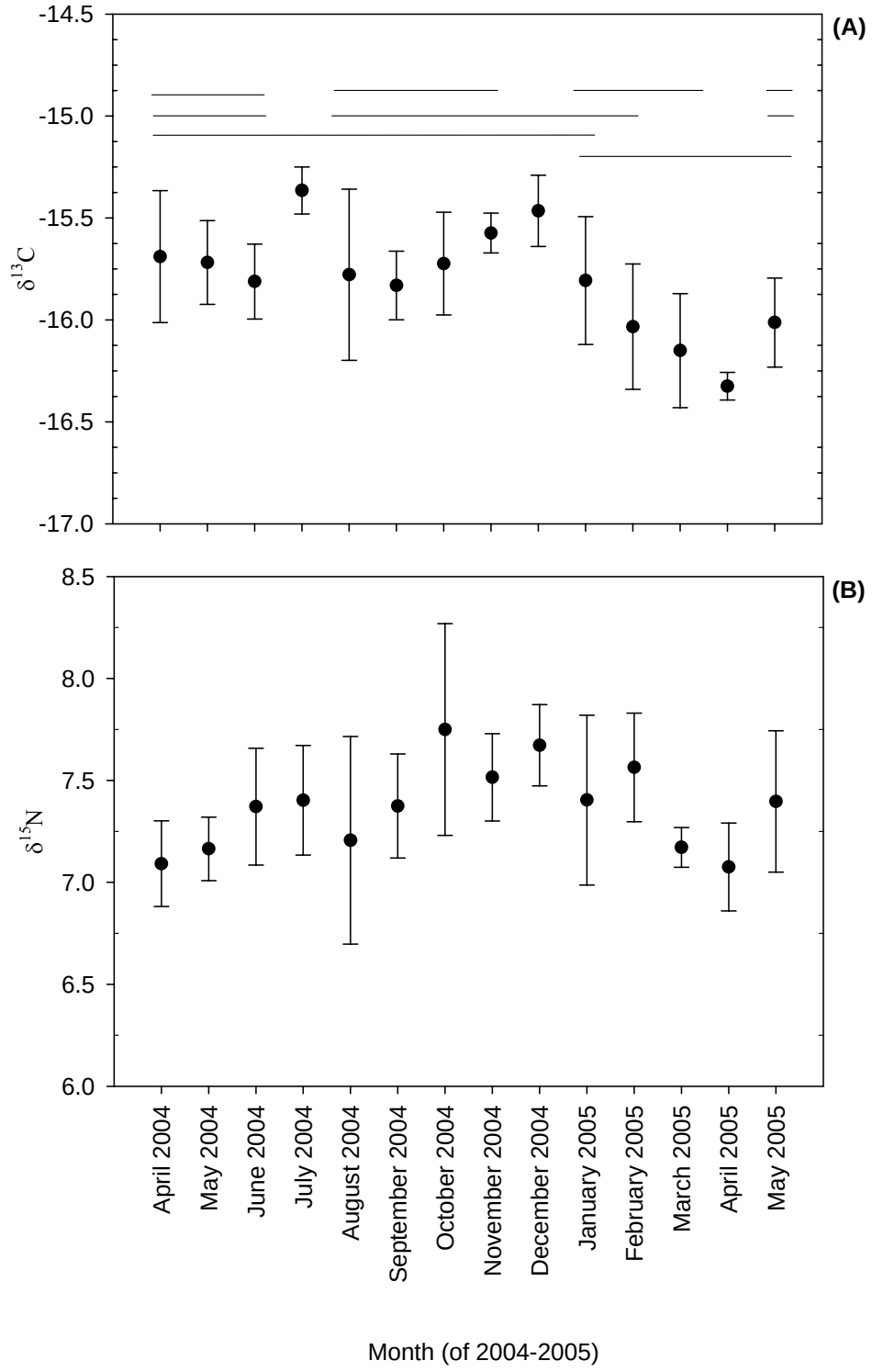

Figure 3.4: (A) Monthly averages (‰ ± 1SD) for $\delta^{13}C$ (A) and $\delta^{15}N$ (B) signatures of adductor tissue of *Perna perna* collected from April 2004 to May 2005. Lines above graph indicate homogenous groups (Newman-Keuls $p < 0.05$).

The two isotope system, three source model, using average $\delta^{13}C$ and $\delta^{15}N$ ratios of nearshore, offshore and estuarine SPM ratios for all months, showed mussels demonstrating over 50% dependence on nearshore SPM for incorporated carbon in 12 out of 14 months (Fig 3.5). IsoSource models reported a range of source proportions that satisfy the isotopic mass balance in the mixing model (Phillips & Gregg 2003), however as some of the source proportions were reported to make contributions along a wide range of percentages (e.g. contributions of nearshore SPM to mussel diet in July 2004 could be between 53 and 100%) I have presented the generated mean graphed with standard deviations.

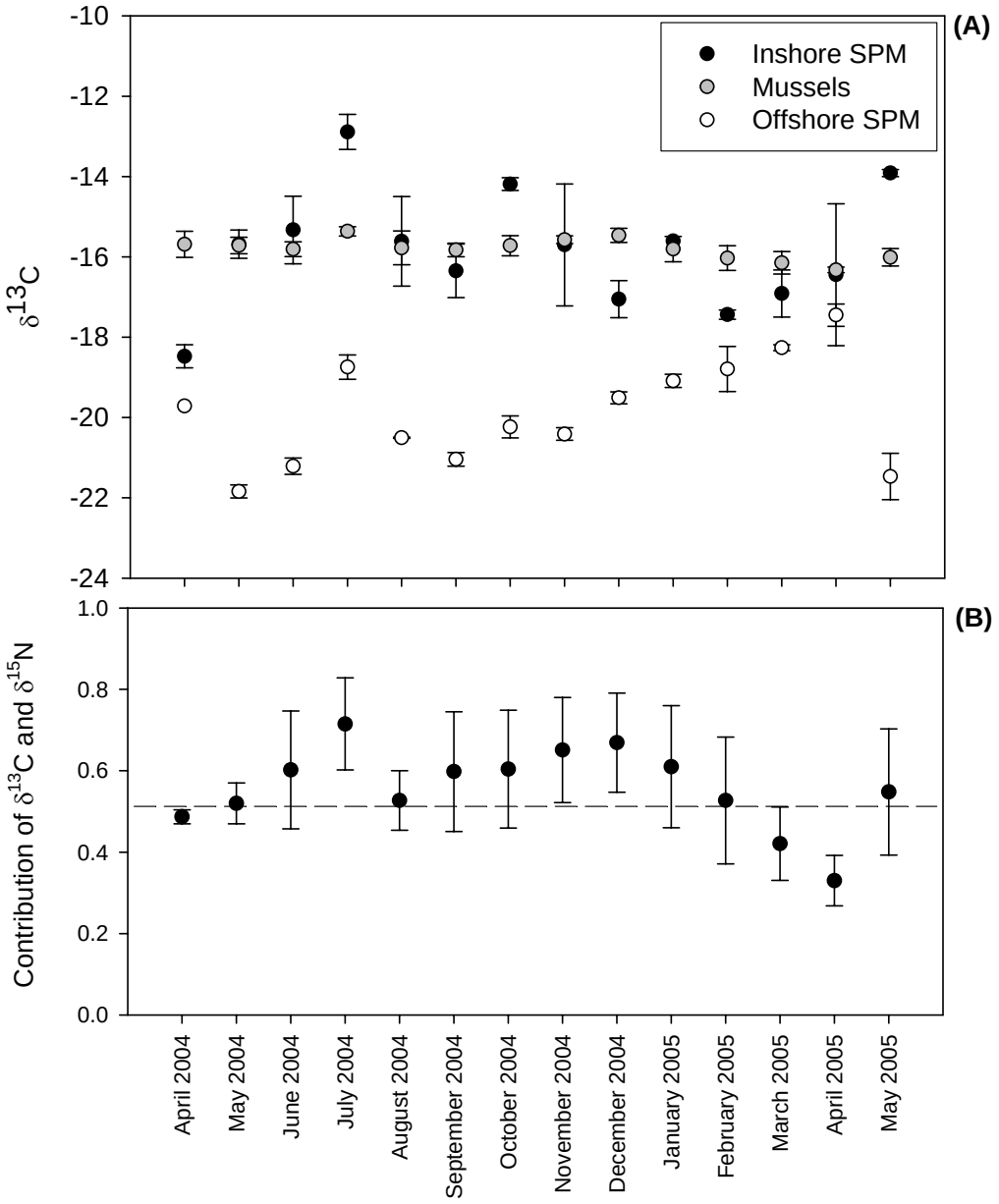

Figure 3.5: (A) $\delta^{13}C$ (‰) relationships among mussels, nearshore and offshore SPM for 2002 to 2004; (B) percentage $\delta^{13}C$ and $\delta^{15}N$ contribution of average nearshore SPM for all months to mussel diet determined using IsoSource modeling (Phillips & Gregg 2003). Models calculated for each month using an increment of 1% and mass-based tolerance of 0.08‰. Values are means ± SD.

3.3.4 Patterns in primary production

Macroalgal signatures lacked clear temporal trends in either $\delta^{13}C$ or $\delta^{15}N$, with inconsistent enrichments and depletions in consecutive months and a wide range in both carbon and nitrogen signatures. Both *Hypnea spicifera* and *Gelidium pristoides,* for example, demonstrated large scale temporal variations, ranging between $\delta^{13}C$ values of -11.60 to -17.67‰ and -12.64 to -17.40‰ and $\delta^{15}N$ values of 5.4 to 6.83‰ and 5.84 to 8.83‰ respectively. Such variation in macroalgal isotopic ratios complicated links between mussel diet and nearshore SPM, but the application of the two-source linear mixing model showed $\delta^{13}C$ of macroalgae accounted for over 50% of nearshore SPM in 10 out of 14 months. For April 2004 and Feb-Apr 2005 this value fell to 24-38%.

Chl-a in all three size fractions showed large inshore variability with February and March 2005 showing high concentrations (mg chl-a m^{-3}) in all three size classes that decreased with distance offshore, however overall there were no consistent temporal trends (Fig 3.6).

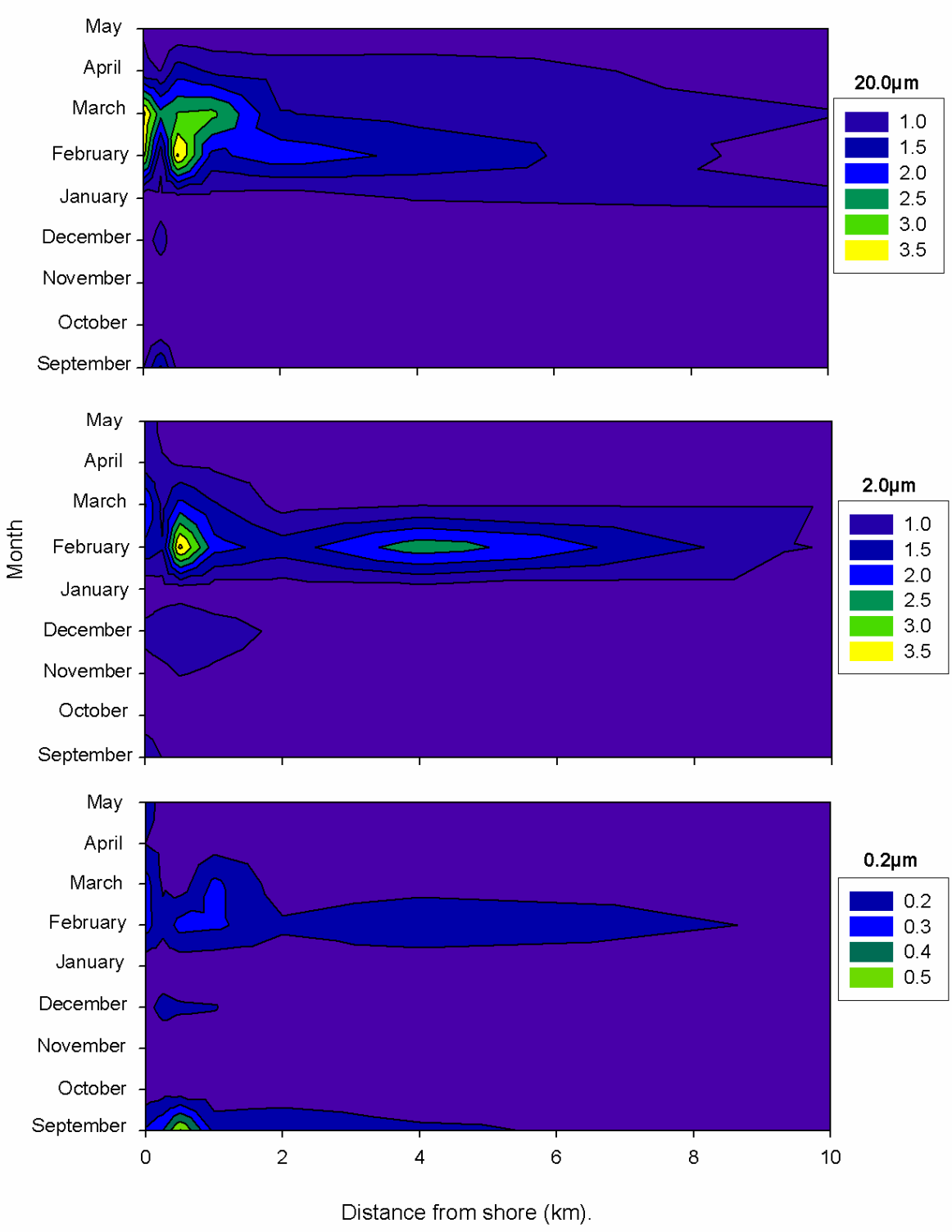

Figure 3.6: Measurements of chlorophyll-a (mg chl-a m^{-3}) for three size fractions along the 10km offshore transect from September 2004 to May 2005.

Monthly SeaWiFS satellite photos of chl-a concentrations (mg chl-a m^{-3}) for each sampling occasion showed seasonal peaks in primary production during austral spring (Sept. – Oct.) and autumn (Apr. - May) around Kenton-on-Sea and also allowed a daily overview of basic coastal hydrography. Satellite photos showed nearshore primary production around Kenton-on-Sea being drawn offshore and south-west along the coastline on four occasions (June – August 2004 and May 2005; Fig 3.7, also available at www.rsmarinesa.org.za). These occasions corresponded to the months that demonstrated projections of enriched nearshore SPM away from the coast.

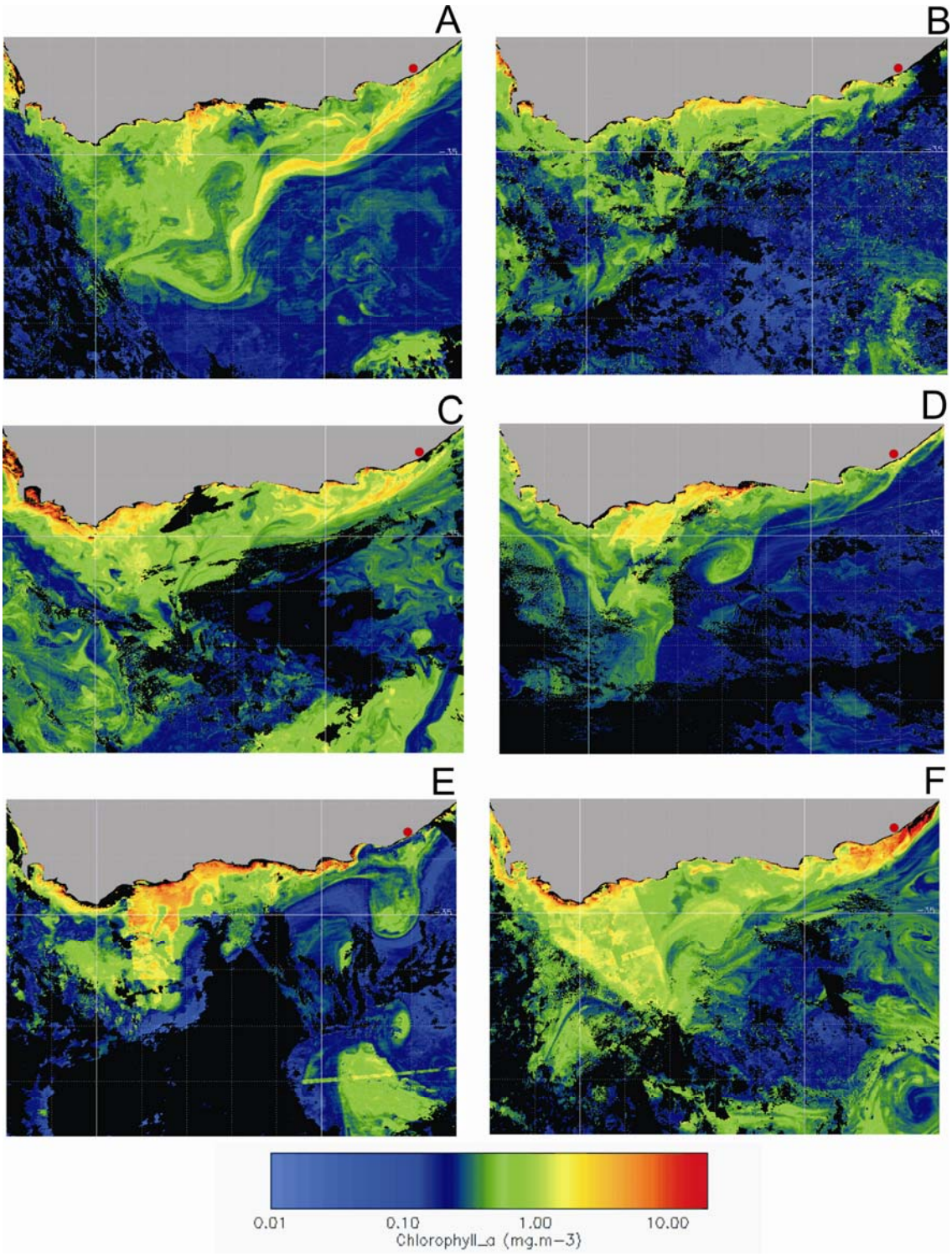

Figure 3.7: (A) SeaWiFS satellite photo (http://www.rsmarinesa.org.za/) of chlorophyll a concentrations (mg chl-a m^{-3}) for the south coast of South Africa in June 2004. (B) July 2004. (C) August 2004. (D) May 2005. (E) May 2004. (F) September 2004. Red dot indicates Kenton-on-Sea (33° 41' 0S, 26° 40' 60E).

Altogether 39 species of phytoplankton were identified and species richness was generally higher for inshore stations. PERMANOVA and subsequent MDS showed a significant effect of time ($F_{1, 41} = 17.4923$, $p_1 < 0.001$) and a significant time x distance interaction ($F_{6, 41} = 1.7216$, $p_1 = 0.0129$) which *a posteriori* t-tests resolved into distinct nearshore (0km; in both Oct. and Nov.) and offshore (10km; Nov. only) communities that were overall significantly different from those at any other distance (0.25 - 4km; Table 3.1, Fig 3.8).

Table 3.1: Monte Carlo probability values (p_2) of PERMANOVA results for phytoplankton species composition for the time x distance interaction.* $p_1 < 0.05$

Distance Comparisons	October 2005	November 2005
0km vs. 0.25km	0.0397*	0.0096*
0km VS 0.50km	0.0067*	0.0111*
0km vs. 1.00km	0.0456*	0.1121
0km vs. 2.00km	0.0499*	0.0296*
0km vs. 4.00km	0.0054*	0.0150*
0km vs. 10.00km	0.0355*	0.0024*
0.25km vs. 0.50km	0.0564	0.1053
0.25km vs. 1.00km	0.1931	0.0255*
0.25km vs. 2.00km	0.1990	0.0743
0.25km vs. 4.00km	0.1469	0.1362
0.25km vs. 10.00km	0.2630	0.0101*
0.50km vs. 1.00km	0.0973	0.0539
0.50km vs. 2.00km	0.5466	0.2342
0.50km vs. 4.00km	0.0719	0.1785
0.50km vs. 10.00km	0.2547	0.0098*
1.00km vs. 2.00km	0.4399	0.4403
1.00km vs. 4.00km	0.3638	0.1042
1.00km vs. 10.00km	0.7005	0.0138*
2.00km vs. 4.00km	0.3738	0.6387
2.00km vs. 10.00	0.6174	0.0264*
4.00km vs. 10.00km	0.5843	0.0493*

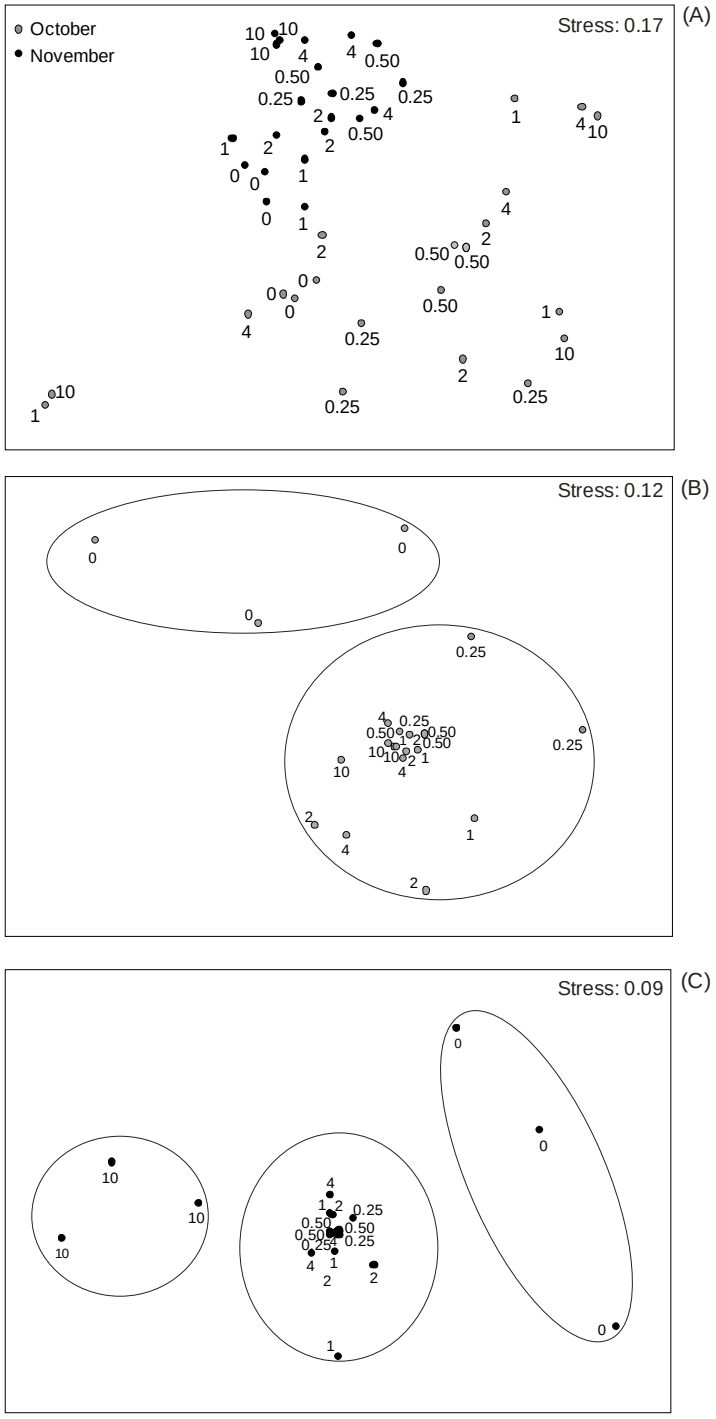

Figure 3.8: (A) MDS plot for both months showing overall effect of time (stress 0.17). (B) MDS plot showing time x distance interaction in October 2005 only (stress 0.12). (C) as (B) for November 2005 only (stress 0.09). Circles indicate significant groups, numbers on the graph represent distances offshore (km).

3.4 Discussion

3.4.1 Large-scale patterns in SPM

The temporal consistency of the observed nearshore to offshore depletions confirms the existence of a constant $\delta^{13}C$ gradient at kilometer scales when moving from nearshore to offshore water. Offshore (10km) signatures (average -19.88‰) fell into the $\delta^{13}C$ range previously reported for oceanic phytoplankton (Sherr 1982; Rau *et al*. 1982; Fry & Sherr 1984; Monterio *et al*. 1991), and appear to be typical of Agulhas Current water as the gradient extends a great distance from the coast; as far as 50km offshore. Similar spatial patterns have also been shown within estuarine ecosystems (Deegan & Garritt 1997), exhibiting strong environmental gradients that may influence food availability within both estuarine and coastal systems. This implies a shift from a nearshore carbon supply to one more representative of an offshore pelagic carbon source in surface water. It was not possible to link these $\delta^{13}C$ values directly to distinct sources of production but the nearshore (0km) $\delta^{13}C$ ratios fell within a range of values previously reported for macroalgae (Smith & Epstein 1971; Bustamante & Branch 1996), and most likely represents inshore primary production. Nearshore water consists of a mixture of phytoplankton, organic and inorganic debris (Stuart 1982; Cranford & Grant 1990; Bustamante & Branch 1996; Levinton *et al*. 2002) and is likely to be dominated by macroalgal detritus (Mann 1988; Duggins *et al*. 1989; Bustamante & Branch 1996; Vizzini *et al*. 2002). Although nearshore carbon signatures also fell within the range of macroalgal values found at Kenton-on-Sea in this study, the two could not be directly linked. This is presumably because SPM samples represent a mixture of phytoplankton plus detritus from different algal species that have signatures modified by degredation and that occur in unknown proportions. However, the applied two-source mixing model showed that macroalgae accounted for over 50% of nearshore SPM in 10 out of 14 months, relative to offshore phytoplankton.

Temporal patterns in carbon enrichment were seen at Kenton-on-Sea during late austral autumn and winter with protrusions of $\delta^{13}C$ enriched nearshore SPM extending up to 2km offshore in both June - August 2004 and May 2005. As the values ranged between -12.88 ± 0.44 and -16.44 ± 0.74‰ and the $\delta^{13}C$ of outgoing water from the Kariega mouth averaged -15.80 ± 0.50 ‰, neither riverine or terrestrial sources can be

discarded as possible contributing sources of carbon. The highly enriched nature of nearshore $\delta^{13}C$ values and the absence of significant correlations with water temperature, rainfall, wind speed or wind direction however, suggest that these seasonal increases in nearshore carbon values are unrelated to terrigenous inputs or pelagic primary production. Local macroalgal communities show strong seasonality in both species biomass, and their biochemical composition (McQuaid 1985a, 1985b) and changes in nearshore SPM signatures presumably reflect changes in the pool of macroalgal detritus.

Monthly (MODIS) satellite photos of SST allowed the determination of the position of the inshore edge of the Agulhas Current (the Agulhas Front) and its distance offshore at Kenton-on-Sea on each sampling occasion, but there was no correlation between the position of the Agulhas Front and SPM values. Brown (1992) demonstrated a cyclical pattern of primary productivity in this part of the southeastern Indian Ocean, using chl-a concentrations as an estimate of phytoplankton biomass. She showed increased phytoplankton production in spring and autumn and decreased production in summer and winter. These seasonal peaks in primary production were also obvious from SeaWiFS satellite imagery for the 14 month sampling period.

The four months which exhibited projections of carbon enriched nearshore water away from the shoreline also showed nearshore primary production being drawn offshore and south-west along the coast in SeaWiFS satellite images, suggesting similar mechanisms of water movement. Although these projections may not be directly related to the position of the Agulhas Front, they are very likely to be related to hydrographic features inshore of the current. During three months there were incursions of offshore water as close as 3km from the shore (May, September 2004 and May 2005; Fig 3.1) and corresponding SeaWiFS satellite images show strong mixing between nearshore and offshore waters in May and September 2004 with offshore water coming particularly close inshore in May 2005. This indicates strong ties between coastal SPM $\delta^{13}C$ signatures and oceanic hydrography.

3.4.2 Small-scale patterns in SPM

Although there was consistent nearshore to offshore carbon depletion in SPM during every month, the slopes of the curves varied among months, implying temporal changes in nearshore SPM carbon. The investigation of nearshore (0km) SPM variation over 24

hour periods showed a cyclical pattern of $\delta^{13}C$ enrichment and depletion that was tightly correlated with the daily tidal cycle. SPM $\delta^{13}C$ was most depleted at low tides and gradually increased to be most enriched at high tides. The range of variability at the tidal scale was much smaller than at the seasonal scale, remaining within 2.0‰. This enrichment/depletion sequence is the opposite of that expected if offshore or estuarine water directly influenced the intertidal zone at high tide. During high tide, when riverine water is forced up the estuary by incoming seawater, nearshore SPM signatures did not reflect high tide estuarine signatures (-18.37 ± 0.14‰), but were more enriched with an average of -15.20 ± 0.25‰, conversely, at low tide when estuarine water (average -15.80 ± 0.50‰) empties into the sea, nearshore SPM values were depleted (average -17.59 ± 1.54‰), suggesting that terrigenous or riverine inputs are not a major contributor to nearshore SPM. These results are not surprising as both the Kariega and the Kowie Rivers are highly saline, impounded estuaries with minimal outputs to the sea, and although the strong flow regime of the Great Fish River contributes large freshwater outputs to the coastal zone which often extend south-west down the coastline (Vorwerk 2007), $\delta^{13}C$ signatures of SPM from the Great Fish river are extremely depleted (approx. -28.0; Froneman 2002). Such depleted carbon signatures clearly did not influencing nearshore water at Kenton-on-Sea. Instead the influence of the tidal cycle on isotopic signatures is believed to reflect the effects of detrital resuspension. For the purpose of comparison, the average isotopic values for mussel tissue over the 14 month period were added to the nearshore SPM graph over 24 hours, showing that the average carbon signature for mussel tissue falls just above the median of those for high and low tides, while the nitrogen signature is much more enriched. The huge differences in spatial scales and resolution between local nearshore measurements, 10km transect measurements and satellite SeaWiFS images introduces a large degree of uncertainty into the interpretation of results. In light of this, the clarity of temporal patterns in SPM at Kenton-on-Sea is remarkable and although, overall there is large variation in isotope signatures at both small (hourly) and large (inter-annual and inter-monthly) temporal scales, this variability appears to be strongly linked to coastal and oceanic hydrography.

3.4.3 Mussels and diet

Recent investigations of bivalve molluscs have shown that these suspension feeders respond to changing food conditions through preferential selection and ingestion of SPM (Newell *et al*. 1989; Ward *et al*. 1998; Milke & Ward 2003). Subsequently, I speculated that mussels with $\delta^{13}C$ signatures reflecting those of nearshore water either feed non-selectively or else preferentially select/assimilate macroalgal detritus, while if mussels demonstrated $\delta^{13}C$ signatures similar to offshore SPM, it could be explained through preferential selection of phytoplankton. During this study, carbon signatures of mussel tissue were more enriched than or similar to the values of nearshore SPM in 11 out of 14 months, while for three months mussel $\delta^{13}C$ ratios fell between those of nearshore and offshore SPM. The species composition of phytoplankton communities in surface waters can differ significantly between nearshore and offshore (Kaneta *et al*. 1985; Caldeira *et al*. 2001) suggesting a third alternative, that mussels may feed non-preferentially or passively on a diet of nearshore phytoplankton dominated by coastal diatom species. PERMANOVA and subsequent MDS of phytoplankton species composition showed dramatic differences between months, with October species composition at all stations significantly different from those in November. This strong temporal effect is likely related to available nutrients and hydrography and although the overall effect of distance was insignificant, *a posteriori* tests showed that within each month, distance offshore played a role in community composition. There were significantly different phytoplankton communities in nearshore (0km) water in both October and November and although offshore (10km) phytoplankton communities showed significant differences from other stations in November only, I suggest that overall, phytoplankton communities can be roughly classified into three approximate groups; nearshore (0km), mid-shore (0.25km - 4km) and offshore (10km) communities. Diatoms predominate in nutrient rich water and are replaced by flagellates after the majority of dissolved silicate is consumed (Tsunogai 1979; Tsunogai & Watanabe 1983). As the Indian Ocean on South Africa's east coast is notoriously nutrient poor (Lutjeharms 2005), we might expect the majority of phytoplankton to consist of flagellates. However, upwelling along the inshore edge of the Agulhas Current (Lutjeharms *et al*. 2000), the presence of localized upwelling cells around Port Alfred and Kenton-on-Sea (Lutjeharms 2005) and inputs from both the Kariega, Kowie and Fish River estuaries may provide sufficient nutrients to support

nearshore diatom communities. Kukert & Riebesell (1998) show distinct isotopic differences between size fractions of SPM, with diatom dominated samples (> 20µm) being 2‰ (up to 5‰ in bloom conditions) heavier than samples dominated by flagellates (< 20µm). Accordingly, I suggest that, while enriched nearshore SPM signatures are likely representative of inshore macroalgal detritus, they may also be partly due to diatom dominated phytoplankton, which are isotopically heavier than their flagellate counterparts seen in nutrient poor water, further offshore (10km).

In some cases, measurements of surface water may not be indicative of food availability to suspension feeders and although it is not unusual for coastal and estuarine waters to be vertically stratified with respect to nutrients and food, along the Eastern Cape, where the continental shelf widens offshore compared to farther North, strong currents, persistent winds and wave action are likely to result in a thoroughly mixed water column along the continental shelf as has been recorded for the west coast (Field *et al*. 1980).

$\delta^{13}C$ signatures of mussel tissue demonstrated a temporal pattern that could not be clearly correlated to season. With the exception of July 2004, carbon signatures seemed to be more depleted in spring (September/October) and autumn (April/May) when phytoplankton blooms are expected (Brown 1992), but the results of *post hoc* tests were not clear cut. Moreover, mussel and SPM signatures did not show consistent 1‰ carbon fractionation (DeNiro & Epstein 1978; Fry & Sherr 1984; Peterson & Howarth 1987). The ambiguity of this relationship illustrates the significance of integrated isotope assimilation in consumers, demonstrating that time integrated tissue does not reflect short term changes in $\delta^{13}C$, with important implications for food web studies. Isotopic ratios of primary producers are subject to more variation than higher trophic levels as variability in coastal hydrography may drive changes in nutrient sources and/or the isotope composition of the macroalgae themselves at short temporal scales (O'Reilly *et al*. 2002). Consequently, temporal integration must be considered when comparing samples which average isotopic signatures over different time periods (O'Reilly *et al*. 2002; Phillips & Eldridge 2006). The situation is complicated by the finding of Lorrain *et al*. (2002) that seasonal metabolic variations in consumers may cause isotopic changes in their composition that are unrelated to diet. As adductor muscle tissue has a lengthy turnover

time (see chapter 2), they are unlikely to reflect the instantaneous nature of monthly SPM measurements.

Clearly temporal variation occurs at different scales in consumers and SPM. Accordingly, the application of IsoSource modeling (Phillips & Gregg 2003) used SPM values averaged over all months to investigate the percentage contribution of nearshore organic carbon relative to offshore and estuarine sources to the $\delta^{13}C$ and $\delta^{15}N$ ratios of each mussel sample, allowing the assessment of the overall importance of nearshore SPM to mussel diet. In all but two months, mussels at Kenton-on-Sea showed more than 50% dependence on nearshore SPM for their organic carbon and nitrogen, demonstrating stronger ties to nearshore than offshore or estuarine carbon sources, and indicating a high degree of dependence on nearshore primary production.

3.4.4 Summary and conclusions

Sandy beaches often possess a well developed surf zone, with the combined intertidal/surf zone acting as a semi-autochthonous ecosystem driven largely by primary production by the surf zone diatom *Anaulus birostratus* (Brown & McLachlan 1990). Such systems support at least three (interstitial, microbial and macroscopic) food chain systems, and act as a self-contained ecosystem that provides an interface between oceanic and coastal communities. I propose that in the same fashion, the rocky intertidal zone represents a moderately self-sustaining ecosystem that typically exports materials to adjacent ecosystems, and is only poorly dependant upon offshore inputs. The factors affecting rocky shore primary production may however be influenced by local hydrographic processes that will affect nutrient availability. Although much work has been done on ecosystem regulation through top-down or bottom up controls (Menge 1992; Hunter & Price 1992; Menge 2000; Seitz & Lipcius 2001; Posey *et al*. 2006), current research has begun to recognize the importance of oceanographic processes in ecosystem dynamics (Menge *et al*. 1997; Menge *et al*. 1999; Menge *et al*. 2003; Blanchette *et al*. 2006). With strong $\delta^{13}C$ links between mussels and nearshore SPM, and overall $\delta^{13}C$ patterns of SPM at both small (hourly) and large (inter-annual and inter-monthly) temporal scales associated with coastal hydrography, I conclude that local hydrographic processes play a major role in the ecosystem dynamics of intertidal rocky shores.

CHAPTER 4: BIOGEOGRAPHIC AND NEARSHORE/OFFSHORE TRENDS IN ISOTOPE RATIOS OF INTERTIDAL MUSSELS AND THEIR FOOD SOURCES AROUND THE COAST OF SOUTHERN AFRICA.

4.1 Introduction

Ecosystem dynamics in rocky shore communities have long been a focus of attention to intertidal ecologists with respect to community structure and trophic level analysis on both a regional and a global scale. Early research primarily emphasized the effects of locally operating forces such as predation, disturbance and competition (Menge 1992), but more recent work has suggested the importance of large scale oceanographic factors (e.g. nutrient regimes and hydrodynamics) in shaping intertidal systems (Menge *et al*. 2003; Schiel 2004). Variation in coastal hydrography for example, plays an important role in intertidal community ecology. Nearshore conditions of coastal waters have significant effects on intertidal benthic organisms (Blanchette *et al*. 2006), and several studies have shown strong correlations between coastal oceanography and nearshore benthic community structure (e.g. Menge *et al*. 1999; Nielsen & Navarrete 2004). Because of biogeographic variation of nearshore hydrography, the influence of coastal water extends beyond the effects on recruitment rates that are a present focus of attention (Alexander & Roughgarden 1996; McQuaid & Lawrie 2005), and includes effects on the nature of the food available to primary consumers, especially suspension feeders. This coupling of oceanographic conditions and nearshore community structure has significant implications for the South African shoreline, which is influenced by both the Benguela and the Agulhas Current systems (McQuaid & Payne 1998), implying biogeographic differences in the food resources available to intertidal communities. The Agulhas Current, along the east and south coasts, brings warm, oligotrophic water from the Mozambique Channel (Lutjeharms *et al*. 2000), while the Benguela current flows northwards along the west coast bringing cold eutrophic water of Antarctic origins (Andrews & Hutchings 1980).

Broad geographic and possibly temporal differences therefore exist in the oceanography, primary production (Andrews & Hutchings 1980; Brown 1992) and macroalgal species that generate detritus (Bolton *et al*. 2004) along the South African

coastline, implying that intertidal filter feeders in particular may experience differences not only in the quantity, but also the quality of food available to them.

Stable isotope analysis has proven to be a powerful tool for resolving trophic relationships and several studies have shown the importance of biogeography in relation to isotope signatures. Rau *et al*. (1982) reported changes in plankton $\delta^{13}C$ values with latitude that were attributed to phytoplankton through either carbon fractionation or changes in biosynthesis and metabolism. Burton & Koch (1999) presented evidence that pinnepeds demonstrate latitudinal differences in collagen carbon values, indicating variation in lower trophic levels. Similarly, in other marine mammals, carbon isotope signatures vary with geographical position (Hobson *et al*. 2002), allowing the identification of movement over large latitudinal ranges, by identifying different sources of primary production (Burton *et al*. 2001). $\delta^{15}N$ has also been shown to change biogeographically; with phytoplankton showing highly depleted nitrogen ratios in oligotrophic relative to eutrophic waters (Waser *et al*. 2000) and zooplankton demonstrating nitrogenous enrichment with increasing exchange between euphotic zone and deeper water (Mullin *et al*. 1984). Despite these studies, there has been limited research on biogeographic variation in isotopic composition of marine consumers and their food, especially within intertidal benthic communities.

The aims of my study were to investigate the effects of biogeographic and temporal variation in coastal hydrography on food resources of intertidal mussels along the South African coastline by means of stable isotope analysis, examining the $\delta^{13}C$ and $\delta^{15}N$ ratios from suspended particulate matter (SPM), mussels and macroalgae. These were analyzed to establish: (1) if the nearshore to offshore isotopic gradients seen in chapter 3 vary on a biogeographic and/or seasonal scale and if they can be linked to distinct sources of production (2) if the isotopic signatures of intertidal consumers and producers vary biogeographically and/or seasonally and (3) the relative importance of pelagic primary production (i.e. phytoplankton) versus coastal detritus in the diet of intertidal mussels.

4.2 Methods

4.2.1 Sample collection

SPM samples were obtained from five-liter surface water samples taken at twelve rocky shore sites along the southern African coastline, from Walvis Bay in Namibia to Sodwana Bay, near the Moçambique border (Fig 4.1), in austral summer Jan. - Feb. 2002, Nov. - Dec. 2003, and austral winter Jun. - Jul. 2004. At each site, duplicate water samples were collected along a transect running perpendicular to the coast at distances of 0, 0.25, 0.5, 1, 2, 4 and 10km (offshore). In 2002 samples were collected at only five sites, the Port Nolloth transect was collected once in Jun. - Jul. 2004 and the Walvis Bay transect was collected once in Nov. - Dec. 2003. Due to logistic constraints, only two of the samples collected were between zero and ten kilometres at Walvis Bay. Five mussels (either *Perna perna* or *Mytilus galloprovincialis* depending on the site) and samples of the most abundant macroalgae species (n = 2 – 8 spp. depending on availability) were collected at each of the SPM transect sites and two additional sites: Zinkwaze Beach and St. Helena Bay (Fig 4.1).

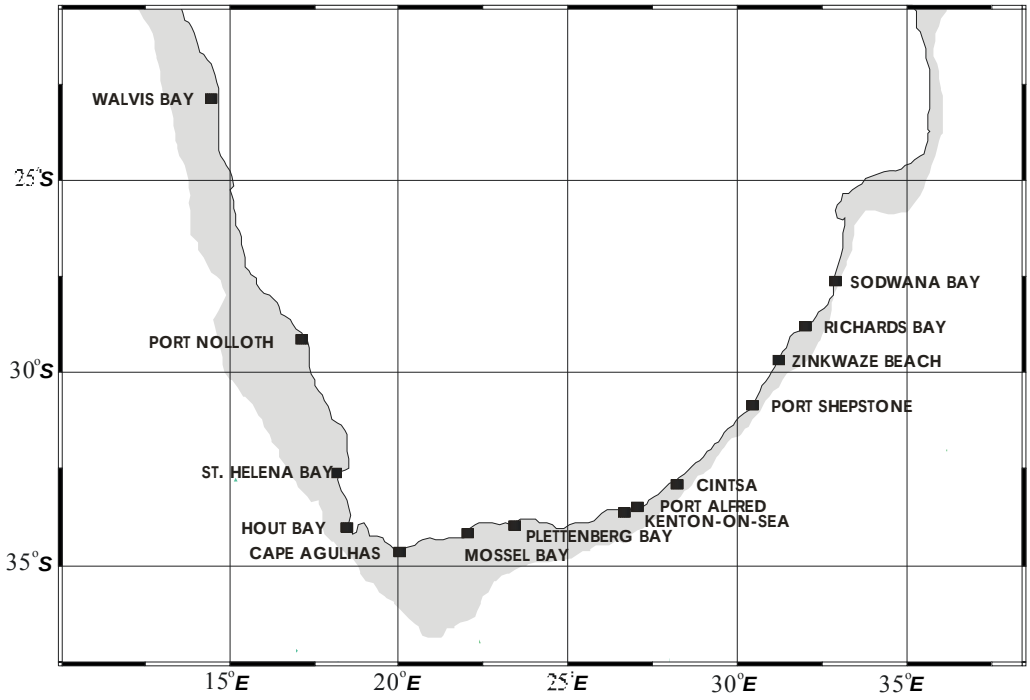

Figure 4.1: Map of southern Africa, showing sample sites along the coastline, shaded area = continental shelf (≤ 500m depth).

Mytilus galloprovincialis is an invasive exotic species found on the west coast and rare on the east coast, while *Perna perna* is an indigenous species missing from the Benguela region. Because different mussel species occur around the coast, fifteen individuals of each species were collected from the same shore and intertidal zone, near Port Alfred, where the two co-occur. These animals were analyzed to test for interspecific differences in $\delta^{13}C$ and $\delta^{15}N$ signatures. T-tests showed no significant difference between mean isotopic carbon ($p = 0.09$) or nitrogen ($p = 0.125$) values between species (power of test was 0.99).

4.2.2 Sample Preparation

Water samples were filtered through pre-combusted (500°C, 6hrs) GF/F Whatman® filters (0.45µm pore size), using a vacuum pump (≤ 4cm Hg) and then oven dried at 60°C for 24hrs. Zooplankton and other large particles were manually removed under a dissecting microscope at 16x magnification. Mussel adductor muscle tissue was removed,

rinsed in distilled water (dH_20) and oven dried (60°C, 48hrs). Adductor muscle tissue was used in this study as muscle tissue has low turnover rates (chapter 2; Gorokhova & Hansson 1999) and is therefore representative of a time-integrated diet. All macroalgae were rinsed in dH_20, visible epiphytes were removed and algae were also oven dried (60°C, 48hrs). Two replicates of each macroalgal sample were prepared for analysis. As mussel adductor tissue has minimal lipid content (Tieszen *et al*. 1983; Soudant *et al*. 1999; Chu *et al*. 2000), no lipid extractions were performed on any tissue.

4.2.3 Isotopic Analysis

All isotopic analyses were completed as described in Chapter 2.

4.2.4 Data Analysis

$\delta^{13}C$ and $\delta^{15}N$ mussel signatures were analyzed using a k-means cluster analysis based on Euclidian distances and validated using a discriminant function analysis (DFA). All analyses were performed using Statistica v7 (StatSoft Inc. 2004). Carbon and nitrogen contour graphs were created using Ocean Data View 3 (Schlitzer; available at www.awi-bremerhaven.de/GEO/ODV). IsoSource modeling (Phillips & Gregg 2003) as used in chapter 3 requires a minimum of three sources for a 2 element analysis, consequently the two-source linear mixing model (chapter 3; Bustamante & Branch 1996) was used to determine the percentage contribution of organic carbon and nitrogen to mussel diet by nearshore vs. offshore SPM:

$$\% \text{ Isotope} = [(d_{\text{mussel}} - d_{\text{offshore}} - I) / (d_{\text{nearshore}} - d_{\text{offshore}})] \times 100$$

where I is the average fractionation of $\delta^{13}C$ or $\delta^{15}N$ per trophic level and d is the isotopic ratio of the sample (see Fig 4.10A for nearshore, offshore and mussel isotopic ratios at all sites). These mixing models assumed an average $\delta^{13}C$ fractionation value of 1‰ (DeNiro & Epstein 1978; Fry & Sherr 1984; Peterson & Howarth 1987), and a $\delta^{15}N$ trophic correction of 1.7‰ (Hansson *et al*. 1991; Raikow & Hamilton 2001; Moore & Suthers 2005).

4.3 Results

4.3.1 SPM

Suspended particulate matter (SPM) showed highly enriched $\delta^{15}N$ ratios (>10.0‰) between zero and four kilometres at Port Alfred and Mossel Bay in 2003, but otherwise lacked clear geographic, spatial or temporal trends (Figs 4.2A & Fig 4.2B).

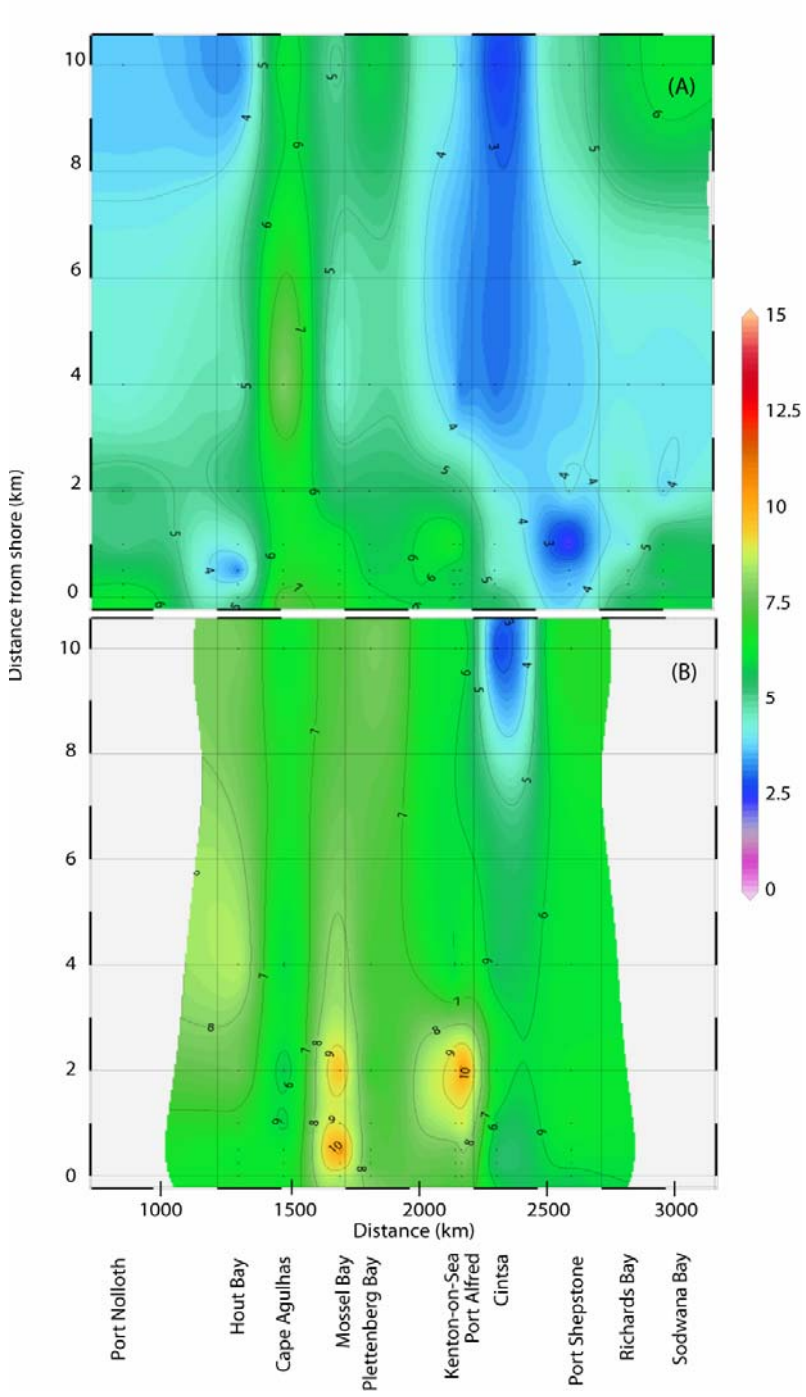

Figure 4.2: Contour plot of $\delta^{15}N$ (‰) signatures from SPM transects in winter 2004 (A) and summer 2003 (B). Graph excludes Walvis Bay as only two points in the transect fell between 0 and 10km. Distance is in km along the coast from Walvis Bay, Namibia. No data were collected for Port Nolloth or Sodwana Bay in 2003.

In contrast, $\delta^{13}C$ signatures exhibited spatial trends of depletion from nearshore to offshore water along the entire coastline (Walvis Bay is excluded as only 2 samples fell between the zero and ten kilometer range). Five sites displayed clear depletion patterns in all years (Port Shepstone, Kenton-on-Sea, Mossel Bay, Cape Agulhas and Port Nolloth; Figs 4.3A-B & Fig 4.5A-B). Three sites showed patterns that were less clear but conformed to the general depletion trend in at least one of three years (Richards Bay, Plettenberg Bay, and Hout Bay; Fig 4.3C-D). Finally, three sites demonstrated enrichments on some occasions between 4 and 10km offshore (Sodwana Bay, Cintsa and Port Alfred; Fig 4.4).

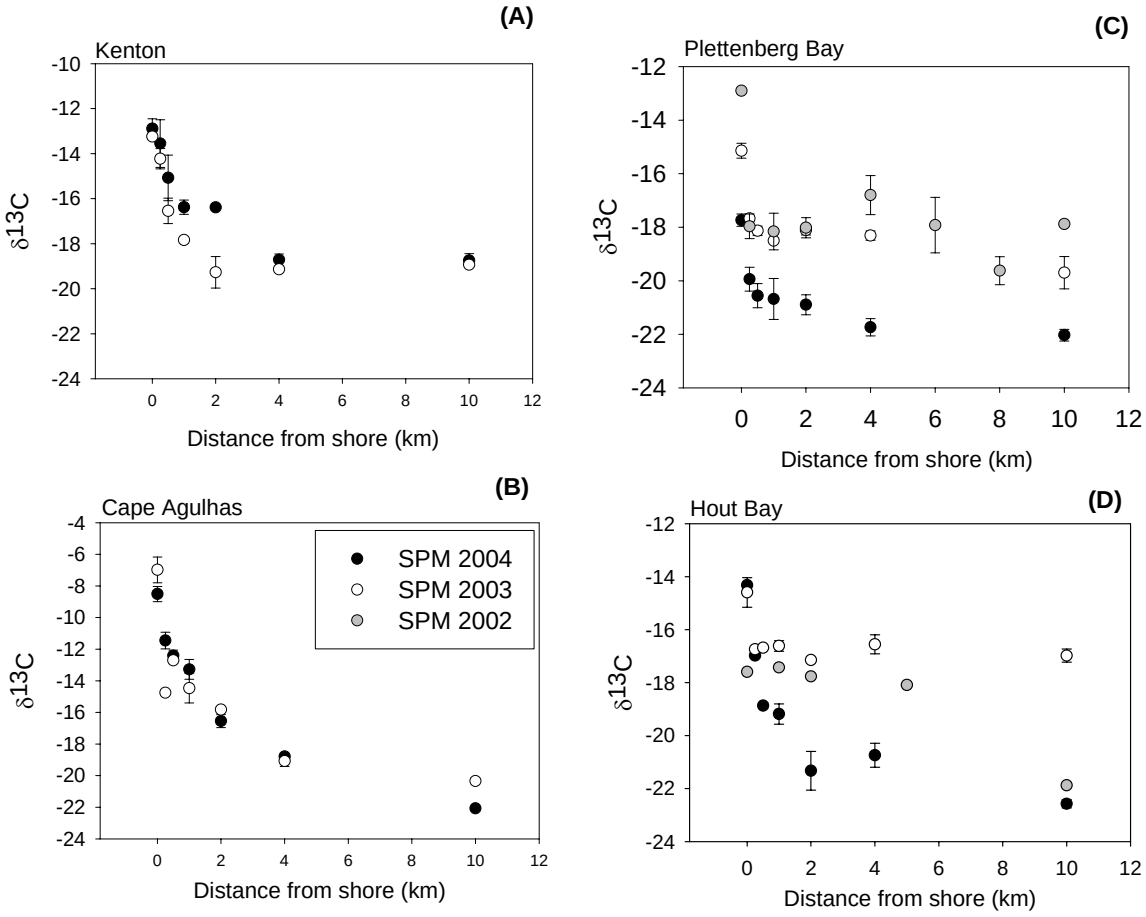

Figure 4.3: Examples of nearshore to offshore $\delta^{13}C$ (‰) gradients from sample sites along the coastline showing clear (A & B) and weaker (C & D) depletion trends. Values are means ± SD.

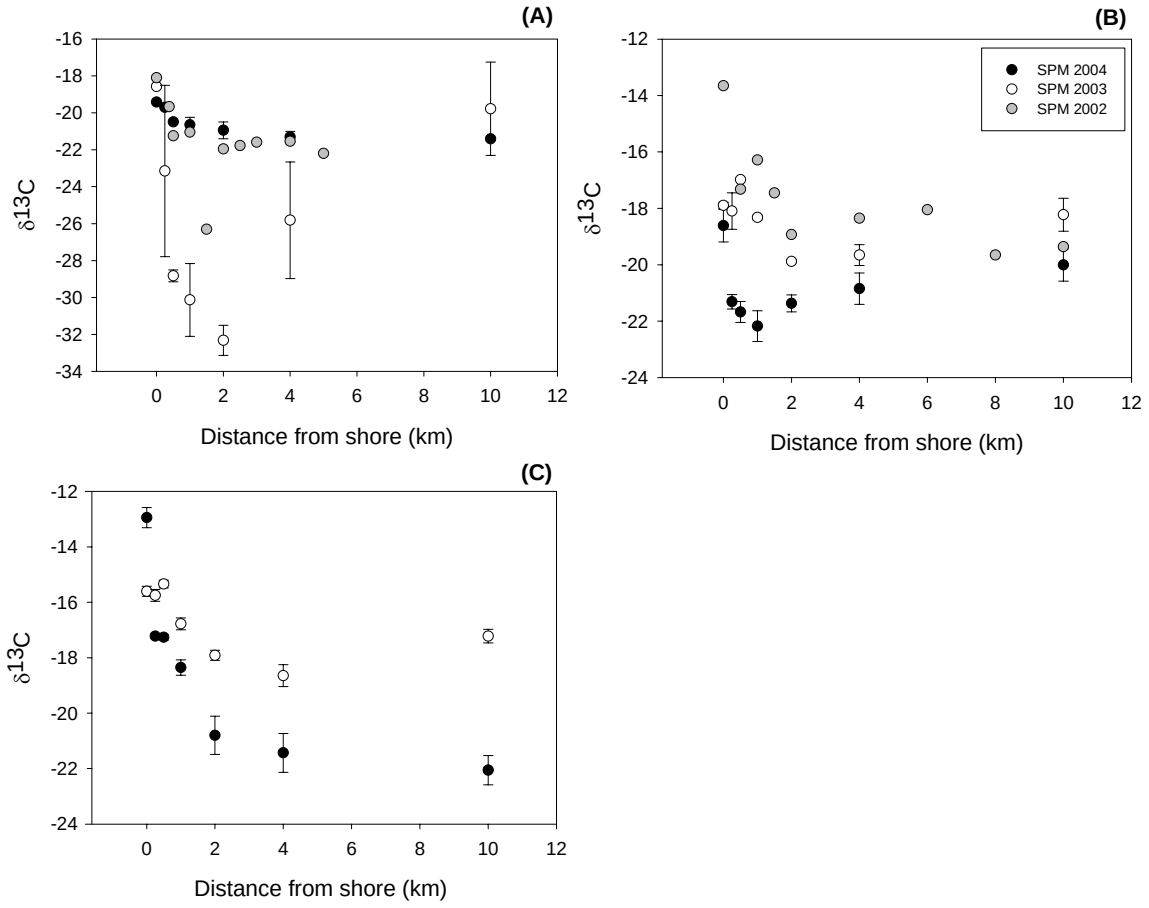

Figure 4.4: Three deviations from the overall nearshore to offshore $\delta^{13}C$ (‰) trends; (A) Sodwana Bay; (B) Cintsa; (C) Port Alfred. Values are means ± SD.

Values for nearshore (intertidal, 0km) water ranged from highly enriched carbon values (mean ± SD) of -6.99 ± 0.81‰ (Cape Agulhas) to much more depleted values of -19.4 ± 0.01‰ (Sodwana Bay), with an average of -15.4 ± 3.06‰, while offshore (10km) waters displayed less variation, ranging between -16.98 ± 0.25‰ (Hout Bay) and -23.58 ± 0.09‰ (Port Nolloth), with an average of -20.36 ± 1.84‰. $\delta^{13}C$ depletion curves were more enriched at five sites (Richards Bay, Cintsa, Port Alfred, Plettenberg Bay and Hout Bay) in 2003 than in other years, ranging from a 2-4‰ increase depending on site and sample, suggesting overall enrichment in austral summer, but with no obvious geographic

trends. Overall patterns in $\delta^{13}C$ for all sites in 2003 - 2004 are represented in figs 4.5A and 4.5B.

Along the coastline, six sites (Sodwana Bay, Richards Bay, Cintsa, Plettenberg Bay, Mossel Bay, Port Nolloth) showed nearshore SPM $\delta^{13}C$ values lower than the midpoint between nearshore and offshore signatures (< -17.88‰). The remaining five sites showed nearshore SPM values higher than the midpoint (> -17.88‰) and these pockets of enriched nearshore SPM extended out to a maximum of 2km from the shoreline (Figs 4.5A & Fig 4.5B).

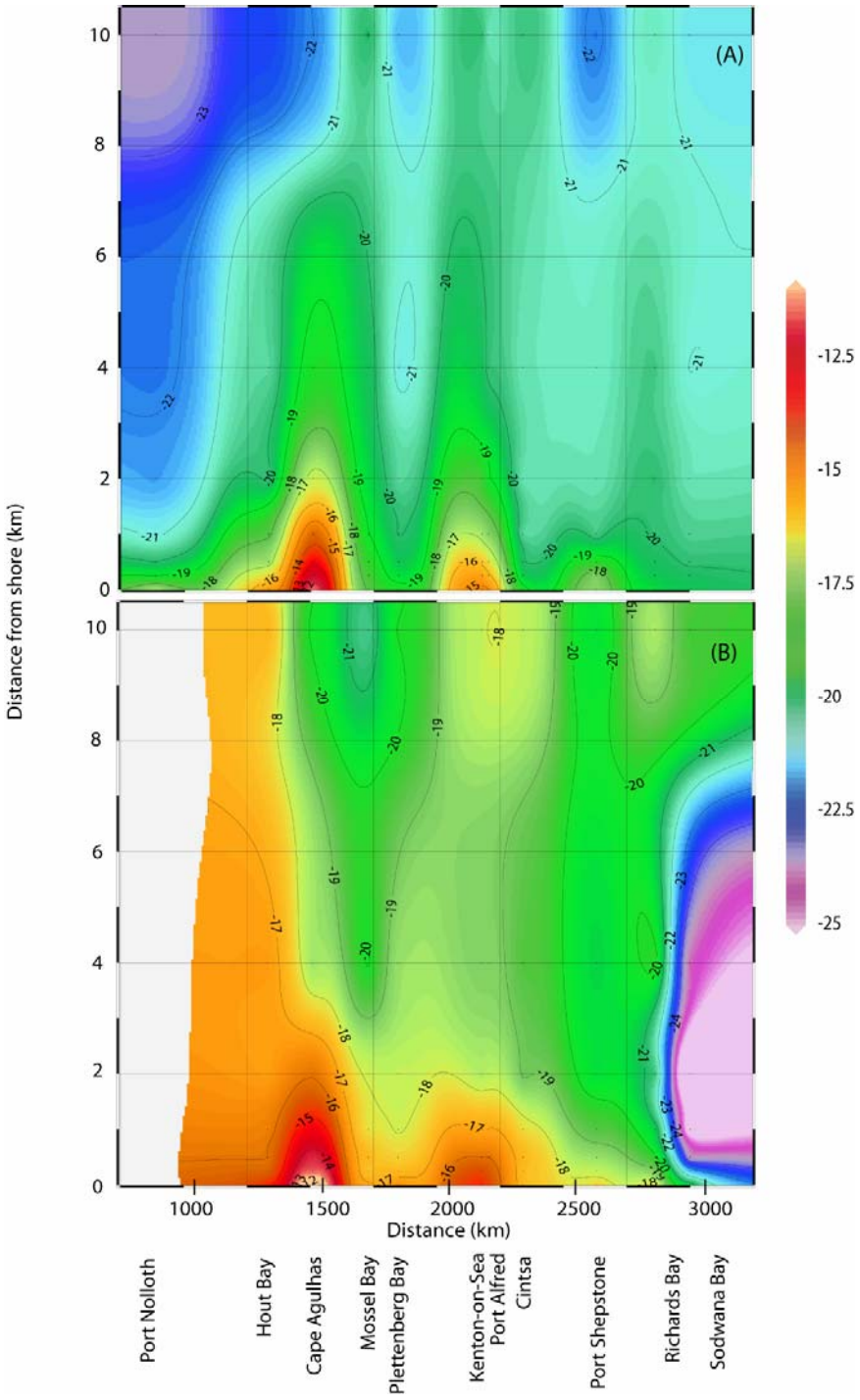

Figure 4.5: Contour plot of $\delta^{13}C$ (‰) signatures from SPM transects in winter 2004 (A) and summer 2003 (B). Graph excludes Walvis Bay as only two points in the transect fell between 0 and 10km). Distance is in km along the coast from Walvis Bay, Namibia. No data were collected for Port Nolloth in 2003.

Nearshore SPM demonstrated an overall geographic enrichment in $\delta^{13}C$ from the east coast (Sodwana Bay) with a mean $\delta^{13}C$ of -18.7 ± 0.67‰, to the south coast (Cape Agulhas) with a mean $\delta^{13}C$ of -7.8 ± 1.08‰, while offshore water remained relatively constant at an average of -20.36‰ ± 1.84‰ (Fig 4.6).

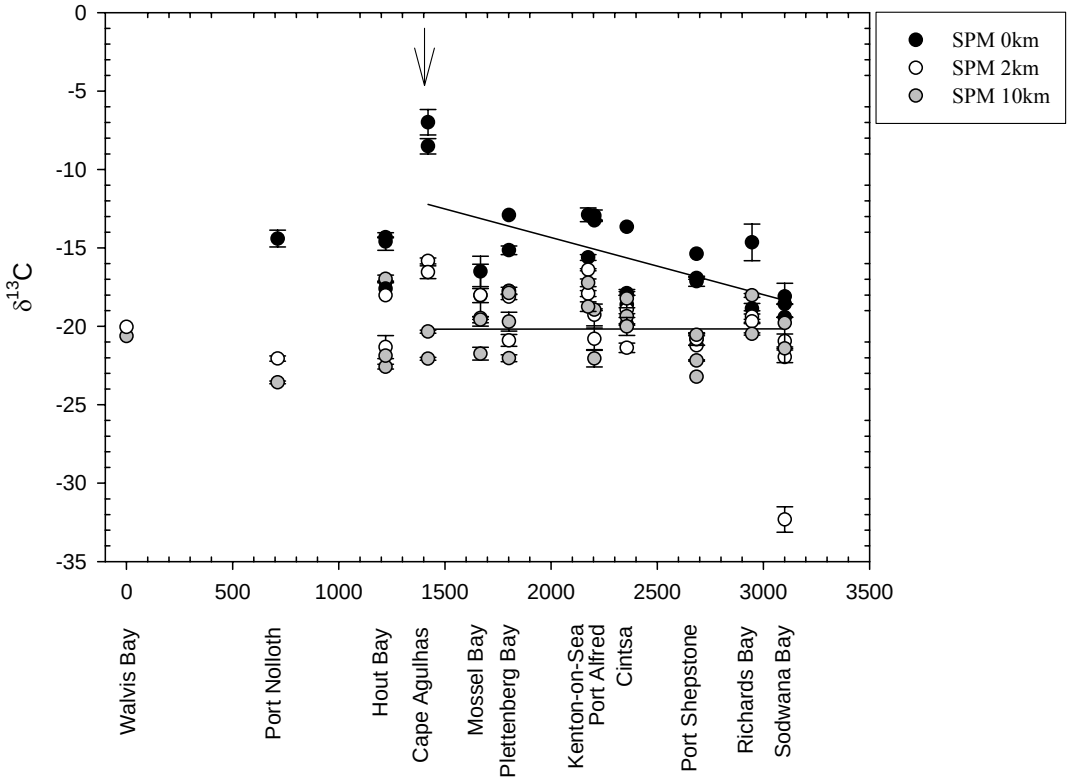

Figure 4.6: $\delta^{13}C$ (‰) of SPM along the coastline, regression excludes values to the west of Cape Agulhas, i.e. to the left of the arrow. Offshore regression: $y = 1^{-05x} - 20.2$, $r^2 = 2^{-05}$, inshore regression: $y = -0.0038x - 6.7763$, $r^2 = 0.3954$. Values are means ± SD and are presented separately for each year.

4.3.2 Macroalgae

Macroalgal signatures showed no consistent geographic or temporal trends in either $\delta^{13}C$ or $\delta^{15}N$, with erratic enrichments and depletions in consecutive years, and most macroalgae showed large ranges in both carbon and nitrogen (Fig 4.7). Both *Ulva* sp. and *Gelidium pristoides,* for example, demonstrated large scale differences along the coastline ranging between $\delta^{13}C$ values of -10.89 to -17.83‰ and -11.49 to -15.66‰ and $\delta^{15}N$ values of 4.39 to 7.20‰ and 5.85 to 7.69‰ respectively. Consequently macroalgal isotopic ratios could not be related to either mussel diet or nearshore SPM.

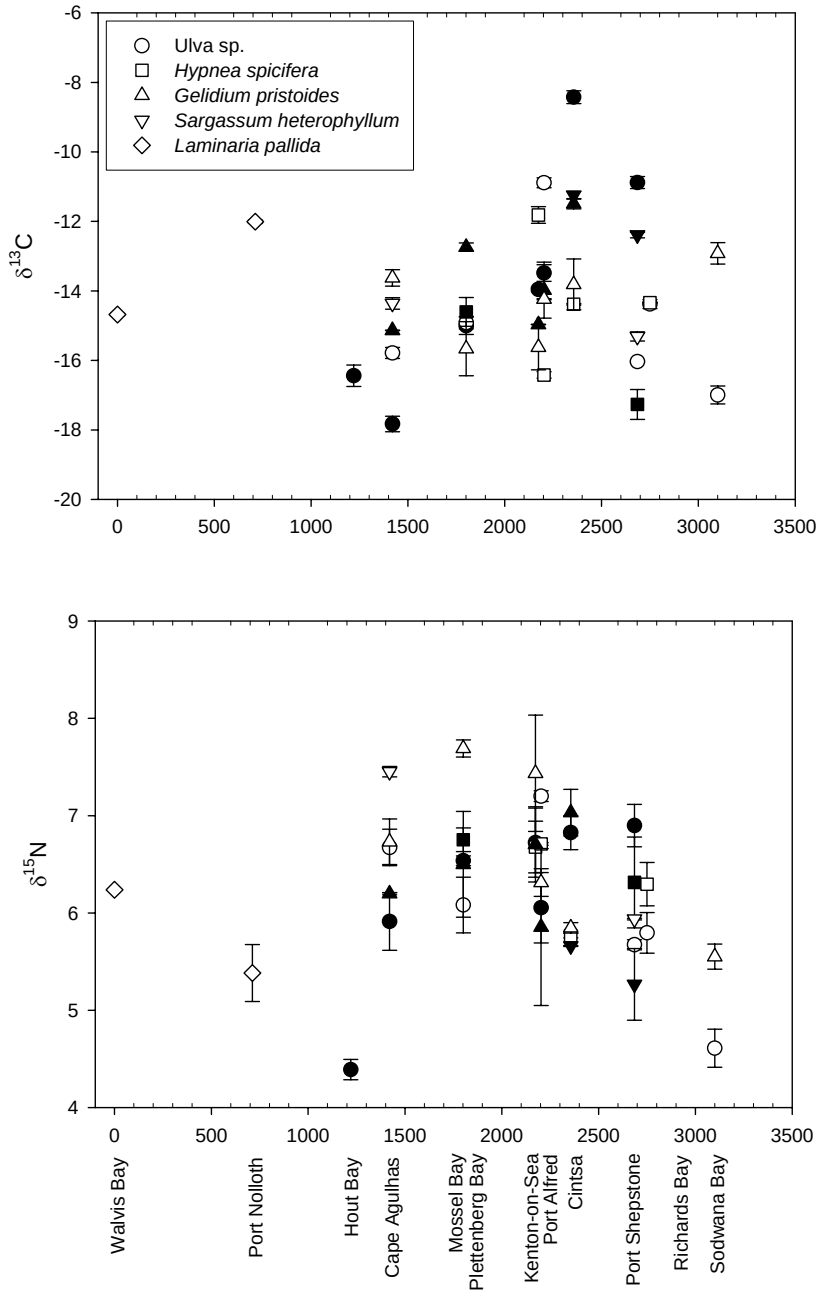

Figure 4.7: $\delta^{13}C$ (A) and $\delta^{15}N$ (B) ratios (‰) for 5 species of macroalgae along the southern African coastline. Black symbols are samples from 2003; white symbols are samples from 2004.

4.3.3 Mussels

Although many sites showed considerable variation in $\delta^{13}C$ values of mussel tissue among sampling occasions, there were no consistent seasonal or interannual trends among sites. Despite this temporal variability within sites, there were distinct geographic patterns, allowing the k-means cluster analysis to classify the mussels into four isotopic groups that represent animals collected from the east, south-east, south-west and west coasts (Fig 4.8). Subsequent DFA showed that these clusters could be identified by their signatures alone with 97-98% accuracy. The east coast group (group A) was characterized by low $\delta^{13}C$ and $\delta^{15}N$ values (-17.63‰ to -16.56‰; 6.31 to 7.78‰), the south-east coast group (group B) by intermediate $\delta^{13}C$ and $\delta^{15}N$ values (-16.19 to -15.37‰; 6.73 to 8.17‰) and the south-west (group C) and west coasts (group D) (-15.85 to –14.45‰; 8.35 to 9.033 and -17.50 to -16.78‰; 8.85 to 9.6‰ respectively) by their high $\delta^{15}N$ values (Fig 4.8). Two exceptions were site eleven (S11: Hout Bay), which fell into the south-east grouping in all three years, and site five (S5: Cintsa) which was grouped with group A in 2002 and 2003, but with group B during 2004. From the east to the south coast, both mussel carbon ($r^2 = 0.71$, $p < 0.01$) and nitrogen ($r^2 = 0.69$, $p < 0.01$) ratios demonstrated enrichment (excluding all sites west of Cape Agulhas) (Fig 4.9). Although it was not possible to associate near/offshore carbon values with distinct sources of production, links between isotopic values of nearshore water and macroalgae, and offshore water and phytoplankton were found. The linear mixing model revealed that mussels demonstrated more than 50% dependence on nearshore water for their organic carbon and nitrogen, at eight and ten out of eleven sites respectively. Two locations in carbon (Cintsa and Hout Bay) and five locations in nitrogen (Cape Agulhas, Mossel Bay, Plettenberg Bay, Cintsa and Sodwana Bay) showed temporal variation (Fig 4.10).

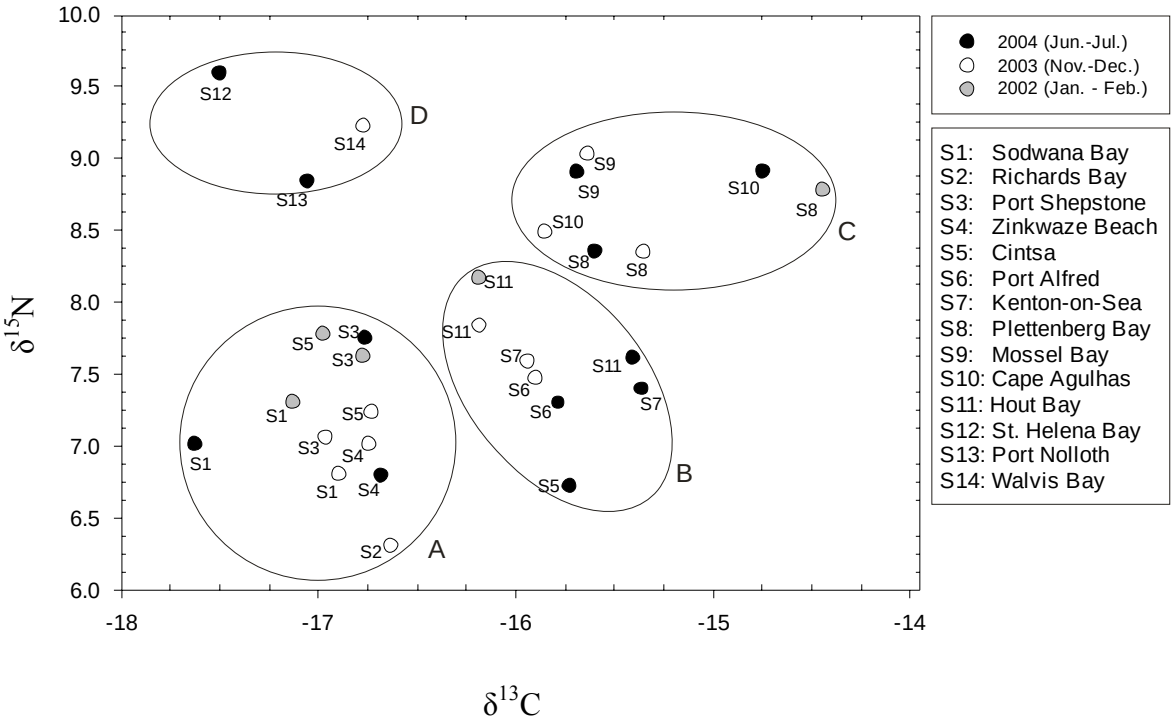

Figure 4.8: $\delta^{13}C$ and $\delta^{15}N$ signatures (‰) of intertidal mussels. Groupings according to k-means cluster analysis: (A) east coast, (B) south-east coast, (C) south-west coast, (D) west coast.

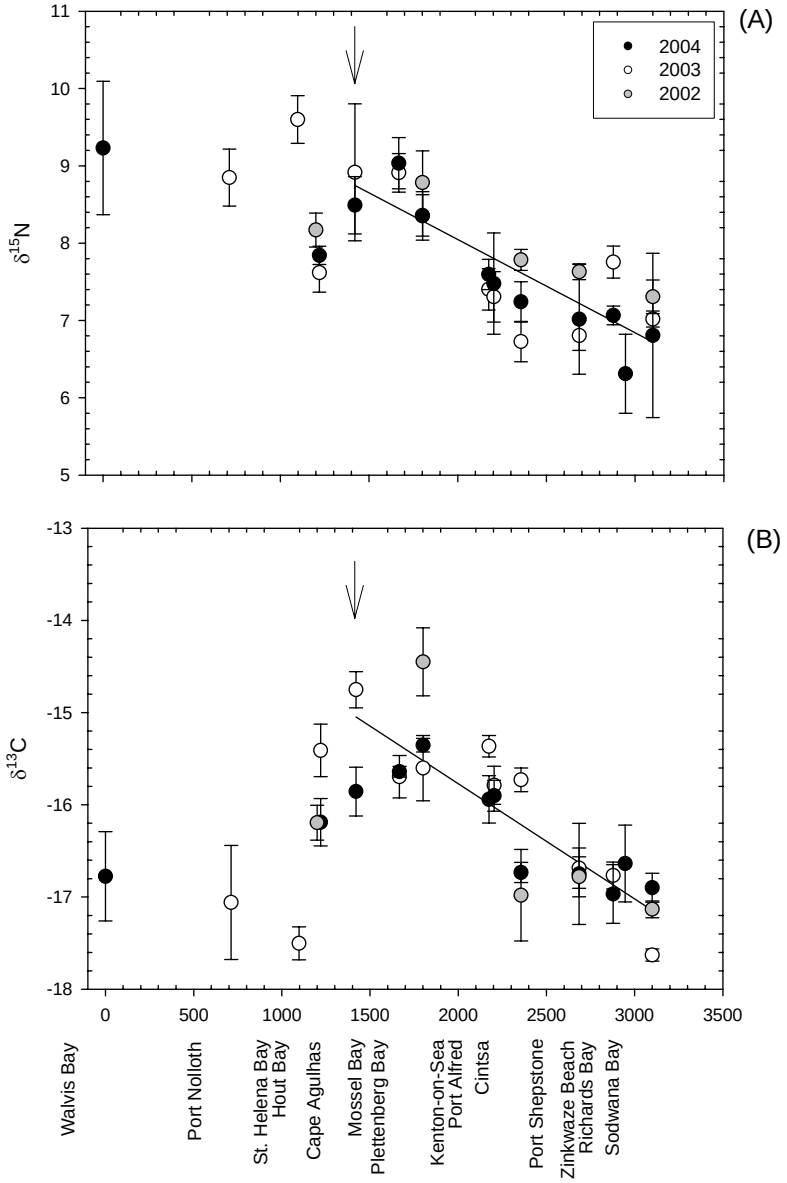

Figure 4.9: $\delta^{15}N$ (A) and $\delta^{13}C$ (B) geographic enrichment (‰) of intertidal mussels. Nitrogen regression: $y = 10.4594 - 0.0012*x$, $r^2 = 0.69$, $p < 0.01$. Carbon regression: $y = -13.2726 - 0.0012*x$, $r^2 = 0.71$, $p < 0.01$. Regressions exclude values to the west of Cape Agulhas, i.e. to the left of the arrows. Values are means ± SD.

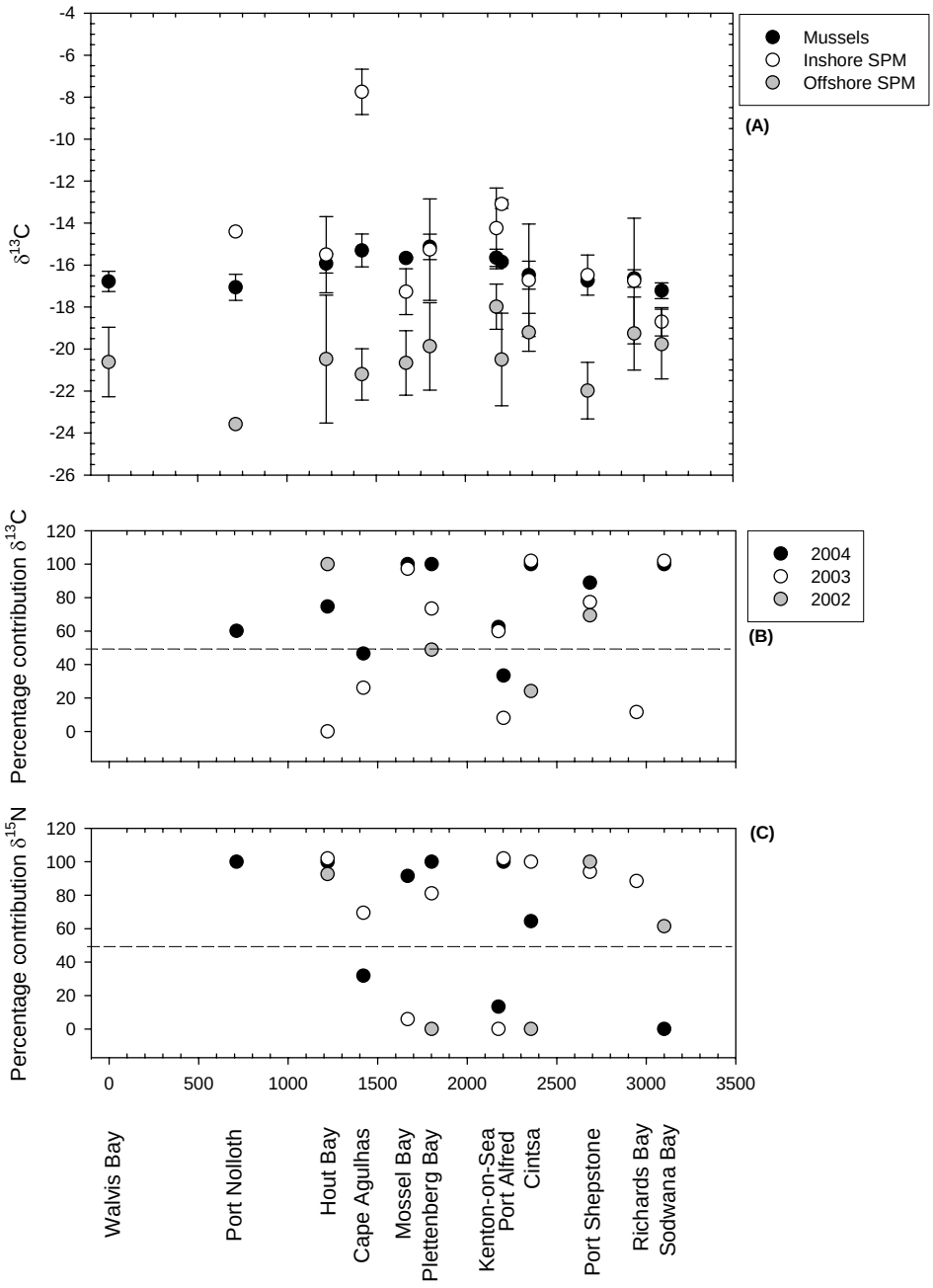

Figure 4.10: (A) Isotopic (‰) relationships between mussels, nearshore and offshore SPM for 2002 to 2004; percentage $\delta^{13}C$ (B) and $\delta^{15}N$ (C) contribution of nearshore SPM to mussel diet.

4.4 Discussion

4.4.1 Spatial and biogeographic gradients

Allowing for processing of material, spatial patterns in carbon signatures ultimately reflect patterns in the distribution or signatures of primary producers, which in turn are likely to reflect environmental gradients and recent investigations have shown variation in carbon isotopes on several physical scales. These include spatial patterns within estuarine ecosystems (Deegan & Garritt 1997), which exhibit very strong environmental gradients, and global latitudinal trends in pinnepeds (Burton & Koch 1999; Hobson *et al*. 2002) and squid (Takai *et al*. 2000). In this study we explored trends in carbon signature along two gradients. The first was a nearshore/offshore gradient likely to reflect a gradient of decreasing macrophyte influence; the second was a biogeographic gradient that reflects the influence of two very different oceanographic regimes, rather than a latitudinal effect.

4.4.1.1 Nearshore/offshore gradients

With few exceptions, $\delta^{13}C$ signatures of SPM became depleted with distance from shore. This trend of depletion suggests a shift from an inshore carbon supply to one more representative of a pelagic carbon source. Although it was not possible to link these $\delta^{13}C$ values directly to distinct sources of production, the nearshore (0km) signatures, with an average of 15.4‰, fell into the range of carbon values previously reported for macroalgae (Smith & Epstein 1971; Bustamante & Branch 1996), and most likely represents inshore primary production (chapter 3, Hill *et al*. 2006). Although nearshore carbon values fell within the range of algal signatures found along the coast in this study, the two could not be directly related to one another. Again this is not surprising, as SPM samples are unlikely to have had the same algal composition as the seaweeds subsampled from the intertidal zone. Offshore (10km) signatures (average -20.36‰) fell into the $\delta^{13}C$ range previously reported for oceanic phytoplankton (Sherr 1982; Rau *et al*. 1982; Fry & Sherr 1984; Monterio *et al*. 1991) and appear to be typical of pelagic production, as carbon signatures at Kenton-on-Sea remain in the range of -19.00 to -21.5‰ as far as 50km offshore (see chapter 3). Chapter 3 also showed temporally consistent nearshore to offshore depletions in carbon, establishing the existence of a constant $\delta^{13}C$ gradient at

kilometer scales when moving from nearshore to offshore at Kenton-on-Sea. These data confirm that such trends not only consistent in time, but are ubiquitous along the coastline of southern Africa and imply a shift from a nearshore carbon supply to one more representative of an offshore pelagic carbon source in surface water (chapter 3, Hill *et al*. 2006). This nearshore to offshore pattern of depletion seen in SPM may permeate higher trophic levels, as a study done by Cherel & Hobson (2007) reported a relationship between enrichment in $\delta^{13}C$ signatures in penguin blood and foraging areas, with inshore foraging species > offshore foragers > polar front foragers.

Three of the twelve sites showed deviations from the general depletion trend (Fig 4.3) with Port Alfred showing slight offshore (10km) enrichment in 2003 and Cintsa showing enrichment from 2km offshore in both 2003 and 2004. These elevated carbon signatures may be representative of localized upwelling resulting in small algal blooms, where increased growth rates subsequently increase $\delta^{13}C$ (Burkhardt *et al*. 1999a). The third deviation was in 2003 at Sodwana Bay, where highly depleted SPM values (-32.0‰) were seen at 2km, eventually reaching a more enriched value of -20.0‰ by 10km offshore. These depleted $\delta^{13}C$ signatures exceed reported values for freshwater/estuarine inputs (Haines & Montague 1979; Reira & Richard 1997) and may be attributable to mixing of SPM from coral reefs or subterranean canyons, both of which are found as close as 6 km from the Sodwana Bay coast (Roberts *et al*. 2006).

Although nearshore to offshore depletion was ubiquitous around the coast, the slopes of the curves varied among sites and years, suggesting not only geographic but also temporal effects on offshore depletion. Previous studies on phytoplankton (a main component of offshore SPM) show that photoperiod, temperature, light intensity and nutrient supply all serve to increase growth rates (Goericke *et al*. 1994; Laws *et al*. 1995; Rau *et al*. 1996; Burkhardt *et al*. 1999a), and hence the $\delta^{13}C$ signature of SPM. Studies investigating phytoplankton biomass in the southwest Indian Ocean using measurements of chl-a have shown increased primary production and $\delta^{13}C$ of SPM in austral winter (Falkowski *et al*. 1998; Machu *et al*. 2005). Our transects started much farther inshore than these studies, overlapping marginally offshore, but showed austral summer (Jan. - Feb. 2002 and Nov. - Dec. 2003) depletion curves that were either more enriched, or in four cases, similar to carbon values found in winter (Jun. - Jul. 2004) suggesting a general tendency for SPM to be more enriched in austral summer than in winter. Unless seasonal

differences exist in phytoplankton species composition of SPM, it is likely that the observed $\delta^{13}C$ enrichment during austral summer was due to local effects, rather than seasonal patterns in oceanic carbon. Variation in position and meandering of the Agulhas Current (Goschen & Schumann 1990), extent of mixing between near and offshore waters and changes in local autochthonous or allochthonous inputs on a regional scale may have a large effect on SPM composition (Megens *et al*. 2001), changing $\delta^{13}C$ signatures on a daily basis.

$\delta^{13}C$ SPM inflection points, where nearshore signatures changed to a more pelagic one, generally occurred between 500m and 1km but occurred further offshore at some sites (e.g. Hout Bay, Cape Agulhas, Kenton-on-Sea, Port Alfred and Port Shepstone; Figs 4.5A & 4.5B). In both 2003 and 2004 Hout Bay/Cape Agulhas and Kenton-on-Sea/Port Alfred showed pockets of enriched nearshore SPM extending out to a maximum of 2km from the shoreline and signatures became typically pelagic (-20.0 to -21.5‰) only at 10km. Samples taken in 2004, at these four sites, were taken during outgoing tides, while in 2003 samples were taken on incoming tides and hence tidal effects may account for the difference in distances that nearshore enriched SPM was seen offshore. Port Shepstone showed a similar peak in $\delta^{13}C$, but on a much smaller scale with both 2003 and 2004 samples taken on an incoming tide. These data indicate that, while water with a pelagic signature may often come close inshore to introduce offshore organic carbon to the intertidal zone at five sites along the coastline, the reverse may also occur, with nearshore water extending to fairly significant distances offshore. For example, Koop *et al*. (1982), working at Oudekraal Bay, in the Cape of Good Hope, showed that mannitol-fermenting bacteria derived from kelp beds within 1-200m of the shore could be found as far as 10km offshore. The Hout Bay/Cape Agulhas and Kenton-on-Sea/Port Alfred extensions of enriched nearshore $\delta^{13}C$ values may represent a forcing of inshore water out to sea, due to wind pressures, circulation and positional patterns of the Agulhas Current (Goschen & Schumann 1990; Boyd & Shillington 1994), and in the case of Kenton-on-Sea/Port Alfred, potential dynamic upwelling (Lutjeharms *et al*. 2000). The smaller extension of carbon enriched water at Port Shepstone may be attributable to a large meander of the Agulhas Current in KwaZulu Natal called the Natal Pulse (Lutjeharms & Roberts 1988; Roberts 2005), which may result in reduced mixing between nearshore and offshore water. As SPM values represent instantaneous measurements of $\delta^{13}C$ however,

they are not necessarily good indicators of the organic carbon generally available in the water column, as this may fluctuate with local weather conditions, tides, currents and/or autochthonous/allochthonous inputs. Nevertheless, these site-specific offshore extensions of enriched $\delta^{13}C$ values appear to be closely related to hydrographic features or events along the coastline.

4.4.1.2 Biogeographic gradients

$\delta^{13}C$ values for suspended particulate matter (SPM) of nearshore water showed an overall geographic enrichment from an average of -18.7 ± 0.67‰ on the east coast to -7.8 ± 1.08‰ on the south coast, while offshore water remained relatively constant at an average of -20.36 ± 1.84‰. This trend of east-south enrichment of nearshore carbon applied only to sites within the Agulhas system and excluded all sites to the west of Cape Agulhas. The remaining sites showed lower carbon values both nearshore and offshore, most likely reflecting the influence of the Benguela current and the geographic shift to a colder water and more nutrient rich ecosystem (Andrews & Hutchings 1980). Nearshore carbon enrichment from east to south reflects changes in coastal hydrography. Where the continental shelf is narrow (east coast; Fig 4.1), the Agulhas Current comes close inshore (Goschen & Schumann 1990; Goschen & Schumann 1994), resulting in nearshore water being well mixed with offshore water. Further south, the shelf begins to widen and the Agulhas moves offshore (Roberts 2005), weakening the mixing effect and ensuring larger carbon differences between nearshore and offshore water. The biogeographic consistency in offshore $\delta^{13}C$ indicates well mixed pelagic waters 10km from shore along the entire coast. Overall $\delta^{13}C$ signatures seem to reflect the existence of known hydrographic features along the coast (i.e. upwelling regions, current meanders, shelf width and shelf abnormalities such as canyons) and should therefore be considered for future use in studying water mixing and coastal hydrography. Nevertheless, instantaneous SPM samples give only a snapshot in time, and may not be the best measure for determining time-integrated carbon source availability and composition.

4.4.2 Mussels and diet

The lack of clear temporal variation in the adductor tissue, of both $\delta^{13}C$ and $\delta^{15}N$ values reflects the slow turnover rate of adductor tissue and using carbon and nitrogen

signatures, mussels around the southern African shoreline could be divided into four groups, with a clear geographic pattern. The east coast group (A) encompassed: Sodwana Bay to Cintsa, the south-east coast group (B): Port Alfred and Kenton-on-Sea; south-west coast group (C): Plettenberg Bay to Cape Agulhas and the west coast group (D): St. Helena Bay to Walvis Bay, Namibia. Two sites did not conform to this biogeographic pattern, the first deviation was site eleven (S11: Hout Bay), which lies on the west coast, near the Cape of Good Hope, but fell into the south-east coast group (group B) in all years, with its $\delta^{15}N$ values lying at the group boundary in two out of three years. The reason for the inclusion of Hout Bay in group B is unknown. The second exception was site five (S5: Cintsa) which showed strong $\delta^{13}C$ enrichment during austral winter, indicating a change in diet in June - July 2004. Cintsa was grouped with its geographic neighbours in group B in the winter sample (2004), but with its other neighbour, the more depleted group A in both summer samples (2002 and 2003). This may suggest that Cintsa lies close to the boundary between the east and south coast carbon sources, that different processes occur there during summer and winter months, or that carbon sources available to mussels in group A are more thoroughly mixed than those from group B.

Among sites, $\delta^{15}N$ exhibited geographic enrichment with a clear gradient from east to south-west, excluding all sites to the west of Cape Agulhas. This reflects the isotopic gradient from oligotrophic to eutrophic conditions, described by Saino & Hattori (1980) and Minagawa & Wada (1984). These isotopic differences are likely to be due to a reliance on recycled nitrogen (especially ammonia) in oligotrophic waters, which is depleted in $\delta^{15}N$ relative to the upwelled nitrate used in eutrophic systems (Miyake & Wada 1967). Mussel tissue exhibited geographic $\delta^{13}C$ enrichment from the east to the south-west coast, again excluding all locations to the west of Cape Agulhas. The west coast sites showed significant carbon depletions, mirroring the trend seen in nearshore SPM. Although a consistent fractionation value of 1.0‰ (DeNiro & Epstein 1978; Fry & Sherr 1984; Peterson & Howarth 1987) was not evident between offshore or nearshore SPM and mussel tissue, the latter two show similar patterns in $\delta^{13}C$ enrichment from east to south-west coast, indicating a close overall link between them. It is unrealistic to expect time integrated mussel tissue to reflect the instantaneous nature of the SPM sample, which is subject to constant interplay between nearshore and offshore water,

varying temporally on a local scale (Goschen & Schumann 1990) and this difference in time-scale integration may mask the relationship between the two.

Mussels are unlikely to depend on a single food source, relying instead on the changing sources of carbon available in the water column, and filtering selectively (Bougrier *et al*. 1997; Ward *et al*. 1998). As in chapter 3, I hypothesized that mussels eating macroalgal detritus would reflect $\delta^{13}C$ values of the nearshore SPM, while those eating phytoplankton would have similar carbon values to offshore SPM. At seven out of ten sites (Walvis Bay lacked nearshore SPM values) mussel tissue showed $\delta^{13}C$ values similar to, or just above those of nearshore SPM. This indicates that mussels at these sites either fed non-selectively, and so reflected available carbon in nearshore SPM, or selected for macroalgal detritus. At the remaining three sites, mussel tissue had intermediate $\delta^{13}C$ values, falling between those for nearshore and offshore SPM (Fig 4.10), suggesting a possible preferential selection of phytoplankton (Bougrier *et al*. 1997; Rouillon & Navarro 2003) from the nearshore SPM, but more likely reflecting local differences in nearshore SPM composition and available organic carbon.

The application of a linear mixing model allowed the determination of the percentage contribution of nearshore and offshore SPM to the overall $\delta^{13}C$ and $\delta^{15}N$ signatures for each mussel sample, investigating the dietary importance of nearshore versus pelagic primary production. Surprisingly, nearshore SPM accounted for over 50% of nitrogen (five sites demonstrated temporal variation) and carbon (two sites demonstrated temporal variation) incorporated by mussels along the coastline at all but one and three sites respectively (Fig 4.10), although there was otherwise little congruence between the two. Investigations into the diet of intertidal bivalves in Rià de Arosa (NW Spain) concluded that the primary source of organic carbon was resuspended microbenthos, with phytoplankton acting only as a supplement during bloom events (Page & Lastra 2003). A number of studies of variability in the $\delta^{13}C$ signatures of marine phytoplankton ascribe lower values to colder water temperatures and increased solubility of CO_2 (Arthur *et al*. 1985; Fry 1996), and this is supported by evidence of depleted carbon values in Antarctic plankton relative to plankton from equatorial waters (Rau *et al*. 1989). In this study however, although we have no information on the composition of the phytoplankton in nearshore and offshore SPM, it is unlikely that such an effect would be caused by a steep CO_2 gradient existing on a scale of 10km. Therefore differences in $\delta^{13}C$ between

nearshore and offshore SPM are attributed to different carbon sources in terms of phytoplankton and macroalgae and this overall reliance on nearshore primary production suggests that overall mussels are feeding unselectively and emphasizes the dietary importance of macroalgal detritus for mussels in the intertidal zone.

It is unrealistic to expect SPM samples to reflect a single macroalgal signature when SPM contains a variable composition of detritus from numerous seaweeds and consequently the links between mussel diet and macroalgae remain ambiguous. The macroalgae themselves showed large variation in isotopic signatures and lacked any temporal or biogeographic trends. Consequently no clear, consistent relationship was apparent between living macroalgae and mussel diet or nearshore SPM. Macroalgal isotopic ratios remain difficult to interpret and various studies have reported wide $\delta^{13}C$ ranges in aquatic plants and ratios that are subject to extensive variation. This includes not only within-species differences, but within fronds, between fronds, between different plant tissues, between populations and even between seasons (Stephenson *et al*. 1984; Fry & Sherr 1984; Gearing 1991). The isotopic discrimination of the carbon fractionation enzyme and the intracellular concentration of CO_2 or HCO_3^- of various macroalgae and their tissues have been suggested as mechanisms explaining this variance (Farquar *et al*. 1982) but as of yet are largely unexplored.

4.4.3 Summary and Conclusions

Overall trends of $\delta^{13}C$ SPM depletion between nearshore and offshore water were seen along the entire coastline, representing changes from nearshore carbon sources that include a component of macrophyte detritus to offshore pelagic carbon sources. This SPM depletion pattern may also manifest in higher trophic levels. Geographically, nearshore SPM became enriched moving from the east to the south coast, while offshore waters remained relatively constant and the extent of mixing of the two carbon sources and the protrusion of nearshore into offshore waters seemed to be closely related to hydrographic features or events along the coastline (i.e. shelf width, pulses, current position and upwelling cells).

Mussel tissues demonstrated geographic variation, particularly in carbon, with differences among east, south and west coasts. Although it was difficult to relate these trends in $\delta^{13}C$ values or carbon values of near/offshore SPM with distinct sources of

production, the linear mixing model revealed a high carbon and nitrogen dependence on nearshore instead of offshore SPM, suggesting that nearshore detritus plays a crucial role in mussel diet. This reliance on nearshore SPM is of further significance if we consider that mussels are a major component of the intertidal biota and are the prey of a wide range of species (Griffiths & Hockey 1987), playing a major part in retaining nearshore primary production that might otherwise be exported. As the importance of autochthonous primary production to intertidal filter feeders is clarified, it becomes important to focus on regional geographic differences in primary production along the southern African coastline to elucidate changes in SPM composition. Further studies into isotopic signatures of water in the Benguela current system and an investigation into the effect of degradation on macroalgal isotopic ratios may help to unravel the links between SPM and intertidal consumers.

CHAPTER 5: BIOGEOGRAPHIC TRENDS AND TROPHIC LEVEL INTERACTIONS WITHIN ROCKY INTERTIDAL COMMUNITIES ALONG THE COAST OF SOUTH AFRICA.

5.1 Introduction

Numerous studies throughout the world have illustrated strong top-down controls (e.g. consumer abundance, intraspecific competition and predation) in rocky intertidal communities (e.g. Paine 1966; Carpenter *et al*. 1985; Menge & Farrell 1989; Power 1992, Menge *et al*. 1999). In contrast, although recognized as a significant influence in terrestrial and freshwater ecosystems, few papers have examined the role of bottom-up effects (nutrients and productivity) in marine rocky shore ecology (e.g. Menge 1992; McQuaid & Lindsay 2000; Menge 2000; Nielsen & Navarrete 2004). The regulation of community structure through bottom-up controls extends beyond the influence of localized processes, and incorporates the effects of oceanography and coastal hydrography (Seitz & Lipcius 2001; Menge *et al*. 2003; Blanchette *et al*. 2006). As mentioned in chapters 3 and 4, the coupling of nearshore hydrography and intertidal community ecology (Menge *et al*. 1999; Nielsen & Navarrete 2004; Blanchette *et al*. 2006) has important implications for the South African shoreline, which is influenced by both the eutrophic Benguela (west coast) and the oligotrophic Agulhas (east-south coast) current systems (McQuaid & Payne 1998), implying biogeographic differences in the food resources available to intertidal communities.

Modeling energy flow through food chains is a step towards understanding trophic relationships in the marine environment (Monterio *et al*. 1991) and combined measurements of $\delta^{13}C$ and $\delta^{15}N$ ratios can provide information on both food web structure and source materials (Fredriksen 2003). The application of stable isotope analysis has contributed to the elucidation of both benthic and pelagic food webs; e.g. determining the primary diet of long finned pilot whales (Abend & Smith 1997); quantifying the importance of benthic phaeophtyes to the Antarctic peninsular fauna (Dunton 2001); elucidating trophic level interactions in an upwelling system in Galicia in NW Spain (Bode *et al*. 2003) and evaluating the dispersal of fish farming waste in the western Mediterranean (Sara *et al*. 2004).

A few studies have also applied this technique to intertidal communities. Bustamante & Branch (1996) investigated the trophic connections between consumer species and the importance of macroalgal detritus in the Groenriver rocky intertidal food web on the west coast of South Africa, suggesting a strong dependence on kelp-derived organic carbon, while Page & Lastra (2003) proposed heavy dependence of intertidal soft substratum bivalves on resuspended microbenthos. Likewise both chapters 3 and 4 of this dissertation have inferred a strong dependence of mussels on nearshore SPM, which contains a large component of detritus that is presumably macroalgal in origin. The trophic links between macroalgal detritus and intertidal communities are however still unclear. Using stable isotope analysis, the aims of this chapter were: (1) to investigate biogeographic variation in trophic level positioning on the rocky shore and to determine if the biogeographic pattern observed in mussel adductor tissue (chapter 4) holds true for other trophic levels and (2) to examine the reliance of intertidal communities on macroalgal-derived organic carbon at four sites along the South African coastline.

5.2 Methods

5.2.1 Sample Collection

SPM samples (x 3) were obtained from five-liter surface water samples taken from the beach (0km), microalgal samples (x 3) were obtained by scraping the rock surface with a razorblade, multiple species of intertidal consumers were collected from different trophic levels and triplicate samples of the most abundant macroalgae species (Table 5.1) were collected at St. Helena Bay, Cape Agulhas, Port Alfred and Port Shepstone (see Fig 4.1). Collection took place in February – March 2006. Due to logistic constraints, *Ralfsia verrucosa* and *Gelidium micropterum* were collected from *Scutellastra longicosta and Scutellastra cochlear* gardens respectively (x 3) in Port Alfred only in September 2006.

Table 5.1: List of species, trophic groups and number of samples for each site along the South African coastline.

Species	Taxon	Trophic group	St. Helena Bay	Cape Agulhas	Port Alfred	Port Shepstone
SPM		Producer	3	3	3	3
Microalgae		Producer	3	3	3	3
Ulva sp.	Chlorophyta	Producer	3	3	3	3
Sargassum heterophyllum	Phaeophyta	Producer	0	3	3	3
Porphyra capensis	Rhodophyta	Producer	3	3	0	0
Gelidium micropterum	Rhodophyta	Producer	0	0	3	0
Ralfsia verrucosa	Encrusting Rhodophyta	Producer	0	0	3	0
Mytilus galloprovincialis	Bivalvia	Filter feeder	3	0	0	0
Perna perna	Bivalvia	Filter feeder	0	3	3	3
Gunnarea capensis	Polychaeta	Filter feeder	3	3	3	0
Octomeris angulosa	Cirripedia	Filter feeder	0	3	3	3
Tetraclita serrata	Cirripedia	Filter feeder	3	3	3	3
Oxystele tigrina	Gastropoda	Microalgal grazer	3	3	3	0
Oxystele variegata	Gastropoda	Microalgal grazer	3	3	3	3
Scutellastra granularis	Gastropoda	Microalgal grazer	3	3	3	3
Helcion pectunculus	Gastropoda	Microalgal grazer	3	3	3	0
Siphonaria capensis	Gastropoda	Microalgal grazer	3	3	3	3
Siphonaria serrata	Gastropoda	Microalgal grazer	0	3	3	3
Scutellastra longicosta	Gastropoda	Macroalgal grazer	0	3	3	0
Scutellastra cochlear	Gastropoda	Macroalgal grazer	3	3	3	3
Parechinus angulosa	Echinoidea	Macroalgal grazer	3	3	3	0
Stomopneustes variolaris	Echinoidea	Macroalgal grazer	0	0	0	3
Burnupena lagenaria	Gastropoda	Scavenger	3	3	3	3
Nucella cingulata	Gastropoda	Predator	3	0	0	0

5.2.2 Sample Preparation

Water samples were filtered through pre-combusted (500°C, 6hrs) GF/F Whatman® filters (0.45µm pore size), using a vacuum pump (≤ 4cm Hg) and then oven dried at 60°C for 24hrs. Zooplankton and other large particles were manually removed under a dissecting microscope at 16x magnification. Muscle tissue from each consumer was removed, rinsed in distilled water (dH_20) and oven dried (60°C, 48hrs). Muscle tissue was used in this study as it has low turnover rates (chapter 2; Gorokhova & Hansson 1999) and is therefore representative of a time-integrated diet. All macroalgae were rinsed in dH_20, visible epiphytes were removed and the algae were oven dried (60°C, 48hrs). As muscle tissue has minimal lipid content (Tieszen *et al*. 1983; Soudant *et al*. 1999; Chu *et al*. 2000), no lipid extractions were performed on any tissue.

5.2.3 Isotopic Analysis

All isotopic analyses were completed as described in chapter 2.

5.2.4 Data Analysis

$\delta^{13}C$ and $\delta^{15}N$ signatures of sampled filter feeders (excluding mussels) were analyzed using a k-means cluster analysis based on Euclidian distances and validated using a discriminant function analysis (DFA). All analyses were performed using Statistica v7 (StatSoft Inc. 2004). A two-source linear mixing model (Bustamante & Branch 1996) using offshore (10km) SPM values determined in chapter 4 (also in Table 5.2) was applied to determine the percentage contribution of organic carbon and nitrogen to filter feeder diet by nearshore SPM:

$$\% \text{ Isotope} = [(d_{\text{mussel}} - d_{\text{offshore}} - I) / (d_{\text{nearshore}} - d_{\text{offshore}})] \times 100$$

where I is the average fractionation of $\delta^{13}C$ or $\delta^{15}N$ per trophic level and d is the isotopic ratio of the sample. This mixing model assumed an average fractionation value of 1‰ for $\delta^{13}C$ (DeNiro & Epstein 1978; Fry & Sherr 1984; Peterson & Howarth 1987), while a $\delta^{15}N$ fractionation of 1.7‰ was used for mussels (Hansson *et al*. 1997; Raikow & Hamilton 2001; Moore & Suthers 2005) and 2.2‰ for the polychaete *Gunnarea capensis*, and the barnacles *Tetraclita serrata* and *Octomeris angulosa* (secondary consumers; Dunton 2001).

The IsoSource model described by Phillips & Gregg (2003) was applied on two separate occasions; firstly as a two isotope system ($\delta^{13}C$ and $\delta^{15}N$) with three sources (*Ulva* sp., *Porphyra capensis* / *Sargassum heterophyllum* and phytoplankton) to quantify the contribution of macroalgae to nearshore SPM, using average offshore SPM (10km) (as determined in chapter 4; also in Table 5.2) as a measure of phytoplankton. Secondly, as a two isotope system ($\delta^{13}C$ and $\delta^{15}N$) with three sources (microalgae, *Ulva* sp. and *P. capensis* or *S. heterophyllum*) to determine the importance (in terms of organic $\delta^{13}C$ and $\delta^{15}N$) of macro vs. microalgae in the diet of two micrograzers, *Siphonaria capensis* and *Scutellastra granularis,* at each site (see Table 5.2 for all isotopic ratios used in chapter 5 mixing models). An average fractionation of 1‰ for $\delta^{13}C$ (as reported by DeNiro & Epstein 1978; Fry & Sherr 1984; Peterson & Howarth 1987) and 2.4‰ for $\delta^{15}N$ (as reported for limpets by Dunton 2001) was used in the second model.

Table 5.2: Isotopic ratios of $\delta^{13}C$ and $\delta^{15}N$ (‰) used in IsoSource mixing models (Phillips & Gregg 2003)

	St. Helena Bay		**Cape Agulhas**		**Port Alfred**		**Port Shepstone**	
	$\delta^{13}C$	$\delta^{15}N$	$\delta^{13}C$	$\delta^{15}N$	$\delta^{13}C$	$\delta^{15}N$	$\delta^{13}C$	$\delta^{15}N$
Nearshore SPM	-15.49	7.00	-10.75	6.42	-15.76	6.70	-18.82	4.77
Offshore SPM	-21.33	4.84	-21.33	4.84	-21.33	4.84	-21.33	4.84
Microalgae	-13.86	9.36	-3.74	1.43	-4.21	2.37	-7.13	3.93
Ulva sp.	-10.31	9.17	-14.37	8.63	-14.62	6.04	-13.00	5.83
Porphyra capensis	-15.97	6.10	-17.72	8.72	-	-	-	-
Sargassum heterophyllum	-	-	-	-	-11.97	6.38	-11.95	6.10
Siphonaria capensis	-11.44	12.45	-11.05	9.43	-15.89	10.15	-12.16	7.35
Scutellastra granularis	-11.71	10.75	-13.10	11.04	-13.28	8.00	-9.90	9.45

5.3 Results

5.3.1 Trophic level fractionation

Each site along the coastline showed similar positioning of trophic groups, with all organisms remaining in the range of -9.0 to -18.0‰ and 4.0 to 14.0‰ for $\delta^{13}C$ and $\delta^{15}N$ signatures respectively. Although no consistent carbon fractionation was seen between lower trophic groups, an overall trend of nitrogen enrichment on the scale of 1-2‰ was observed with increase in trophic level. While the pattern was not perfect for all sites, producers and SPM tended to be the most depleted; followed by macroalgal grazers. Microalgal grazers and filter feeders were comparatively enriched and scavengers were the most enriched in nitrogen at all sites (Fig 5.1). The relative trophic positions of SPM and filter feeders make sense at some sites for some species, as do the relative positions of SPM and macroalgae. Although microalgae were in the same position at three sites, the only consistent trophic positioning across all sites was the scavenger *Burnupena lagenaria*, which demonstrated predictable fractionation (1.0 – 2.0 ‰ $\delta^{13}C$ as reported by DeNiro & Epstein 1978; Fry & Sherr 1984; Peterson & Howarth 1987, 3.0 – 4.0‰ $\delta^{15}N$; as reported by Minagawa & Wada 1984; Post 2002) consistent with a primary diet of filter filters and/or microalgal grazers. Overall, values of standard deviations were larger for carbon signatures than nitrogen.

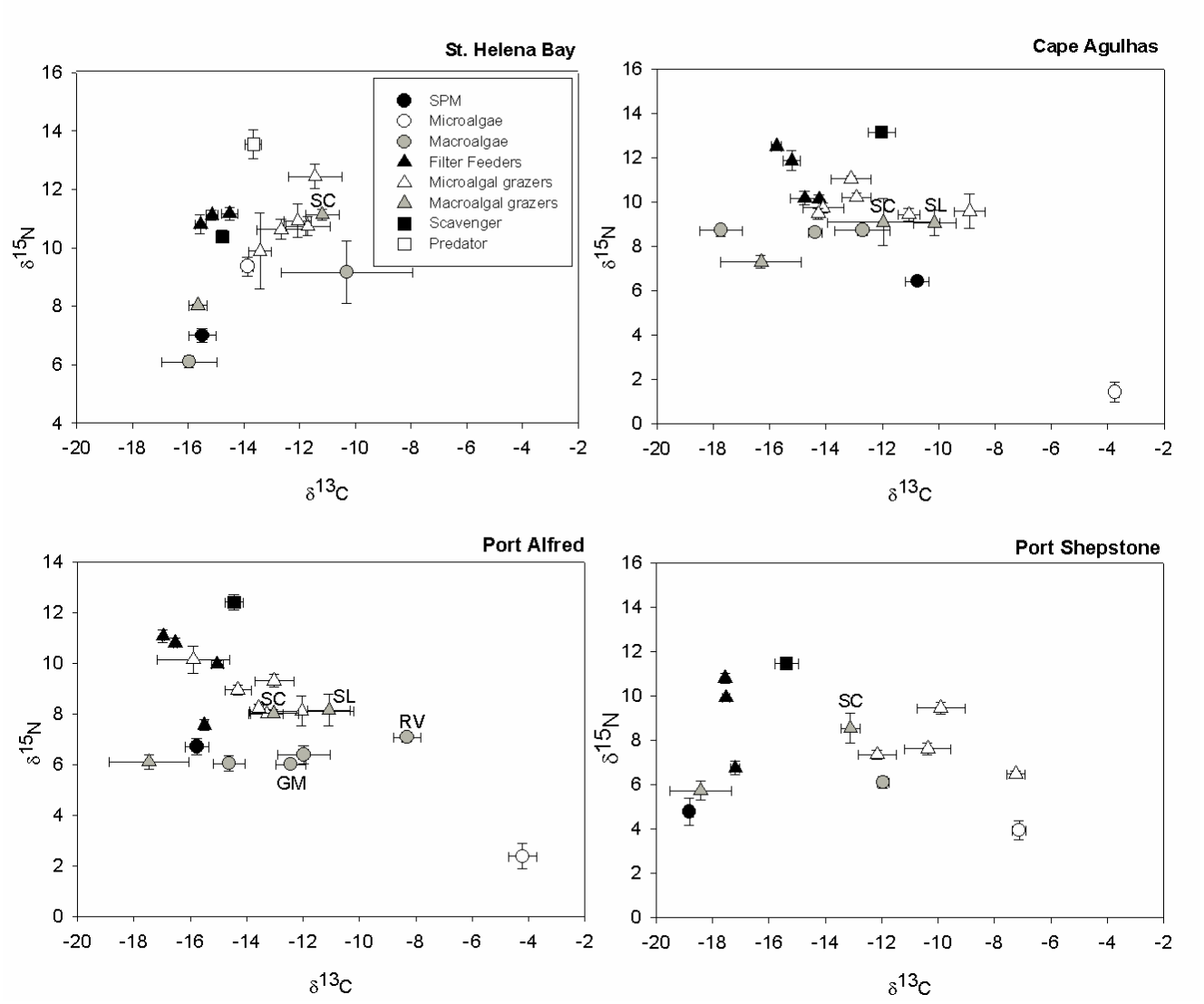

Figure 5.1: $\delta^{13}C$ and $\delta^{15}N$ signatures (‰) of all organisms sampled from each site along the coastline, broken down into trophic groups. SC = *Scutellastra cochlear*, GM = *Gelidium micropterum*, SL = *Scutellastra longicosta*, RV = *Ralfsia verrucosa*. Values are means ± SD.

5.3.2 Biogeographic trends

Most, but not all species common to all four sites showed crude groupings into south-west/west coasts vs. south-east/east coasts (Fig 5.2). This effect was clearer for nitrogen than for carbon. An overall pattern of enriched $\delta^{15}N$ on the south-west and west coasts (Cape Agulhas, St. Helena Bay) and depleted $\delta^{15}N$ on south-east and east coasts (Port Alfred, Port Shepstone) suggests a biogeographic effect on nitrogen sources (Fig 5.2). In the case of $\delta^{13}C$, separation of east/west sites was only clear for three of the six species: *Tetraclita serrata*, *Oxystele variegata* and *Scutellastra cochlear*. For *T. serrata* and *S. cochlear*, specimens from the west coast were depleted relative to east, but for *O. variegata* the reverse was true. No north-south patterns were evident in either $\delta^{13}C$ or $\delta^{15}N$.

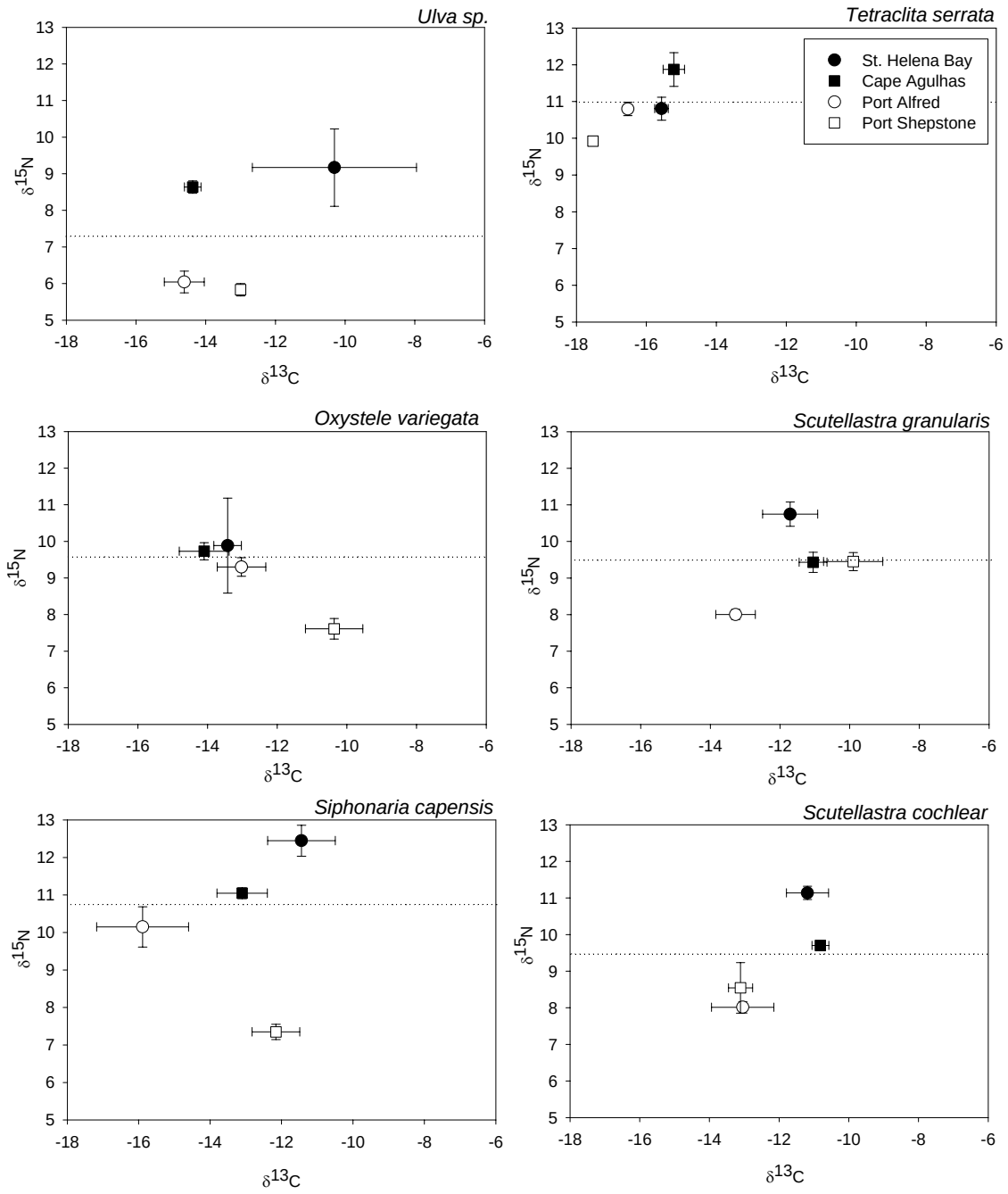

Figure 5.2: $\delta^{13}C$ and $\delta^{15}N$ signatures (‰) of the six organisms found at every site. Dotted line roughly separates east and west sites. Values are means ± SD.

No biogeographic trends were seen in $\delta^{13}C$ among any trophic group as a whole, but the addition of this study's mussel data to fig 4.8 demonstrated that, with the exception of St. Helena Bay, sampled mussel tissue conformed to the biogeographic trends seen in chapter 4 (Fig 5.3A). Although the other filter feeders (i.e. *Gunnarea capensis*, *Tetraclita serrata* and *Octomeris angulosa*) sampled in this study did not fall into the groups previously identified for mussels in chapter 4, they showed a similar biogeographic pattern, with the sole exception of *G. capensis* from Port Alfred. K-means cluster analysis classified them into three isotopic groups that represent animals collected from the east, south-east, and south-west coasts (Fig 5.3B). Subsequent DFA showed that these clusters could be identified by their isotope signatures alone with 93-100% accuracy. Although the biogeographic pattern for these filter feeders was the same as for mussels in chapter 4, the actual values were displaced. Signatures of barnacles and polychaetes were on average depleted by 1‰ in $\delta^{13}C$ and enriched by 3‰ in $\delta^{15}N$ relative to mussels from the same biogeographic regions (cf Fig 5.3A and 5.3B) The two source linear mixing model illustrated over 40% and 50% dependence on nearshore organic carbon and nitrogen respectively, for filter feeder diet (mussels included) at all sites (with the exception of all filter feeders at Port Shepstone for $\delta^{15}N$ only; Fig 5.3C), and IsoSource modeling determined that macroalgae accounted for upwards of 60% of the organic matter (carbon and nitrogen) in nearshore SPM relative to phytoplankton in three out of four sites.

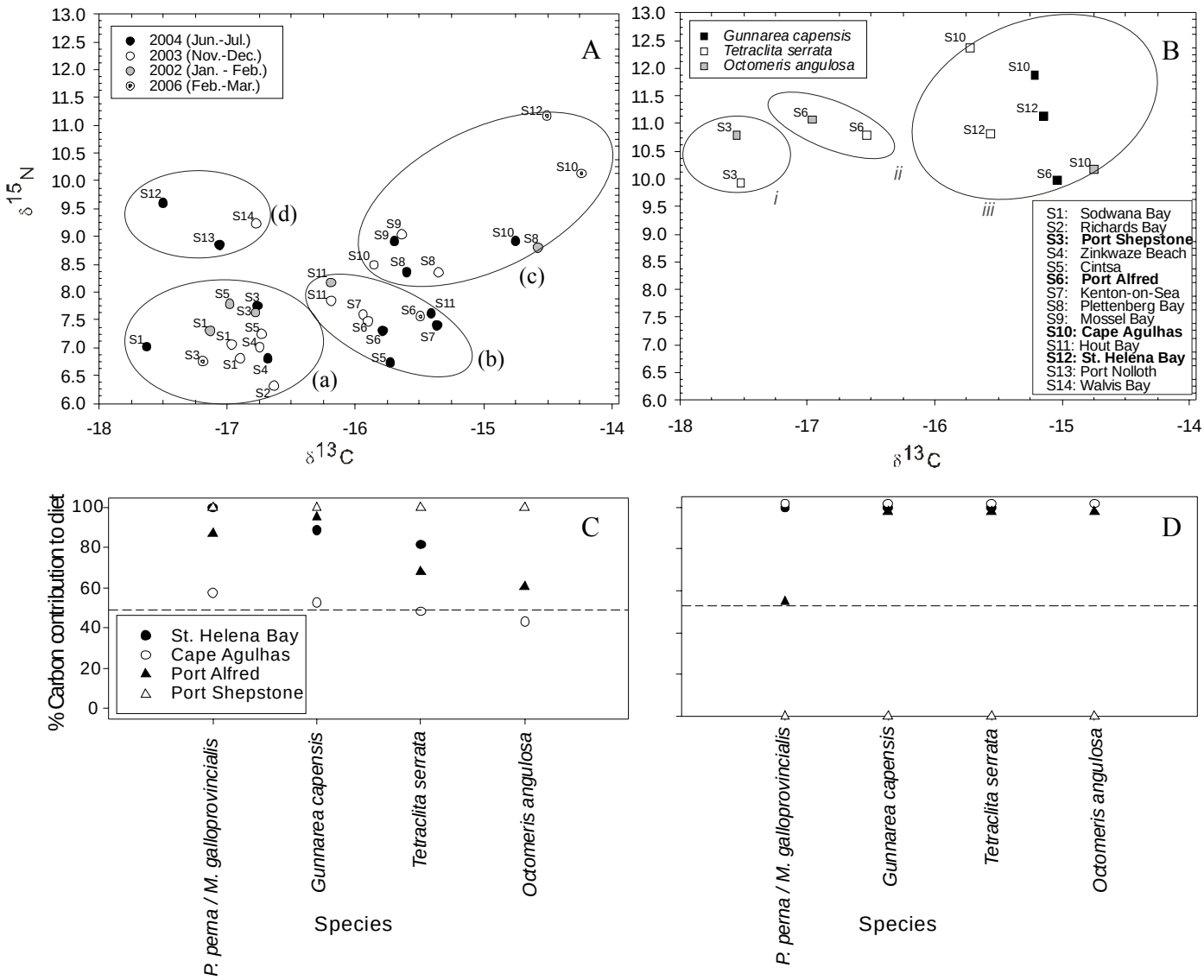

Figure 5.3: (A) $\delta^{13}C$ and $\delta^{15}N$ signatures (‰) of intertidal mussels including samples from this study (Feb – Mar 2006) and those from chapter 4, groupings according to k-means cluster analysis (see chapter 4): (a) east coast, (b) south-east coast, (c) south-west coast, (d) west coast. (B) $\delta^{13}C$ and $\delta^{15}N$ signatures of intertidal filter feeders from this study (excluding mussels) groupings according to k-means cluster analysis: (i) east coast, (ii) south-east coast, (iii) south-west coast. Percentage $\delta^{13}C$ (C) and $\delta^{15}N$ (D) contribution of nearshore SPM to filter feeder diet.

IsoSource modeling also indicated that *Ulva* sp. was the most important contributor to the diet of both microalgal grazers (*Siphonaria capensis* and *Scutellastra granularis*) that were sampled at every site. Dependence on *Ulva* sp. increased when moving east along the coastline (with the exception of *S. granularis* at Port Shepstone; Fig 5.4). IsoSource models reported all possible combinations of each source contribution, examined in small increments (e.g. 1%), that satisfied the isotopic mass balance in the mixing model (Phillips & Gregg 2003), however as some of the source proportions were reported to make contributions along a wide range of percentages (e.g. contributions of *Ulva* sp. to the diet of *S. capensis* at St. Helena Bay could be between 12 and 60%), consequently the generated mean graphed with standard deviations are presented.

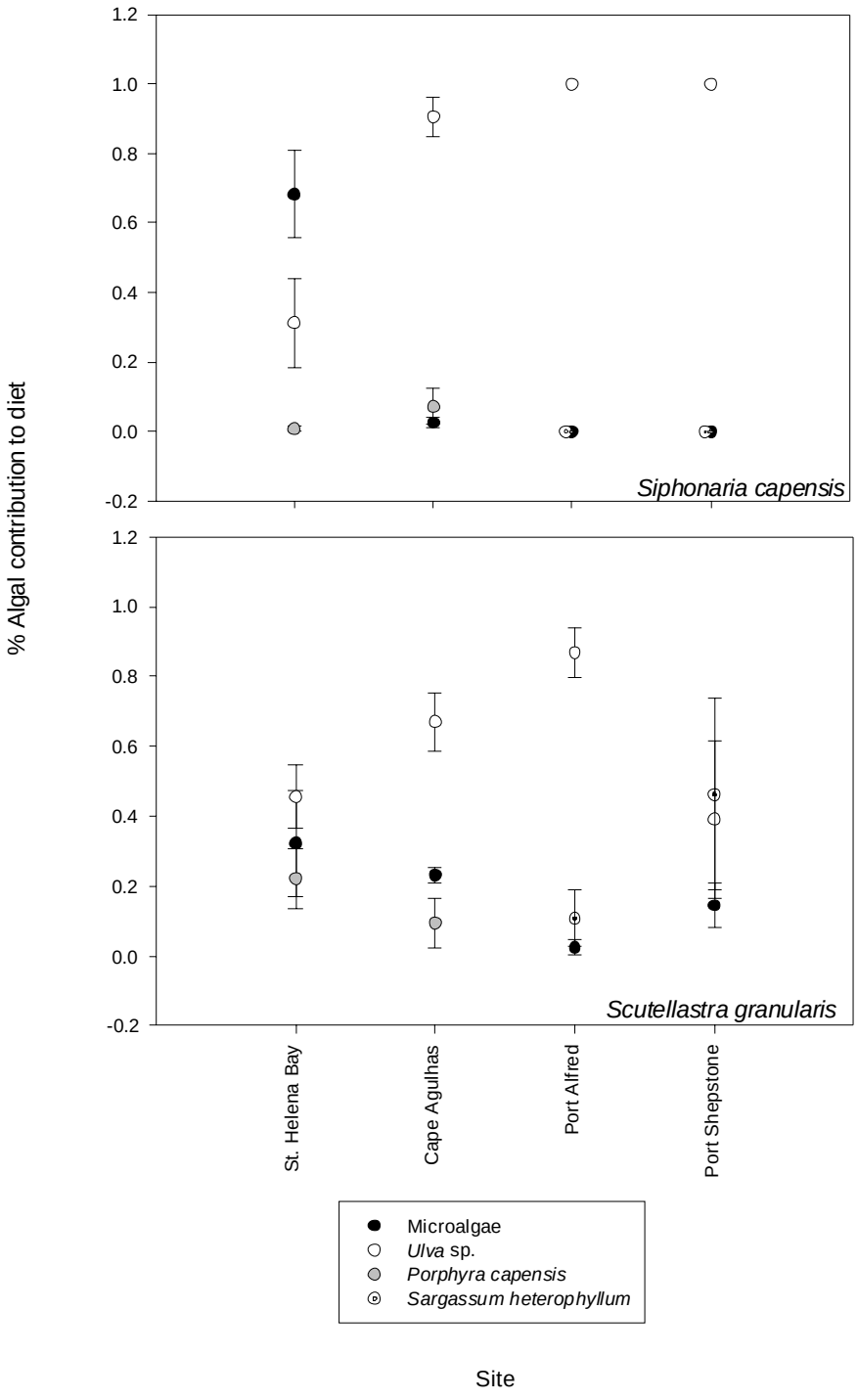

Figure 5.4: Percentage $\delta^{13}C$ and $\delta^{15}N$ micro and macroalgal contribution to the diets of *Siphonaria capensis* and *Scutellastra granularis* determined using IsoSource modeling (Phillips & Gregg 2003). Models calculated for each month using an increment of 1% and mass-based tolerance of 0.5‰.

5.4 Discussion

5.4.1 Photosynthetic isotope fractionation

No consistent $\delta^{13}C$ fractionation or clear dietary links in carbon were observed between producers and primary consumers at any site. In retrospect, this lack of consistent fractionation isn't surprising. Not only could the main diet of intertidal consumers be something other than the sampled producers, or a mixture of multiple macroalgae in unknown proportions, but the isotopic signatures of primary producers (including macro/microalgae and phytoplankton) are subject to large variability due to isotope fractionation during photosynthesis. Early research into photosynthetic isotope fractionation of marine algae returned conflicting results. Rau *et al*. (1989, 1991) described a relationship in phytoplankton between the photosynthetic fractionation factor (ε_p) and $[CO_2]_{aq}$, where increased solubility of CO_{2aq} resulted in depleted algal $\delta^{13}C$ signatures. Further investigation by Goericke & Fry (1994) found weak covariance of ε_p and $[CO_2]_{aq}$, suggesting that $[CO_2]_{aq}$ was not the primary factor behind photosynthetic fractionation. Thompson and Calvert (1994) also contested the (ε_p): $[CO_2]_{aq}$ relationship, and reported influences of irradiance intensity and day length on the $\delta^{13}C$ signatures of natural samples of particulate organic matter (POM). Research by Laws *et al*. (1995) recognized the possibility of an inverse linear relationship in marine algae between ε_p and (μ)/$[CO_2]_{aq}$ (where μ is the algal growth rate), though this was later refuted by Burkhardt *et al*. (1999a, 1999b). Thus the nature of photosynthetic fractionation remains obscured by the contradictory findings of studies seeking to identify its primary mechanism and the numerous potential factors affecting the process.

It is clear however that variation in photosynthetic pathways can cause dynamic changes in ε_p. Fractionation of carbon isotopes occurs at the diffusion/dissolution and carboxylation steps of photosynthesis (Bade *et al*. 2005). In C_3 plants however, carboxylation has been shown to be the principal fractionation step, where carbon is primarily fixed through the enzyme ribulose bisphosphate carboxylase (RUBISCO), with a $\delta\varepsilon_{pCO2}$ of 25 – 29‰ (Wong & Sackett 1978; O'Leary 1988; Goericke *et al*. 1994; Popp *et al*. 1998). During carboxylation, plants preferentially assimilate ^{12}C and consequently have lighter $\delta^{13}C$ signatures than the surrounding pools of dissolved inorganic carbon

(DIC). However, if diffusion of CO_2 into a plant cell becomes rate limiting, or the molecular carbon pool becomes drained (due to changes in pH, growth rates or temperature), then fractionation during carboxylation is reduced (Wong & Sackett 1978). This concept also applies to $\delta^{15}N$, with organisms in nitrogen limited systems demonstrating less fractionation than in unlimited systems (Fry 2006). The reason is that, although both isotopes of carbon/nitrogen react in the same fashion during metabolism and photosynthesis, the lighter isotope ($^{12}C/^{14}N$) reacts faster, and so in unlimited systems organisms assimilate more of the lighter isotopes. Consequently, as CO_2 diffusion is slower in water than air, aquatic plants (especially benthic algae) frequently exhibit less fractionation than their terrestrial counterparts (Finlay *et al*. 1999). Additionally, carbon fixation during light-independent reactions uses the enzyme β-carboxylase instead of RUBISCO and this may influence cellular $\delta^{13}C$ (Thompson & Calvert 1994). Changes in irradiance length and intensity and the re-supply of nitrogen in the form of NH_4^+ instead of NO_3^- during nutrient limitation have been shown to alter the amount of carbon fixed through β-carboxylase within aquatic plants (Descolas-Gros & Fontugne 1985; Guy *et al*. 1989; Thompson & Calvert 1994). Diurnal variation of NH_4^+ and NO_3^- utilization in phytoplankton also suggests that these two factors may interact to influence the proportion of carboxylation via β-carboxylase vs. RUBISCO (Mortain-Bertrand *et al*. 1988; Thompson & Calvert 1994). This suggestion was supported by Burkhardt *et al*. (1999a) who presented evidence of diurnal $\delta^{13}C$ variation in marine phytoplankton on a scale of $\leq 1.5‰$.

Other factors may also affect photosynthetic fractionation in C_3 plants, particularly the presence of carbon concentrating mechanisms (Sharkey & Berry 1985). Some algae can actively obtain CO_2 by accumulating bicarbonate (HCO_3^-) in addition to the passive diffusion of $(CO_2)_{aq}$ (Degens *et al*. 1968; Raven 1970; Wong & Sackett 1978). By packaging RUBISCO within a plant cell, and then converting CO_2 from HCO_3^- at that same site, these algae can produce a locally elevated CO_2 environment that allows rapid RUBISCO carboxylation (Moroney & Somanchi 1999) and, as bicarbonate has a greater affinity for ^{13}C, the fractionation associated with carboxylation decreases. Since many algae have two mechanisms for CO_2 acquisition, the problem lies in discerning whether HCO_3^- or CO_2 is the primary source of inorganic carbon (Laws *et al*. 1997).

Determining characteristic $\delta^{13}C$ signatures for primary producers is further complicated by a number of factors related to growth rate. In order to support consumer biomass, primary producers have comparatively higher growth rates which respond to smaller scale changes in nutrient source and availability (O'Reilly *et al*. 2002). These growth rates are consequently highly variable and plants with faster growth rates show less fractionation than slower growers with the same photosynthetic pathways (Degens *et al*. 1968). Macroalgae may also exhibit tissue-specific growth rates, resulting in a variety of distinct isotopic signatures within a single plant (Fry & Sherr 1984; Gearing 1991; Michener & Schell 1994). Dissolved CO_{2aq} is fractionally identical to atmospheric CO_2 (Degens *et al*. 1968; Mook *et al*. 1974). Therefore if algae obtain CO_2 through passive diffusion, photosynthetic fractionation should correlate closely with tidal cycles. However, CO_{2aq} and $HCO_3^-{}_{aq}$ differ fractionally by 7-9‰ (Degens *et al*. 1968). Therefore the primary mechanism for CO_2 acquisition becomes an important factor in understanding variability in $\delta^{13}C$ signatures. With multiple factors influencing isotope ratios, obtaining consistent and/or characteristic signatures for different algal species is difficult, especially considering that $\delta^{13}C$ variability may be on a scale of >10.0‰. With such dynamic variation in algal isotopes and differences in tissue turnover rates, it is unrealistic to expect clear links between producers and primary consumers, which complicates assessments of macroalgal dependence at higher trophic levels.

5.4.2 Carbon and nitrogen trends in trophic groups

The only consumer to demonstrate predicted fractionation values at all sites was the whelk *Burnupena lagenaria*, which, as a typical secondary consumer, exhibits less variation in isotope signatures than primary producers (O'Reilly *et al*. 2002). Data from all four sites suggest that the primary diet of *B. lagenaria* consists of filter feeders and/or microalgal grazers. The general positioning of trophic groups was comparable across all sites, with animals from the same trophic level grouping together. The only exceptions to this overall trend were the producers and macroalgal grazers, whose variable signatures, as mentioned above, may be explained by isotope changes during photosynthetic fractionation, or, in the latter group, through assimilation of unique species (or proportions) of macroalgae. Although there is much evidence that *Scutellastra cochlear*

gardens *Gelidium micropterum* in a mutualistic relationship (Branch 1975; Branch 1981; Hughes *et al*. 1991), the $\delta^{13}C$ signatures of the limpet were more depleted than those of the algae, suggesting that *G. micropterum* is not a primary component in the diet of *S. cochlear*. This is supported by a number of studies which report strong dietary associations between the pear limpet and the encrusting algae *Spongites yendoi* (Branch 1971, 1976; Maneveldt *et al*. 2006). There is also well documented evidence that *Scutellastra longicosta* is a monospecific feeder grazing on *Ralfsia verrucosa* (Branch 1971; McQuaid & Froneman 1993; Lasiak 2006) and yet Port Alfred samples showed *S. longicosta* to be more depleted in carbon than *R. verrucosa* and to be relatively enriched by only 1.0‰ in $\delta^{15}N$. The lack of predicted fractionation between *S. longicosta* and *R. verrucosa* is puzzling, and this discrepancy in carbon ratios may be a consequence of algal isotope variability or supplementary feeding by *S. longicosta*. Investigation of the contribution of carbon and nitrogen by micro and macroalgae to the diets of *Siphonaria capensis* and *Scutellastra granularis* revealed that *Ulva* sp. contributed the most organic content to their diet (with the exception of *S. granularis* at Port Shepstone), suggesting that macroalgal sporelings and/or small macroalgal plants may be assimilated in addition to microalgae. In the case of *S. capensis* this is supported by a study done by Ocaña & Fa (2003) who described the use of fine-toothed radulae by *Siphonaria* spp. for grazing epilithic and superficial algae, including spores and emerging germlings. They suggested this feeding strategy would reduce competition with other patellogastropod limpets, which tend to scrape deeper into the rocky substratum while foraging, and such a feeding strategy would also explain the lack of dependence seen on sampled microalgae. The minimal contribution of *Porphyra capensis* to the diet of either limpet may be attributable to its strong seasonality (McQuaid 1985a) and that of *Sargassum heterophyllum* can be explained in a spatial context, as *S. heterophyllum* is found much further down on the rocky shore, and is usually confined to mid-intertidal rock pools (De Clerck *et al*. 2005).

5.4.3 Biogeographic trends

With the exception of St. Helena Bay, isotopic signatures of newly sampled mussel tissue in this study conformed to the biogeographic pattern of mussel signatures seen in chapter 4 (Figs 4.8 & 5.3A). A similar pattern, but with a shift in both carbon (1‰

depletion) and nitrogen (3‰ enrichment) emerged in the remaining filter feeders, suggesting that fractionation within trophic levels varies significantly between species. Multiple studies have confirmed preferential particle selection in bivalves in response to changing nutrient conditions (Newell *et al*. 1989; Ward *et al*. 1998; Ward & Shumway 2004), and there is some evidence that sedentary polychaetes may also be selective in terms of particle size and coating (Horng & Taghon 1999; Mahon & Dauer 2005). The efficiency of particle selection might explain the consistency in biogeographic patterns within suspension feeders relative to other trophic levels. The application of a two-source linear mixing model (Bustamante & Branch 1996) showed over 40% contribution of nearshore organic carbon to the diet of all sampled filter feeders at all sites, inferring a strong dependence on inshore vs. offshore SPM. In contrast, nearshore SPM contributed upwards of 50% organic nitrogen to all filter feeders at only three out of four sites, suggesting a separate source of nitrogen at Port Shepstone. $\delta^{15}N$ showed trophic level enrichment, but with less fractionation than predicted values (3.0 – 4.0‰ as reported by Minagawa & Wada 1984; Post 2002). Research by Bustamante & Branch (1996) at Groenrivier on the west coast of South Africa, showed similar results in carbon and nitrogen among trophic groups, although many of their $\delta^{15}N$ signatures were significantly more depleted than any values found in these studies.

The six organisms found at all four sites also demonstrated an overall pattern of $\delta^{15}N$ enrichment when moving from east to west coast, which presumably represents a biogeographic shift in nitrogen sources. Documented isotopic gradients seen between oligotrophic and eutrophic conditions (Saino & Hattori 1980; Minagawa & Wada 1984) likely represent a reliance on recycled nitrogen (especially ammonia) in oligotrophic waters, which is depleted in $\delta^{15}N$ relative to the upwelled nitrate used in eutrophic systems (Miyake & Wada 1967). These biogeographic and trophic level gradients suggest significant bottom-up effects that may regulate processes further up the food chain in rocky intertidal ecosystems. Investigations into the diet of *Siphonaria capensis* and *Scutellastra granularis* revealed a clear trend of increasing dependence on *Ulva* sp. when moving from west to east coast in *S. capensis*, suggesting a biogeographic shift in primary food source between St. Helena Bay and Cape Agulhas, which may correlate to physicochemical differences or nutrient availability between the Atlantic and Indian

Oceans. Patterns in *S. granularis* show a similar biogeographic increase in *Ulva* sp. dependence from west to east coast, but with a sharp decrease at Port Shepstone, the reasons for which are still unclear. Although three of the six species found at all sites also demonstrated crude $\delta^{13}C$ separations between east and west coasts, the filter feeder (*Tetraclita serrata*) and macroalgal grazer (*Scutellastra cochlear*) demonstrated west coast enrichments in $\delta^{13}C$ while the microalgal grazer, *Oxystele variegata*, showed west coast depletion. The reasons for this are unclear, but may again be a reflection of differential fractionation between macroalgae (including macroalgal detritus) and microalgae during photosynthesis. No biogeographic patterns were evident among the remaining species or trophic levels.

5.4.4 Summary and conclusions

Understanding the mechanisms behind isotopic variation in marine algae is the key to elucidating intertidal food webs, and a variety of algal factors influence stable isotope signatures and so complicates trophic level analyses in rocky shore communities. Photosynthetic pathways, carbon concentrating mechanisms, irradiance, growth rates and the capacity for sustained photosynthesis in air, all interact to alter algal isotopic composition. Stable isotope analysis remains a useful tool when applied between organisms with consistent signatures and comparable tissue turnover rates; however, although this technique has proven constructive in the trophic analysis of pelagic ecosystems, the undetermined link between SPM and primary producers, variation in macroalgal signatures, generalized omnivory and differences in producer vs. consumer growth rates, reduce its efficacy in resolving rocky shore food webs. In light of this, the clarity in biogeography of filter feeders is remarkable and demonstrates very strong bottom-up effects of the physical environment on nitrogen sources etc.

Although the importance of macroalgal-derived organic carbon in filter feeder diet is clear, the direct links between living macroalgae, nearshore SPM and consumers are still uncertain. Nevertheless $\delta^{15}N$ appears to be a reliable indicator of trophic level within rocky shore ecosystems and a biogeographic enrichment in nitrogen indicates strong ties to regional oceanography and resulting nutrient availability. In the previous three chapters, understanding how the diet of intertidal consumers relates to available sources

of organic carbon has been complicated by the difficulty in linking nearshore SPM with living macroalgae. The following chapter examines the isotopic changes in macroalgae during the process of degradation in hopes of putting the relationship between macroalgae and intertidal consumers into perspective.

CHAPTER 6: EFFECTS OF DEGRADATION ON $\delta^{13}C$ AND $\delta^{15}N$ SIGNATURES OF MACROALGAE

6.1 Introduction

At temperate and high latitudes, extensive beds of kelp (e.g. *Ecklonia* spp., *Laminaria* spp., *Macrocystis* spp.) are frequently associated with cold water upwelling systems and range along the coast, with productivities comparable to the most productive terrestrial ecosystems on earth (Barnes & Hughes 1982). Despite this there appear to be very few animals that directly utilize this material and consequently the primary production of kelps vastly exceeds herbivorous consumption (Newell & Lucas 1981). The low biomass of kelp-grazing animals suggests that minimal amounts of kelp primary production enter the food web through grazing (Field *et al*. 1977), with the bulk entering community food webs through the detrital food chain (e.g. Newell & Lucas 1981; Mann 1988; Duggins *et al*. 1989; Kaehler *et al*. 2000; Fredriksen 2003). Thus kelps contribute large amounts of organic carbon to the ecosystem as suspended particulate and dissolved organic matter (SPM, DOM). On the west coast of South Africa for example, immense forests of *Ecklonia maxima* and *Laminaria pallida* account for upwards of 65% of the detritus found in intertidal SPM (Bustamante & Branch 1996).

In the same fashion, primary production by salt marsh plants (e.g. *Spartina alterniflora*) can be very high, with only a small percent of the above-ground vegetation being consumed by grazers (e.g. Hackney & De La Cruz 1980; Valiela *et al*. 1985; Buth 1987; Benner *et al*. 1987; Currin *et al*. 1995); the rest degrades and enters the detrital food web. Many studies have underlined the dependence of salt marsh and estuarine ecosystems on *Spartina* spp. detritus (e.g. Haines 1977; Haines & Montague 1979; Hughes & Sherr 1983; Paterson & Whitfield 1997). Studies of algal-grazer interactions in the intertidal zone indicate that macroalgal biomass and distribution can be set by grazers (e.g. Southward 1964; Hawkins & Hartnoll 1983; Williams 1993). Nevertheless, many benthic grazers in both marine and freshwater systems feed on the periphyton of macrophytes, or on epilithic microalgae (McQuaid 1996; Hillebrand *et al*. 2002) which show much higher turnover rates and contain less refractory material than macrophytes. Consequently, as with saltmarshes and kelp beds, the bulk of macroalgal production is

likely to enter a detrital food chain, so that understanding the process of macrophyte decomposition becomes central to elucidating food webs in communities.

The majority of macrophyte detritus is fibrous and nitrogen poor, but the process of decomposition includes microbial colonization (Stuart *et al*. 1981), which results in reduced fiber content and increased available nitrogen, making the previously indigestible detritus highly nutritious for detritivores and many intertidal filter feeders (Mann 1988; Levinton *et al*. 2002). Consequently the $\delta^{13}C$ and $\delta^{15}N$ values of macrophyte detritus will not necessarily reflect those of the alga itself, but rather a combination of signatures from the decomposing alga, plus the various microbial communities colonizing the alga (Newell & Lucas 1981; Newell *et al*. 1982). In addition the signature of the alga itself will be altered by the gradual leaching of chemical contents (e.g. polyphenolics), some of which may deter the herbivorous grazing of living plants (Hay 1996; Levinton *et al*. 2002). If fractionation accompanies macrophyte decomposition, then consumer stable isotope signatures may bear no resemblance to those of living primary producers and the process of degradation may be seen as a critical step in explaining the isotopic link between primary producers and consumers.

Although a number of studies have addressed the isotopic effects of degradation in aquatic vegetation, the focus has been on angiosperms and conflicting results have emerged. Schweizer *et al*. (1999) found no change in ^{13}C signatures in decomposing tropical grasses and legumes and Machás *et al*. (2006) showed no changes in $\delta^{13}C$ or $\delta^{15}N$ during the early phases of decay in the leaves of *Zostera noltii*. Fenton & Ritz (1988), Zieman *et al*. (1984) and Stephenson *et al*. (1986) described little change in $\delta^{13}C$ signatures during the decomposition of seagrasses (*Thalassia testudinum* and *Zostera marina*), although Zieman *et al*. (1984) showed a marked reduction in $\delta^{15}N$ of degrading mangroves. The differences between seagrasses and mangroves in the behaviour of $\delta^{15}N$ during degradation were attributed to characteristics of the original plant matter, which consequently affect its availability to microbial communities. As living mangroves have lower nitrogen content than seagrasses, the additional nitrogen required for microbial degradation probably originated from exogenous sources and consequently exhibited different $\delta^{15}N$ values (Zieman *et al*. 1984). In contrast, investigations into the decomposition of *Spartina alterniflora* showed distinct depletions in ^{13}C during

diagenesis in salt marsh sediments (Benner *et al*. 1987; Alberts *et al*. 1988; Fogel *et al*. 1989; Benner *et al*. 1991) Currin *et al*. (1995) also reported $\delta^{15}N$ depletions ranging from 2.0 – 3.0‰ and Fellerhoff *et al*. (2003) described high $\delta^{15}N$ variability (± 6.0‰) during degradation of seven species of tropical angiosperms, though neither Currin *et al*. (1995) nor Fellerhoff *et al*. (2003) found significant changes in $\delta^{13}C$.

In comparison to angiosperms, little work has been done on shifts in the isotopic ratios of carbon or nitrogen in decomposing macroalgae, with only Fenton & Ritz (1988) reporting both small enrichments and depletions in carbon during decomposition and Stephenson *et al*. (1986), describing a lack of carbon fractionation during kelp decomposition. As a consequence, the isotopic differences between living macroalgae and their detritus are poorly understood. The south coast of South Africa is too warm to support extensive kelp beds, but maintains a high biomass of intertidal and subtidal algae that may contribute large amounts of organic carbon to intertidal community food webs. Although there are beds of *Zostera capensis* that occur in nearby estuaries, these estuaries are few and small, so that the contribution of angiosperm production to the intertidal zone is thought to be minimal (Taylor & Allanson 1995). A large percentage of intertidal SPM is then likely to be detritus of macroalgal origin. The aims of this study were, firstly, to conduct a preliminary investigation of isotopic variation of different tissue types within a single plant and secondly to elucidate changes in C: N ratios, $\delta^{13}C$ and $\delta^{15}N$ signatures during decomposition of two abundant intertidal rhodophytes: *Gelidium pristoides* and *Hypnea spicifera*. I hypothesized that both degrading macroalgae and SPM produced from macroalgal detritus would be enriched in $\delta^{13}C$, depleted in $\delta^{15}N$ and would demonstrate decreased C: N ratios relative to healthy plants as a result of microbial mineralization during decomposition.

6.2 Methods

6.2.1 Study Site

Algal samples were collected from Port Alfred (33°27’21 S, 26°52’29 E), an exposed rocky shore on the south coast of South Africa. The macroalgae *Gelidium pristoides* and *Hypnea spicifera* were chosen for decomposition studies as they are important

contributors to macroalgal biomass and primary production on this coast (Dower 1990), and are likely to contribute large amounts of SPM to the intertidal zone.

6.2.2 Sample Design

6.2.2.1 Differences between tissues

Ten samples each of healthy fronds, senescent fronds and holdfasts (*Gelidium pristoides*) or rhizomatous holdfasts (*Hypnea spicifera*) were dissected from ten specimens of each species collected in the field. Samples of the midsection of the frond, between frond tip and rhizomatous holdfast, were also collected from *H. spicifera* only.

6.2.2.2 Field Study

Twenty holes were drilled into the rocks in the sub-littoral fringe of the intertidal zone and eye-hooks were secured in the holes using plastic anchors. Twenty mesh bags (150µm mesh) were each filled with 100g of freshly collected macroalgae which were subsequently cut up into small pieces to induce decomposition, either *Gelidium pristoides* (10) or *Hypnea spicifera* (10) and attached to eye-hooks using zip-ties. Bags were left on the rocks for 60 days and the degrading algae were sampled at time zero, day 30 and day 60.

6.2.2.3 Laboratory Study

Since most physical changes in algal structure were seen within the first few weeks of the field study, a shorter-term laboratory experiment was undertaken. 100g of fresh, mechanically damaged *Gelidium pristoides* (10) or *Hypnea spicifera* (10) were added to each of twenty 10L buckets filled with seawater, strongly aerated (to maintain agitation) and left to decompose for 30 days. Algae were sampled at time zero and day 30. Initial SPM samples were obtained from five-liter surface seawater samples (i.e. same as water in buckets) and later SPM samples were obtained from each bucket on day 30. A microbial film developed on the walls of all the buckets as the experiment progressed and this material (biofilm) was sampled from each bucket on day 30 only. Water temperature throughout the experiment was ambient, ranging between 16-20°C.

6.2.3 Sample Preparation

All macroalgae were rinsed in distilled water (dH_20), visible epiphytes were removed and algae were oven dried (60°C, 48hrs). Water samples were filtered through pre-combusted (500°C, 6hrs) GF/F Whatman® filters (0.45µm pore size), using a vacuum pump (≤ 4cm Hg) and then oven dried at 60°C for 24hrs. Zooplankton and other large particles were manually removed under a dissecting microscope at 16x magnification. Biofilm was rinsed with dH_20 and also oven dried (60°C, 48hrs).

6.2.4 Isotopic Analysis

All isotopic analyses were completed as described in Chapter 2.

6.2.5 Data Analysis

6.2.5.1 Differences between tissues

Separate one-way ANOVAs were performed for *Gelidium pristoides* and *Hypnea spicifera* to assess the effect of tissue type on $\delta^{13}C$, $\delta^{15}N$ and C: N.

All ANOVA analyses were Model I and performed using Statistica v7 (StatSoft Inc. 2004). In the event of significant effects, Newman-Keuls post hoc comparisons were used to determine homogeneous groups. The prerequisites for parametric analyses were tested using Kolmogorov-Smirnov's and Lilliefor's tests for normality and Levene's test for homogenous variances. Data transformation was not required for any analysis.

6.2.5.2 Field Study

Two-way ANOVAs were performed separately to assess species specific differences in $\delta^{13}C$, $\delta^{15}N$ and C: N ratio through time, using species and time as fixed factors.

6.2.5.2 Laboratory Study

Separate two-way ANOVAs were performed for *Gelidium pristoides* and *Hypnea spicifera,* in order to assess the effects of time and source (alga, SPM) on $\delta^{13}C$, $\delta^{15}N$ and C: N ratios. A two-way ANOVA was performed for day 30 only, using species and source as fixed factors to assess species-specific differences in $\delta^{13}C$, $\delta^{15}N$ and C: N ratios between SPM, algae and biofilm.

6.3 Results

6.3.1 Differences between tissues

The holdfast of *Gelidium pristoides* was significantly more depleted in $\delta^{13}C$ than both the healthy and senescent fronds ($F_{2, 14} = 16.17$, $p < 0.0001$). $\delta^{15}N$ signatures of tissues decreased in the sequence holdfast > healthy fronds > senescent fronds ($F_{2, 14} = 32.06$, $p < 0.0001$). No significant differences were seen in C: N ratios. $\delta^{15}N$ signatures of *Hypnea spicifera* showed healthy and senescent fronds > mid-frond > holdfast ($F_{3, 19} = 19.63$, $p < 0.0001$) with C:N ratios of healthy fronds being significantly lower than all other tissues ($F_{3, 19} = 6.01$, $p < 0.0001$). No significant differences were seen in $\delta^{13}C$ (Fig 6.1). Patterns in tissue-specific signatures differed between species, but clearly indicated significant differences in $\delta^{13}C$, $\delta^{15}N$ and C: N ratios among tissue types within a single plant. For this reason only frond tips were used in the decomposition experiments.

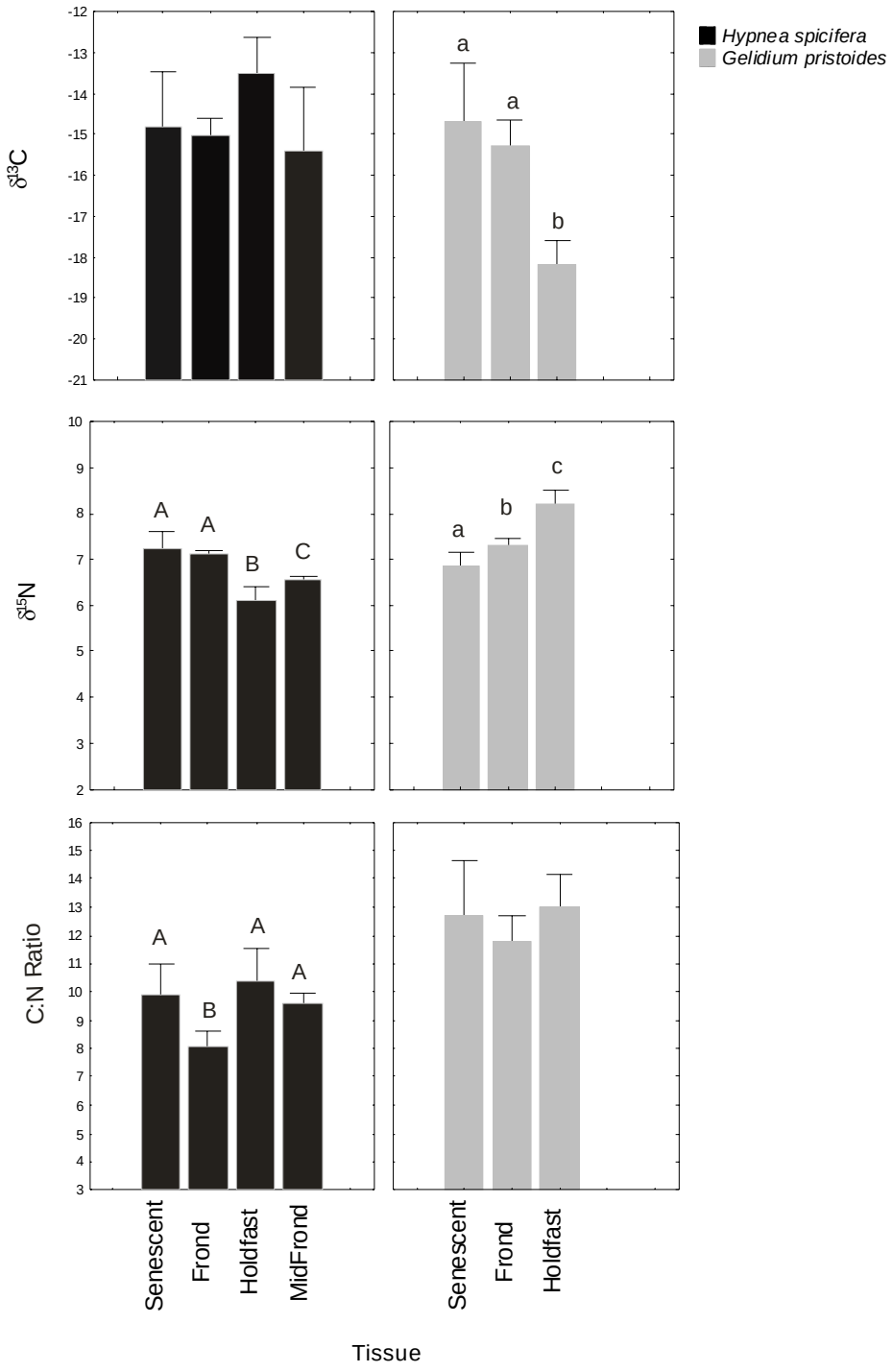

Figure 6.1: Tissue specific differences in *Gelidium pristoides* and *Hypnea spicifera* for $\delta^{13}C$, $\delta^{15}N$ (‰) and C: N ratios. Values are + means SD. Uppercase letters indicate homogenous groups within *H. spicifera* and lowercase letters indicate homogenous groups within *G. pristoides* (Newman-Keuls, $p < 0.05$).

6.3.2 Field Study

Two-way ANOVA indicated significant time x species interactions for both $\delta^{13}C$ and $\delta^{15}N$, while C: N ratios showed only a species effect (Table 6.1).

For both species, $\delta^{13}C$ was significantly depleted (1-2.0 ‰) at days 30 and 60, relative to day zero, however, the effect was stronger for *Hypnea spicifera* (Fig 6.2A). $\delta^{15}N$ was similarly significantly depleted (approx. 1‰) after 30 and 60 days in *H. spicifera*, but not in *Gelidium pristoides*. Both $\delta^{13}C$ and $\delta^{15}N$ differed significantly between species after, but not before degradation. In all cases values were 1-2.5‰ ($\delta^{13}C$) or 1-1.5‰ ($\delta^{15}N$) higher in *G. pristoides*.

Neither species showed any effect of time on their C: N ratios, which were consistently, significantly higher in *Gelidium pristoides* (mean 13.3 ± 1.12 SD) than in *Hypnea spicifera* (8.4 ± 0.40).

6.3.2 Laboratory Study

The results differed between species. *Gelidium pristoides* (Table 6.2): for both $\delta^{15}N$ and C: N ratios, all effects were significant, including the time x source interaction. For $\delta^{13}C$ the interaction was non-significant, $\delta^{13}C$ enriched over time in both algae and SPM, although the effect was not significant for algae. *Hypnea spicifera* (Table 6.3): for both $\delta^{13}C$ and C: N ratio, time and source had significant effects (both decreased over time, although decrease was less for SPM than algae), but $\delta^{15}N$ showed no significant effects.

Table 6.1: 2-way ANOVA assessing species-specific differences in $\delta^{13}C$, $\delta^{15}N$ and C: N ratios in the field, through time. DF = Degrees of Freedom, MS = Mean Square, F = F-Ratio, p = probability value. * $p < 0.05$

		$\delta^{13}C$			$\delta^{15}N$			C:N Ratios		
	DF	**MS**	**F**	**p**	**MS**	**F**	**p**	**MS**	**F**	**p**
Time	2	23.65	18.29	< 0.0001*	1.869	6.999	0.0023*	0.314	0.408	0.6678
Species	1	21.45	16.58	< 0.0001*	10.307	38.606	< 0.0001*	289.132	374.714	< 0.0001*
Time x Species	2	6.40	4.95	0.0115*	1.347	5.046	0.0106*	0.291	0.377	0.6880
Error	44	1.29			0.267			0.772		

Table 6.2: 2 way ANOVA for *Gelidium pristoides* in the laboratory, assessing the effect of time and source on $\delta^{13}C$, $\delta^{15}N$ and C: N ratios. DF = Degrees of Freedom, MS = Mean Square, F = F-Ratio, p = probability value. * $p < 0.05$

		$\delta^{13}C$			$\delta^{15}N$			C:N Ratios		
	DF	**MS**	**F**	**p**	**MS**	**F**	**p**	**MS**	**F**	**p**
Time	1	19.2	17.71	0.0001*	8.161	5.834	0.0209*	70.692	47.084	< 0.0001*
Source	1	8.371	7.723	0.0086*	13.335	9.533	0.0039*	369.511	246.11	< 0.0001*
Time x Source	1	1.662	1.534	0.2236	9.132	6.529	0.0150*	26.060	17.357	0.0002*
Error	36	39.02			1.399			1.501		

Table 6.3: 2-way ANOVA for *Hypnea spicifera* in the laboratory, assessing the effect of time and source on $\delta^{13}C$, $\delta^{15}N$ and C: N ratios. DF = Degrees of Freedom, MS = Mean Square, F = F-Ratio, p = probability value. * $p < 0.05$

		$\delta^{13}C$			$\delta^{15}N$			C:N Ratios		
	DF	**MS**	**F**	**p**	**MS**	**F**	**p**	**MS**	**F**	**p**
Time	1	9.94	9.47	0.0040*	10.137	3.9158	0.0555	31.261	43.016	< 0.0001*
Source	1	18.92	18.04	0.0001*	0.028	0.0107	0.9182	20.146	27.722	< 0.0001*
Time x Source	1	0.85	0.81	0.3750	2.659	1.0271	0.3176	2.345	3.226	0.0808
Error	36	1.05			2.589			0.727		

6.3.2.1 Effect of time

After thirty days of decomposition, $\delta^{13}C$ signatures of *Hypnea spicifera* tissue were on average 1.5‰ more depleted than fresh samples, and the C: N ratios of both *Gelidium pristoides* and *H. spicifera* decreased significantly from day 0 to day 30. No other significant changes were seen in either species. SPM collected from the *G. pristoides* buckets showed signatures at day 30 to be enriched in $\delta^{13}C$ but depleted in $\delta^{15}N$ relative to day 0, while C: N ratios showed no significant change. SPM from the *H. spicifera* buckets showed the reverse: a decrease in C: N ratio from day 0 to day 30, but no change in $\delta^{13}C$ or $\delta^{15}N$ (see Tables 6.2, 6.3 and Fig 6.2B).

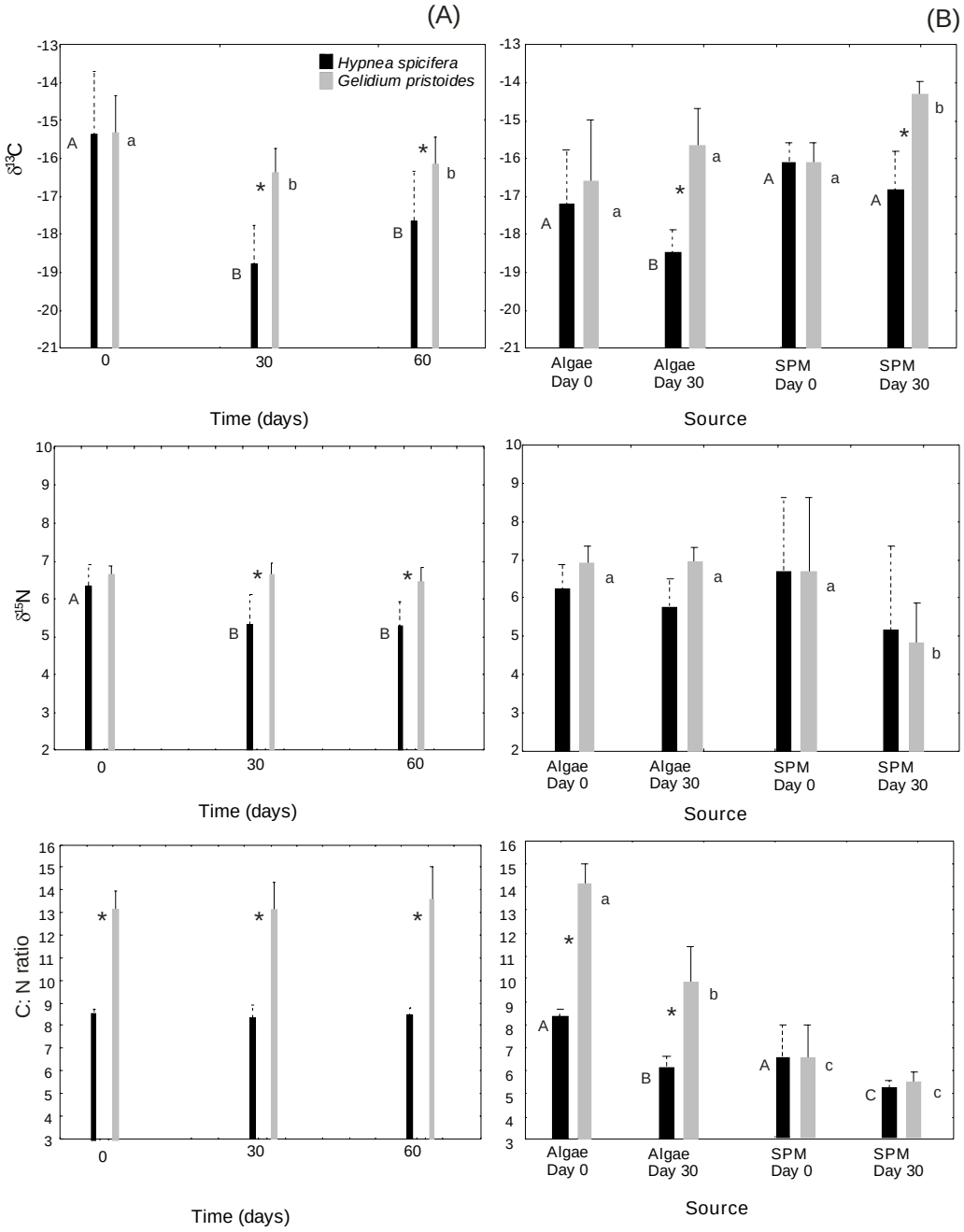

Figure 6.2: Effects of decomposition of *Gelidium pristoides* and *Hypnea spicifera* on $\delta^{13}C$, $\delta^{15}N$ (‰) and C: N ratios in the field (column A) and laboratory (column B). Uppercase letters indicate homogenous groups within *H. spicifera* and lowercase letters indicate homogenous groups within *G. pristoides* (Newman-Keuls, $p < 0.05$). * indicates significant differences between species (Newman-Keuls, $p < 0.05$). Values are means + SD.

For both species $\delta^{13}C$ did not differ between algal tissue and SPM at day zero, but was significantly higher for SPM on day 30. For $\delta^{15}N$, SPM and tissue values differed only for *Gelidium pristoides* on day 30, when SPM was depleted relative to tissue. The results for C: N ratios were more complex. Algal tissue and SPM differed only on day 30 for *Hypnea spicifera* and only on day zero for *G. pristoides*. In both cases SPM had lower C: N ratios.

The two-way ANOVA for day 30 allowed the inclusion of biofilm as a source (see Fig 6.3). At day 30 *Gelidium pristoides* showed significant differences among all three source types, with $\delta^{13}C$ signatures of SPM > algae > biofilm ($F_{2, 29}$ =12.77, $p < 0.01$) and $\delta^{15}N$ signatures of algae and biofilm > SPM ($F_{2, 29} = 17.76$, $p < 0.01$). Significant differences were also seen between source types in *Hypnea spicifera* at day 30, with SPM and biofilm $\delta^{13}C$ signatures > algae ($F_{2, 29} = 12.25$, $p < 0.01$). In both *G. pristoides* and *H. spicifera* the algal C: N ratio > SPM and biofilm ($F_{2, 29} = 18.22$; $F_{2, 29} = 15.11$ respectively, $p < 0.01$; Fig 6.3).

6.3.2.2 Differences between species

Although the two-way ANOVA for day 30 showed significant species x source interactions, we deal here only with species-specific differences. Carbon and nitrogen signatures of SPM and algal tissue from both species were similar at day 0 (Fig 6.2), but *Gelidium pristoides* SPM and tissue were significantly enriched in $\delta^{13}C$ relative to those of *Hypnea spicifera* at day 30 ($F_{1,54} = 51.04$, $p < 0001$). No species-related differences were found in $\delta^{15}N$ ($F_{1,54} = 0.06$, $p = 0.81$). Algal C: N ratios of *G. pristoides* at day 0 were higher than those of *H. spicifera* (Fig 6.2) and at day 30 both tissue and biofilm from *G. pristoides* were significantly higher than those from *H. spicifera* ($F_{1,54} = 32.54$, $p < 0001$; see Fig 6.3).

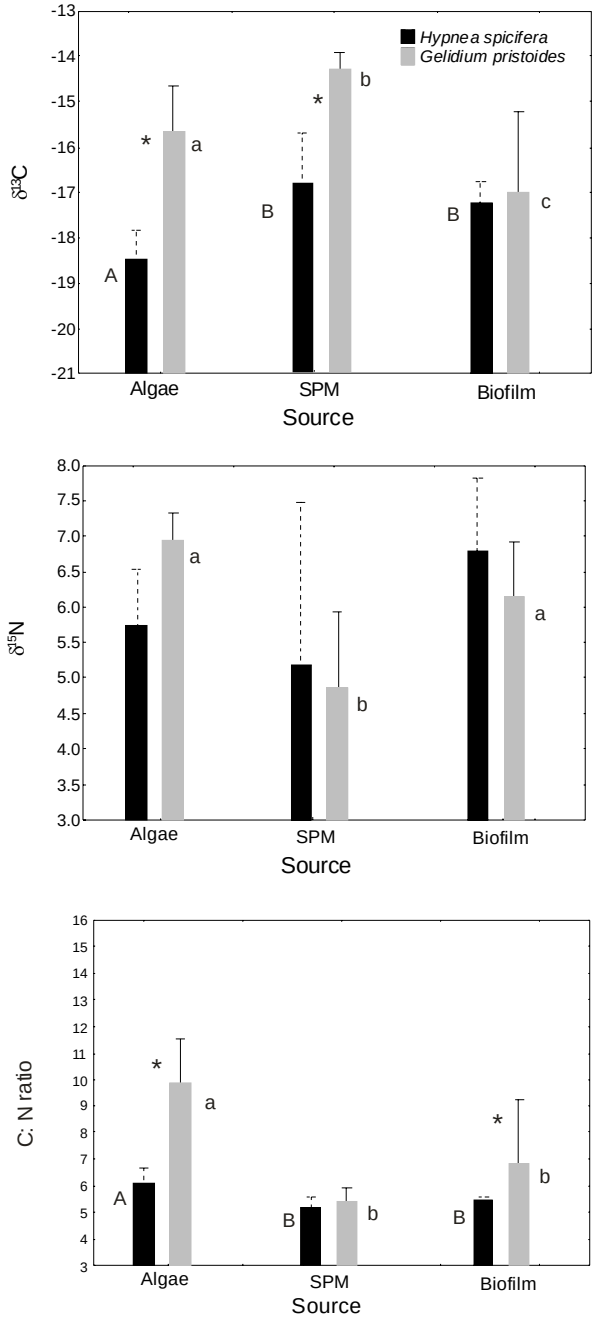

Figure 6.3: Species specific differences in *Gelidium pristoides* and *Hypnea spicifera* for $\delta^{13}C$, $\delta^{15}N$ (‰) and C: N ratios in the laboratory at day 30. Values are means + SD. Uppercase letters indicate homogenous groups within *H. spicifera* and lowercase letters indicate homogenous groups within *G. pristoides* (Newman-Keuls, $p < 0.05$). * indicates significant differences between species (Newman-Keuls, $p < 0.05$).

6.3.4 Links to consumer diet

Other than a reasonable enrichment from *Hypnea spicifera* SPM at day 30 to average mussel tissue (from Kenton-on-Sea, chapter 3), no overall trophic pattern or consistent fractionation of $\delta^{13}C$ (1.0 – 2.0 ‰ as reported by DeNiro & Epstein 1978; Fry & Sherr 1984; Peterson & Howarth 1987), or $\delta^{15}N$ (1.7 – 2.5‰; as reported by Raikow & Hamilton 2001; Fry 1988, 2006) emerged from the carbon or nitrogen ratios seen in decomposing algae or SPM compared to mussel signatures (Fig 6.4).

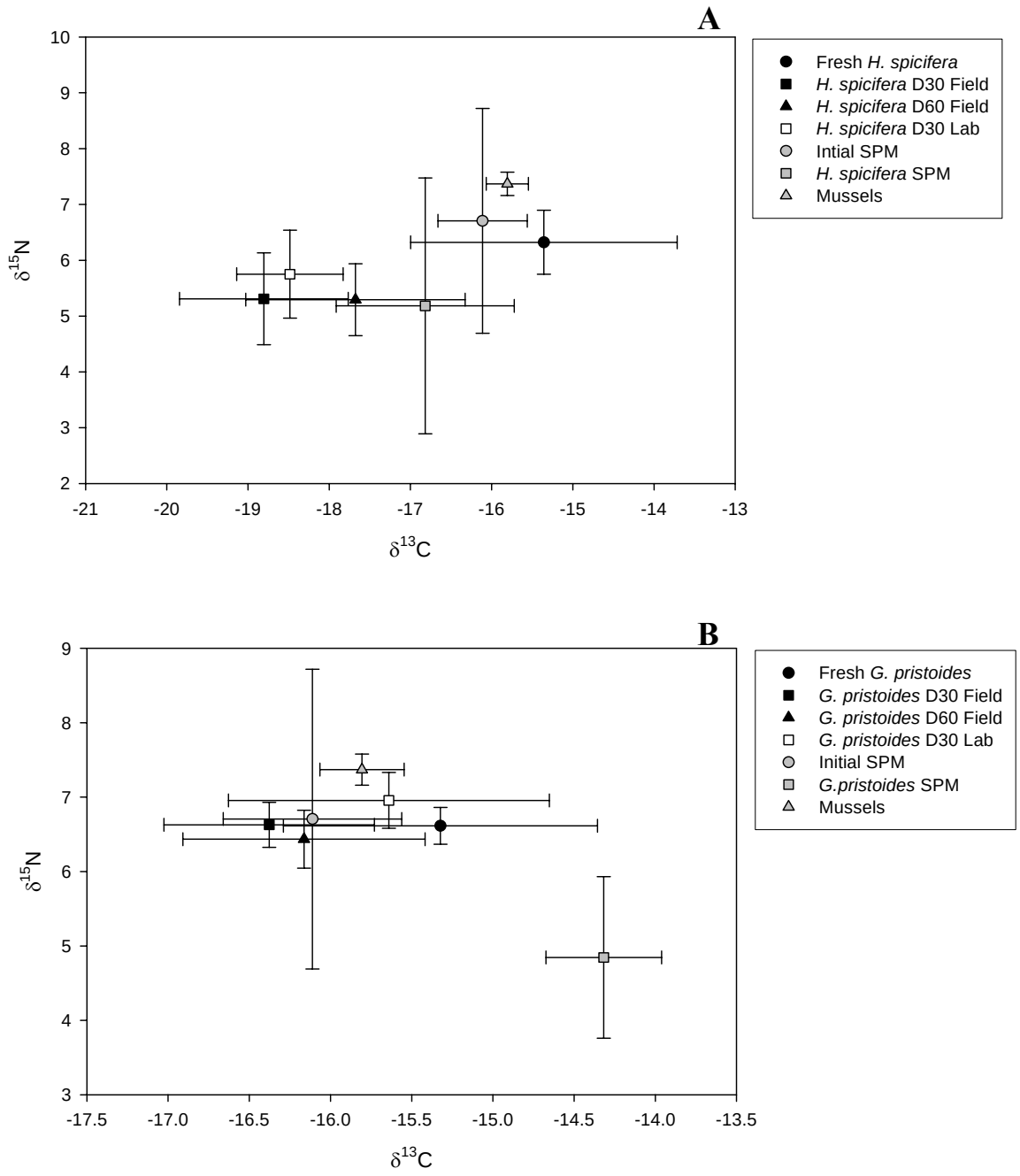

Figure 6.4: Average $\delta^{13}C$ and $\delta^{15}N$ plot (‰) of measured variables for *Hypnea spicifera* (A) and *Gelidium pristoides* (B) in the field and laboratory, compared with average mussel signature from Kenton-on-sea (chapter 3). Initial SPM was taken from the sea, algal SPM (i.e. *H. spicifera* and *G. pristoides* SPM) was sampled from the buckets at day 30.

6.4 Discussion

Some of the trends observed were non-significant, but, with the exception of Hypnea spicifera δ13C, the laboratory results conformed closely to the predicted hypotheses, while the field results did not (see Table 6.5).

Table 6.4 Predicted and observed changes in isotope composition of algal tissue and tissue-derived SPM. – indicates no clear trend, * denotes a significant change.

	Hypotheses	**Field**		**Laboratory**			
	Both Algae	*G. pristoides*	*H. spicifera*	*G. pristoides*		*H. spicifera*	
				Algae	SPM	Algae	SPM
$\delta^{13}C$	↑	↓*	↓*	↑	↑*	↓*	↓
$\delta^{15}N$	↓	-	↓*	↓	↓*	↓	↓
C: N ratio	↓	-	-	↓*	↓	↓*	↓*

6.4.1 $\delta^{13}C$

Overall, the observed isotopic changes of decomposing macroalgae did not conform to the predictions for carbon. $\delta^{13}C$ signatures of *H. spicifera* (lab and field) and of *Gelidium pristoides* in the laboratory showed up to 2.0‰ depletion after thirty and sixty days of decomposition, while in the laboratory *G. pristoides* carbon was non-significantly enriched. Fenton & Ritz (1988) also observed changes in carbon over a 60-day decomposition experiment but these were in the order of less than 1.0‰, and of the six macroalgae they examined, only the kelp *Ecklonia radiata* remained depleted by day 60. Combined with our findings, this suggests that carbon changes during decomposition are largely species-specific. Shifts in carbon similar to those observed here were seen during early diagenesis of the angiosperm *Spartina alterniflora* by Benner *et al*. (1987, 1991) with large depletions (up to 4.0‰) that were attributed to polysaccharide removal through leaching, resulting in decomposed material having lower ^{13}C due to enrichment in lignin-derived carbon (Benner *et al*. 1991). Although other studies have shown that decomposition does not affect $\delta^{13}C$ ratios in vascular plants or angiosperms (Currin *et al*. 1995, Schweizer *et al*. 1999, Fellerhoff *et al*. 2003, Machás *et al*. 2006), we suggest that macroalgae undergo leaching during decomposition, with leached compounds having

species-specific isotopic signatures and/or leaching rates that result in depleted algal signatures during degradation. In contrast to algal tissue, SPM signatures for *G. pristoides* in the laboratory behaved as predicted, showing significant carbon enrichment over time. Leaching would be greater under field conditions due to constant water replenishment as well as greater agitation. Thus, while changes in the laboratory would be due to the microbial effects with weak leaching, results from the field would include microbial effects plus strong leaching.

6.4.2 $\delta^{15}N$

Changes in nitrogen were species and/or source specific, but the observed trends conformed remarkably closely to predictions, albeit some of these trends were non-significant. Under virtually all conditions, $\delta^{15}N$ was depleted during degradation (max 1.0‰) in algal tissue and SPM derived from both species. The exceptions were the absence of marked changes in *Gelidium pristoides* tissue signatures in either the laboratory or the field. Although there are no comparable published data for macroalgae, ^{15}N depletion of 2-3‰ during degradation has been reported in angiosperms (Currin *et al*. 1995), with up to 10.0‰ depletion reported for mangroves (Zieman *et al*. 1984). The observed depletion in the nitrogen of both algal tissue and SPM nitrogen during decomposition accords with our hypothesis and may be explained by the colonization of organic matter by heterotrophic bacteria. Macko & Estep (1984) suggested that microbial communities assimilate ^{15}N enriched dissolved organic or inorganic nitrogen and could preferentially retain organic nitrogen during biochemical processing. They reported microbial alteration of the $\delta^{15}N$ composition of organic substrates by the marine bacterium *Vibrio harveyi*, which showed bacterial fractionation between 0 - 22‰ depending on the amino acids or sugars available in the substrate. The effects of bacterial colonization could therefore play a large role in isotopic shifts in nitrogen during macroalgal decomposition. In natural environments, it is likely that species and substrate diversity will influence bacterial populations and Macko & Estep (1984) and Fellerhoff *et al*. (2003) suggest that large isotope fractionations may then cancel each other out, resulting in a more consistent $\delta^{15}N$ signature. However, in cases where a homogenous

microbial community dominates the organic substrate (e.g. high sulphur, anaerobic marine sediments), isotopic signatures will be significantly altered. This is of particular importance to macroalgae decomposition as there is indirect evidence that algal species differ in the bacterial communities they support (Šyvokeine *et al*. 1988). Macroalgae are also known to excrete defensive chemicals (Hay & Fenical 1988) in times of stress and proteinaceous enzymes (e.g. alkaline phosphatase) to aid in the uptake of phosphorus (Weich & Graneli 1989). Penhale & Capone (1981) provided evidence that exuded organic matter may act to stimulate N-fixing bacteria living on macroalgal thalli. Thus, nitrogen and carbon may be lost as a byproduct of exudation while the consequent stimulation of N-fixing bacteria may in turn affect the overall isotopic signatures of decomposing material.

6.4.3 C: N ratio

If the C: N ratio of an animal is constant, then limitations of available nitrogen may restrict C utilization (Hessen 1992), resulting in compensation for low food quality through increased feeding rates (Cruz-Rivera & Hay 2000, Norderhaug *et al*. 2003). However, there may be an upper limit to the C: N ratio above which an alga will be unable to support consumer growth (Hessen & Bjerkeng 1997). Russel-Hunter (1970) estimated that consumers require a diet with a C: N ratio of 17 or less. Since some macroalgae (e.g. kelp) often have C: N ratios above this limit (Sjøtun *et al*. 1996, Elser *et al*. 2000, Norderhaug *et al*. 2003), the effects of decomposition on C: N ratios become important. Both *Gelidium pristoides* and *Hypnea spicifera* had C: N ratios well below 17, but the ratio of *H. spicifera* was lower than that of *G. pristoides*, suggesting that it is the better food. Indeed, there is little evidence of direct grazing on *G. pristoides*, while *H. spicifera* often shows signs of fish cropping (pers obs). Also algal fronds, which are the parts consumed, have better C: N ratios than holdfasts. Although we saw no significant changes in C: N ratios under field conditions, in the laboratory both macroalgae showed the predicted decline in C: N ratios in algal tissue and SPM during decomposition (for *G. pristoides* SPM the decrease was not significant). Overall, this will make degraded material a better food source than live algae. The mechanisms underlying changes in C: N ratios are likely to be tied to those responsible for isotopic changes. Potential

explanations include: bacterial colonization and subsequent nitrogen fixation during macrophyte decay (Thornton & McManus 1994, Fellerhoff *et al*. 2003), increased carbon loss through microbial respiration or mineralization (Fellerhoff *et al*. 2003), or the removal of carbohydrates (containing only C, H and O) during the initial stages (first 100 days) of decomposition (Fogel & Tuross 1999).

6.4.5 Differences between species

Although fresh samples of *Gelidium pristoides* and *Hypnea spicifera* had comparable isotopic values, there were clear differences between them after 30 and 60 days of decomposition. *G. pristoides* had consistently more enriched carbon and nitrogen isotope ratios throughout the experiments, and demonstrated higher C: N ratios. This may simply reflect the biochemical composition of each species and their subsequent ease of decomposition, with *H. spicifera* experiencing more rapid leaching and microbial colonization than *G. pristoides*. A more complex explanation would be the species-specific support of colonies of unique microflora (Macko & Estep 1984, Šyvokeine *et al*. 1988, Hamilton & Lewis 1992). Carbon and nitrogen signatures of biofilm samples were very similar between species, which suggests that the signatures of developing biofilm may have more to do with microbial colonies than the algae themselves and the higher C: N ratio of *G. pristoides* would accordingly indicate either higher microbial mineralization in *G. pristoides* or higher colonization by nitrogen fixing bacteria in *H. spicifera*. Interpreting differences between species can be further complicated by the potential for macroalgae to exhibit tissue-specific growth rates, resulting in a variety of distinct isotopic signatures within a single plant (Fry & Sherr 1984, Gearing 1991, Michener & Schell 1994). Such variations in tissues are likely to be due to isotopic routing, and/or differential metabolic fractionation and the allocation of assimilated carbon among tissues (Thompson & Ballou 1956, Libby *et al*. 1964, Tieszen *et al*. 1983, Gorokhova & Hansson 1999, Sweeting *et al*. 2005). For example in animals where rare or limiting nutrients are required for synthesis in particular tissues, they may be mobilized from long-term stores rather than from the available food sources (Bearhop *et al*. 2002).

The two species showed tissue-specific trends that were largely opposite. *Hypnea spicifera* showed no significant differences in $\delta^{13}C$, while these were marked in *Gelidium*

pristoides, holdfasts having the lowest ratios. Patterns in $\delta^{15}N$ were exactly opposite between species, being minimal in the fronds of *G. pristoides* and the holdfasts of *H. spicifera*. C: N ratios in both algae were lowest in the fronds. Living plants of *G. pristoides* often include fronds that are senescent at the tips that demonstrated enriched $\delta^{13}C$ and depleted $\delta^{15}N$ signatures relative to healthy fronds (nitrogen only) and holdfast tissue (carbon and nitrogen). Although the opposite to the field results for $\delta^{13}C$, this result supports the laboratory findings and suggests that $\delta^{13}C$ changes associated with decomposition start in living plants (senescent fronds are visibly discoloured) and are completed in the water column during the formation of SPM.

6.4.6 Implications for SPM

Because decomposing macroalgae are likely to contribute substantially to SPM, our results suggests that SPM signatures are influenced by: the species contributing to the detrital pool and their proportions; the tissue types predominating; the ages of the detrital components. A further complication is that detritivores assimilate microflora with 50-100% efficiency, while assimilation rates of algal material are typically less than 5% (Lopez *et al*. 1977, Cammen 1980), so that detritivores derive a large proportion of their nutritional requirements from the microbial component of detritus. However, the implications of this differ between elements. The bacteria associated with detritus and SPM generally supply only a small fraction (10-30%) of the carbon requirements of consumers, but a high proportion (>90%) of their nitrogen requirements (Cammen 1980, Newell *et al*. 1982, Newell & Field 1983), providing nitrogen, vitamins and essential amino acids, while the plant tissue is assimilated at lower efficiencies as a carbon and energy source (Zieman *et al*. 1984).

Our results suggest that unexplored mechanisms of carbon and nitrogen change are at work at tissue/species-specific levels. Both *Gelidium pristoides* and *Hypnea spicifera* showed tissue-specific differences in values within individual plants and distinct changes in isotope and C: N ratios related to time during decomposition. The general patterns in isotopic change were similar in both species, but for carbon they differed between the lab and the field, while the rates of change in signatures appear to be species-specific. All this indicates a high level of complexity in the relationship between living macroalgae

and SPM. Hill *et al*. (2006) for example, found that filter feeding bivalves acquired a large percentage of their isotopic signatures from nearshore SPM (a single source), but the isotopic signature of that single source is complex and shows high temporal variability (unpubl. data). Clarifying the links between primary producers and SPM, which is likely to include detritus from numerous macroalgae as well as other sources, remains a difficult task.

CHAPTER 7: GENERAL DISCUSSION

7.1. Isotopic links to coastal hydrography

The primary aim of this dissertation was to investigate the temporal and biogeographic variation in isotopic signatures of marine intertidal consumers and their food sources, with a focus on the importance of phytoplankton versus macroalgal-derived production in the diet of intertidal mussels. As a result of these studies a number of important isotopic patterns have emerged, which have furthered our understanding of the role played by nearshore and coastal hydrography in rocky intertidal ecosystems. The emergence of these trends suggests that biogeochemical approaches can be powerful tools for understanding patterns in biogeography and ecosystem functioning. Furthermore, the nested spatial scale design employed in this dissertation provided a means by which to link nearshore/offshore ecosystem components and elucidate highly dynamic local and coastal trophic relationships. The isotopic patterns seen throughout these studies, between sites and trophic levels, also serve to emphasize the importance of the biogeochemical role in niche theory and food-web analyses.

The first set of isotopic patterns in this dissertation became evident over a 14 month sampling period at Kenton-on-Sea (chapter 3) and established the existence of a depletion gradient in carbon at kilometer scales (extending up to 50km off the coast) when moving from nearshore to offshore waters. These patterns were further substantiated by the investigations of chapter 4 which showed nearshore to offshore carbon depletions along the coastline of southern Africa in two years, confirming that such trends are ubiquitous around the coast as well as persistent in time. These trends of depletion suggested a shift from an inshore carbon supply to one more representative of a pelagic carbon source in surface water. Although it was not possible to link these $\delta^{13}C$ values directly to distinct sources of production, nearshore (0km) signatures fell into the range of carbon values previously reported for macroalgae (this dissertation; Smith & Epstein 1971, Bustamante & Branch 1996), and offshore (10km) signatures were similar to those previously reported for oceanic phytoplankton (Sherr 1982; Rau *et al*. 1982; Fry & Sherr 1984; Monterio *et al*. 1991). Furthermore, mixing model applications (Bustamante & Branch 1996, Phillips & Gregg 2003) confirmed that macroalgal signatures accounted for more

than 50% of nearshore SPM (see chapters 3 and 5), and at Kenton-on-Sea, offshore SPM remained in the typical $\delta^{13}C$ range for phytoplankton (-20.00 to -21.5‰) as far as 50km offshore (chapter 3). All of this suggests that these nearshore/offshore isotopic patterns reflect an SPM gradient of decreasing macrophyte influence. However, it is also important to note that there are significantly different communities of phytoplankton in nearshore and offshore waters (chapter 3), which may also contribute to differences in SPM signatures. Diatoms predominate in nutrient rich water, and are replaced by flagellates once the majority of dissolved silicate is consumed (Tsunogai 1979; Tsunogai & Watanabe 1983) and Kukert & Riebesell (1998) reported diatom dominated POM samples to be 2.0‰ (up to 5.0‰ in bloom conditions) more enriched than those dominated by flagellates. Although there was no direct evidence for this, fig 3.6 suggests that larger phytoplankton are found in higher quantities inshore and accordingly, while enriched nearshore SPM signatures are likely representative of inshore macroalgal detritus, they may also be partly due to the phytoplankton being dominated by diatoms, which are isotopically heavier than their flagellate counterparts seen in nutrient poor water, further offshore (10km).

With the basic differences between isotopically enriched and depleted surface water sketched out in terms of organic carbon production, the second set of isotopic patterns emerged when considering surface water signatures with respect to nearshore hydrography. $\delta^{15}N$ signatures of SPM were largely uninformative, due to a complete lack of clear temporal or biogeographic trends, as well as a small range of variation at the temporal and spatial scales examined, and these are not discussed further. In contrast, patterns in $\delta^{13}C$ provided ample evidence of the effects of coastal hydrography. Firstly, although nearshore/offshore depletion was consistent in every month at Kenton-on-Sea and with few exceptions along the South African coastline, the slopes of the curves varied among sites and over time, suggesting not only geographic but also temporal effects on offshore depletion. Carbon patterns at Kenton-on-Sea showed strong associations with surface water movement visible from SeaWiFS satellite images of chl-a, which were likely to be related to hydrographic features inshore of the Agulhas Current. Secondly, overall carbon patterns among coastal sites within the Agulhas Current system demonstrated nearshore $\delta^{13}C$ enrichment of SPM when moving from the

east to the south coast. This is presumably directly related to the width of the continental shelf, which widens from east to west, and the position of the Agulhas Current, which roughly follows the 100m isobath (Goschen & Schumann 1990; Goschen & Schumann 1994; Roberts 2005). $\delta^{13}C$ values from sites within the Benguela system however, remained much lower, reflecting a geographic shift to colder water and a more nutrient rich ecosystem (Andrews & Hutchings 1980), where the injection of ^{12}C-enriched upwelled materials results in depleted $\delta^{13}C$ signatures (Naidu & Niitsuma 2003). More importantly, $\delta^{13}C$ signatures also reflected the existence of known hydrographic features along the coast, upwelling regions, current meanders, shelf width and shelf abnormalities such as canyons. The close spatial correlation between carbon signatures and these hydrographic features highlights the strong ties between coastal SPM $\delta^{13}C$ signatures and nearshore hydrography.

Along with spatial, geographic and large-scale temporal variation in SPM $\delta^{13}C$, there is a high potential for daily change in isotope signatures due to variation in the extent of mixing between near and offshore waters, the position/meandering of the Agulhas Current (Goschen & Schumann 1990), and regional variation in local autochthonous or allochthonous inputs (Megens *et al*. 2001). Accordingly, an investigation into daily variation in nearshore (0km) SPM was undertaken; revealing a third set of isotopic patterns which could also be linked with very nearshore hydrography. Enrichment - depletion sequences in $\delta^{13}C$ that were tightly correlated with tidal cycles were evident from data collected at Kenton-on-Sea, and as inputs from nearby estuaries are considered to be minimal, the tidal influence is thought to result from detrital resuspension at high tide. These studies bear out the utility of using stable isotope analysis in a biological context, linking physical oceanography to intertidal environments and consequently representing a powerful tool for the study of water mixing and coastal hydrography in relation to food-web analyses.

7.2 Sources of uncertainty

A number of factors however presented stumbling blocks to the interpretation of some results and complicated the resolution of intertidal food webs on the South African rocky shore. Firstly, SPM signatures of nitrogen throughout this dissertation were highly

variable and lacked any discernable biogeographic or temporal patterns. According to recent literature, the main sources of large $\delta^{15}N$ variation in marine SPM appear to be the availability of nitrate in surface waters (Benner *et al*. 1997), isotope fractionation during nitrate uptake (Wada 1980; Altabet *et al*. 1991; Wu *et al*. 1997) and the residence time of N in SPM (Waser *et al*. 2000). The interpretation of $\delta^{15}N$ particulate nitrogen in oceanic SPM is further complicated by the fact that, unlike dissolved inorganic carbon, the concentration of dissolved inorganic nitrogen can vary significantly at the ocean surface. This in turn can cause variations in the $\delta^{15}N$ of the N source due to Rayleigh fractionation (Waser *et al*. 2000). As phytoplankton growth increases with available nitrate, so does fractionation, and consequently temporal changes (on both large and small scales) in nitrate availability may affect $\delta^{15}N$ signatures of SPM. Although $\delta^{15}N$ may be a reliable indicator of trophic level in marine consumers (Fry 1988), the high variability of SPM signatures in marine environments reduces the efficacy of stable isotope analysis in primary producer – consumer relationships. Secondly, of all sampled macroalgae from the biogeographic, temporal and coastal studies demonstrated extremely erratic signatures, with single species spanning wide isotopic ranges in both carbon and nitrogen (see chapters 3-5). Thirdly, no discernable temporal or biogeographic trends were seen in any one species. These dynamic changes in isotopic signatures are likely to be due to variation in photosynthetic fractionation at the individual, population and species levels. Growth rate, for example, can have far reaching isotopic consequences, as primary producers have high growth rates that respond to small-scale changes in nutrient source and availability (Gartner *et al*. 2002; O'Reilly *et al*. 2002) and plants with faster growth rates show less fractionation than slower growers with the same photosynthetic pathways (Degens *et al*. 1968). Photosynthetic fractionation can also result from, but is not limited to: primary method of CO_2 acquisition and submersion time (Degens *et al*. 1968; Mook *et al*. 1974; Dring & Brown 1982); irradiance day-length/intensity (Guy *et al*. 1989, Thompson & Calvert 1994; Burkhardt *et al*. 1999a, 1999b) and the type of pathway (C_3, C_4 or CAM) used in photosynthesis (Johnson *et al*. 1974; Dring & Brown 1982; Laws *et al*. 1997). As a result, obtaining consistent and/or characteristic signatures for different algal species is a complex problem, especially considering that $\delta^{13}C$ variability due to photosynthetic fractionation may be on a scale of >10.0‰.

Such erratic variation in isotope signatures made it difficult to link living macroalgae with mussel diet or SPM, especially as SPM samples represent a mixture of phytoplankton and detritus from different algal species that have signatures modified by degredation and that occur in unknown proportions. Mussels will assimilate macroalgae from the detrital pool rather than from living plants, and this prompted an investigation into the isotopic effects of degradation on two macroalgal species (*Gelidium pristoides* and *Hypnea spicifera*) with high intertidal biomass on the south coast of South Africa. Rather than clarifying the trophic links between mussels, macroalgae and SPM however, the investigation concluded that not only do macroalgae demonstrate species-specific isotopic decomposition effects, but individual algae also exhibit tissue-specific fractionation in both $\delta^{13}C$ and $\delta^{15}N$ (see chapter 6). These tissue-specific signatures in both the macroalgae and *Perna perna* were likely to be due to the allocation of assimilated nutrients among tissues (i.e. isotopic routing) as well as differential fractionation through metabolism (Thompson and Ballou 1956; Libby *et al*. 1964; Tieszen *et al*. 1983; Gorokhova & Hansson 1999; Sweeting *et al*. 2005). The species-specific decomposition effects are harder to explain, but are probably related to the differential leaching of isotopically dissimilar compounds, the bacterial species present on different algae (Simon & McQuaid 1999) as well as the degree of microbial mineralization (Macko & Estep 1984).

Similar complications arose during an investigation into equilibration rates of four different mussel tissues from the brown mussel *Perna perna* (adductor, gill, gonad and mantle; chapter 2). This revealed that carbon and nitrogen fractionation differed not only between tissues but also between diets. This is of considerable concern when using stable isotopes as indicators of food source, as it means that not only food quality but also the tissue sampled will have strong influences on isotopic signatures. Isotopic equilibration rates also differed between diets and tissues, although the overall pattern in $\delta^{13}C$ equilibration was similar, with adductor tissue taking the longest to turn over, followed by gill and mantle tissue, while the turnover times of gonad tissue were harder to determine due to the close relationship between the synthesis of mantle tissue and subsequent gonad development. However, metabolic rates in captive animals can be considerably different from wild-caught individuals (Hobson & Clark 1992, 1993; Nagy

1987) and relationships derived from laboratory studies may not be an accurate representation of the natural environment. Unfortunately the natural environment presents too many variables at ecological, biological and biochemical levels to obtain reliable measurements. $\delta^{15}N$ signatures of adductor tissue from laboratory maintained mussels were comparable to those collected from the field (chapters 3-5), $\delta^{13}C$ signatures from laboratory mussels however, were somewhat depleted (0.5 – 1.0‰) relative to field animals (chapters 3-5). If we consider that over the 14 month sampling period at Kenton-on-Sea (chapter 3) mussels demonstrated up to 1.0‰ variation in $\delta^{13}C$, the difference between adductor tissue measurements in the laboratory and field becomes minimal. Thus, adductor muscle represented the most time-integrated tissue, with relatively low lipid content (Gilbane 2005), which made it ideal for comparisons among trophic levels, and it was consequently used in further chapters as an overall descriptor of mussel diet. However, the long tissue turnover time presented difficulties in identifying temporal changes in mussel tissue, as well as relating tissue signatures to those of the measured food sources (i.e. spot measurements such as SPM samples and living macroalgae).

7.3 Functional biogeography

Due to these sources of complexity, it was difficult to relate the sampled food sources (i.e. macroalgae and SPM) directly to mussel diet, but the application of two mixing models (Bustamante & Branch 1996; Phillips & Gregg 2003) determined that mussels (chapter 3-4) and other filter feeders (chapter 5) depend on nearshore SPM for more than 50% and 40% on average respectively, of their organic carbon and nitrogen (mussels only). Aside from a strong dependence on nearshore SPM, both mussels (chapters 4-5) and other filter feeders (chapter 5) also demonstrated a biogeographic gradient that probably reflects the influence of two very different oceanographic regimes. Using carbon and nitrogen signatures, mussels and filter feeders around the southern African shoreline could be divided into four site groupings, with a strong geographic pattern. The east coast group encompassed animals from Sodwana Bay to Cintsa, the south-east coast group from Port Alfred and Kenton-on-Sea, the south-west coast group from Plettenberg Bay to St. Helena Bay and the west coast group included only Port Nolloth and Walvis Bay, Namibia. There were three exceptions to this pattern and the discrepancies between

June and February mussel samples at St. Helena Bay (eventually included in the south-west geographic grouping) were more complex and are considered in Chapter 5.

All sampled mussels (chapters 4-5) exhibited geographic enrichment in $\delta^{15}N$ with clear gradients from east to south-west. Similarly, most, but not all species common to all four sites in chapter 5, showed crude enrichment gradients in nitrogen between south-east/south-west sites. These biogeographic gradients in primary producers and consumers reflect an isotopic gradient from oligotrophic to eutrophic conditions (Saino & Hattori 1980; Minagawa & Wada 1984), in which isotopic differences are due to a reliance on recycled nitrogen (especially ammonia) in oligotrophic waters, which is depleted in $\delta^{15}N$ relative to the upwelled nitrate used in eutrophic systems (Miyake & Wada 1967). Mussel tissue in chapters 4 and 5 also exhibited geographic $\delta^{13}C$ enrichment from the east to the south-west coast, excluding all locations to the north of St. Helena Bay. These remaining (west coast) sites showed significant carbon depletions, mirroring the trend seen in nearshore SPM (chapter 4). Although a consistent fractionation value of 1.0‰ (DeNiro & Epstein 1978; Fry & Sherr 1984; Peterson & Howarth 1987) was not evident between offshore or nearshore SPM and mussel tissue, the latter two showed similar patterns in $\delta^{13}C$ enrichment from east to south-west coast, indicating close overall links between them. Allowing for processing of material, spatial patterns in $\delta^{13}C$ signatures ultimately reflect patterns in the signatures or distribution of primary producers, which accordingly are likely to reflect environmental gradients (Deegan & Garritt 1997; Takai *et al*. 2000)

7.4 Overall summary and future considerations

This dissertation showed reasonably strong isotopic signals at large spatial scales (i.e. biogeographic and nearshore/offshore gradients) but the isotopic patterns at smaller temporal and spatial scales (degradation, tissue-specific signatures etc.) were subject to large variability. The nearshore/offshore depletion trends seen throughout this dissertation, whether due largely or just in part to macroalgal production, became a central part of unraveling the dynamics of an ecosystem that has long been thought to be dependent on offshore inputs for nutrient delivery. The heavy dependence seen in mussels and other filter feeders on nearshore SPM suggests that the rocky intertidal zone

represents a moderately self-sustaining ecosystem that is only weakly dependant upon offshore inputs and typically exports materials to adjacent ecosystems. This concept has been developed for sandy beaches (Brown & McLachlan 1990), but is less expected for rocky shores which are zones of erosion rather than deposition. Rocky shore primary production however may be influenced by local hydrographic processes (which can be closely correlated with $\delta^{13}C$ signatures of surface water) that will affect nutrient availability. Direct relationships between primary producers and consumers were complicated by variable fractionation in both macroalgal tissues and species as well as long turnover times in consumer tissues. Overall, this work highlights three main features of rocky shore ecosystems: firstly filter feeders are strongly linked to nearshore SPM rather than offshore inputs, which suggest a semi-closed/semi-autochthonous system, secondly biogeography has an important influence as biogeographic shifts in $\delta^{13}C$ were especially clear among filter feeding groups, likely because of their close ties to nearshore SPM, and lastly that biogeographic shifts are also evident not just in carbon signatures, but mixing models indicate also in the diet of some grazers.

Many natural populations are composed of individuals that exploit different subsets of available resources (e.g. Roughgarden 1974; Van Valen 1965; Bolnick *et al*. 2003; Araújo *et al*. 2007). Individuals within a population may use different resources because they are different ages (Persson 1983; Polis 1984), different sexes (Slatkin 1984; Shine *et al*. 2002) or because they occupy different microhabitats (Durell 2000). Moreover, individuals can also exhibit niche partitioning within a sex or age class, or within a single time or site. This individual specialization means that some individuals use a considerably smaller set of resources than the population as a whole (Bolnick *et al*. 2003; Araújo *et al*. 2007) and such differential exploitation of resources may have significant ecological implications that may ultimately affect population dynamics (Lomnicki 1992). Quantifying individual-level feeding habits is easily done through the analysis of gut contents, but often overestimates the amount of dietary variation (Araújo *et al*. 2007), and is not necessarily representative of assimilated nutrients. Stable isotope analysis on the other hand, is a measure of time integrated dietary assimilation, but often struggles to provide information on shorter term and individual-level changes in diet. The complications of variable time-integration between food resources and consumers

suggests that future investigations into trophic ecology should combine gut-content and stable isotope analysis to give a comprehensive overview of consumer diet.

To maintain sample integrity, $\delta^{13}C$ signatures of both plant and animal tissues in this dissertation were compared without chemical lipid extraction, as such techniques often cause fractionation in $\delta^{15}N$ (Pinnegar & Polunin 1999; Sotiropoulos *et al*. 2004). However, as previously mentioned, variation in lipid content has the potential to introduce significant bias into $\delta^{13}C$ measurements, and despite the potential for introduced error in measurements, there is no consensus on the need to account for lipid influence on carbon signatures. Post *et al*. (2007) however, investigated the necessity of accounting for lipid bias in trophic ecology and their results indicated that it is most important to account for lipid content when it is variable among consumers of interest, or when the difference in $\delta^{13}C$ signatures between food sources and consumers is < 10-12‰. As this clearly encompasses the vast majority of marine food webs, future studies must be prepared to account for lipid content in order to resolve trophic links successfully. Although chemical extraction has significant drawbacks, the application of mathematical normalization as proposed by McConnaughey & McRoy (1979), which uses C: N ratios and a difference estimate (*D*) to produce a post-hoc lipid-normalized $\delta^{13}C$ signature for each sample, could account for lipid content while maintaining sample integrity (Post *et al*. 2007). Unfortunately, although a number of studies have applied various versions of mathematical normalization (e.g. Alexander *et al*. 1996; Schmidt *et al*. 2003; Sweeting *et al*. 2006), there has yet to be any rigorous testing of the robustness of this technique. Nevertheless, mathematical normalization has the potential for resolving lipid-associated bias in stable isotope analysis and should be considered in future isotope investigations into marine ecology.

Although decades of research have been devoted to the use of stable isotopes in the fields of hydrology and geochemistry, the application of stable isotope analysis in the field of marine ecology is still relatively new. The general approach has always been to evaluate food-web links through relationships or by simple mixing models which determine what percentage of organic carbon or nitrogen was derived from a number of food sources of constant composition. These kind of approaches however, are vast simplifications of highly complex systems, and largely ignore the heterogeneity (or

"noise") seen in the isotopic ratios of the majority of organisms. If we consider the "noise" due to variable fractionation seen amongst all living and decomposing macroalgal species sampled throughout this dissertation, it becomes clearly inappropriate to refer to any one species of macroalga as an isotopically constant food source. Yet this is a basic assumption underpinning the use of isotopes in trophic ecology. Neither can SPM be considered an isotopically constant food source, as we have seen predictable but highly variable changes on both temporal and spatial scales.

Fractionation effects are clearly seen at tissue, individual, species and likely even at population levels, all of which must be accounted for in stable isotope studies in trophic ecology. In the field of isotope hydrology for example, hydrologists have documented considerable heterogeneity in the isotopic compositions of groundwater, solute sources, rain and soil (Kendall & Doctor 2003), and have begun to recognize that the degree of variability is highly dependant on the frequency of sampling (Kendall & McDonnell 1998; Kendall & Doctor 2003). To obtain an accurate understanding of relationships within a food-web, more effort must be placed on event-based studies with high-frequency sampling (Kendall & McDonnell 1998) and this dissertation emphasizes the importance of quantifying or calibrating the isotopic fractionation of the animal in question, including tissue (chapter 2), population and species (chapter 4) used, along with the variability and fractionation of the available food sources, prior to the interpretation of results.

The biggest challenge to stable isotope ecology in marine systems is to discover how to deal with sample heterogeneity and to discover a way to use isotopic "noise" as signals in order to open up new avenues of exploration. As ecological isotope techniques develop, fractionation heterogeneity may resolve itself into more complete pictures of marine ecosystems and help to elucidate patterns of variability within those systems.

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APPENDIX 1: Reported ingredients of fish food and kelp diets

AquaPlus Baby Fish Food:

Fish meal, maize, rice, wheat, wheat germ, maize gluten, dehulled soybean meal, lysine, methionine, lime, dicalcium phosphate, Vitamins A, D, E, K, B1, B2, B6, B12, C, biotin, folic acid, pantothenic acid, nicotinic acid, inositol, minerals, colourants, spirulina and immune stimulants, vegetable fats with natural antioxidants, betadine

Bettaway Kelp:

Kelp, dicalcium phosphate, lactose, maize, sucrose, corn syrup, silica, magnesium stearate