Isotopic Ecosystem Studies in the KwaZulu-Natal Bight

by

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As the candidate's supervisor I have approved this thesis for submission.

Signed.....Name:.....Date: .....

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PREFACE

The experimental work described in this Ph.D. thesis was carried out in the School of Life Sciences, University of KwaZulu-Natal, Durban, from January 2009 to May 2012, under the supervision of Dr Albertus J. Smit.

These studies represent original work by the author and have not otherwise been submitted in any form for any degree or diploma to any tertiary institution. Where use has been made of the work of others it is duly acknowledged in the text.

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DECLARATION 1 - PLAGIARISM

I, **Ander Martinez de Lecea** declare that

1. The research reported in this thesis, except where otherwise indicated, is my original research.
2. This thesis has not been submitted for any degree or examination at any other university.
3. This thesis does not contain other persons' data, pictures, graphs or other information, unless specifically acknowledged as being sourced from other persons.
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DECLARATION 2 - PUBLICATIONS

DETAILS OF CONTRIBUTION TO PUBLICATIONS that form part and/or include research presented in this thesis are as follows:

Publication 1

De Lecea, A.M., Smit, A.J. and Fennessy, S. 2011. The effects of freeze-thaw periods and drying methods on isotopic and elemental carbon and nitrogen in marine organisms. *Rapid Communications in Mass Spectrometry*. 25: 1853

Publication 2

De Lecea, A.M., Omarjee, A., Cooper, R. and Smit A.J.2011. The effects of preservation methods, dyes and acidification on the isotopic values ($\delta^{15}N$ and $\delta^{13}C$) of two zooplankton species from the KwaZulu-Natal Bight, South Africa. *Rapid Communications in Mass Spectrometry*. 25: 3640

Publication 3

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Publication 4

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Publication 5

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Contribution from co-authors other than my supervisor Dr A.J. Smit:

Dr Sean Fennessy collected samples, identified species and research funding.
Miss Aadila Omarjee assisted me with sample collection and zooplankton sorting.
Miss Rachel Cooper assisted with zooplankton sorting and important editorial comments.

Signed: ……

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THESIS OVERVIEW

The study area, the KwaZulu-Natal Bight, is an oceanographically important area, which, regardless of having two of the most important fisheries off the east coast of South Africa, has received little research attention regarding its biological functioning. Until now chiefly oceanographic processes have been considered the drivers of this generally oligotrophic system. This study seeks to understand which of three important processes, a topographically induced oceanic upwelling cell near Richards Bay, a cyclonic eddy near Durban, or fluvial fluxes centred around the Thukela River, forces ecological functioning through their nutrient or organic matter input. The overall aim of the thesis is to understand the pelagic and benthic ecosystems of the Bight in terms of these drivers through the use of stable isotope ($\delta^{13}C$ and $\delta^{15}N$) analyses of a range of biotic and abiotic samples. These were collected on board of a number of research cruises – forming predominantly part of the larger African Coelacanth Ecosystem Programme suite of studies – in the wet and dry seasons of 2010.

Isotopic analyses found distinctions between fluvial and oceanic particulate organic matter and indicate that upwelling was not occurring in either sampling season. Organic matter originating from the Thukela River did not play a significant role in the wet season, although it dominated the planktonic pelagic food web in the dry season. The organic matter of the most productive region in the Bight, the Middle Shelf, was of riverine origin in the dry season, but of indeterminate origin in the wet season when it may have been an artefact of an old upwelling event which had previously occurred to the north of the Bight. There is, however, some evidence suggesting that this organic matter may rather have been of riverine origin, with its $\delta^{13}C$ signals subsequently having been modified by the diatom bloom occurring there.

In the demersal ecosystem, sediment isotopic data show organic matter to be well-mixed throughout the Bight in both seasons, with riverine organic matter dominating most of the Bight except its northern and southern edges, where oceanic organic matter increases in importance. Sediment organic matter (most likely via the macrobenthic biota) was deemed an important food source for demersal animals and omnivory an

important feeding strategy. Seasonal studies from 2008 to 2010 in the Thukela Bank area indicate that the demersal animals' stable isotope signatures responded to the seasonal isotopic changes in riverine organic matter, indicating the cross-seasonal importance of this food source to the demersal ecosystem.

Parallel methodological studies examined how routine isotopic sample handling procedures could have affected the results of the ecological studies. These studies suggest that i) effects of preservation/fixation methods and the use of dyes are species-dependant; ii) acidification has no effect on zooplankton isotopic signatures, and that iii) drying methods alone and interactively with multiple thawing and refreezing of samples affect the stable isotope values offish muscle tissues. Recommendations are made for further improvements in methodology and considerations to be taken when processing samples.

Overall, it is concluded that riverine input to the Bight has a more important biological role than previously thought, and that organic matter from this source is an important driver of ecosystems within the Bight throughout the year for the demersal and pelagic ecosystems.

CHAPTER 1

Introduction

1 Introduction

The KwaZulu-Natal Bight (henceforth, "the Bight") as a system has not received as much research attention as some other marine areas in South Africa. Having said this, it must be pointed out that the little research done in the Bight has not equally focused on all types of marine research. The oceanography of the system, which has largely focused on a series of important oceanographic processes found in the Bight such as a topographically induced upwelling cell and the occurrence of a cyclonic lee eddy (Pearce et al. 1978, Malan & Schumann 1979, Carter & d'Aubrey 1988, Flemming & Hay 1988, Lutjeharms & Roberts 1988, Schumann 1988b, Lutjeharms et al. 1989, de Ruijter et al. 1999, Lutjeharms et al. 2000b, Meyer et al. 2002, Green & Luke Garlick 2011), has received a considerably greater research effort than the biology (Ayers & Scharler 2011) or geology (Flemming 1981, Flemming & Hay 1988, Bosman et al. 2007). We know from the oceanographic processes that the Bight provides unique research opportunities, having well defined nutrient sources in a generally oligotrophic system. This has led to a series of interesting multidisciplinary research questions under the scope of the African Coelacanth Ecosystem Program (ACEP) II. It is the aim of ACEP II that the processes described above must have an effect on the biology of the Bight. This thesis comprises a small sub-component of ACEP II.

The central theme for ACEP II is to understand to what extent nutrients from the Thukela River, the St Lucia upwelling cell, and the Durban cyclonic lee eddy drive the structure and function of biological communities in the Bight. Within this broad aim ACEP II therefore seeks to identify i) the oceanographic processes driving these inputs, ii) the physico-chemical signatures of each input, iii) what are the phytoplankton responses to these inputs in terms of structure, productivity, photophysiology, nutritional ecophysiology, and bio-optical characteristics, iv) phytoplankton-zooplankton interactions and v) what drives the benthic and demersal communities. My thesis aims fall within aims ii, iv and v.

In this context my thesis aims to provide insight into the food-webs of the Bight using stable isotopes as ecological tracers of food web pathways. Specifically, stable isotope techniques will be used to establish links between the ecosystem 'compartments',

provide the input for an ecosystem model developed in a parallel study within ACEP II, and will greatly improve the general understanding of ecosystem functioning within the Bight.

This review starts with drawing the reader's attention to the broader scale aspects of the Agulhas Current (Fig. 1.1), specifically its oceanography, as it manifests along the East Coast of South Africa, before focusing on smaller scale features of the continental shelf Bight system, which is the spatial focus of this thesis. At the finer scale of the Bight, I will provide an overview of existing knowledge to date of the biota, as contextualised within and shaped by the framework of the oceanographic features to the east of the Bight, and the fluvial (terrestrially-mediated) features, which form the landward boundary conditions of the system. I will then look at similar systems in other areas of the world as well as examining how isotopes can assist in addressing the research questions of this thesis. At the end of this chapter I will present the reader with a thesis aim and hypothesis layout.

1.1 Oceanography of the KwaZulu-Natal Bight

The Agulhas Current (Fig. 1.1) is a wind-driven current that forms the western limb of the anti-cyclonic South Indian Ocean Current (Lutjeharms 2006a, Speich et al. 2006). It is a relatively narrow current (~100 km) that carries warm subtropical water with a maximum average temperature of 27°C in the summer and a minimum of 22°C in the winter (Lutjeharms 2006a). Its inception is off the coast of Northern KZN / Mozambique at about 27°S, and once properly constituted, it follows the continental shelf closely along the southeast African coast between Delagoa Bay (Maputo) and Port Elizabeth (Schumann 1988b, Lutjeharms et al. 2000b, Lutjeharms 2006a, Speich et al. 2006)..

Figure 1.1. The warm Agulhas Current travelling South close to the east coast of South Africa as visualised by Lutjeharms (2006a)

From the northerly inception point the continental shelf itself has a steep slope and is narrow, not exceeding 25 km between the coast and the 200 m isobath. The steepness of the slope causes the Agulhas Current to follow a stable trajectory parallel with the coast (Fig. 1.1.) (Lutjeharms 2006a). From Richards Bay to just south of Durban (more or less the latitudinal extent of the Bight) the shelf widens, thus causing some disturbances in the current, resulting in topographically-induced upwelling in places. The north-to-south distance of this section of the shelf is approximately 160 km; the shelf is about 45 - 50 km wide at its broadest point offshore of the Thukela River (Lutjeharms & de Ruijter 1996, Lutjeharms 2006a, Bosman et al. 2007). Where the shelf widens a well-formed shelf break is evident and the Agulhas Current is pushed offshore along the widening Bight (Pearce et al. 1978, Schumann 1982, 1988b, Lutjeharms 2006a). The continental shelf narrows again offshore of Durban causing the

Agulhas Current to meander from 15 km to as much as 100 km from the coast, only becoming more stable again south of Port Edward (Heydorn et al. 1978, Schumann 1982). Once the Agulhas Current reaches the Agulhas Bank it once again becomes less stable and overshoots the continental shelf at approximately 23°E, producing a return current known as the Agulhas Retroflection (Lutjeharms et al. 2003, Lutjeharms 2006a). Further detail on the processes of the Agulhas Current south of Durban is not provided here for the sake of brevity, since the focus of this thesis is on the Bight (for a detailed discussion see reference Shillington 1993, Quartly & Srokosz 2003, Lutjeharms 2006a, b, Speich et al. 2006).

Within the Bight two of the major oceanographic processes are i) the topographically-induced upwelling event found in the north near Richards Bay and ii) the cyclonic lee eddy occurring slightly south east of Durban (Heydorn et al. 1978) (Fig. 2.2.). These processes transfer water from the Agulhas Current onto the shelf, giving water masses in the Bight their tropical and subtropical characteristics (Schumann 1988b, Lutjeharms 2006a). The water within the Bight is considered to be generally well-mixed, with the movement of the upper layer being well correlated with synoptic winds (Pearce et al. 1978).

Being topographically-induced and persistent, the upwelling that occurs within the area from Richards Bay to St Lucia is entirely independent of wind conditions (Gill & Schumann 1979, Lutjeharms et al. 1989, Lutjeharms et al. 2000a, Lutjeharms & Machu 2000). The water-type in this upwelling cell consists mainly of South Indian Subtropical Surface Waters and there is no indication that South Indian Central Water is drawn into it (Lutjeharms 2006a). The water from the upwelling cell is carried from the core of Agulhas Current onto the shelf and is usually colder and more nutrient-rich than the surrounding waters (Lutjeharms et al. 2000a). As such, this upwelling is thought to have a very strong influence on the physical water characteristics of the entire Bight in terms of nutrients, biota and primary productivity (Lutjeharms et al. 2000b). Indeed, the Bight water has long been known to have a lower temperature, with airborne radiation indicating a mean sea surface temperature of 1.5 °C lower (Gründlingh 1974) than the surrounding water. The salinity of the upwelling cell is also lower than anywhere else within the Bight (Pearce 1977). Lutjeharms *et al.* (2000b) showed a strong

concentration of nitrate around and slightly north of the Richards Bay area, as well as fluctuations in temperature of as much as 8°C in a time scale of days. Satellite images have shown a correlation between primary production and cold water plumes in the area, with a greater concentration of chlorophyll-*a* between Cape St Lucia and Richards Bay than anywhere else in the Bight (Lutjeharms et al. 1989). Filaments of water from the upwelling cell can occasionally be found as far south as Durban forming small cyclonic eddies (Lutjeharms 2006a), which may have important consequences for the biology of the entire Bight. Additionally, the area of active upwelling is known to be a point source of nutrients for the entire Bight, which has the highest primary productivity of the entire Bight region (Lutjeharms 2006b). Despite the significant input of nutrients from the upwelling cell, the entire East Coast of South Africa, including the Bight, remains oligotrophic (Bustamante et al. 1995).

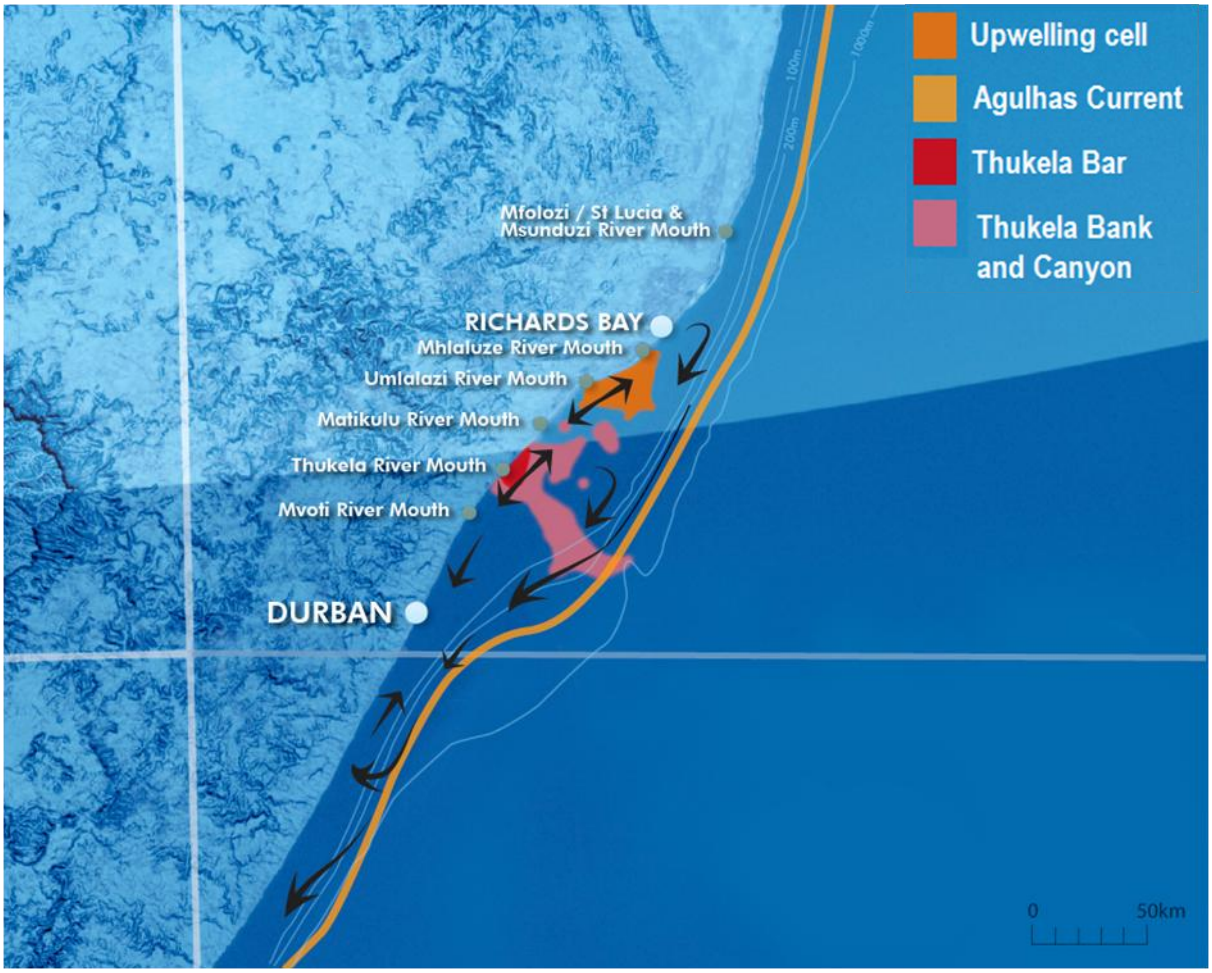

Figure 1.2. Fluvial and oceanographic processes ocurring within the Bight as visualised by Schumann (1987); Flemming and Hay (1988); Lutjeharms (2006b) and Bosman et al. (2006).

Another important oceanographic process is the cyclonic gyre (or cyclonic lee eddy sensu, Schumann 1988b) off Durban. It has a diameter of 20 to 30 km and forms where the continental shelf narrows south of Durban (Schumann 1982, 1988b). At this point the Agulhas Current overshoots the shelf, producing some instability and pushing some of the water from the Agulhas Current northwards to produce the cyclonic gyre just offshore of Durban (Pearce et al. 1978, Schumann 1982). Schumann (1982) suggested that the water from the cyclonic lee eddy may remain trapped for a period of time in the Bight, circulating on the shelf before being reintroduced into the Agulhas Current. The cyclonic gyre causes the major persistent north-eastward flow through the water column off Durban and it is not present anywhere else along this coastline (Lutjeharms 2006a, Guastella et al. 2011). It is not yet known how stable and persistent the Durban cyclonic lee eddy is (Lutjeharms 2006a). This oceanographic feature is considered an important feature as far as the rationale for this thesis is concerned, because similar eddies are known to force nutrients into shallower waters resulting in an enhancement in local productivity (Siegel et al. 1999). I hypothesise that this feature may be another driver of the Bight's primary productivity.

In addition to these oceanographic processes, fluvially-mediated physical, chemical and geological processes also occur within the Bight. A total of 73 catchments discharge their loads directly or indirectly into the sea along the KwaZulu-Natal coastline (Begg 1978). The largest of these originate in particular from the Thukela River, the third largest river in southern Africa (Bosman et al. 2007). It produces more than 35 % of the freshwater entering the Bight with an annual run-ff of 3,865 × 10^6 m^3 and sediment discharges estimated around 6.79 x 10^6 m^3 yr^{-1} sediment directly into the Bight (Begg 1978, Birch 1996, Whitfield & Harrison 2003, Hutchings et al. 2010). The Thukela River subsurface nutrient data show that the river outflow extends approximately 25 km from the shore (Meyer et al. 2002). The high sediment input from the Thukela River, aided by a complex current system over the shelf, has over time created the Thukela Bank (Fig. 2.2.) (Bosman et al. 2007), a mud bank important for fisheries (Lamberth & Turpie 2003).

The Thukela River is by no means the only river entering the Bight, with the Mgeni, Mvoti, Matigulu, Mlalazi and Mhlathuze all discharging into the Bight (DEA 2001).

Hutchings et al. (2010) estimated from the literature that the total nitrogen (N) entering the system through these estuaries was on average 2,333 t y^{-1}, which appears minor in comparison to their estimated value for the upwelling cell of 289,154 t y^{-1} N. However, the upwelling N concentration and flux were calculated from the relatively limited nutrient studies of the Bight. Overall, it has been suggested that the contribution of river run-off for the entire Bight is relatively minor compared to that of the oceanographic processes (Lutjeharms 2006a, Lutjeharms 2006b, Hutchings et al. 2010). Consequently, the nutrient characteristics of the Bight water masses are thought to be influenced by both the Agulhas Current, through a series of oceanographic processes, and to a lesser extent, the entry of nutrients from numerous rivers in the region (Heydorn et al. 1978). Meyer et al. (2002), in their study on the nutrient characteristics of the Bight, concluded that the upwelled water was the source of nutrients for the entire Bight, while the influence of the Thukela River was noticeable but small. However, the authors also agreed that in the case of a large flooding event the riverine influence could then be substantial.

Studies on nutrient characteristics have shown that the concentrations of nitrate generally range from 1.0 to 7.0 µmol l^{-1} across the Bight (Oliff 1973). In the south of the Bight, the Durban cyclonic lee eddy brings colder, nutrient-rich water into the Bight from the deep (Carter & d'Aubrey 1988). The nitrate concentrations at the Durban cyclonic lee eddy are greater than in the surrounding non-upwelling area. Nitrate levels 7 km off Durban have been found to be 2.3 µmol l^{-1} (Burchall 1968, Carter & d'Aubrey 1988), while those in the non-upwelling central part of the Bight have been observed at around 1.01 – 1.86 µmol l^{-1} nitrate (Meyer et al. 2002). In the northern part of the Bight, Meyer et al. (2002) found nitrate levels as high as 16 µmol l^{-1} at the Richards Bay upwelling cell (for a comprehensive study on the nutrients of the Bight see: Meyer et al. 2002). Clearly, significant nutrient inputs to the Bight occur in the upwelling areas, which would conceivably perturb the otherwise generally oligotrophic water of the Bight. It is worth noting, however, that the samples in the Meyer et al. (2002) study were collected in July, the dry season in KwaZulu-Natal region (Day 1981), when riverine input of nutrients is likely to be at its lowest.

The current paradigm is that the upwelling cell dominates the Bight nutrient sources and hence drives primary productivity of the region. Estuarine fluxes of water and associated nutrients should nevertheless not be overlooked as potentially important nutrient sources to the Bight, and are hypothesised to be a dominant ecosystem driver with seasonal characteristics, at least for more inshore portions of the Bight. This is an interesting hypothesis which will receive considerable attention in the body of this thesis.

1.2 The biology of the Bight

Unlike the oceanography of the Bight, its biology has received very little detailed research focus (for some insides into the biology of the Bight see: Heydorn et al. 1978, Carter & Schleyer 1988, Van der Elst 1988, Beckley et al. 2002, Barlow et al. 2008). Ayers and Scharler (2011), who are presently producing a biological model for the Bight, have described the area as data-poor. Apart from South Africa's only prawn fishery (Fennessy & Groeneveld 1997) and a line fishery, the most important fishery on the Bight (Lamberth et al. 2009), the Bight does not support large fisheries, which has resulted in limited research in the area.

The East Coast of South Africa, including the Bight, is generally oligotrophic (Bustamante et al. 1995), which consequently impacts the primary productivity of the region. Mitchell-Innes (1967) concluded from a three year study (1961 – 1963) that the primary productivity of the Bight ranged from 0.1 – 3.1 g C m^{-2} d^{-1}. In a more recent study, Barlow *et al*. (2010) observed that the primary productivity of the Bight spans an order of magnitude from 0.3 – 3.7 g C m^{-2} d^{-1}, with considerable spatial variability. Despite large spatial differences, overall primary production for the entire Bight has been described as low (Burchall 1968, Carter & Schleyer 1988, Bustamante et al. 1995). In terms of chlorophyll-*a* concentrations Barlow et al. (2008) found that in the northeast at the upwelling cell and to the southwest at the Durban Cyclonic Eddy there were zones with chlorophyll-*a* levels ranging from 1.5 – 3.2 mg m^{-3} and 1.7 – 2.8 mg m^{-3} respectively. These values agree with those from another study for the northern part of the Bight (Meyer et al. 2002), but not for the southern part, which was found to be 0.1

mg m^{-3} (Meyer et al. 2002). Chlorophyll-*a* levels in the central part of the Bight ranged from 0.1 – 0.5 mg m^{-3} (Meyer et al. 2002). As with the primary productivity, chlorophyll-*a* concentration also appear to be spatially variable, with the highest concentration found at both end of the Bight.

There is a paucity of studies on zooplankton within the Bight. As such, zooplankton is one of the main focal groups of ACEP II. The earliest record on zooplankton studies that I could find for the Bight is as early as 1960 (Zoutendyk 1960). Since then very little research has been conducted on zooplankton from this region and in most cases, they have been one off studies that do not provide a comprehensive understanding of the system (Shipley & Zoutendyk 1964, Carter 1968, Zoutendyk & Sacks 1969, Carter 1977, Schleyer 1977, Carter & Schleyer 1988). More recent studies have been carried out on ichthyoplankton of the Bight (Olivar & Beckley 1994). Beckley and van Ballegooyen (1992), listed the oceanographic processes within the Bight as a mechanism by which ichthyoplankton being transported south by the Agulhas Current become trapped. Hutchings et al. (2002) highlighted the important role played by the Bight in allowing ichthyoplankton and eggs being transported south to mature, increasing their chances of survival. The authors also describe the importance of the Bight in generating recruitment for the entire shelf region south of the Bight, by offering shelter from the Agulhas Current. However, there are no studies examining the links of the aforementioned oceanographic and fluvial processes to zooplankton responses. Regardless of the little research attention that zooplankton of the Bight has received, it has become clear that there is high zooplankton diversity but low biomass (Gibbons & Hutchings 1996).

Higher trophic levels in the Bight have also received limited research attention and with the exceptions of the sardine run and its associated top predators during its occurrence in the Bight (Fennessy et al. 2010, Hutchings et al. 2010, O'Donoghue et al. 2010, Van der Lingen et al. 2010) little work has been done on the general ecology of the system. The majority of work done on the biology of the Bight tends to be species specific, such as on the top predators caught in the anti-shark nets placed along the KZN coastline (Cockcroft 1990, Dudley & Cliff 1993, Dudley & Cliff 2010), species collected in game fishing studies (Pradervand & van der Elst 2008) and to a small

extent, studies on a few large pelagic species (Beckley & Leis 2000, Beckley et al. 2002). Furthermore, demersal species in the Bight, one of the foci of this thesis, have received little to no attention other than in fisheries research on the few economically important species or as associated by-catch species in the prawn fisheries or on the biology of individual species (Fennessy & Groeneveld 1997, Fennessy 2000, Olbers & Fennessy 2007, Turpie & Lamberth 2010). Fennessy et al. (1994) studying the bycatch species of the prawn fisheries found that the majority of demersal teleost species were permanent inhabitants of the region.

Consequently, little information exists on the relative importance of riverine and oceanographic processes on these higher trophic levels. Oceanographic studies, as mentioned, have suggested that fluvial input plays a small role. However, it is known from elsewhere that rivers can play an important role in shaping the biology of the adjacent coast, supplying sediments and nutrients to the system and consequently controlling the ecology (Martin & Meybeck 1979, Milliman & Heade 1983, Milliman & Syvitski 1992, Gillanders & Kingsford 2002, Kristiansen & Hoell 2002). In the Bight, a series of authors have shown that the inshore fishery in the Thukela Bank, a shallow water mud bank formed off the Thukela River, is dependent on the riverine input (Lamberth & Turpie 2003, Lamberth et al. 2009, Turpie & Lamberth 2010). It has been further demonstrated that increases in water input from the Thukela increased the prawn catches considerably (Turpie & Lamberth 2010) suggesting that anthropologically-induced changes in water regime have the potential to affect line fishery catches (Lamberth et al. 2009). This appears to suggest that riverine input may play a more important role in regulating the biology of the system than suggested by oceanographic studies.

This thesis addresses the question of the importance of oceanographic and riverine processes in shaping the Bight ecosystem as a whole, through developing an understanding of the food-webs, trophic levels and biophysical processes operating in the Bight.

1.3 Concise overview on ecosystems and food-webs ecology

In its simplest form, an ecosystem is a place where organisms and the environment interact (Post et al. 2007a). Lindeman (1942) argued over 60 years ago that a better approach to study plants and animals was a more "bio-ecological" approach, in which the living organisms would "co-act" with each other and "re-act" with the non-living environment. Lindeman also argued in favour of animals being placed in trophic levels, such as primary producers, primary consumers, secondary consumers etc. on the basis of energy flows. A vast array of organisms consume other organisms and in most cases are preyed upon by other organisms, except for those high up the food chain, which become food for decomposers at the point of death. As such, through the consideration of energy pathways and matter transfer, each organism's trophic position within an ecosystem can be deduced (Paine 1988). At present ecology appears to be moving towards a more holistic approach where all species and the large network of interactions are considered (Bascompte 2009).

Some important considerations in understanding food webs were highlighted by Pimm (1980), which he described as four parameters that needed to be chosen to produce a stable ecosystem. These were as follows 1) species can be omnivorous and feed on several trophic levels; 2) prey selection in general should not be separated by more than one trophic level; 3) some systems due to their own dynamics, would permit longer food chains and 4) there should not be compartments within the food web separating species from other species in a different compartment.

With time, ecological research has shown that food-webs in nature are extremely complex with hundreds of links and thousands of species (For more details see, Polis & Strong 1996). Because ecosystem processes are scale dependant, the choice of the boundaries of an ecosystem will have a very important impact on the formulation, the scope and validity of the questions being asked within an ecosystem (O'Neill et al. 1986, Post et al. 2007a). In light of this complexity, food-web studies must make some simplifying assumptions with inherent limitations, but they are invaluable and provide important insights into the functioning of ecosystems and their innate complexity. Food-web studies are statistical representations of a community, snapshots of an

ecosystem at a certain time and place, or an aggregation of trophic relationships over broader spatial and temporal scales (Polis et al. 1995).

Some authors have further argued that the trophic level is an overly simplistic view of food-webs, e.g. a tertiary (or higher level) consumer could prey on multiple levels below it, and even on species within its own trophic level (Polis et al. 1995, Polis & Strong 1996). Therefore, omnivory has to be considered in trophic studies as it potentially has important effects on community, resources, and ultimately food-web structure (Polis & Strong 1996); omnivory can be considered to occur when an organism feeds on trophic levels nonadjacent to its own, at the same time or during different life stages (Pimm 1982). Sprules and Bowerman (1988) studied the plankton community of a glacial lake and reported a high level of complexity due to a *Mysis* species that had a strong effect on the composition of the entire community. This specific species would feed on all zooplankton communities in the studied lake, feeding at more than one trophic level, from primary consumer to tertiary consumers, as well as on some early juvenile stages of algae (Sprules & Bowerman 1988). They also appeared to show some cannibalistic behaviour as most adults would feed on their own species' naupliar larvae, which is a fairly common occurrence in aquatic environments (Sprules & Bowerman 1988). This illustrates the potential complexity of food-webs and the effects of omnivory, or multiple connections, in just one food-web for just a single consumer.

Just as the connections between consumers in a food-web can be used to elucidate the flow of matter, similarly the flows of matter and energy through a food-web can be used to deduce the connections (Paine 1988). In the last decades a novel method to identify and understand ecosystems and trophic structure has appeared due to developments in stable isotope technology, particularly in the isotopes of nitrogen and carbon ($^{14}N/^{15}N$ and $^{13}C/^{14}C$), allowing these deductions of food-web connections to be made.

1.4 On the use of stable isotopes for ecosystem studies

Stable isotopes can be and have been applied in a great variety of ecological studies, such as food-webs structure (e.g. Fry 1991, Angradi 1994, Vander Zanden et al. 1999, Kaehler et al. 2000), seasonal changes in diets (e.g. Ben-David et al. 1997), soil analyses (e.g. Bremmer & Edwards 1965), identification of migratory animals such as birds (e.g. Chamberlain et al. 1997), species invasions (e.g. Vander Zanden et al. 1999) and phytoplankton turnover rates (e.g. Slawyk et al. 1977), amongst many other applications (For introductory reading and reviews see: Fry 2006, Michener & Lajtha 2007, Post et al. 2007b, Martínez del Rio et al. 2009, Wolf et al. 2009, Layman et al. 2011). As such, stable isotopes are a particularly useful technique in their own right or as a tool to support data collected by other methods.

As mentioned earlier, one of the primary interests of the thesis is to understand the functioning of the Bight ecosystem. To understand ecosystem function it is necessary to determine what processes drive the ecosystem and the associated food web. In the case of the marine environment this can be done by identifying the source of OM and/or nutrients entering the system. Several studies have successfully implemented stable isotope techniques to identify i) the origin of particulate organic matter (POM) in diverse aquatic environments (e.g. Schell et al. 1998, Grey et al. 2000, Grey et al. 2001, Goñi et al. 2006, Waite et al. 2007, Schmidt et al. 2010, Govender et al. 2011, Stowasser et al. 2011), ii) the influence estuarine POM (input) has on the nearby shelf ecosystem through the analysis of the stable isotope signatures of surface sediments (e.g. Goñi et al. 1998, Darnaude et al. 2004, Krull et al. 2009), iii) the use of POM by benthic organisms (e.g. Iken et al. 2001, Iken et al. 2010) and iv) elucidate POM seasonal changes and their importance for the associated ecosystems (e.g. Vizzini & Mazzola 2003, Olin et al. 2011). These studies are possible due to the fact that the nutrients and/or POM entering the system from different sources generally have distinctive isotopic signatures.

For example, the POM entering through an estuary would be expected to primarily contain detrital plant material entering with terrestrial runoff and consequently have an isotopic signature dominated by such. Depending on the area the detrital material

could consist mainly of Calvin-Benson (C_3) or Hatch-Slack (C_4) cycle plants (O'Leary 1988). C_3 plants $\delta^{13}C$ values range between -22.00 and -33.00 ‰, while C_4 plants $\delta^{13}C$ values range from -9.00 to -16.00 ‰ (O'Leary 1988, Huang et al. 2000), while both C_3 and C_4 plants have $\delta^{15}N$ ranging from -7 to 7 ‰ (Kelly 2000). These values are very different from marine phytoplankton signatures, which range from -19.1 to -22 ‰ for $\delta^{13}C$ and 3 to 12 ‰ for $\delta^{15}N$, while those of riverine phytoplankton range from -35 to -5.9 ‰ for $\delta^{13}C$ and -2.1 to 12.8 ‰ for $\delta^{15}N$ (Gearing et al. 1984, Owens 1987, Vuorio et al. 2006), making it more difficult to identify. Furthermore, organic matter with a mixture of C_3 and C_4 plant matter could give the misleading isotopic signature, and therefore conclusion, that the organic material originated from marine algae (Goñi et al. 1998). Thus results must be carefully analysed and interpreted. Nevertheless with careful interpretation the known isotopic signatures for various categories of primary producers provide the possibility to identify the source of OM or nutrients entering the system, and consequently to act as natural tracers to track it's (the OM) flow through the food-web.

Food-webs and how these link to the OM available have successfully been used in a diversity of aquatic systems (Mullin et al. 1984, Hobson et al. 1995, Schell et al. 1998, Lara et al. 2010, Pomerleau et al. 2011). This type of application can therefore provide an understanding of processes, connections and energy flow pathways in ecosystems (Smit et al. 1998). One of the goals of this thesis is to provide a preliminary insight into the food-webs and potential diets for a range of animals within the Bight. This is a further possible application of stable isotope techniques (Newsome et al. 2007).

Animal dietary studies are possible because food sources have characteristic isotopic signatures (Hobson et al. 1997), as mentioned earlier. The preferred isotopes for these types of studies are $^{14}N/^{15}N$ and $^{13}C/^{14}C$, both used as natural tracers. By analysing $^{14}N/^{15}N$ it is possible to differentiate the trophic position for each organism; consumers reflect the nitrogen isotope ratio of their food/prey item with a substantial positive shift (generally enriched by up to +3.4 ‰), *i.e.* enriched (positive δ-values) (Peterson & Fry 1987, Toda & Wada 1990, Ponsard & Amlou 1999, Post 2002, Smit et al. 2005). Similarly, $^{13}C/^{14}C$ can be used to track the flow of OM through organisms in a food-web, with the carbon signatures of consumers also being enriched compared to that of

their diet (enriched by up to +0.5 to +1.0‰) (Peterson & Fry 1987, Fry 1991, Edwards et al. 2002, Post 2002, McCutchan Jr et al. 2003, Smit et al. 2005). The assumption that an animal is in equilibrium with its diet can lead to false data interpretation, especially in the case of larger, more mobile organisms. The isotopic values of the tissue can take a period of time to reach equilibrium with that of the diet (Martínez del Rio et al. 2009, Wolf et al. 2009). This delay in isotopic signature response is due to tissue fractionation. Fractionation is the period of time that the animal tissue takes to assimilate the isotopic signature of the food item the animal is feeding on (Fry & Arnold 1982, Hesslein et al. 1993, Vanderklift & Ponsard 2003).

A series of analytical/statistical methods have evolved in order to be able to use isotope data to answer specific questions about trophic linkages. By using the above enrichment values alongside the stable isotope data obtained in the study mixing models, a type of appropriate analytical/statistical method, can be used, which aids in developing an understanding of the ecosystem. Layman et al (2011) produced a very comprehensive review on the analytical tools available for trophic linkage analysis including the primary strengths and the primary weaknesses for each one of these tests and from the early usage of basic statistics, to the complex Bayesian statistics used in most mixing models today.

Bayesian statistics provide an advantage over other, less powerful statistical methods due to the fact that they a) are subject to the concept of probability, or priors, along with the data, b) do not require large sample sizes and c) their input parameters are a random variable with a distribution (Albert 2009). Bayesian mixing models enable the user to portray the most likely proportional contribution of different prey items to predators (Layman et al. 2011). Another advantage is that various Bayesian-based models are freely available on the internet (Moore & Semmens 2008, Semmens et al. 2009a, Semmens et al. 2009b, Parnell et al. 2010, Ward et al. 2010, Ward et al. 2011).

1.5 Case studies: the oligotrophic Smokey Cape and South Brazil Bight

The KwaZulu-Natal Bight is not the only example of an ecosystem where upwelling is generated from a western boundary current interacting with bathymetric features. Other examples include the area between Smokey Cape and Diamond Point in the East Australian Current (EAC) (Rochford 1984, Oke & Middleton 2000), an upwelling cell at Cape Blanco in the Gulf Stream Current next to the Oregon coastline (Dale 2001), and upwelling areas and cyclonic eddies along the Japanese coast produced by the Kuroshio Current (Ito et al. 1995). There are also other examples from the Agulhas Current including the coastal upwelling of the East Madagascar Current or the Port Alfred upwelling cell (Lutjeharms & Machu 2000). The South Brazil Bight (SBB) also has similar features, however, the upwelling in the SBB is also partly influenced by the wind (Gonzalez-Rodriguez et al. 1992).

A good case study is that of the EAC where upwelling is caused by narrowing of the shelf (<20 km) at Lauriton (Roughan & Middleton 2004). As with the Bight, the EAC upwelling plays a very important role of localised enrichment of nutrients for the oligotrophic New South Wales coastal waters (Roughan & Middleton 2002). Unlike the Bight, the upwelling region in the EAC has a relatively small riverine input, as such the main input of nutrients into the coastal area is through upwelling (Macdonald et al. 2009). Oke and Middleton (2001) studied the nutrient enrichment of coastal waters near Port Stephens and concluded that the EAC upwelling was the main source of nutrients for phytoplankton blooms and the cause of enhanced primary productivity that occurred in the area. This findings correlate the findings of Pritchard et al. (2003) on the importance of oceanic nutrient sources to coastal productivity in the area. However, Gaston and Suthers (2004), working on stable isotopes of planktivorous fish collected from sewage affected and non-affected areas around the EAC upwelling, concluded that continuous sewage discharge, and not just upwelled nutrients, played an important role in the source of nutrients to the fish where the sewage discharge was present. If this is considered in the context of the Bight it adds credibility to this thesis hypothesis of greater importance of the fluvial nutrient fluxes entering the Bight, at least in localised areas.

The SBB, on the south east of Brazil, is another example of an oligo-mesotrophic region in which localised upwelling occurs (Teixeira & Tundisi 1981). Despite the general oligotrophic nature of the region, this area has the most important fishery off the Brazilian coast due to the localised input of upwelled nutrients and the freshwater discharges of the La Plata River and Patos Lagoon (Ciotti et al. 1995). During upwelling periods high chlorophyll-*a* and primary production have been measured around the Ubatuba region, while during non-upwelling periods organic detritus from the rivers maintains the food-web (Gonzalez-Rodriguez et al. 1992). Sumida et al. (2005) found that the biological response to upwelling could be measured in benthic communities months after the upwelling event had occurred. On the other hand, Muto and Soares (2011) found seasonal variability in the stable isotope signatures from hake (*Merluccius hubbsi*) collected between Cabo Frio and Ubatuba; however, they were unable to match it to the upwelling events. The authors suggested that the results for hake showed spatial, temporal and ontogenetic variations. Once again, this could indicate that the upwelling cell is not the sole source of nutrients in these oligotrophic waters, adding further support to my hypothesis of the greater importance of the fluvial nutrient fluxes entering the Bight. Overall, these studies suggest that isotopic changes in response to seasonal upwelling events may be detected, but not necessarily for all trophic levels. This could be due to the period of time that the animal tissue takes to assimilate the isotopic signature of the food item, and this (i.e. tissue fractionation) needs to be taken into account (Fry & Arnold 1982, Hesslein et al. 1993, Vanderklift & Ponsard 2003). In addition, riverine inputs will be more or less constant over time compared to the upwelling cell, therefore providing a constant food supply with a characteristic signature.

What appears to be common from the literature for most oligotrophic waters where upwelling occurs is that the upwelling nutrient input provides a large boost to productivity of the area. This appears to be the case in the Bight, the EAC and the SBB. However, in the SBB the temporal consistency of the riverine output, although not measured, was also suggested to be a significant source of nutrients (Gonzalez-Rodriguez et al. 1992), as was the constant sewage discharge in the EAC (Gaston & Suthers 2004). Similar suggestions of the possible importance of the (seasonal) riverine input of nutrients to certain trophic levels of the Bight have been suggested by some

fisheries studies in the area (Lamberth et al. 2009, Turpie & Lamberth 2010). Overall, it appears that in many oligotrophic waters, upwelled nutrients are not the sole driver of biological responses.

1.6 Issues with stable isotope sample preservation

Despite the widespread and valuable applications of isotope techniques in ecology, there are weaknesses, as with any other technique, worth acknowledging. One major drawback is that of the effect of sample preservation and preparation on the isotopic results. Most preservation and storage techniques appear to have an effect on the isotope value of the tissues that are being preserved (see below for a list of studies). In terms of accuracy, this could have serious consequences for dietary and food-web studies such as the ones discussed above. As such, there are an increasing number of studies seeking to understand and address the effects of preservation on isotope values. As part of this thesis, I sought to add to this body of knowledge and elucidate the potential effects that the various storage methods, and also drying methods, could have on the isotope ratios for a variety of samples encountered in the main body of research presented here.

In many situations samples are collected far afield and instantaneous drying and processing of tissue samples for isotopic analysis is not possible (Hobson et al. 1997, Ponsard & Amlou 1999, Kaehler & Pakhomov 2001, Carabel et al. 2009), which creates the need to preserve samples. Several preservation and fixation methods have been used for animal tissues, including freezing (Bosley & Wainright 1999, Kaehler & Pakhomov 2001, Edwards et al. 2002, Bugoni et al. 2008), instant oven drying (Mullin et al. 1984, Kaehler & Pakhomov 2001), freeze-drying (Hobson et al. 1997), fixation in formalin (Kaehler & Pakhomov 2001, Edwards et al. 2002, Kelly et al. 2006), preservation in ethanol (Kaehler & Pakhomov 2001, Kelly et al. 2006, Bugoni et al. 2008), the use of formalin-ethanol mixtures (Bosley & Wainright 1999, Carabel et al. 2009), storage in solutions of saturated sodium chloride (Fábián 1998, Ponsard & Amlou 1999, Bugoni et al. 2008), and storage in ethylene glycol (Ponsard & Amlou 1999).

All of the storage methods, except for immediate oven drying, have been reported to alter $^{15}N/^{14}N$ and $^{13}C/^{12}C$ ratios of biological samples. Of course it cannot be known if even immediate oven drying alters isotope ratios, because moisture-containing samples cannot be analysed. The mechanism by which alteration in isotopic signatures occurs is not fully understood, but some of the suggested mechanisms are the extraction of lipids (Syväranta et al. 2008), exchange of light and heavy isotopes between the organism and the preservative (Hobson et al. 1997, Edwards et al. 2002), or the hydrolysis of proteins during preservation (Arrington & Winemiller 2002, Sarakinos et al. 2002). Some studies have attempted to ascertain a correction factor for the isotopic values of preserved samples, but found that the use of data from preserved samples could give an erroneous outcome in the mixing models or that the change in isotope signatures for different preservation methods is species specific (Bosley & Wainright 1999, Carabel et al. 2009).

Bosley and Wainright (1999) reported that ideally the only method of preservation and storage that should be used in order not to affect stable isotope ratios, until further investigation, should be freezing if immediate drying is not possible. Unfortunately in some situations, especially where samples are collected for multiple purposes and not just isotope analysis alone, it is not possible to use either of these methods. Faced with this issue for my study, the best possible solution is to try and understand how the stable isotope signatures of the species examined varied under different preservation and desiccation treatments.

1.7 Thesis layout, aims and hypotheses

Following the introductory Chapter 1, Chapter 2, 3 and 4 focus on the drivers and the ecosystem functioning of the Bight. Concretely, Chapter 2 seeks to identify the main sources of nutrients entering the pelagic system and driving the pelagic zooplankton food-web in both the wet (austral summer) and dry (austral winter) seasons. I hypothesise that; i) oceanic and fluvial sources differ in terms of isotope ratios of their N- and C-bearing materials and ii) that these isotopic signals are heterogeneously distributed across the Bight. Answered to these hypotheses are achieved through the

isotopic ($\delta^{13}C$ and $\delta^{15}N$) analysis of Total Suspended Solids (TSS) and zooplankton collected within the Bight. Subsequently Bayesian mixing models will be use to analyse from the isotope signature which TSS source is the main pelagic zooplankton food-web driver.

Chapter 3 examines whether the oceanographic and/or fluvial processes are driving the benthic ecosystem and how dependent demersal animals are on the local benthos for food. The broad aim of the study is to determine what is driving the local benthos and present a first attempt at producing a food-web reconstruction for demersal organisms of the Bight. This will be done within a framework of the underlying hydrography and sedimentary material distribution. Three major hypotheses underpin this study, i) that estuarine biological drivers, which occur throughout the year, play an important role in this environment, contrary to the current paradigm, ii) that the isotopic signatures of sediment organic matter (OM) vary spatially but that these variations are consistent with the occurrence of the biological drivers in the Bight (*i.e.* the upwelling cell, the cyclonic eddy and riverine sources close to the shore) and iii) that isotopic signatures of demersal organisms vary spatially and resemble the isotope signatures of the OM in the sediments (*i.e.* the same as those for hypothesis ii). For this stable isotope techniques and Bayesian mixing models are again used, this time to analyse surface sediment samples collected throughout the Bight for organic C and total N and to determine the percentage of organic C and N matter in the marine sediment derived from the estuaries and from the marine environment. Stable isotopes will also be used in the analysis of demersal organisms collected at several locations in the Bight to produce a preliminary understanding of trophic interactions in the benthic communities. These results will be supported with a literature review of the diets of the species collected or from closely related species.

Chapter 4 focuses on a smaller area of the Bight known as the Thukela Bank, an important fishing ground and the location of South Africa's only prawn fishery. This region is closest to the Thukela River and therefore the most likely site to be influenced by seasonal fluctuations in rainfall and resultant river runoff. The aim of this chapter is to determine if seasonal differences exist in the riverine TSS and marine fauna stable isotopes signatures between the wet and dry season. I hypothesise that the continuous

input of OM from the Thukela River, i) has strong seasonal differences in its OM stable isotope signatures and ii) if these seasonal stable isotope changes can also be measured for the demersal organisms from the Thukela Bank. This is achieved by isotopically analysing Thukela riverine TSS and a diverse selection of marine fauna from the Thukela Bank to determine whether marine faunal stable isotope signatures change in parallel with those of the TSS for the 2008, 2009 and 2010 wet and dry seasons.

Chapter 5 and 6 are methodological rather than ecological studies; they were completed in order to help understand and improve knowledge on how sample handling can affect final isotopic data. These chapters were designed to provide insight into how the standard sample preparation applied in the ecological chapters of the thesis might have affected final isotopic results and therefore conclusions, as samples for these studies were collected at the same time as the samples for the main body of the thesis. For the broader audience, though, the studies were designed to raise questions and provide some advice on how samples to be used for stable isotope analysis should be handled, due to the formal lack of agreement on the matter. Both Chapters (5 and 6) have already been published in *Rapid Communication in Mass Spectrometry*.

Specifically, Chapter 5 examines i) the effect of freezing, ethanol and formalin as preservatives/fixatives on the stable isotope signatures of zooplankton, ii) the effect of preservation time in these media on measured stable isotope signatures and iii) effects of sample acidification for $CaCO_3$ removal, through the example of two species (*Euphausia frigida* and *Undinula vulgaris*). This study is also thought to be the first to examine the effects of dyes on zooplankton: it examines how two different dyes, which are sometimes used to separate OM from non-OM in zooplankton sample processing, can alter the stable isotope signature zooplankton concerned. I hypothesize that i) the effects of preservation on zooplankton tissue are preservative dependant, ii) the isotopic signature of zooplankton in the preservation media is affected by time, iii) acidification causes a significant effect on $\delta^{15}N$ isotopic ratios, but not $\delta^{13}C$ isotopic ratios of zooplankton and iv) dyes affect the $\delta^{13}C$ isotopic ratios of the organisms.

Following on from the finding that freezing is probably the least likely method to affect samples, in Chapter 6 experimental studies are carried out to determine the effect of

freezing protocol and desiccation method on stable isotope values. Specifically, this chapter examines how multiple freeze-thaw events, which occur in freezer malfunction or routine transport and subsampling, could consequently affect the stable isotope signatures of muscle tissue from six different demersal organisms. It also compares the effect on stable isotope values of oven-drying *vs.* freeze-drying as a desiccation method and makes subsequent recommendations on protocol improvement and considerations for future work. I hypothesize that different desiccation methods and multiple freeze-thaw events i) have an effect on the $\delta^{13}C$ and $\delta^{15}N$ isotopic ratios, ii) decrease the percentage content of carbon and nitrogen due to the leaching of organic compounds from the muscle tissue, iii) also decrease the C:N ratios due to carbon leaching, and iv) that these methods have a constant effect across species.

Finally, Chapter 7 evaluates the outcomes of each chapter and attempts to link and explain the relevance of these outcomes to the bigger picture of the entire Bight marine ecosystem, including the role played by each of the nutrients sources in the biology of the Bight. Conclusions, recommendations and suggestions for future research are then given; including ways in which such studies can be improved.

1.7.1 In summary, the aims of the study are:

1) The general aim is to contribute towards our understanding of the KwaZulu-Natal Bight ecosystem through the use of stable isotopes. The sub-aims are;

- Aim 1: To determine the origin of the nutrients and OM entering the ecosystem, these being oceanographic and/or fluvially induced processes, and assessing their relative importance.
- Aim 2: To establish which of the above processes is the main driver of the pelagic planktonic food-web.
- Aim 3: To establish which of the above processes is the main driver of the demersal food-web.
- Aim 4: To determine if the Thukela River OM isotopic values vary seasonally.

Aim 5: To determine if the Thukela River OM isotopic seasonality is visible in isotopic signatures of the Thukela Bank's demersal organisms.

2) In support of the above questions, two methodological studies were completed to help increase our understanding of how the handling and preservation of samples can have an effect on the stable isotope signature of organisms. The aims of these studies are;

Aim 1: To examine the effect of different preservation/fixation methods on zooplankton.

Aim 2: To determine the effect of dyes, often used in estuarine samples to separate organic from non-organic material, on zooplankton isotopic signals.

Aim 3: To study the effect that acidification, used for the removal of calcareous body components, has on zooplankton isotope ratios.

Aim 4: To establish the effect that long term preservative storage has on zooplankton isotope signals.

Aim 5: To understand how several thawing events affect the isotopic and elemental values of the muscle tissue of demersal organisms.

Aim 6: To study the effect that oven- and freeze-drying has on the isotopic and elemental values of the muscle tissue of demersal organisms.

ECOSYSTEM STUDIES

CHAPTER 2

Zooplankton trophic-linkages of the KwaZulu-Natal Bight and assessment on spatial variations and bio-physical coupling dynamics using $\delta^{13}C$ and $\delta^{15}N$

2 Zooplankton trophic-linkages of the KwaZulu-Natal Bight and assessment on spatial variations and bio-physical coupling dynamics using $\delta^{13}C$ and $\delta^{15}N$.

2.1 Abstract

The KwaZulu-Natal Bight in South Africa has received little biological research attention. This study aims to elucidate how different sources of organic matter drive its ecological functioning in terms of zooplankton food webs. I hypothesize that i) the localised fluvial and a series of oceanographic processes are the main nutrient inputs into the oligotrophic Bight, and that ii) these nutrient sources differ substantially in the isotope ratios of their nitrogen and carbon bearing materials. The relative importance of these materials was then assessed using mixing models. Samples were collected at five locations along the Bight during two seasons in 2010 and subjected to isotopic and mixing model analysis. All locations had unique physical-chemical characteristics, but it was mainly Mid Shelf and to some extent Richards Bay North and Richards Bay South total suspended solids were biologically important as primary carbon sources for zooplankton in the wet season. While in the dry season total suspended solids from Richards Bay North and South were the main biological drivers. Overall the important primary carbon and nitrogen sources were largely marine-dominated, while riverine inputs played a minor role. I concluded that the Bight's zooplankton food web and likely the entire pelagic system are primarily driven by oceanic total suspended solids. I also found that the zooplankton spatial isotopic values matched the underlying hydrography of the region, but more research is needed to fully understand these findings.

2.2 Introduction

The South African east coast continental shelf, in particular the area off KwaZulu-Natal (KZN), is a complex neritic region influenced by a range of physical processes. The Agulhas Current impinges upon the coast and strongly influences the oceanography of the generally very narrow continental shelf region in the area (de Ruijter et al. 1999). The shelf widens at the northern end of the KwaZulu-Natal Bight (the "Bight") and local bathymetry induces upwelling (Gill & Schumann 1979, Lutjeharms et al. 1989, Lutjeharms et al. 2000a, Lutjeharms & Machu 2000, Lutjeharms 2006a). To the south of the Bight near Durban a cyclonic gyre (or cyclonic lee eddy *sensu* Schumann 1988b) is suggested to trap water for a period of time, circulating it within the shelf before reintroducing it into the Agulhas Current (Schumann 1982). Inshore, fluvially-mediated physical, chemical and geological processes originating in particular from the Thukela River mouth region (Bosman et al. 2007) during the austral summer season produce a net movement of dissolved and particulate nitrogen (N)- and carbon (C)-containing material onto and over the Bight. All these water sources are coupled with wind- and wave-driven shelf processes, and drive the Bight's ecosystem. Although nutrients do enter the Bight system, the east coast of South Africa is nevertheless described as oligotrophic (Bustamante et al. 1995).

The Bight has not received as much research attention as other South African marine areas. However, the most important water movements occurring within the Bight or in its vicinity have been well studied (Pearce et al. 1978, Malan & Schumann 1979, Carter & d'Aubrey 1988, Flemming & Hay 1988, Lutjeharms & Roberts 1988, Schumann 1988b, Lutjeharms et al. 1989, de Ruijter et al. 1999, Lutjeharms et al. 2000b, Meyer et al. 2002, Green & Luke Garlick 2011). Unlike the oceanography, biological studies of the Bight, particularly those involving zooplankton, have not been extensive (Heydorn et al. 1978, Carter & Schleyer 1988, Van der Elst 1988, Beckley et al. 2002, Barlow et al. 2008).

In this paper I report on a series of studies that aim to present biological community data within a framework of the underlying ocean hydrography. My aim is to present a subset of this information – that pertains to the zooplankton of the region – and so provide a deeper understanding of how different particulate material sources (total

suspended solids, TSS) drive the Bight's ecological functioning and biodiversity. Firstly, I propose that localised fluvial fluxes, which occur episodically and predominantly during the wet season associated with the austral summer, are one of the forcing agents of food webs in the Bight. Secondly, the upwelling cell in the north of the Bight, and a cyclonic eddy at the south, are suggested to function as the oceanic food web drivers. Mechanistically, these coastal and oceanic drivers import OM and nutrients that are used by and incorporated into the isotopic signature of phytoplankton and subsequently zooplankton. Conveniently, at the level of particulates and above, these drivers are easily measured in the whole ecosystem using naturally-occurring isotopes of ^{15}N and ^{13}C as currencies; this approach has been used successfully to differentiate water masses in other ocean regions (Mullin et al. 1984, Hobson et al. 1995, Schell et al. 1998, Lara et al. 2010, Pomerleau et al. 2011). Additional information comes from zooplankton community composition, which is also water mass specific (Mackas 1984, Froneman & Pakhomov 1998, Llinas et al. 2009). Over the last decade it has become increasingly important to understand the biological and physical processes occurring in the aquatic environment (Vander Zanden & Rasmussen 1999, Schindler & Scheuerell 2002), because of consequences for ecosystem structure and function. Zooplankton communities in particular have been shown to be influenced by changes in the physical environment, whether these are induced by climatic perturbations such as El Niño events (Peterson et al. 2002), climatic change (Burrows et al. 2011), or from riverine inputs into the marine system (Bosley et al. 2004). With strong responses to changes in the physical environment, it is plausible that the isotopic signatures of zooplankton communities would match that of the water masses they occupy.

The first aim of this study is to determine the ^{15}N and ^{13}C content of TSS entering the Bight at the three key areas. In this regard, I hypothesise that the organic fractions of oceanically- and fluvially-sourced TSS differ in terms of their $\delta^{13}C$ and $\delta^{15}N$ signals. Further, because the fluvial contribution of TSS can be expected to be strongly seasonal, I predict that riverine inputs on the inshore, terrestrially dominated portion of the Bight will decrease during the dry season (austral winter). The second objective is to determine which of the OM input mechanisms dominate in the Bight as far as its importance in sustaining zooplankton biomass in the region is concerned. To this end, I

constructed a series of mixing models to assess the relative importance of TSS from different regions (*i.e.* upwelling cell, cyclonic eddy and freshwater input) as food source to zooplankton collected in the same regions. I expect that zooplankton at the inshore region is strongly seasonally linked to fluvial C and N, while zooplankton towards the ocean edge of the Bight is increasingly and consistently reliant on non-oceanic C and N.

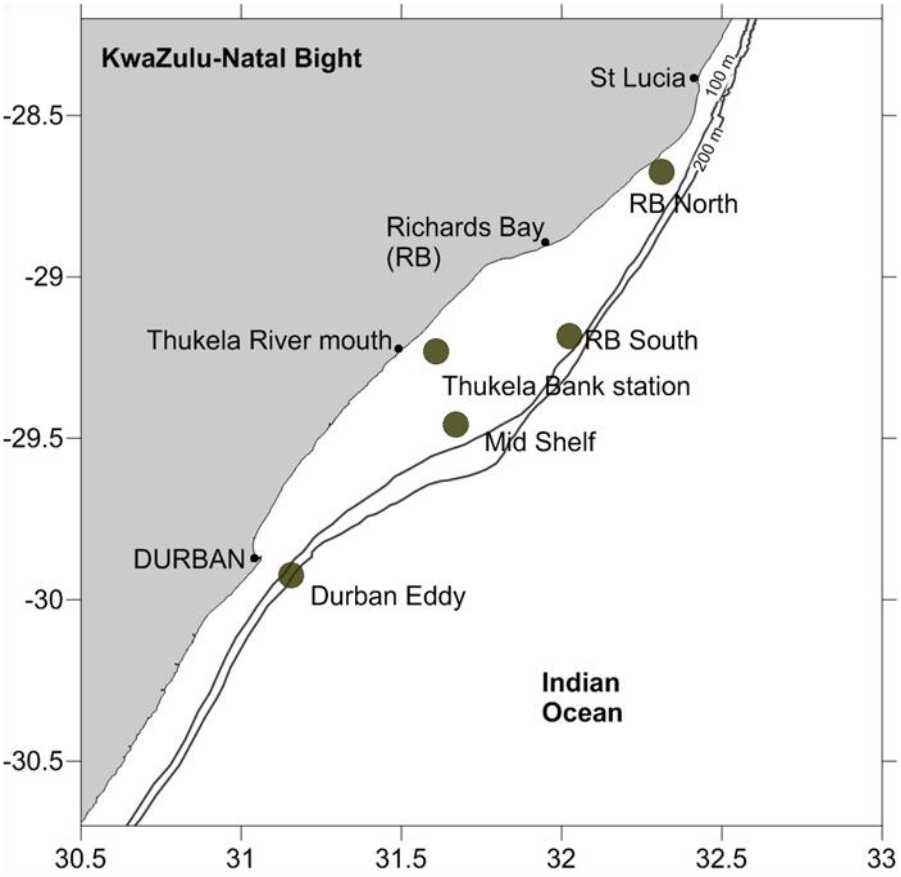

Figure 2.1. The KwaZulu-Natal Bight with sampling localities matching the oceanographic and fluvial processes in the Bight (Refer to Chapter 1 and Figure 1.2 for more information on these).

2.3 Material and methods

2.3.1 Study area

Three mechanisms through which nutrients are brought into the system are presumed to occur in the Bight (for more information refer to Chapter 1 Section 1.1). Firstly, a persistent topographically-induced upwelling cell is situated in the area around Richards Bay and St Lucia at the northern extent of the Bight (Lutjeharms et al. 2000b). Secondly, a cyclonic gyre (Schumann 1982) is situated just south of Durban. This water, which is also considered a potential source of nutrients to the Bight, may remain trapped for a period of time, circulating within the shelf before being reintroduced into the Agulhas Current. Barlow et al. (2008) described chlorophyll-*a* concentrations at both the northeast, upwelling cell, and southwest, Durban cyclonic eddy, reporting ranges of 1.5 – 3.2 mg m^{-3} and 1.7 – 2.8 mg m^{-3} respectively. Thirdly, the Thukela River, the third largest river in southern Africa (Bosman et al. 2007), with an annual flux of 3,865 × 10^6 m^3 (Hutchings et al. 2010), is presumed to be a source of nutrients and N-, C- and P-containing materials at the coastal portion of the central Bight. A sampling programme was designed with the intention to capture TSS and zooplankton from across the Bight, with emphasis on the areas where hydrographic patterns reflect the regions of nutrient input mentioned above.

2.3.2 Sample collection and processing

Samples were collected at five sites in the Bight (Fig. 2.1) during the wet (austral summer, February 2010) and dry (austral winter, August 2010) seasons on board of the *FRV Algoa*. The sites correspond to the three locations where physical processes are thought to play a major role in driving the productivity of the Bight through the nutrient input mechanisms that operate there, *viz*., i) the Durban Eddy (DE) (Lat -29.9239; Lon 31.1545), representative of the cyclonic lee eddy; ii) the Thukela Bank station (TM) (Lat -29.2312; Lon 31.6075), representative of the region where fluvial materials are thought to dominate ecosystem processes; iii) the Richards Bay South (RS) (Lat -29.6735; Lon 32.3117) and iv) Richards Bay North (RN) (Lat -29.1825; Lon

32.0250) sites, reflecting the region where topographically-induced upwelling is believed to take place; and lastly, v) the Mid Shelf (MS) (Lat -29.4572; Lon 31.6703), which represents an area of anomalously high levels of primary productivity which was serendipitously encountered at the time of sampling. This high productivity phenomenon was again observed in the winter season, suggesting that it might be a permanent or common feature of the Bight. Sampling was replicated on consecutive days at each site (n = 4 to n = 5). A sixth site, the Thukela River mouth, was sampled at the same time as sites i) to v), but only for TSS to determine an estuarine isotopic signature. At this site sampling was carried out during ebb tide to ensure that the samples were of estuarine and not of marine origin.

For all other sites (i to v), on each sampling occasion, temperature and salinity were measured using a Sea-Bird 911 *plus* CTD (Sea-Bird Electronics, Inc., Bellevue, Washington, USA), and oxygen and nutrients determined from samples taken with 12 PVC Niskin bottles of 5 liter capacity, each attached to the rosette housing the CTD. Water samples for TSS and PON were collected at F_{max} and surface depths. Chlorophyll-*a* biomass was measured using a WET Labs ECO-fluorometeter (Philomath, USA), which was integrated with the CTD. To determine TSS, water was collected from the surface and the fluorescence maximum (F_{max}). Water volumes of 500 ml were filtered through pre-combusted (4 hr at 450 ^{o}C) 25 mm diameter Whatman GF/F, and the mass of TSS determined on a dry mass basis. The filters were frozen at -20 ^{o}C and stored for the determination of total TSS organic fraction as well as its particulate organic N fraction, PON. PON was determined from the GF/F by digestion using a wet oxidation method according to Raimbault et al. (1999) and analysed on a Skalar SAN++ continuous flow analyser. For the Thukela River mouth only TSS stable isotope signatures were determined (*i.e.* neither total organic TSS nor PON were calculated). Nutrient samples were analysed using a standard Technicon Autoanalyzer II method adapted to an Astoria Nutrient Analyser (Astoria-Pacific Int., Clackamas, U.S.A.).

Zooplankton samples were collected using a double oblique bongo net (200 µm and 300 µm mesh) lowered to a few meters from the recorded bottom. Zooplankton collected in the 200 µm mesh net were immediately preserved in 4% formalin and

stored in plastic jars. All samples were size fractionated within 2 months of collection through 1000 µm, 500 µm and 200 µm Nitex meshes, with the largest size fraction separated to species level under a dissecting microscope. Only species with sufficient numbers or biomass were retained for isotope analysis. Size fractions of 500 and 250 µm were each analysed as a whole, *i.e.* animals were not sorted into species or feeding guilds.

2.3.3 Stable isotope analysis

For the TSS filters, $CaCO_3$ was removed by acidification with a 2 % HCl solution to prevent it affecting carbon δ-values. Filters were then placed into tin capsules (OEA Laboratories, Callington, UK) and sent for analysis at two isotope laboratories. Samples collected during winter were sent to the University of Cape Town (Stable Light Isotope Laboratory, Archeometry Research Unit) and combusted in a Flash EA 1112 series elemental analyser (Thermo Electron, Milan, Italy). The gases were passed to a Delta Plus XP isotope ratio mass spectrometer (Thermo Electron, Bremen, Germany) via a Conflo III gas control unit (Thermo Electron, Bremen, Germany). Merck Gel – a proteinaceous gel produced by Merck (Darmstadt, Germany) – was used as standard and was calibrated against IAEA (International Atomic Energy Agency, Vienna, Austria) standards. The analytical precision of the instrument was 0.07 ‰ for $^{15}N/^{14}N$ and 0.03 ‰ for $^{13}C/^{12}C$. TSS samples taken during summer were analysed at IsoEnvironmental Isotope Facility at Rhodes University, Grahamstown, South Africa, using an ANCA SL Elemental Analyser coupled to a Europa Scientific 20-20 IRMS (Sercon Ltd. Crewe, UK). To ensure the quality of the results, each batch of 96 combustions contained 34 known standards: these included 29 beet sugar and ammonium sulphate (in-house standards) and five certified protein standard casein (calibrated against IAEA-CH-6 and IAEA-N-1). The analytical precision of the instrument was 0.17 ‰ for $^{15}N/^{14}N$ and 0.09 ‰ for $^{13}C/^{12}C$.

As suggested in Chapter 5, zooplankton samples were sorted within two weeks of collection and were not acidified to avoid further increasing the uncertainty surrounding the measurement of the isotope ratios. Lipid extraction has been shown to enrich ^{13}C and ^{15}N and increase variability (Post 2002, Sotiropoulos et al. 2004).

Following the Beaudoin et al. (2001) lipid removal method using 1:1 chloroform:methanol, I decided to do lipid removal on five species/group, *viz*. *Flaccisagitta enflata, Undinula vulgaris, Euphausia* sp, *Euphausia frigida* and mixture size 500 µm (de Lecea and Smit, unpublished). There was no significant difference between the control and the lipid-removed samples for either $\delta^{13}C$ or $\delta^{15}N$ values and it was therefore decided not to apply lipid removal to any of the remaining samples. Zooplankton samples were dried for 24 hours at 50 °C, homogenised and weighed into tin capsules (SANTI® Analytical, Teufen, Switzerland); 0.5 – 0.6 mg dry mass was encapsulated. Samples were analysed on the Thermo Electron instrument specified above.

$\delta^{15}N$ is expressed in terms of their value relative to atmospheric N_2, while $\delta^{13}C$ is expressed in terms of its value relative to Pee-Dee Belemnite (vPDB). Isotope values resulting from both instruments are expressed in the usual δ-notation (Epstein et al. 1953):

$$\delta\text{-value } (‰) = (R_{\text{sample}} / R_{\text{standard}} - 1) \times 1000$$

where R is the ratio of $^{15}N:^{14}N$ or $^{13}C:^{12}C$ in the sample (R_{sample}) and in the standard (R_{standard}), expressed relative to the international standard (Sulzman 2007).

2.3.4 Statistical analyses, trophic positions and mixing models

Parametric statistical analyses of TSS, nutrients and zooplankton isotope signatures, were executed after data were confirmed to be normally distributed and homoscedastistic. Analyses were accomplished using *R* version 2.12.0 (R Development Core R Development Core Team 2010). Data not conforming to the aforementioned criteria were subjected to a Kruskal-Wallis non-parametric one-way ANOVA. TSS, the only primary C source collected in this study, was sampled at two different depths, and a two-way ANOVA was therefore used to examine the effects of the factors depth and location and the interactive effect of these. Seasons were not compared statistically as samples were only collected for one summer and one winter. Those stations in the dry season where not enough replicates were collected were excluded from the

statistical analysis. Because no seasonal statistical comparisons were done, this had no effect on the overall results.

I assumed that as C and N move through the food web changes in ^{13}C enrichment are small (~1 ‰) and that of ^{15}N larger (~3.4 ‰) (Peterson & Fry 1987, Smit et al. 2005, Ng et al. 2007). The trophic position (TP) of zooplankton was calculated following the method proposed by (Hobson & Welch 1992, Govender et al. 2011):

$$TP = 1 + (\delta^{15}N_{consumer} - \delta^{15}N_{source}) / \Delta^{15}N$$

where $\delta^{15}N_{consumer}$ is the $\delta^{15}N$ of individual consumer, $\delta^{15}N_{source}$ is the $\delta^{15}N$ of the main nutrient source in the consumer diet, and $\Delta^{15}N$ is the enrichment factor, which was set at 3.4 ‰ (Toda & Wada 1990, Smit 2001, Post 2002). TSS was assigned trophic position 1.

Using mixing models I i) determined the food source of each zooplankton species at each location, and ii) determined the area within the Bight which was most favoured by zooplankton species. To accomplish this, consumers were firstly compared with organisms from lower TPs and TSS from the same site in order to assess the importance of the TSS towards the diet of the consumer. Secondly, TSS from all five sites and from both depths were used as possible food sources for each of the consumer species or size fractionations sampled at each site. To calculate the proportional contribution, I used the Bayesian mixing model MixSIR (Moore & Semmens 2008) version 1.0.4 with uninformative priors. This model is designed to estimate the probability distribution of source contributions to a consumer, but at the same time account for uncertainty with multiple sources of food items or preys and fractionation values can be set. Previously published fractionation values of 0.5 ± 0.13 ‰ for ^{13}C (McCutchan Jr et al. 2003) and 3.4 ± 1.1 ‰ for ^{15}N (Toda & Wada 1990) were used for this study. The maximum importance ratio was below 0.001 suggesting that the models were effective in estimating the true posterior density (Moore & Semmens 2008). Results for MixSIR are presented as median and the 5th and 95th credibility intervals.

A principal component analysis (PCA) was conducted in Primer 6.0 (Plymouth Marine Laboratory, UK) (Clarke 1993) using the mixing model results, for all TSS as a possible diet source, to assess the feeding pattern of zooplankton within the Bight.

2.4 Results

2.4.1 Physico-chemical characteristics

Salinity had little between-site variability in the wet season, with the highest values found at the Mid Shelf station at 48 m deep (35.43 ± 0.02) while the lowest values were at Durban Eddy at 192 m deep (35.18 ± 0.07). There was less variability during the dry season (Fig. 2.2). Temperature did not differ greatly between sites except at Richards Bay South in the wet season, which on average was 4.26 °C warmer than at the next highest site, Richards Bay North. In the dry season all sites had similar temperatures, with the highest temperature at Richards Bay North being only 0.82 °C higher than that at other sites (Fig. 2.2). As expected, temperature decreased with depth for all stations in the wet season, and only for Richards Bay South and Durban Eddy in the dry season.

Chlorophyll-*a* biomass was higher in the wet season than the dry. During the wet season the Mid Shelf had the highest F_{max} of all sites at 4.69 ± 1.64 mg m^{-3} at 19 m depth, while Richards Bay South had the lowest chlorophyll-*a* biomass from surface to about 45 m (Fig. 2.2). In the dry season the F_{max} was more difficult to distinguish for all sites due to the high variability of chlorophyll-*a* biomass with depth within sites. In this season Richards Bay South had the highest F_{max} values of 1.84 ± 0.62 mg m^{-3}. As with the other environmental factors, chlorophyll-*a* biomass decreased with depth for all stations except for Richards Bay South in the wet season. In this station chlorophyll-*a* biomass increased close to the seafloor.

Concentrations of most nutrients were significantly different between sites and depths, and an interactive effect of the two was generally present in both seasons (two-way ANOVA and Kruskal-Wallis, Table 2.1). Generally the pattern of nutrient

concentration for sites that emerged in the wet season was that the Thukela Bank station had the highest nutrient concentrations at both surface and the F_{max} (Surface: silicate 1.60 ± 0.89 µmol l^{-1}; phosphate 0.20 ± 0.06 µmol l^{-1}; nitrate 0.50 ± 0.69 µmol l^{-1}; nitrite 0.41± 0.51 µmol l^{-1}; F_{max}: silicate 4.84 ± 1.08 µmol l^{-1}; phosphate 0.42 ± 0.04 µmol l^{-1}; nitrate 3.43 ± 0.58 µmol l^{-1}; nitrite 0.37± 0.10 µmol l^{-1}). In the dry season, however, the Thukela Bank station and Richards Bay South both had the second highest nutrient concentrations at the surface, the Mid Shelf generally having higher nutrient concentrations at this depth (silicate 3.33 ± 0.19 µmol l^{-1}; phosphate 0.44 ± 0.08 µmol l^{-1}; nitrate 1.78 ± 0.06 µmol l^{-1}; nitrite 0.23 ± 0.07 µmol l^{-1}). Richards Bay South had higher nutrient concentrations at F_{max} during the dry season (silicate 4.55 ± 1.50 µmol l^{-1}; phosphate 0.45 ± 0.15 µmol l^{-1}; nitrate 1.85 ± 0.28 µmol l^{-1}), with the exception of nitrite (Mid Shelf nitrite 0.24 ± 0.12 µmol l^{-1}). In terms of depth, nutrient concentrations were generally higher at the F_{max} than at the surface in the wet season, but concentrations were generally similar between depths in the dry season (Fig. 2.3).

Table 2.1. Statistical analysis of nutrient concentrations with respect to Site, Depth, and the interaction between these terms. Two-way ANOVA is indicated by no symbol, ++ indicates that data were square-root converted prior to analysing using a two-way ANOVA; # - Kruskal-Wallis non-parametric test used.

Nutrient / Season	Test	*df*	*SS*	*MS*	*F* / $\chi^{2\#}$	*p*
Wet Season						
Silicate	Site	4	35.60	8.90	11.71	< 0.0001
	Depth	1	25.39	25.39	33.42	<0.0001
	Site*Depth	4	15.08	3.77	4.96	<0.01
Phosphate #	Site	4			21.54	<0.001
	Depth	1			15.50	<0.0001
Nitrate ++	Site	4	4.32	1.08	5.31	<0.001
	Depth	1	10.03	10.03	49.36	<0.0001
	Depth*Site	4	2.51	0.62	3.09	<0.01
Nitrite #	Site	4			24.31	<0.0001
	Depth	1			7.79	<0.01
Dry Season						
Silicate	Site	4	21.04	5.26	13.43	<0.0001
	Depth	1	2.26	2.26	5.76	<0.05
	Depth*Site	4	6.85	1.71	4.37	<0.01
Phosphate	Site	4	0.28	0.07	9.96	<0.0001
	Depth	1	0.001	0.001	0.14	0.71
	Depth*Site	4	0.16	0.04	5.86	<0.001
Nitrate ++	Site	4	3.46	0.87	15.02	<0.0001
	Depth	1	0.34	0.34	5.96	<0.05
	Depth*Site	4	1.33	0.06	5.77	<0.0001
Nitrite #	Site	4			52.55	<0.0001
	Depth	1			0.86	0.36

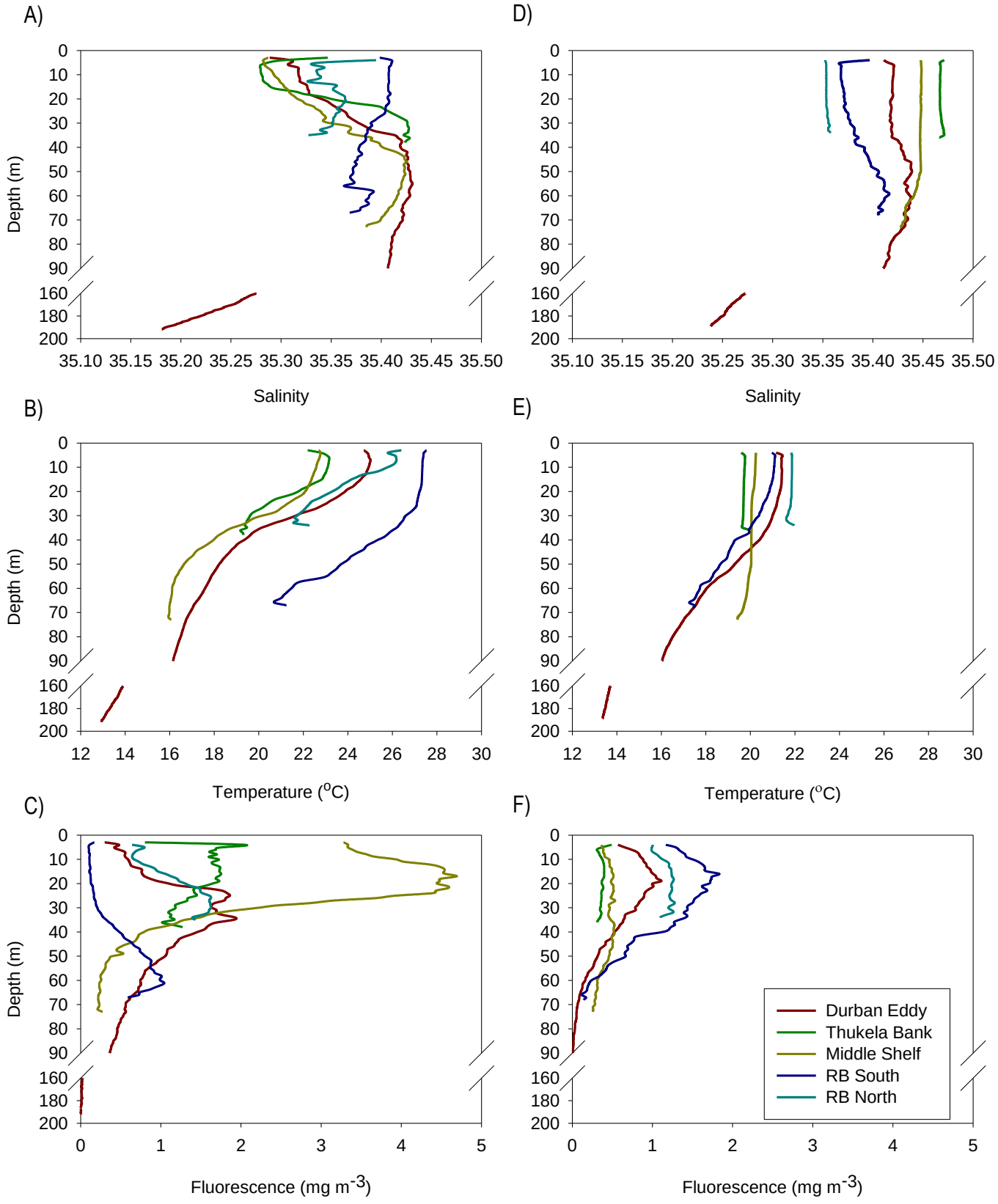

Figure 2.2. Average depth profiles from CTD data of salinity, temperature and chlorophyll-*a* biomass (fluorescence). Letters A, B and C represent the wet season; D, E and F represent the dry season.

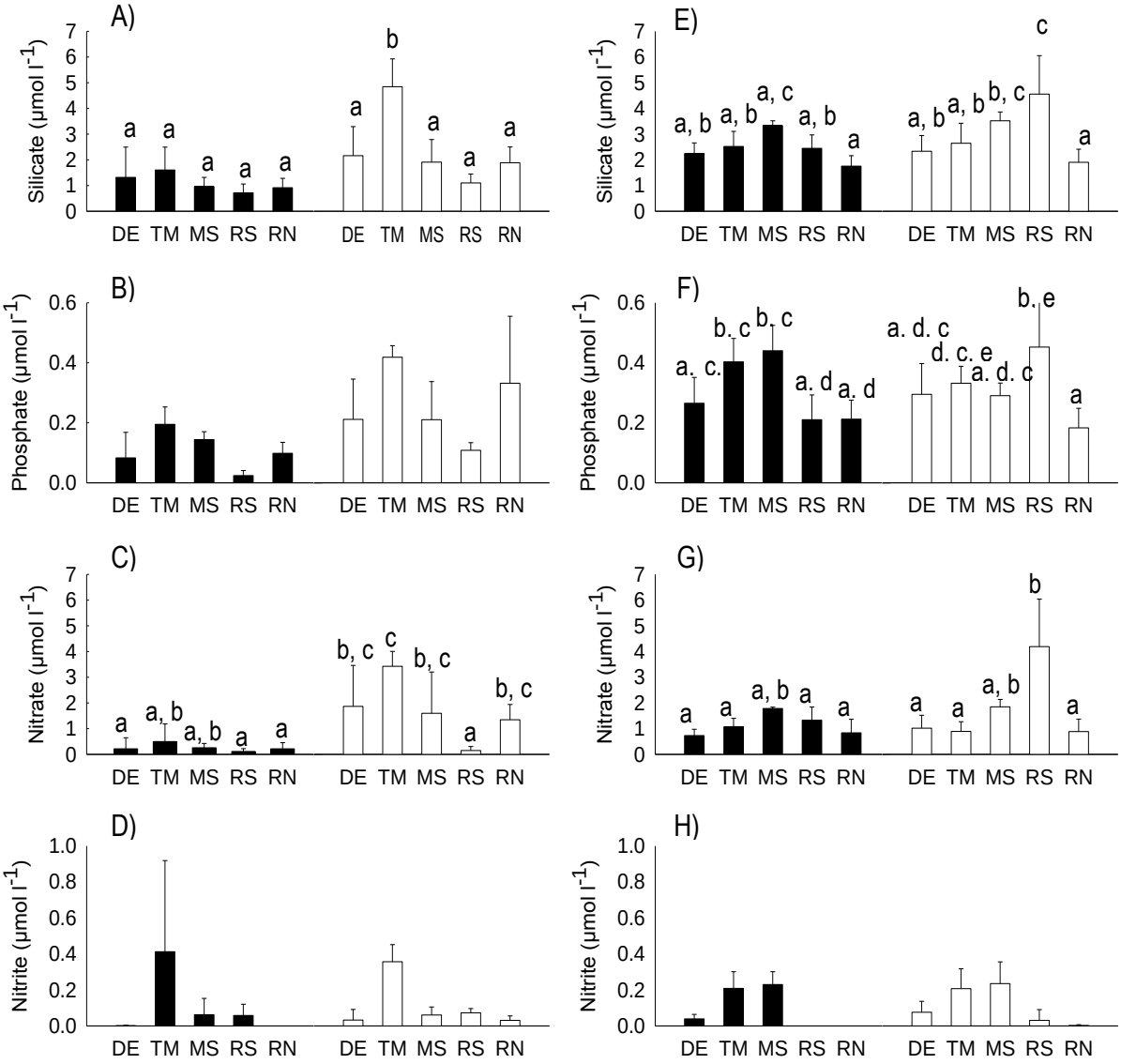

Figure 2.3. Nutrient concentrations for surface water (black) and fluorescence maximum (F_{max}) (white) for each site. Panels A), B), C) and D) represent the wet season; panels E), F), G) and H) represent the dry season. Sample locations are given as DE (Durban Eddy), TM (Thukela Bank), MS (Mid-Shelf), RS (Richards Bay South) and RN (Richards Bay North). Where parametric tests were possible letters indicate significant differences between sites (Tukey's HSD *post-hoc*, $p < 0.05$). Refer to Table 2.1 for statistical analysis of differences between Stations and Depths. For depth of F_{max}, refer to Figure 2.2. Mean ± SD is shown.

2.4.2 Total suspended solids

TSS concentrations were low in both seasons, with surface and F_{max} waters seldom reaching levels of 0.02 g l^{-1} (Fig. 2.4). A two-way ANOVA for TSS data collected during the wet season indicates a significant effect of Site and a significant interactive effect of Site and Depth (Fig. 2.4) (*df* = 4, *SS* = 0.00008, *MS* = 0.00002, *F* = 4.13, *p* = 0.01; *df* = 4, *SS*

= 0.00006, *MS* = 0.00001, *F* = 3.11, *p* = 0.04 respectively). Tukey's *post-hoc* test showed that the only significant difference was for Richards Bay North. During the dry season TSS was more homogenous, with no significant differences in TSS concentration for either Depth or Site nor the interaction term (Fig. 2.4).

PON within the TSS varied greatly in the wet season, with only Site having a significant influence on its concentration (Fig. 2.4 C) (two-way ANOVA *df* = 4, *SS* = 226,.00, *MS* = 56.65, *F* = 4.33, *p* = 0.01); the Thukela River mouth and Mid Shelf region had the highest surface PON concentrations of 0.68 ± 0.14 and 0.97 ± 0.06 µmol l^{-1} respectively, while Mid Shelf and Durban Eddy sites had the highest PON values at F_{max} depth, 1.07 ± 0.22 and 0.80 ± 0.50 µmol l^{-1} respectively (Fig. 2.4). Richards Bay South and North were the only significantly different stations at surface and F_{max} (Fig. 2.4 B). During the dry season neither site, depth nor the interaction term was significant as far as PON was concerned (Fig. 2.4).

2.4.3 $\delta^{13}C$

There was a significant spatial variation in TSS $\delta^{13}C$ during summer (two-way ANOVA *df* = 5, *SS* = 29.51, *MS* = 5.90, *F* = 11.96, *p* < 0.0001). Looking at the results in more detail it was confirmed that only Richards Bay South and North surface and F_{max} $\delta^{13}C$ values were homogeneous to those from the Thukela River mouth (Tukey's *post-hoc* results; Fig. 2.5 A) The $\delta^{13}C$ values of TSS taken during summer ranged from -22.98 ± 0.49 ‰ in Richards Bay South surface waters to -20.14 ± 0.54 ‰ in Mid Shelf F_{max} waters, while the $\delta^{13}C$ signal of Thukela River mouth samples was -24.01 ± 0.67 ‰ (Fig. 2.5 A). $\delta^{13}C$ data for TSS collected during the winter differed with Site but not Depth (Fig. 2.5 C) (two-way ANOVA *df* = 4, *SS* = 22.22, *MS* = 5.56, *F* = 7.80, *p* < 0.0001; data for Richards Bay South were omitted due to insufficient replication). Tukey's *post-hoc* test confirmed that the Thukela River mouth TSS $\delta^{13}C$ values in the dry season were different to Durban Eddy and Richards Bay North surface waters and to all stations at F_{max}. Winter TSS $\delta^{13}C$ ranged from -20.74 ‰ at Richards Bay South to -24.96 ± 0.67 ‰ in the Thukela River mouth.

Although zooplankton exhibited a wide range of $\delta^{13}C$ across the five sites for both seasons, the differences were most prominent for samples collected during summer (Tables 2.2 and 2.3). However, only one species, the Chaetognatha *Flaccisagitta enflata,* occurred in all sites and in both seasons, with $\delta^{13}C$ values ranging from -21.64 ‰ to -17.91 ‰ during summer and -20.61 ‰ to -18.70 ‰ during winter. Other species that occurred during both seasons and sites were the Copepoda, *Undinula vulgaris* and *Subeucalanus monachus*, as well as the mixed zooplankton size classes 500 and 250 µm.

2.4.4 $\delta^{15}N$

$\delta^{15}N$ of TSS collected during summer ranged from 6.31 ± 0.52 ‰ at the Mid Shelf to 3.06 ± 0.64 ‰ at Richards Bay North for surface water. TSS taken at the F_{max} ranged from 5.08 ± 0.92 ‰ at Thukela Bank station to 3.44 ± 0.23 ‰ at Richards Bay South. A two-way ANOVA highlighted Site rather than Depth as having a significant influence on TSS $\delta^{15}N$ values (Fig. 2.5 B) (df = 5, SS = 26.12, MS = 5.23, F = 7.87, $p < 0.001$). Tukey's *post-hoc* results showed that Thukela River TSS $\delta^{15}N$ values were significantly higher only from those taken at the Durban Eddy and Richards Bay South and North surface waters (Fig. 2.5 B). There were no significant differences between the shelf sites and those of the Thukela River mouth as far as $\delta^{15}N$ at the F_{max} was concerned. In the wet season the Thukela Bank station and the Mid Shelf TSS surface $\delta^{15}N$ values were more enriched than those at the Durban Eddy, Richards Bay South and Richards Bay North (Fig. 2.5 B). The dry season $\delta^{15}N$ of TSS was not significantly different with Site or Depth (Fig. 2.5 D), but nevertheless ranged from 4.44 ± 1.41 ‰ at Richards Bay North to 2.38 ± 0.38 ‰ at the Thukela Bank station site in the surface waters; at the F_{max} $\delta^{15}N$ values were more homogenous with Richards Bay North having the highest value at 4.33 ± 1.14 ‰ and the Mid Shelf the lowest at 3.95 ± 0.81 ‰. Zooplankton $\delta^{15}N$ signatures ranged widely from 4.38 ‰ to 9.66 ‰ during the summer and 5.29 ‰ to 9.41 ‰ during the winter, while within species variations were small (Tables 2.2 and 2.3).

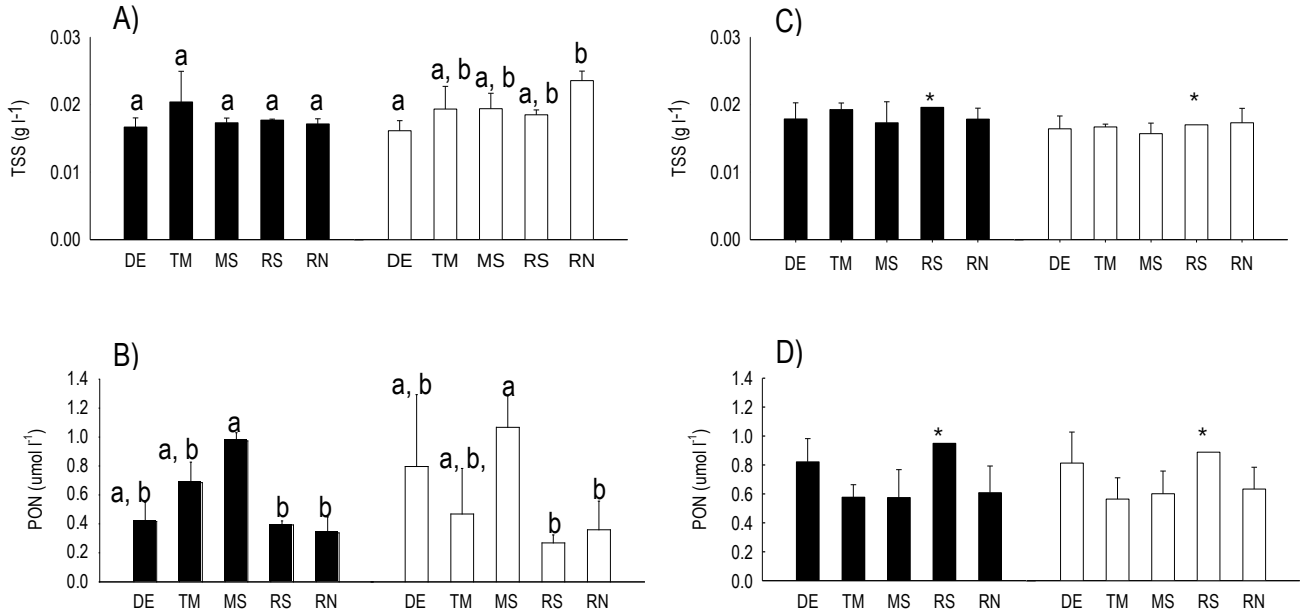

Figure 2.5. TSS and PON concentrations during the wet (A, B) and dry (C, D) seasons. Black bars represent surface water and white bars the F_{max} depth. Refer to text for details regarding statistical treatment of the data. Sample locations are represented as DE (Durban Eddy), TM (Thukela Bank), MS (Mid-Shelf), RS (Richards Bay South) and RN (Richards Bay North). Significant differences (Tukey's HSD *post-hoc*, $p < 0.05$), no letters indicate no significant differences and * indicates there were not enough replicates for inclusion in statistical analysis. For F_{max} depth refer to Figure 2. Mean ± SD shown.

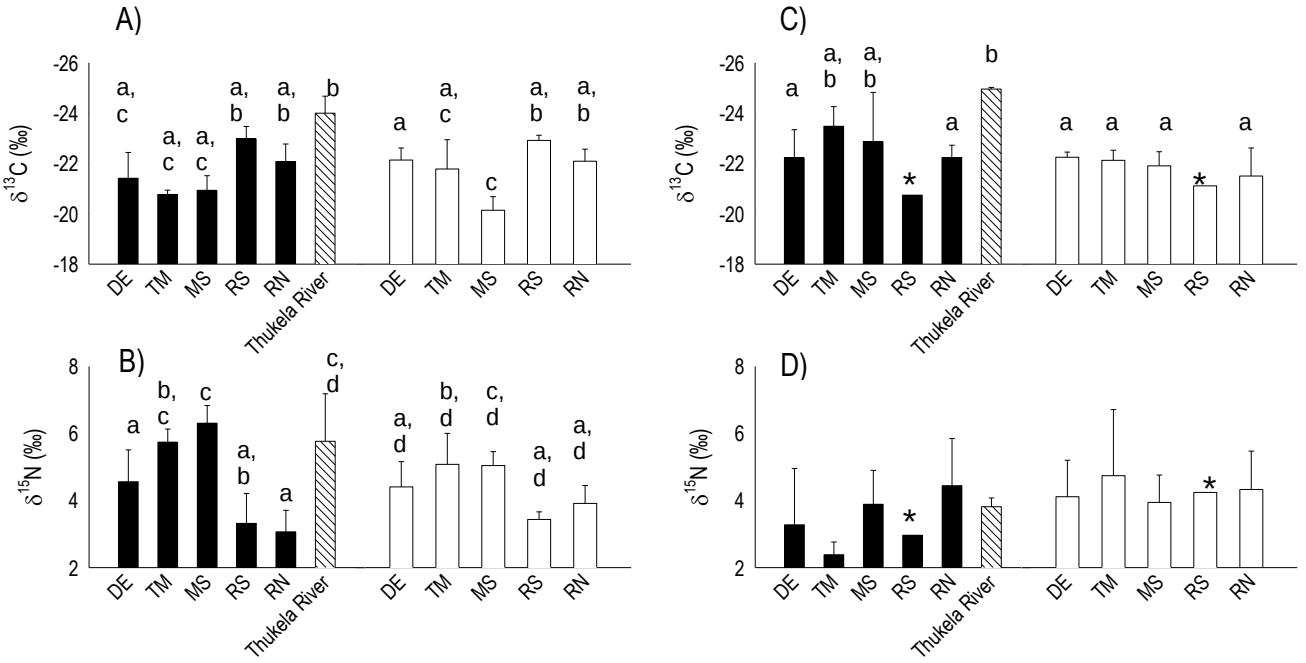

Figure 2.4. TSS $\delta^{13}C$ and $\delta^{15}N$ values for the wet season (A, B) and the dry season (C, D). Black lines represent surface water, white bars represent the F_{max} depth. Striped bars represent Thukela River mouth. Refer to text for ANOVA results, letters indicate Tukey's *post-hoc* differences ($p < 0.05$); the absence of letters indicates the absence of significant differences. Asterisk (*) indicates that not enough replicates were collected. For F_{max} depth refer to Figure 2.2. Mean ± SD shown.

Table 2.2. Zooplankton species sampled in the wet season, grouped by location (DE – Durban eddy; TM – Thukela Bank; RS – Richards Bay North, RS – Richards Bay South; MS – Mid Shelf), including $\delta^{15}N$ and $\delta^{13}C$, trophic position (TP) and mixing model outcomes. For the mixing models each species/taxon was analysed against all other species/taxa with lower TP, as well as against TSS. In all situations, TSS was the dominant food source; therefore only mixing model results for TSS are shown here. Results for MixSIR are presented as median and the 5th and 95th credibility intervals and represent the outcomes for TSS from the location where the zooplankton was collected. Zooplankton from lower trophic levels were also included as possible prey items, but for the sake of being concise, and due to the overwhelming dominance of TSS as a food source, their negligible contributions are not presented here.

Location	Species/Taxon	*n*	$\delta^{15}N$	$\delta^{13}C$	C:N	TP	Surface TSS	F_{max} TSS
DE								
	Cephalopoda larva	1	8.63	-20.31	4.25	2.2	0.22 (0.02 - 0.48)	0.27 (0.02 - 0.54)
	Ferosagitta sp.	2	8.60 ± 0.04	-19.67 ± 0.24	4.17 ± 0.14	2.2	0.14 (0.01 - 0.37)	0.21 (0.03 - 0.40)
	Flaccisagitta enflata	2	8.34 ± 0.60	-19.65 ± 0.54	4.08 ± 0.38	2.1	0.44 (0.33 - 0.55)	0.10 (0.02 - 0.23)
	Penaeus sp.	1	7.42	-21.03	5.01	1.9	0.43 (0.04 - 0.89)	0.19 (0.02 - 0.67)
	Subeucalanus monachus	11	6.87 ± 0.37	-19.90 ± 0.56	4.57 ± 0.59	1.7	0.67 (0.50 - 0.81)	0.26 (0.01 - 0.34)
	Ostracoda	1	6.86	-20.87	4.91	1.7	0.30 (0.02 - 0.72)	0.46 (0.03 - 0.78)
	Undinula vulgaris	7	6.84 ± 0.29	-19.92 ± 0.60	4.12 ± 0.48	1.7	0.65 (0.43 - 0.80)	0.12 (0.01 - 0.39)
	Lucifer typus	4	6.70 ± 0.23	-20.27 ± 0.19	4.83 ± 0.05	1.7	0.11 (0.01 - 0.60)	0.62 (0.16 - 0.77)
	Euphausia sp.	25	6.64 ± 0.39	-19.98 ± 0.42	3.69 ± 0.21	1.7	0.71 (0.56 - 0.87)	0.17 (0.03 - 0.35)
	Mixture 500	12	6.42 ± 0.17	-20.36 ± 0.33	4.32 ± 0.21	1.6	0.07 (0.01 - 0.27)	0.77 (0.63 - 0.87)
	Mixture 250	12	5.95 ± 0.13	-20.44 ± 0.33	4.50 ± 0.15	1.5	0.05 (0.00 - 0.16)	0.86 (0.75 - 0.95)
	Euphausia frigida	7	5.65 ± 0.73	-20.09 ± 0.34	3.91 ± 0.04	1.4	0.86 (0.03 - 0.95)	0.06 (0.00 - 0.87)
	Larvacea	1	5.42	-21.50	3.78	1.3	0.32 (0.02 - 0.96)	0.68 (0.04 - 0.98)
	Creseis sp.	1	5.39	-20.18	3.41	1.3	0.52 (0.04 - 0.97)	0.48 (0.03 - 0.97)
	Cephalopoda larva	1	5.32	-21.02	6.84	1.3	0.27 (0.02 - 0.95)	0.73 (0.05 - 0.98)
	Thalia democratica	9	5.16 ± 0.51	-20.63 ± 0.48	4.36 ± 0.30	1.2	0.02 (0.00 - 0.11)	0.94 (0.86 - 0.98)
TM								
	Ostracoda	1	8.94	-19.78	4.86	2.1	0.12 (0.01 - 0.43)	0.39 (0.22 - 0.58)
	Flaccisagitta enflata	7	8.86 ± 0.64	-18.58 ± 0.70	3.72 ± 0.30	2.1	0.15 (0.02 - 0.35)	0.64 (0.14 - 0.77)
	Scyphozoa	2	8.47 ± 0.06	-19.43 ± 0.10	4.12 ± 0.07	2.0	0.79 (0.29 - 0.98)	0.21 (0.02 - 0.71)
	Liriope sp.	2	8.46 ± 0.04	-19.37 ± 0.03	4.07 ± 0.00	2.0	0.23 (0.02 - 0.49)	0.20 (0.02 - 0.43)
	Lucifer typus	2	7.57 ± 0.10	-19.40 ± 0.17	4.79 ± 0.13	1.7	0.30 (0.13 - 0.53)	0.23 (0.02 - 0.87)
	Mixture 500	5	7.55 ± 0.33	-19.36 ± 0.13	4.19 ± 0.04	1.7	0.49 (0.28 - 0.61)	0.08 (0.01 - 0.53)
	Undinula vulgaris	2	7.24 ± 0.33	-19.05 ± 0.38	4.47 ± 0.04	1.6	0.32 (0.03 - 0.98)	0.40 (0.02 - 0.65)
	Mixture 250	5	7.23 ± 0.23	-19.36 ± 0.11	4.21 ± 0.08	1.6	0.51 (0.35 - 0.64)	0.07 (0.01 - 0.73)
	Creseis sp.	1	6.61	-19.37	4.16	1.5	0.45 (0.04 - 0.98)	0.12 (0.01 - 0.49)
	Thalia democratica	4	6.07 ± 0.20	-19.90 ± 0.23	4.29 ± 0.06	1.3	0.07 (0.00 - 0.23)	0.93 (0.77 - 0.99)
	Diacavolinia longirostris	2	5.68 ± 0.71	-19.51 ± 0.30	4.03 ± 0.03	1.2	0.09 (0.01 - 0.95)	0.20 (0.01 - 0.60)
MS								
	Flaccisagitta enflata	2	8.86 ± 0.05	-18.76 ± 0.47	4.00 ± 0.06	2.1	0.42 (0.05 - 0.61)	0.13 (0.01 - 0.60)
	Squilla sp. larval stage	1	8.84	-20.11	4.91	2.1	0.64 (0.12 - 0.96)	0.36 (0.04 - 0.88)
	Siriealla sp.	1	8.84	-19.99	4.11	2.1	0.13 (0.01 - 0.36)	0.40 (0.05 - 0.89)
	Liriope tetraphylla	1	8.70	-19.48	4.04	2.0	0.17 (0.01 - 0.42)	0.15 (0.01 - 0.42)
	Leptostraca larva	1	8.06	-19.54	4.72	1.8	0.40 (0.05 - 0.90)	0.61 (0.10 - 0.96)
	Lucifer typus	2	7.43 ± 0.04	-19.33 ± 0.07	4.64 ± 0.13	1.7	0.18 (0.02 - 0.63)	0.33 (0.03 - 0.56)
	Subeucalanus monachus	1	7.23	-19.00	4.67	1.6	0.22 (0.02 - 0.84)	0.17 (0.01 - 0.55)
	Mixture 500	5	7.12 ± 0.10	-19.19 ± 0.48	3.90 ± 0.43	1.6	0.07 (0.01 - 0.22)	0.93 (0.78 - 0.99)
	Cephalopoda larva	1	7.04	-20.02	4.85	1.6	0.37 (0.02 - 0.95)	0.63 (0.05 - 0.98)
	Mixture 250	4	6.74 ± 0.19	-19.85 ± 0.29	4.18 ± 0.17	1.5	0.04 (0.00 - 0.20)	0.96 (0.80 - 1.00)
	Undinula vulgaris	7	6.50 ± 0.25	-18.72 ± 0.56	3.69 ± 0.48	1.4	0.02 (0.00 - 0.09)	0.79 (0.04 - 0.85)
	Larvacea	1	6.32	-20.99	3.78	1.4	0.16 (0.01 - 0.45)	0.13 (0.01 - 0.43)

Table 2.2. Continuation

	Euphausia frigida	2	6.16 ± 0.16	-19.55 ± 0.18	4.28 ± 0.26	1.3	0.06 (0.00 - 0.24)	0.28 (0.01 - 0.59)
RS								
	Ferosagitta sp.	3	7.89 ± 0.32	-19.88 ± 0.46	4.00 ± 0.13	2.2	0.22 (0.03 - 0.50)	0.16 (0.02 - 0.48)
	Mysida	1	7.57	-20.92	3.83	2.1	0.74 (0.63 - 0.80)	0.11 (0.06 - 0.50)
	Flaccisagitta enflata	3	7.23 ± 1.32	-19.94 ± 0.55	4.08 ± 0.34	2.0	0.57 (0.07 - 0.79)	0.11 (0.06 - 0.70)
	Pterotrachea sp.	1	6.78	-19.70	3.78	1.9	0.10 (0.01 - 0.49)	0.90 (0.51 - 0.99)
	Undinula vulgaris	1	6.56	-20.97	4.21	1.9	0.29 (0.05 - 0.47)	0.26 (0.01 - 0.57)
	Subeucalanus monachus	4	6.17 ± 0.71	-20.49 ± 0.48	4.49 ± 0.49	1.7	0.09 (0.01 - 0.28)	0.92 (0.72 - 0.99)
	Mixture 500	3	6.03 ± 0.57	-20.55 ± 0.23	4.17 ± 0.08	1.7	0.69 (0.19 - 0.91)	0.24 (0.03 - 0.77)
	Lucifer typus	1	5.43	-20.63	4.48	1.5	0.31 (0.03 - 0.94)	0.69 (0.06 - 0.98)
	Mixture 250	3	4.91 ± 0.51	-20.30 ± 0.71	4.44 ± 0.25	1.4	0.05 (0.00 - 0.19)	0.95 (0.81 - 1.00)
	Euphausia frigida	1	4.56	-19.95	3.89	1.3	0.10 (0.01 - 0.91)	0.90 (0.08 - 0.99)
	Polychaeta	1	4.42	-21.53	5.31	1.3	0.60 (0.04 - 0.97)	0.40 (0.03 - 0.96)
RN								
	Ferosagitta sp.	2	7.48 ± 0.11	-19.70 ± 0.17	4.00 ± 0.03	2.2	0.36 (0.07 - 0.57)	0.28 (0.02 - 0.96)
	Flaccisagitta enflata	3	7.47 ± 0.17	-20.38 ± 1.10	5.01 ± 1.80	2.2	0.36 (0.08 - 0.59)	0.31 (0.03 - 0.97)
	Subeucalanus monachus	1	6.56	-20.48	4.54	1.9	0.49 (0.07 - 0.93)	0.51 (0.07 - 0.93)
	Euphausia sp.	1	6.13	-20.42	3.91	1.8	0.54 (0.07 - 0.94)	0.46 (0.06 - 0.93)
	Mixture 500	4	6.05 ± 0.40	-20.99 ± 0.51	4.71 ± 0.72	1.8	0.12 (0.01 - 0.49)	0.16 (0.01 - 0.62)
	Copepoda	1	5.50	-20.13	3.85	1.6	0.66 (0.07 - 0.97)	0.37 (0.03 - 0.93)
	Mixture 250	4	5.01 ± 0.59	-20.49 ± 0.57	4.59 ± 0.33	1.5	0.04 (0.00 - 0.15)	0.77 (0.01 - 0.95)

2.4.5 Trophic positions and mixing models

Mixing models were used to aid in the selection of primary sources of C and N entering the zooplankton trophic web. Zooplankton occupied a wide range of trophic positions, especially during summer. For *Flaccisagitta enflata*, the only species occurring throughout the Bight in both seasons, the TP varied from 2.2 at Richards Bay North to 2.0 at Richards Bay South in summer (Table 2.2), and 2.5 at Richards Bay South to 1.9 at Richards Bay North during winter. Overall, the TPs ranged from 2.2 for unidentified Teuthoidea larvae, and to 1.2 for *Diacavolinia longirostris* in the wet season (Table 2.2). Zooplankton TPs during the dry season was generally higher than those measured during the wet season, the lowest TP being 1. 6 for the zooplankton mixture size 250 µm (mix 250) to 2.5 for *F. enflata*.

Due to a wide range of TPs for zooplankton, a variety of consumer-prey interactions were modelled for each location and species. In every single interaction attempted, the primary C source taken up by zooplankton was TSS (Table 2.2 and 2.3 for mixing

models results for TSS). Some outcomes suggested a strong preference for surface water TSS and in other instances for that at F_{max} (see Tables 2.2 and 2.3).

In this light, it was decided that each species from each location should be tested against TSS isotope ratios from surface and F_{max} from all locations across the Bight to determine if the animals had been feeding in other areas of the Bight before being transported to the sampling location due to the prevailing hydrodynamics in the region (Fig. 2.6, see also Appendix A wet season; Appendix B dry season).

A series of PCA's allowed for the relative importance of each TSS source to be established for each of the species This confirmed that the organisms in the Bight did indeed prefer TSS from other localities within the Bight for both wet and dry seasons (Fig. 2.7 and 2.8 respectively). In the wet season the most important TSS sources were the Mid Shelf, Richards Bay North and Richards Bay South stations, with the other stations playing a smaller role. In the dry season the Richards Bay South and Richards Bay North stations remained the most important TSS sources.

Table 2.3. Zooplankton species sampled in the dry season grouped by location (see explanation in caption to Table 2.2), including $\delta^{15}N$ and $\delta^{13}C$, trophic position and mixing model outcomes. Asterisk (*) indicates that only one replicate for each depth was collected. In this situation the values for both depths were pooled together. See caption to Table 2.2 for additional information.

Location	Species/Taxon	*n*	$\delta^{15}N$	$\delta^{13}C$	C:N	TP	Surface TSS	F_{msx} TSS
DE								
	Flaccisagitta enflata	9	8.11 ± 0.66	-20.07 ± 0.32	4.49 ± 0.76	2.42	0.24 (0.05 - 0.40)	0.34 (0.13 - 0.53)
	Heteropoda	2	7.70 ± 0.09	-19.70 ± 0.79	3.84 ± 0.16	2.30	0.37 (0.14 - 0.55)	0.10 (0.01 - 0.33)
	Euphausia mixture	3	7.56 ± 0.42	-20.32 ± 0.21	4.19 ± 0.23	2.26	0.40 (0.06 - 0.65)	0.25 (0.03 - 0.51)
	Euphausia sp.	4	7.43 ± 0.85	-19.86 ± 0.39	3.81 ± 0.11	2.22	0.39 (0.10 - 0.58)	0.17 (0.02 - 0.46)
	Subeucalanus monachus	7	7.40 ± 0.25	-19.92 ± 0.33	4.91 ± 0.69	2.22	0.31 (0.07 - 0.50)	0.28 (0.06 - 0.51)
	Mixture 500	8	7.09 ± 0.72	-20.32 ± 0.34	4.17 ± 0.12	2.12	0.32 (0.06 - 0.54)	0.43 (0.15 - 0.67)
	Undinula vulgaris	9	7.02 ± 0.68	-20.27 ± 0.50	4.26 ± 0.24	2.10	0.46 (0.26 - 0.64)	0.27 (0.06 - 0.49)
	Scyphozoa	1	7.01	-20.10	4.03	2.10	0.77 (0.49 - 0.98)	0.23 (0.03 - 0.51)
	Mixture 250	8	6.48 ± 0.47	-20.55 ± 0.38	4.29 ± 0.15	1.94	0.49 (0.27 - 0.75)	0.51 (0.25 - 0.73)
	Creseis sp.	2	5.85 ± 0.17	-20.07 ± 0.99	4.14 ± 0.07	1.76	0.89 (0.69 - 0.99)	0.11 (0.01 - 0.31)
TM								
	Flaccisagitta enflata	5	8.61 ± 0.71	-18.96 ± 0.18	3.98 ± 0.08	2.41	0.06 (0.01 - 0.23)	0.33 (0.09 - 0.44)
	Undinula vulgaris	6	8.33 ± 0.60	-19.39 ± 0.44	4.28 ± 0.24	2.33	0.26 (0.02 - 0.41)	0.18 (0.01 - 0.54)
	Subeucalanus monachus	3	7.87 ± 0.48	-19.10 ± 0.20	4.62 ± 0.47	2.19	0.09 (0.01 - 0.31)	0.34 (0.05 - 0.51)
	Mixture 500	6	7.87 ± 0.38	-19.23 ± 0.14	4.22 ± 0.19	2.19	0.08 (0.01 - 0.27)	0.40 (0.14 - 0.53)
	Mixture 250	6	7.46 ± 0.34	-19.22 ± 0.18	4.09 ± 0.13	2.07	0.01 (0.00 - 0.03)	0.99 (0.97 - 1.00)
MS								
	Flaccisagitta enflata	2	8.81 ± 0.08	-19.81 ± 0.01	4.14 ± 0.14	2.44	0.26 (0.04 - 0.43)	0.11 (0.01 - 0.34)
	Undinula vulgaris	2	7.70 ± 0.80	-18.94 ± 0.04	3.97 ± 0.01	2.11	0.31 (0.03 - 0.54)	0.14 (0.01 - 0.56)

Table 2.3. Continuation

	Mixture 500	2	6.62 ± 0.97	-19.28 ± 0.05	3.98 ± 0.16	1.80	0.48 (0.02 - 0.73)	0.18 (0.01 - 0.73)
	Mixture 250	2	6.53 ± 0.30	-19.36 ± 0.27	4.18 ± 0.06	1.77	0.51 (0.02 - 0.75)	0.18 (0.01 - 0.72)
RS*							**Surface and F_{max} TSS combined**	
	Flaccisagitta enflata	4	8.79 ± 0.16	-19.69 ± 0.68	3.96 ± 0.07	2.53	0.72 (0.56 - 0.85)	
	Undinula vulgaris	3	7.21 ± 0.20	-19.89 ± 0.21	4.39 ± 0.27	2.06	0.70 (0.55 - 0.83)	
	Euphausia sp.	1	7.13	-20.65	4.07	2.04	0.84 (0.64 - 0.98)	
	Mixture 500	5	7.04 ± 0.87	-20.48 ± 0.17	4.48 ± 0.38	2.01	0.91 (0.87 - 0.95)	
	Subeucalanus monachus	3	6.89 ± 0.58	-19.94 ± 0.16	5.21 ± 0.56	1.97	0.75 (0.62 - 0.86)	
	Euphausia frigida	1	6.05	-19.98	3.88	1.72	0.95 (0.83 - 0.99)	
	Mixture 250	5	5.76 ± 0.43	-20.57 ± 0.14	4.37 ± 0.17	1.64	0.94 (0.88 - 0.97)	
RN								
	Subeucalanus monachus	5	7.22 ± 0.25	-20.01 ± 0.19	5.39 ± 0.44	1.93	0.38 (0.13 - 0.55)	0.35 (0.05 - 0.62)
	Mixture 500	5	6.43 ± 0.30	-20.42 ± 0.22	4.72 ± 0.12	1.70	0.61 (0.29 - 0.80)	0.30 (0.04 - 0.64)
	Mixture 250	4	5.96 ± 0.30	-20.78 ± 0.64	5.04 ± 0.86	1.56	0.73 (0.13 - 0.94)	0.22 (0.02 - 0.81)

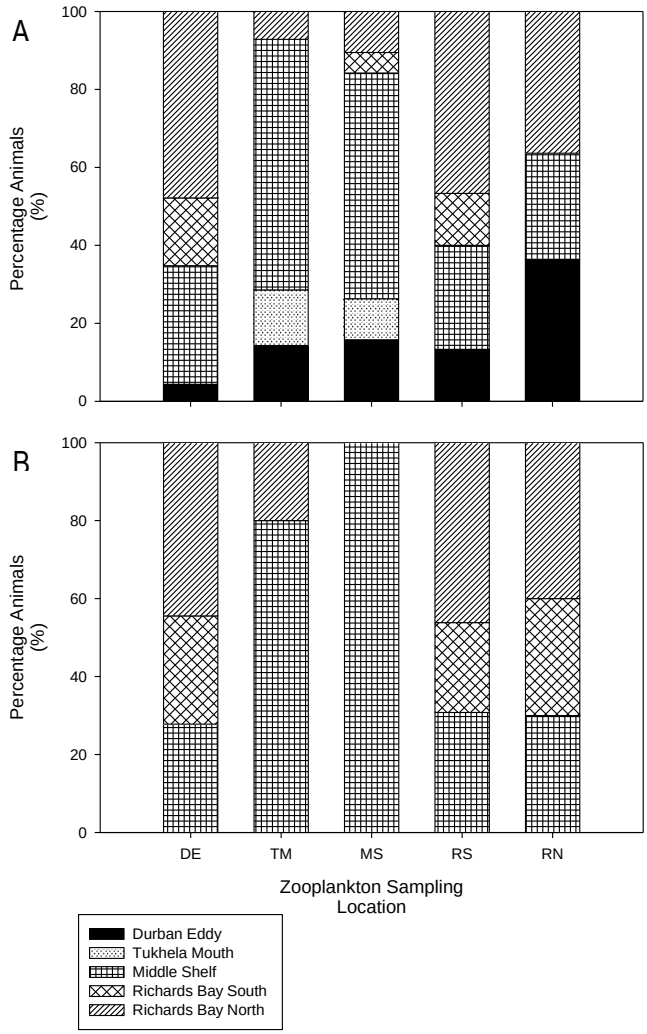

Figure 2.6. The proportion (%) of zooplankton at each sampling location feeding on the TSS from that and all other locations; during the A) wet season, and B) dry season. The proportions were calculated as the fraction of zooplankton species/taxa found at the sampling site that had fed on TSS from the surface and the F_{max} at the location of collection and all other sampling sites. Only MixSIR results showing a primary C source preference greater than 10 % were accounted for. See Appendix A and B for more details.

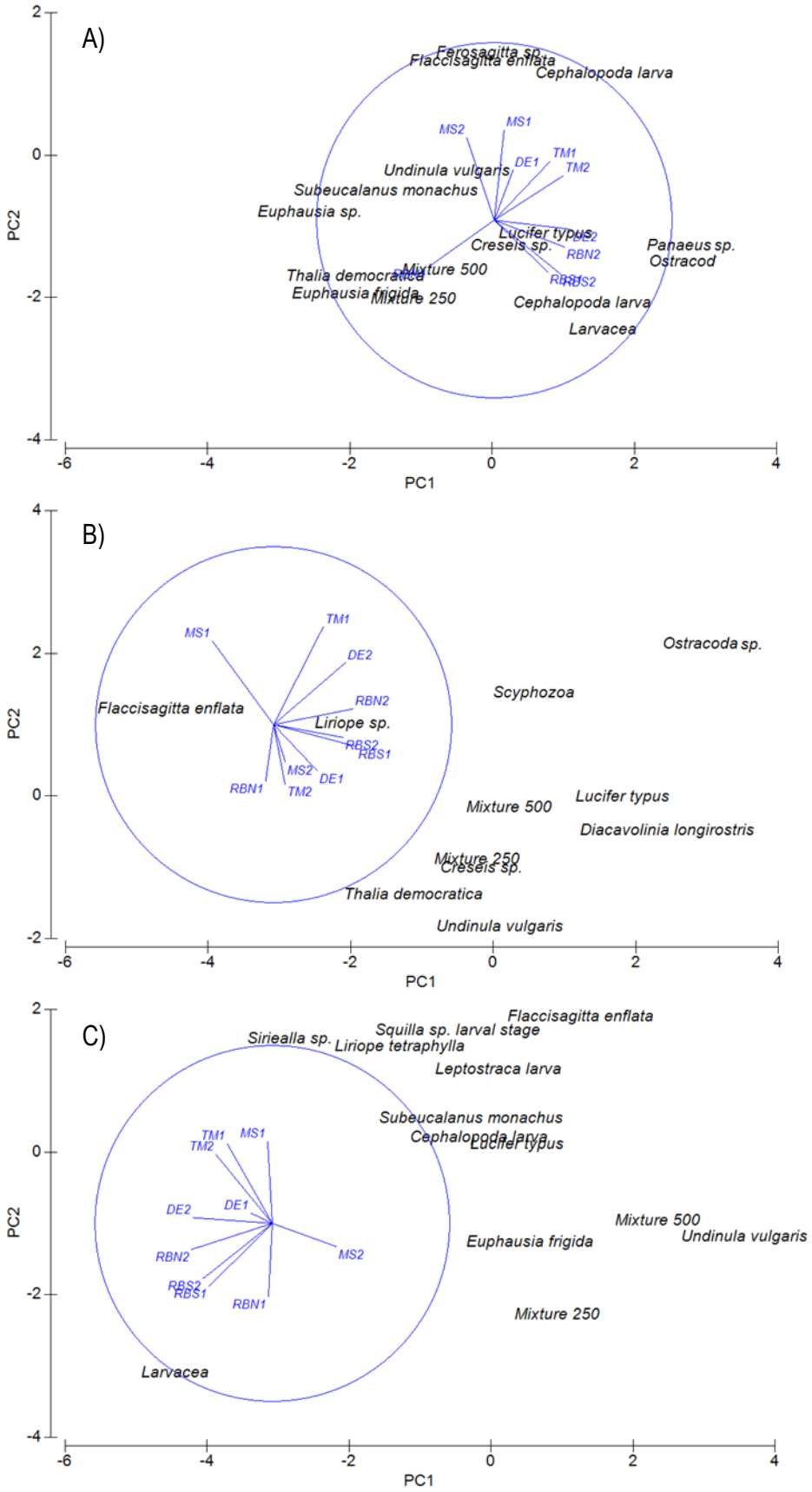

Figure 2.7. PCA analysis illustrating zooplankton feeding preferences in the wet season at each sampling location A) Durban Eddy; B) Thukela Mouth and C) Mid Shelf in support of the findings from the mixing model. See Appendix A for more details.

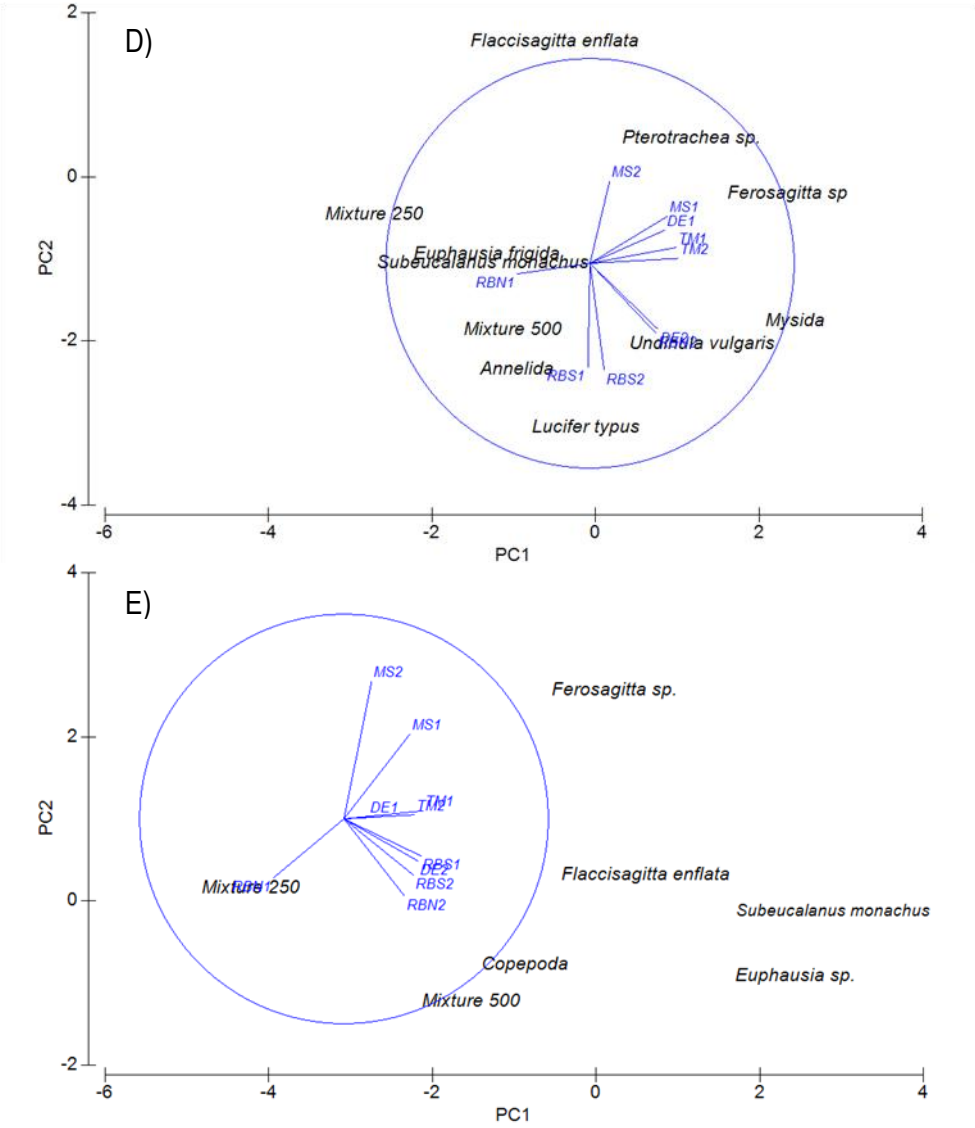

Figure 2.7. (Continuation) D) Richards Bay South and E) Richards Bay North. See Appendix A for more details.

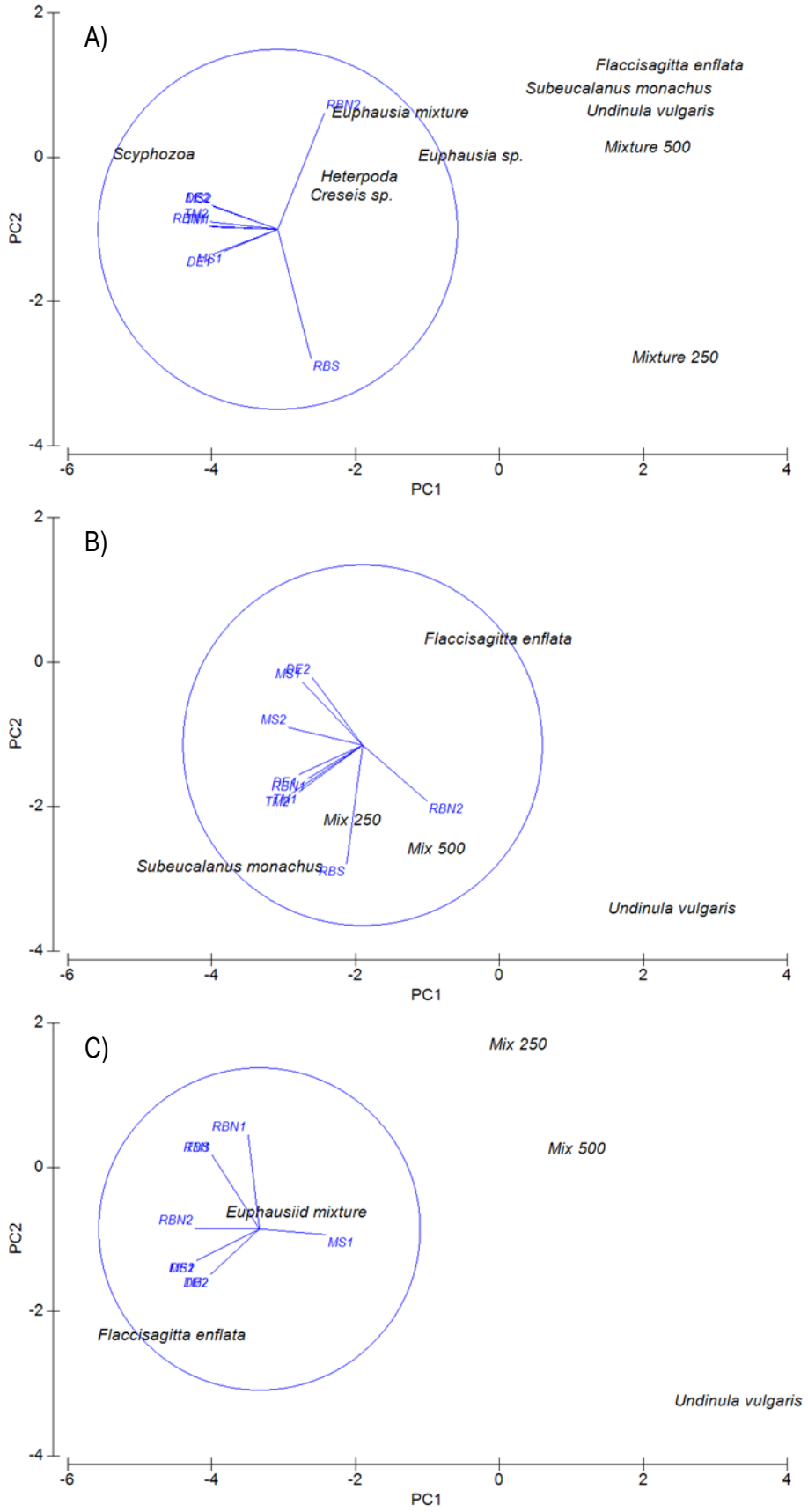

Figure 2.8. PCA analysis illustrating zooplankton feeding preferences in the dry season at each sampling location A) Durban Eddy; B) Thukela Mouth and C) Mid Shelf in support of the findings from the mixing model. See Appendix B for more details.

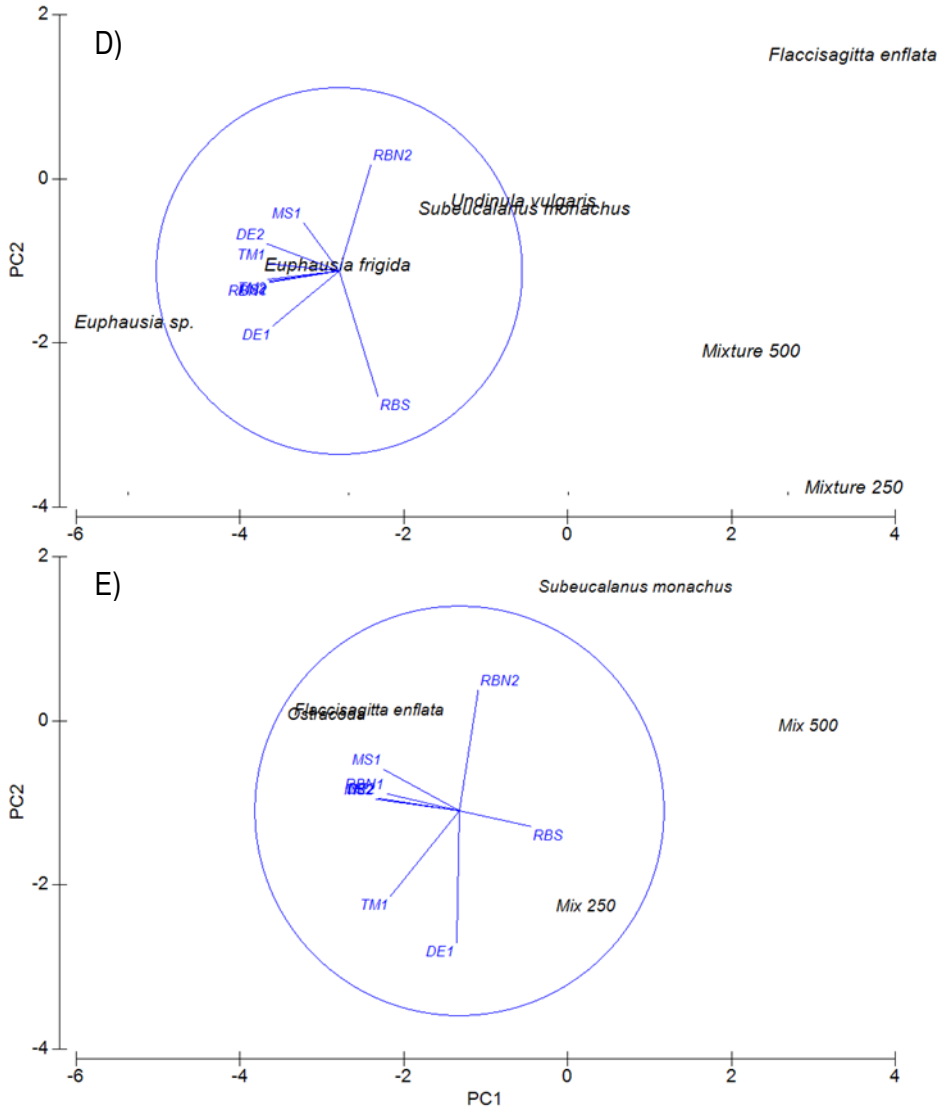

Figure 2.8. (Continuation) D) Richards Bay South and E) Richards Bay North. See Appendix B for more details.

2.5 Discussion

This study provides the first account of zooplankton trophic studies in the KwaZulu-Natal Bight. A striking finding of the research was that the water movement patterns in the Bight inferred from spatial zooplankton feeding patterns on TSS seem to match that of the literature on the physical oceanographic investigations in the region. This was demonstrated by the zooplankton mixing model results that indicated that feeding had occurred in other areas of the Bight prior to the zooplankton being transported to the locale where they were sampled. It serves to inform our understanding of the trophic linkages of zooplankton in an oceanographically important area, the KwaZulu-Natal Bight, and provides strong supporting evidence for oceanographic studies (Roberts et al *in prep*.[1]) concurrently undertaken in the Bight.

A range of physico-chemical and biological variables were analysed to establish distinctions between water masses at the sampling sites. There were no observable distinctions in the salinity, temperature or chlorophyll-*a* biomass between sites, with the exception of the Durban Eddy and the Mid Shelf in the wet season, which had the highest chlorophyll-*a* biomass. However, chlorophyll-*a* biomass for the Mid Shelf station was low in the dry season, closely matching values for the Thukela Bank. Richards Bay South exhibits the opposite pattern, with this station having the lowest chlorophyll-*a* biomass in the wet season and the highest in the dry season. In the wet season, the Durban Eddy exhibited a clear and previously described pattern of salinity with depth (Lutjeharms et al. 2000b), with a low salinity from the surface to a depth of ~20 m, where it increased abruptly with depth, remaining at a higher salinity to a depth of ~100 m, from where the salinity again rapidly decreased. Lutjeharms et al. (2000b) described this salinity profile as evidence of the doming caused by the Durban cyclonic eddy. Despite this evidence of doming at the time of sampling, the Durban Eddy site displayed slightly lower concentrations of nitrate, silicate and phosphate in both seasons compared to previous studies on the eddy (Burchall 1968, Carter & d'Aubrey 1988, Meyer et al. 2002) and instead had similar nutrient levels to other stations within the Bight at time of sampling.

[1] Dr. Mike Roberts, Department of Environmental Affairs, South Africa

Nutrient concentrations at the surface were lower in the wet season than the dry season at all sites, except for nitrite at the Thukela bank station. This was most likely due to the uptake of nutrients by primary producers, which are known to bloom during the wet, austral summer months of the KwaZulu-Natal region (Hunter 1988). Nutrient levels in the dry season were higher than those of the wet season for both surface and $F_{max,}$ probably due to the lower sun radiation during this season. Nutrient concentrations measured in this study for the Thukela Bank station and Mid Shelf area are similar to those recorded by Meyer et al. (2002) (3.50 – 4.69 µmol l^{-1} silicate, 0.48 – 0.72 µmol l^{-1} phosphate and 1.01 – 1.86 µmol l^{-1} nitrate in the central Bight). In contrast to previous studies, which described the Richards Bay upwelling as being persistent (Lutjeharms et al. 1989), I did not find nutrient concentrations anywhere near as high as those anticipated for the upwelling area for either season (Carter & d'Aubrey 1988, Meyer et al. 2002). This would indicate that the upwelling phenomenon was not occurring at the time of sampling.

Quantities of TSS were virtually the same across all sites and in both seasons, while $\delta^{13}C$ signatures differed spatially. $\delta^{13}C$ values are a useful and strong indicator of the origin OM (Rubenstein & Hobson 2004), allowing the water masses at different sites to be distinguished from one another. $\delta^{13}C$ values were of a similar range to previous studies on TSS in other regions of the ocean (Fry & Sherr 1984, Fry & Wainright 1991, Smit et al. 2006, Allan et al. 2010). Richards Bay South and North summer TSS δ-values indicate that its most likely origin was from the Thukela River in the wet season. This substantiates the suggestion that upwelling was not occurring at the time of sampling. During the same season, the results indicated that TSS from the mid- and south region of the Bight, including the Thukela Bank station, were not of riverine origin. However, in the dry season, the period when the Thukela River mouth water should have had less influence, $\delta^{13}C$ values indicated that the Thukela River mouth, Thukela Bank station and Mid Shelf station signatures were statistically similar, indicating that these waters could have come from the same source. It is therefore safe to say that the Thukela River isotopic signature, and hence water, was measured in the central region of the Bight.

During the wet season the most ^{13}C- enriched samples were those from the Thukela Bank station (-20.77 ± 0.17 ‰) and Mid Shelf (-20.93 ± 0.58 ‰) sites. At the Mid Shelf this most likely resulted from the diatom *Thalassionema itzschiodes*, which formed a bloom in the area at the time of sampling (Johan S. van der Molen pers. comm. [2]). Diatoms are known to be enriched in ^{13}C (Fry & Wainright 1991). The cause of this diatom bloom has been suggested to be the result of an upwelling event occurring near Richards Bay and transported south by the prevailing hydrodynamics at the time (Dr Tarron Lammont, unpublished.[3]).

Unlike $\delta^{13}C$ signatures, $\delta^{15}N$ signatures in the wet season seemed to suggest that the TSS samples collected in the Thukela Bank station and Mid Shelf are isotopically similar to the TSS from the Thukela River mouth. Nevertheless, the similarities between the Mid Shelf and the Thukela River mouth could be coincidental, because in N limited environments, N becomes recycled as ammonia resulting in marine plankton reaching $\delta^{15}N$ values of 6 – 7 ‰ (Wada & Hattori 1991, Muto & Soares 2011). The values I sampled at the Mid Shelf were in order with these values, indicating that such N recycling could be occurring and obscuring the isotopic values results.

Mixing models confirmed that TSS was an important source of food underpinning the zooplankton food web, even when other possible food items, such a lower TP zooplankton, were provided in the mixing models. This was the case for all organisms, including predatory organisms such as the arrow worms (i.e. *Ferosagitta* sp. and *Flaccisagitta enflata*), although dependence on TSS consumption for the arrow worms was relatively lower than for other species. The mixing model finding that predatory zooplankton fed on TSS most likely reflects an indirect consumption of TSS through their consumption of smaller, TSS-dependent zooplankton. It therefore suggests that even if I only sampled one possible primary C source, TSS was a very important primary C source in the Bight's water masses. TSS has also been considered important in other studies elsewhere (Govender et al. 2011, Stowasser et al. 2011). The $\delta^{15}N$ values of zooplankton indicated that they occupy up to two TPs, with values ranging from 1.18 to 2.41. The greatest range of TPs was in the wet season, which coincided with and

[2] Dr. Johan van der Molen (2012) Council for Scientific and Industrial Research, South Africa
[3] Dr. Tarron Lammont (2012), Department of Environmental Affairs, South Africa

was likely due to the fact that the greatest diversity of organisms was collected at this time. In general, it has been reported that the east coast of South Africa was a low productivity area (Bustamante et al. 1995), with the zooplankton of the east coast of South Africa exhibiting the greatest diversity within the Agulhas Current and the lowest closest to the shore (Talbot 1974, Gibbons & Hutchings 1996). The zooplankton communities from the East coast of South Africa have been described as having high zooplankton species diversity and very low biomass (Gibbons & Hutchings 1996). This was also the case during the seasons sampled in this study, with biomass decreasing even more in winter (Dr Jenny Huggett *pers. comm.* [4]). Chaetognatha along with Copepoda were the most common organisms occurring in all five sites in my samples.

2.5.1 Spatial patterns and processes

Results seem to indicate that hydrodynamics within the Bight correspond well to isotope ratios of TSS and zooplankton; to the best of my knowledge, this study is the first to have shown such a relationship. Further data collected by the Department of Environmental Affairs will strongly strengthen the relationship explained below. The data was not made available to be added to this thesis but it will most likely be made available for publication.

Using inductive reasoning based on the following key facts, I wish to present my train of thought leading up to this conclusion:

1. There was a causal link between water movement patterns and zooplankton transport in the Bight – Schleyer (1977) suggested that zooplankton mass transport occurs along the east coast of South Africa due to the Agulhas Current bringing with it a number of species from the Indian Ocean.

2. The Bight has formerly been identified as an ideal nursery ground for ichthyoplankton larvae (Beckley & Van Ballegooyen 1992, Hutchings et al. 2002). This was due to ichthyoplankton and plankton being trapped within the

[4] Dr. Jenny Huggett, Department of Environmental Affairs, South Africa

Bight, allowing for the survival of these organisms (Beckley & Van Ballegooyen 1992, Hutchings et al. 2002).

3. From tissue turnover times we may infer feeding localities of zooplankton prior to them being sampled – Due to a delay in consumer tissue isotopic readjustment following assimilation of new, isotopically unique food sources, their isotopic signatures reflect that of food sources earlier fed upon that at a different locale, which are characterised by different isotopic signatures from the location where zooplankton were eventually sampled (Stowasser et al. 2011).

These principles allow me to paint a picture of the dynamic nature of spatial patterns in zooplankton distribution across the Bight. Point 1 and 2 are firmly established (Schell et al. 1998, Rolff 2000, Pagès et al. 2001, Coyle 2005, Pomerleau et al. 2011), but conclusive support for my hypothesis was unfortunately difficult due to paucity in suitable isotopic fractionation time studies (e.g. studies on the isotopic turnover rates of different animal tissues). There are very few laboratory studies on zooplankton tissue turnover rates (Fry & Arnold 1982, Frazer et al. 1997, Grey et al. 2001, Mayor et al. 2011). Field studies on zooplankton tissue turnover are difficult and as such few have been attempted; however one pattern that appears clear was that $\delta^{15}N$ signature varies very little in the species studied over time and in some cases the effect of metabolic turnover has been described as negligible (Matthews & Mazumder 2005, Matthews & Mazumder 2007, Ventura & Catalan 2008). Fry and Arnold (1982), demonstrated that *Panaeus aztecus* tissue turnover rate was growth dependant, and it took up to 23 days for tissue turnover to be completed. In contrast, Frazer et al. (1997) reported that *Euphausia superba* took as long as 10 weeks for tissue to show the isotopic signature of the new diet. O'Reilly et al (2002) found that the full development of copepods from Lake Tanganyika was 31 to 45 days, and therefore their isotopic signatures represent a mixture of phytoplankton digested pre- and post-upwelling events. Slow turnover, and therefore changes in zooplankton $\delta^{15}N$ and $\delta^{13}C$ signatures, means that zooplankton are likely to reflect the source food isotope ratios for some time before their signatures change to reflect the source isotope ratios of the new locale.

Most of the zooplankton species sampled here live long enough to potentially reflect the isotopic signatures of the water masses they previously inhabited. For example, some Euphausids can live well over a year (Pillar & Stuart 1988); *E. frigida*, considered in this study, is known to have a life span of two years (Siegel 1987). The most common copepod found, *Undinula vulgaris*, has a life cycle of roughly 35 days (Webber & Roff 1995). Although records on the life history of the other most common copepods encountered are not available, that of *S. monachus*, which attains a larger size than *U. vulgaris*, can be assumed to have a life expectancy at least as long. Also no records of the life span of the Chaetognatha *F. enflata* were found, but it has been suggested that other members of the Sagitta family species may live close to a year (Terazaki 2004). Although no specific turnover ratio exists for the species in this study, it seems justifiable to assume that the tissue turnover times of organisms encountered in this study are similar to those presented above. Given this, organisms should get transported within the Bight sufficiently fast for the zooplankton to retain the isotopic signature from where they first started feeding and prior to them being transported to and collected from a different locale. It therefore seems plausible that zooplanktonic isotopic signatures can be used as tracer of hydrodynamic patterns in the Bight.

Indeed, I found that zooplankton at most sampling sites favoured TSS from other sites, indicating that they had been feeding elsewhere in the Bight before being carried through mass transport of water to the location where they were collected. This would have lead to the isotopic signatures of those collected at a single focus site reflecting isotopic preference for TSS from a variety of the different sites they passed through including that of the TSS at the collection site. The Mid Shelf yielded the most common TSS source for zooplankton from the entire Bight in the wet seasons according to the mixing models. This was unanticipated at the start of the study, but given the benefit of hindsight, not surprising as the area had a large chlorophyll-*a* biomass at the time of sampling in the wet season. On the other hand, Richards Bay South and North yielded the most common TSS source during the dry season, as shown by the mixing models and PCA. This was to be expected as in this season Richards Bay South had the highest chlorophyll-*a* biomass. The TSS coming out of the Thukela River mouth, that of the Durban cyclonic eddy and that formed by the upwelling centre all played minor roles as primary C sources. There was evidence suggesting that upwelling was not occurring

at the time of sampling in either season, as such its relative importance as a driver of the food source could not be measured. However, as explained previously, the high productivity in the Mid Shelf station was most likely from an old upwelling event being transported south.

The currents described by Schumann (1987) and Lutjeharms (2006a) indicate a possible trajectory for the zooplankton that explains the isotope mixing model results (Fig. 2.7). A near-shore current likely transports zooplankton northwards from Durban cyclonic eddy. The eddy and this near-shore current have been well studied, and are considered important features of the Bight circulation (Schumann 1981, 1982, 1987, 1988b, Shillington 1993, Lutjeharms et al. 2000b, Meyer et al. 2002). Additionally, a northward flow close to the shore in the centre of the Bight was described as early as 1964 (Harris 1964, Schumann 1981, 1987, Lutjeharms et al. 2000b). Based on the evidence suggesting that zooplankton collected at Richards Bay North favoured TSS from the Mid Shelf and Durban Eddy I infer a northward flowing current. This was presumably the reversal current between Richards Bay South and North described by Gründlingh (1974). From the mixing model it also seems likely that in the centre of the Bight has a two-way exchange of water, occurring between the Mid Shelf area and the Thukela Bank station in the wet season; a one-way zooplankton movement (Mid Shelf to Thukela Bank station) was present in the dry season. There is some previous oceanographic evidence to support this; Schumann (1988b) suggested the presence of a small eddy in the centre of the Bight off the Thukela River, while Flemming and Hay (1988) suggested a shoreward bottom current occurring in the centre of the Bight.

The Agulhas Current reaches speeds of 2 m s^{-1} (de Ruijter et al. 1999), while rates for the northward inshore currents have been estimated at ~0.25 m s^{-1} (Harris 1978, Schumann 1988b). It seems plausible that zooplankton was transported across the Bight well within their life span and less than the time it takes for isotopic readjustment to new food source to occur. The aim of a future paper is to integrate isotopic findings amassed in this study with outcomes of a parallel physical oceanographic study of the Bight (Roberts et al. *in prep.* [5]), so as to conclusively link data on the dynamic nature of

[5] Dr. Mike Roberts, Department of Environmental Affairs, South Africa

zooplankton spatial distribution to the underlying patterns in oceanic currents in the study area.

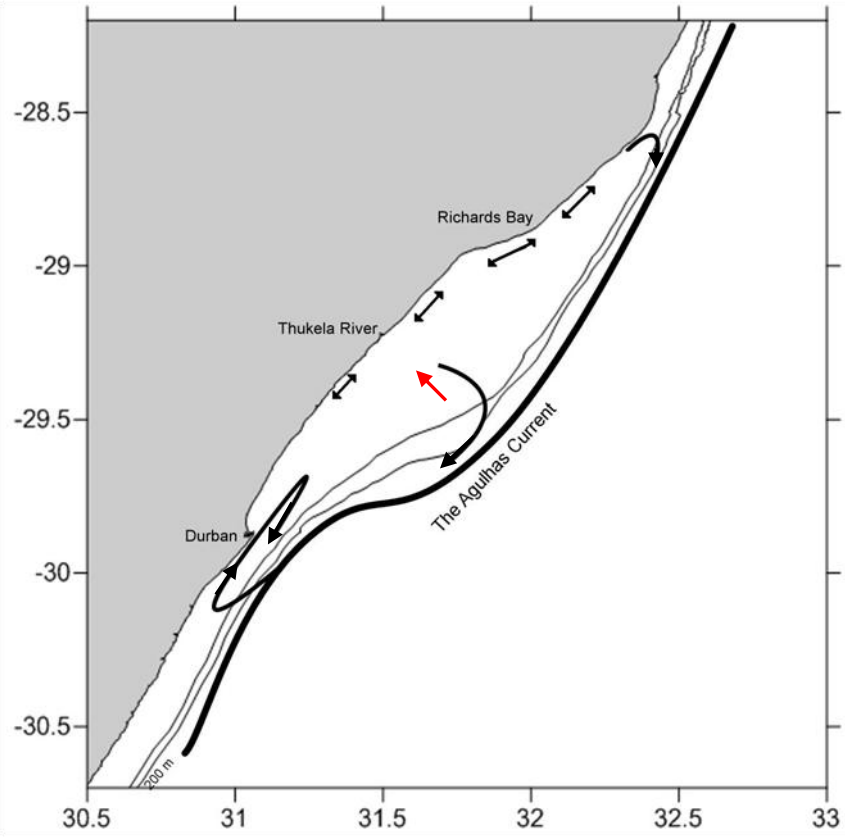

Figure 2.9. The probable direction of movement of currents within the Bight as conceptualised by Schumann (1987). Red arrow represents a near bottom current

2.6 Conclusion

Mixing models confirmed TSS as an important food source, directly grazed upon by of zooplankton biomass in the Bight. All the regions sampled in the Bight during the wet season had unique environmental, biological and isotopic characteristics with the Mid Shelf and Richards Bay North sites being biologically important as TSS sources. The diatom bloom at the Mid Shelf was implicated as the major contributor to the TSS pool of this region and the Bight as a whole. It has been suggested that the diatom bloom formed from an upwelling process, previous to sampling, in the Richards Bay area and was being transported south. Under this light, the important TSS sources were marine-dominated, while riverine inputs played a minor role in the wet seasons, whereas in the dry season the Thukela River appears to dominate surface waters at the centre of the Bight. Isotopic similarities found between the Thukela River mouth and the Mid Shelf were attributed to the recycling of nitrogen into ammonia causing $\delta^{15}N$ values of plankton to become enriched.

There is support for the notion that oceanographic hydrological patterns may be inferred from the zooplanktonic isotope data and their relations with the TSS in the region. In this light, zooplankton appears to be transported among sites by the Mid Shelf–Thukela exchange and the reversal current between Richards Bay South and North. Bio-physical coupling, as I have demonstrated here, is an important approach which can be of great assistance to those studying purely physical processes, such as trajectories of currents and their mixing proportions.

I highlight the need for an increase in zooplankton food web studies, which are increasingly recognised as being important to the understanding of marine ecosystems. Isotopic tissue fractionation studies of zooplankton might assist in studies such as this one in elucidating the elapsed time since the isotopic signature was gained and, therefore, the time since feeding in the previous locale. Isotopic changes following dietary shifts could be used in conjunction with measurements of current flow rates, with each set of measurements interpreted within the context of the other.

2.7 Acknowledgements

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CHAPTER 3

On the use of stable isotopes to understand the processes controlling a benthic food-web of an oligotrophic bight, KwaZulu-Natal, South Africa

3 On the use of stable isotopes to understand the processes controlling a benthic food-web of an oligotrophic bight, KwaZulu-Natal, South Africa

3.1 Abstract

The KwaZulu-Natal Bight is considered to be oligotrophic/mesotrophic with distinct sources of nutrients entering the system by a series of oceanographic processes, including an upwelling cell and several estuaries, of which the Thukela River is the most important. It has been suggested that the upwelling cell is the main factor controlling the biology of the Bight. My aim is to describe the main nutrient/organic matter (OM) source driving the benthic system of the Bight and to produce a food-web to aid in understanding the trophic interactions occurring in the demersal ecosystem. For this, marine and riverine sediment samples, total suspended solids and marine demersal organisms were collected across the Bight and analysed $\delta^{13}C$ and $\delta^{15}N$ composition during two seasons. My results based on $\delta^{13}C$, $\delta^{15}N$, % organic carbon, % nitrogen and carbon:nitrogen ratios, suggest that the OM in the sediments is mainly dominated by riverine input and the benthic food-web is controlled mainly by riverine total suspended solids input. Future studies should look at importance of bacteria degradation of terrestrial OM and its role in the food-web of the Bight. Omnivory appears to be a wide spread strategy for demersal organisms throughout the Bight a finding supported by the lack of clear $\delta^{15}N$ enrichment between prey and predators and the low variability of trophic positions across a wide array of organisms.

3.2 Introduction

The KwaZulu-Natal Bight (the Bight) on the oligotrophic east coast of South Africa (Bustamante et al. 1995), is recognised as an oceanographically important area highly influenced by the Agulhas Current (Lutjeharms 2006a, Lutjeharms 2006b). The biology of the Bight is dominated by the available shallow-water (shelf) benthic system, and hence is of importance to a limited number of fisheries. However, little work has been done on the biology of the (demersal) system and how it is driven by the nutrient inputs associated with the complex hydrography of the Bight.

Nutrients are introduced into the Bight by a series of well-described oceanographic and fluvial processes (Fig. 3.1) (see Chapter 1, Section 1.1 for full description). Topographically induced upwelling close to the Richards Bay area is regarded as the most important source of nutrients into the Bight (Gill & Schumann 1979, Schumann 1988b, Lutjeharms et al. 1989, Lutjeharms et al. 2000a, Lutjeharms & Machu 2000, Lutjeharms 2006a, Roberts et al. 2010). A cyclonic lee eddy off the coast of Durban is thought to be another source of oceanic nutrients to the system (Schumann 1982, Lutjeharms et al. 2000b), but this was not found to play any significant role in the pelagic ecosystem of Bight (Chapter 2). The central-offshore region of the Bight was found to be the main driver of planktonic systems with a diatom (*Thalassionema itzschiodes*) bloom dominating in the wet season during 2010 (Chapter 2).

In addition to the oceanic sources, the KZN province also has a total of 73 fluvial sources (rivers), with six major estuaries and several smaller estuaries providing fluvial materials to the Bight (Begg 1978). Of these, the Thukela River generates more than 35% of the freshwater entering the KZN near shore environment, with an annual run-off of 3,865 × 10^6 m^3 and a total sediment input of 6.79 x 10^6 m^3 yr^{-1} (Begg 1978, Birch 1996, Whitfield & Harrison 2003, Hutchings et al. 2010). Nevertheless, it has been suggested that the role of river run-off in shaping the biological aspects of the Bight is relatively small (Lutjeharms 2006a). In Chapter 2 it was shown that riverine total suspended solids dominated the northern region of the Bight in the Richards Bay area, in the dry season the Thukela River influenced could be measured in the central region of the Bight. Nonetheless, little is known about the biology of the system and how it is

driven by the oceanic and fluvial dissolved and particulate material inputs associated with the complex hydrography of the Bight.

The oligotrophic nature of the Bight means that it does not support a wide range of fisheries, as in other regions of the country, but it does host South Africa's only prawn fishery (Fennessy & Groeneveld 1997), and a line fishery, which is the most valued fishery on the east coast (Lamberth et al. 2009). Due to the limited commercial fisheries interest in the region compared to the much larger west coast fisheries, the relatively small amount of research in the Bight has mainly focused on economically important prawn species and associated by-catch (Fennessy et al. 1994, Fennessy & Groeneveld 1997, Olbers & Fennessy 2007, Turpie & Lamberth 2010). These studies focus on the shallow Thukela Banks fisheries and there are no studies for greater depths or areas which are not trawled. A few studies look at the effect of riverine flux on fisheries catches (Lamberth & Turpie 2003, Lamberth et al. 2009, Turpie & Lamberth 2010). These studies suggest that any flow reduction in freshwater, mainly from the Thukela River, into the Bight will negatively affect the fisheries catches, highlighting the important role that the riverine input can have on the fishing grounds of the Bight.

The broad aim of this study is to present an understanding of the ecosystem and food web data for demersal organisms within a framework of the underlying hydrography and sedimentary material distributions, through the use of stable isotopes from which inferences about food web structure and function will be derived. Firstly, I aim to identify the main source of carbon (C) and nitrogen (N) to the Bight benthic ecosystem, *i.e.* the origin of the sediment OM. I hypothesise that OM in riverine TSS being delivered to the Bight, which occurs throughout the year and predominantly during the wet austral summer, plays a more important role as a determinant of benthic food-webs than previously thought. My second hypothesis is that sediment OM stable isotope signature vary spatially and that the variations and consistent with the occurrence of the biological drivers in the Bight (*i.e.* fluvial, upwelling and/or cyclonic eddy). Iken et al. (2010) found that the stable isotope signatures of macrobenthic organisms resembled those of the OM in the overlying water masses. As macrobenthos will mainly feed on the OM on the sediments, the surface sediments should also resemble the stable isotope signature of the OM in the overlaying water masses. My

third hypothesis is to determine whether the isotopic signatures of demersal organisms vary spatially with the signatures of the associated sediments (Hobson et al. 1995, Iken et al. 2010).

The premise of this research is that the oceanographic processes, mainly the upwelling cell, in the Bight provide nutrients/organic matter that have their unique isotopic characteristics (specifically $\delta^{13}C$ and $\delta^{15}N$), and this allows me to use this ecological tool to address the questions at hand. Stable isotopes have successfully been used to describe the origin of OM in aquatic systems and the food-webs associated with them (Mullin et al. 1984, Hobson et al. 1995, Schell et al. 1998, Lara et al. 2010, Pomerleau et al. 2011). Ultimately, the intention of this research is to produce an understanding of likely trophic pathways involving demersal organisms and their dependence on the benthos, pelagos or riverine effluvium, through the generation of a demersal food-web of the Bight.

3.3 Material and methods

3.3.1 Study site

The Bight (Fig. 3.1) extends for 160 km from St Lucia to an area just south of Durban on the East Coast of South Africa. It is about 50 km wide at its broadest point, offshore of the Thukela River (Lutjeharms & de Ruijter 1996). Three major processes have been suggested as possible drivers for primary productivity in the Bight. Firstly topographically induced upwelling off Richards Bay (Gill & Schumann 1979, Lutjeharms et al. 1989, Lutjeharms et al. 2000a, Lutjeharms & Machu 2000); secondly a cyclonic lee eddy off of the coast of Durban (Pearce et al. 1978, Schumann 1982); and thirdly a series of fluvially induced processes dominated mainly by the Thukela River (Bosman et al. 2007).

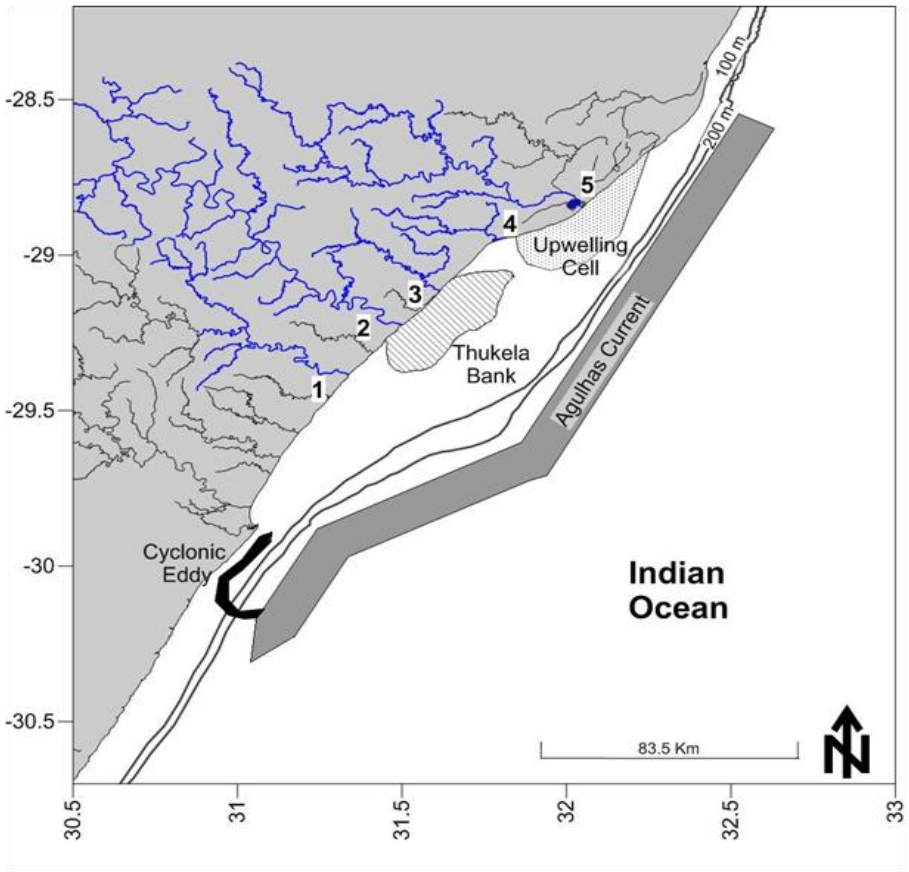

Figure 3.1. Map of the KwaZulu-Natal Bight indicating the oceanographic and riverine phenomena affecting the water masses of the area. In blue are the rivers sampled for this study and their catchment area, in black are other rivers entering the Bight but that were not sample for this study. The sampled rivers are: 1) uMvoti River, 2) Thukela River, 3) Amatikulu River, 4) uMlalazi River and 5) Mhlatuze river.

3.3.2 Sample collection and storage

Demersal samples were collected on board the industrial crustacean trawler *Ocean Spray* during two seasons, a wet (austral summer) and a dry (austral winter) season in 2010. The trawl locations were matched with those from the African Coelacanth Ecosystem Programme (ACEP) focus locations (Fig. 3.2 A). A total of 22 and 20 trawls were successfully deployed in the wet and dry seasons respectively, ranging in depth from 29 to 569 m in the wet season and 27 to 563 m in the dry season. Within each trawl only species for which at least three individuals could be collected were included in the analysis, with the exception of species of "interest" (those species common in commercial trawl catches, but that were not collected commonly in my trawls), where at least two individuals were required per trawl. For each trawl, species were individually bagged, a maximum of five individuals from each species per bag, and

immediately frozen at -20 °C. Samples were processed in the laboratory within two weeks of collection.

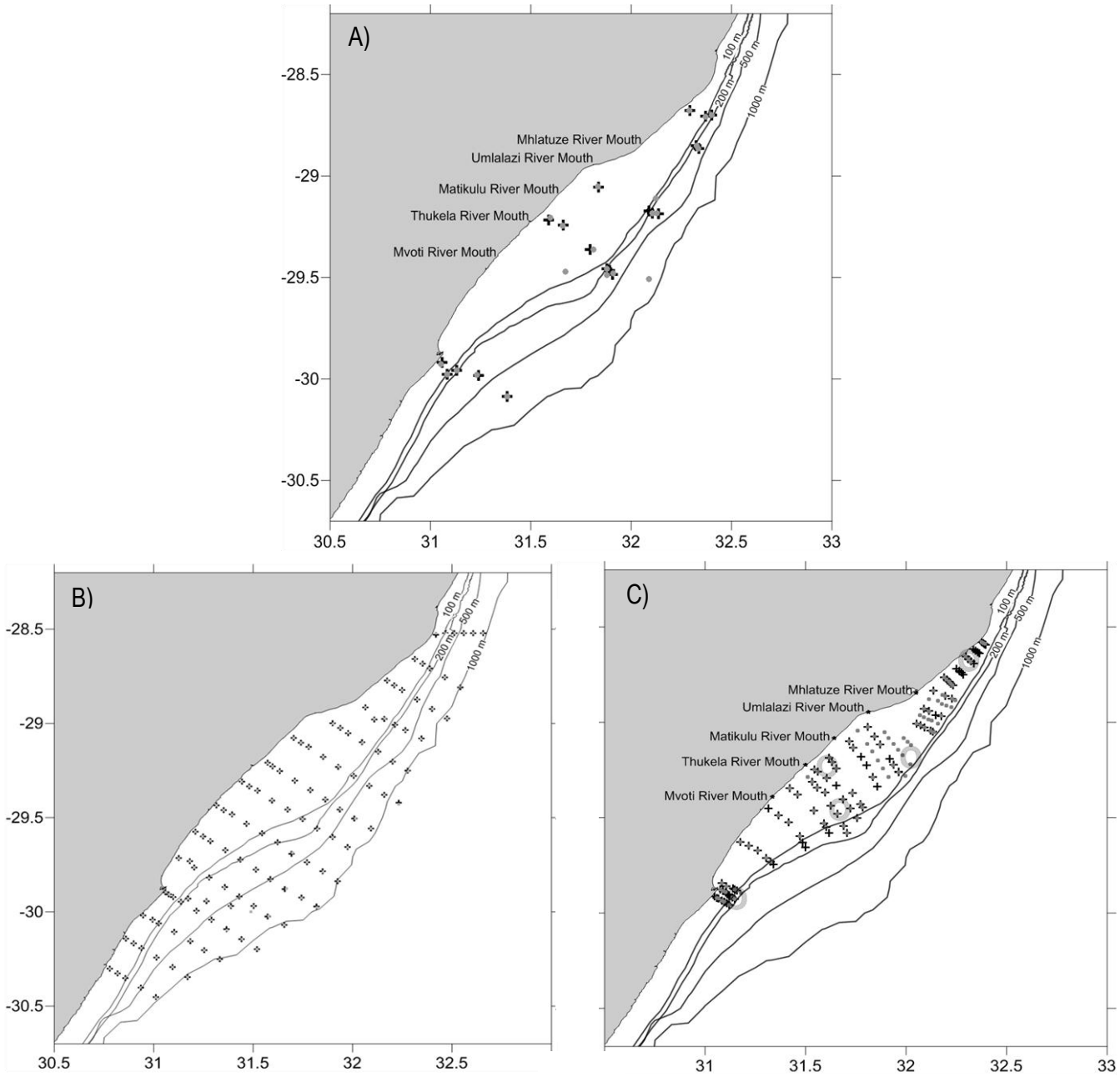

Figure 3.2. The sampling stations throughout the Bight for A) the trawling stations, B) the CTD casts and C) the sediment stations and marine TSS stations. Grey dots indicate summer stations, black crosses indicate winter stations, grey circles indicate marine TSS collection points for both winter and summer. Only sampled river mouths are named, these were sampled in both seasons.

During the same seasons, January/February (wet season) 2010 and July/August (dry season) 2010, physico-chemical variables, total suspended solids (TSS) and sediment were sampled on board of the research vessel *FRV Algoa*. A Sea-Bird 911 plus CTD and oxygen meter (Sea-Bird Electronics, Inc., Bellevue, Washington, USA) with 12 PVC Niskin bottles of 5 litres each attached to a rosette, were used to measure temperature, salinity and oxygen across the Bight (Fig. 3.2 B). For this study, only CTD data from the bottom, just above the sediments, were considered. TSS sample preparation involved filtering 500 ml water from the Niskin through pre-combusted (4 hr at 450 ^{o}C) 25 mm diameter Whatman GF/F to collect TSS. The filters were frozen at -20 ^{o}C and stored for later isotopic analysis. $CaCO_3$ was removed by acidification with a 2 % HCl solution to prevent it affecting organic $\delta^{13}C$ values (Lorrain et al. 2003). A modified VanVeen grab was used to collect sediment samples from across the Bight (Fig. 3.2 C). A spoonful of sediment was immediately collected from the top layer of the grab, placed in a zip sealed bag and frozen at -20 °C for later isotopic analysis. Because of the proximity and speed of the Agulhas Current (core speeds of up to 2 m s-1; de Ruijter et al. 1999), the sediment grab could not be deployed deeper than ~180 m. Sediment and TSS samples for the Mhlatuze, Mlalazi, Matikulu, Thukela and Mvoti river mouths were obtained concurrently for both seasons (Fig. 3.1 C). For marine sediment only one sample per station was collected, for riverine sediment three replicates were collected for each river. These samples were treated the same as explained above for the marine sediments. TSS samples from the river mouths were collected during low tide to ensure riverine and not marine TSS was collected.

3.3.3 Sample preparation and stable isotope analysis

Demersal organisms were partially defrosted prior to tissue sampling to keep leaching of tissue liquids to a minimum. Muscle tissue was taken from the back of the head of Cephalopods, from the caudal peduncle on the same side of the fish at all times for the Elasmobranch and Teleosts, and from the muscular foot of the Gastropods and a species of Bivalve. For Decapods muscle tissue collection was more varied: for the Natantia the shell was removed from the abdomen from where tissue was collected, while for the Brachyurans leg muscle tissue from inside the carapace was collected. Echinoderms were also collected, but were lost due to a freezer power failure. Great

care was taken to ensure that non-muscle tissue (skin, bone, exoskeleton, intestine) was excluded from the samples.

Following the suggestions of Boecklen et al. (2010), I decided not to perform any chemical lipid removal on the muscle tissue to avoid increasing uncertainties in $\delta^{15}N$ values, since an increasing number of authors are questioning the need for lipid removal (Mintenbeck et al. 2008, Iken et al. 2010). This decision was further supported by the findings of a concurrent study on the effect of different chemical lipid removal techniques on some of the demersal species used in this study (De Charmoy et al. unpublished); preliminary results indicated that chemical lipid removal produced no significant isotope differences from untreated samples. Lipid removal models were consequently deemed unnecessary.

Muscle tissue samples were immediately transferred to an air circulating oven for drying (60 °C for 48 hours). Dried samples were homogenised and weighed into tin capsules (SANTI® Analytical, Teufen, Switzerland); ~1.00 mg dry mass was required to yield sufficient nitrogen and C for isotope analysis. The samples were analysed at IsoEnvironmental Isotope Facility at Rhodes University, Grahamstown, South Africa, using an ANCA-SL Elemental Analyser coupled to a Europa Scientific 20-20 IRMS – Isotope-Ratio Mass Spectrometer (Sercon Ltd. Crewe, UK). Each batch of 96 combustions contained 34 known standards; these were, 29 beet sugar and ammonium sulphate (in-house standards) and five certified protein standard casein (calibrated against IAEA-CH-6 and IAEA-N-1). The analytical precision of the instrument was 0.09 ‰ for $^{15}N/^{14}N$ and 0.08 ‰ for $^{13}C/^{12}C$.

Sediment samples were placed in an air circulation oven at 50°C for 24 hours, ground to ensure homogenisation, acidified using 2% HCl solution to be able to obtain the isotopic signature of the organic matter (OM) within the sediments, rinsed with Milli-Q water and returned to the oven for desiccation. Samples were processed at the Archaeometry Laboratory, University of Cape Town. They were combusted in a Flash EA 1112 series elemental analyser (Thermo Finnigan, Milan, Italy); the gases were passed to a Delta Plus XP IRMS (Thermo electron, Bremen, Germany), via a Conflo III gas control unit (Thermo Finnigan, Bremen, Germany). Merck Gel, a proteinaceous gel produced by Merck (Darmstadt, Germany), was used as standard and was calibrated

against IAEA (International Atomic Energy Agency, Vienna, Austria) standards. The analytical precision of the instrument was 0.06 ‰ for $^{15}N/^{14}N$ and 0.06 ‰ for $^{13}C/^{12}C$.

The isotope ratios of $^{15}N/^{14}N$ and $^{13}C/^{12}C$ are expressed in terms of their value relative to atmospheric N_2 and to Pee-Dee Belemnite (vPDB), respectively. δ-notation is used to express the differences (Epstein et al. 1953):

$$\delta\text{-value } (‰) = (R_{sample}/R_{standard} - 1) \times 1000$$

where R is the ratio of ^{15}N:^{14}N or ^{13}C:^{12}C in the sample (R_{sample}) and in the standard ($R_{standard}$), expressed relative to the international standard (Sulzman 2007).

3.3.4 Statistical analysis, trophic positions and mixing models

Benthic contour maps were produced for salinity, oxygen, temperature and sediment isotope values ($\delta^{15}N$ and $\delta^{13}C$), $\%C_{org}$, %N and C:N ratios using Surfer 9. Data were analysed for normality and homoscedasticity, transformed where appropriate and ANOVA and Tukey's *post-hoc* tests were run in *R* version 2.12.0 (R Development Core Team 2010). Because of the lack of replication for the sediment stations, ANOSIM analyses were run in Primer 6 (Plymouth Marine Laboratory, UK) (Clarke 1993) on the $\delta^{15}N$ and $\delta^{13}C$ sediment data. TSS $\delta^{15}N$ and $\delta^{13}C$ isotope values were used to represent "riverine" (those collected at the mouth of the rivers during low tide, these values were entered in the model as an average value ± SD) and "marine" (those collected from the marine focus stations, these values were entered in the model as an average value ± SD) sources in a MixSIR analysis of the sediments, which allowed determination of the relative proportions of marine and riverine sediments in the sites that had mixed-sediments.

Based on the assumption that as C and N are transferred along the food-web enrichment of ^{13}C is small (~1 ‰) and that of ^{15}N larger (~3.4 ‰) (Peterson & Fry 1987, Post 2002, Smit et al. 2005), the trophic position was calculated using the following method (Hobson & Welch 1992, Govender et al. 2011):

$$TP = 1 + (\delta^{15}N_{consumer} - \delta^{15}N_{source}) / \Delta^{15}N$$

where $\delta^{15}N_{consumer}$ is the $\delta^{15}N$ of the individual consumer, $\delta^{15}N_{source}$ is the $\delta^{15}N$ of the main nutrient source in the consumer diet, and $\Delta^{15}N$ is the enrichment factor of 3.4 ‰ (Post 2002). Primary C sources were assigned a trophic position of 1.

To calculate the proportional contribution of possible diets for the demersal organisms, I use the Bayesian mixing model MixSIR (Moore & Semmens 2008) version 1.0.4 with uninformative priors. Fractionation values of 0.4 ± 1.3 ‰ for ^{13}C and 3.4 ± 1 for ^{15}N were used for this study (Post 2002). The maximum importance ratio was below 0.001 suggesting that the models were effective in estimating the true posterior density (Moore & Semmens 2008). Results for MixSIR are presented as median and the 5th and 95th credibility intervals.

Using mixing models, a possible food-web of the demersal organisms of the Bight was constructed. A literature review of the possible diet of these organisms was completed (Appendix C) and used as guidance on the possible prey items for each organism. Due to limited biological research in the Bight no stomach content studies or diet studies existed for the majority of organisms in my study. Where possible local diets for the species were obtained; alternatively diets of the same species or related species were obtained from other parts of the world. Possible prey species listed in the literature were offered as potential dietary items for the relevant predator in the mixing models only if either i) the predator and prey occurred in the same trawl, or ii) if the possible prey item was found in another trawl from the same area and in the known (from literature) depth-range of the predator (Appendix C), making it possible for the predator and suspected prey item to interact. Some animals occurred at more than one site in the Bight. In these cases, mixing models were run for each site independently. From the results of these analyses, prey species that comprised 10% or more of the diet at any one site were included as potential prey items in a further dietary site-combined mixing model analysis. For these widely occurring animals the final site-combined diet was used in generating the general food-web of the Bight. For known zooplanktivores (from the literature), zooplankton isotopic data used in the mixing models were taken from animals from Chapter 2.

Using the results of the mixing model analyses two possible food-webs were produced. A "shallow" food-web containing animals found from 20 – 200 m and a "deep" food-

web, containing animals from 201 – 600 m, with certain ubiquitous organisms occurring in both. Because no sediment samples were collected from depths greater than 180 m, for the deep food-web interpolated isotope values were calculated from the closest stations to the trawl locations.

3.4 Results

3.4.1 Environmental variables

As expected, bottom temperatures declined with depth in both seasons (1st order polynomial relationship, R^2 = 0.95) (Fig. 3.3 A, D). The only visible difference between the two seasons was that in the summer there was a warm water mass close inshore (<100 m depth) in the northern part of the Bight reaching temperatures greater than 27 °C. Bottom salinity clearly decreased below the 200 m isobaths in both seasons (2nd order polynomial relationship, R^2 = 0.97), however, this decrease was not greater than 1.4 (Fig. 3.3 B, E). Bottom oxygen levels for summer and winter increased below the 200 m isobath, but decreased again between the 500 m and 1000 m isobaths (3rd order polynomial relationship, R^2 = 0.55) (Fig. 3.3 C, F). During the winter higher bottom oxygen levels were observed in the shallower areas, around the Thukela Bank. Overall the major physical differences were between the shelf and the area below the 200 m isobath.

3.4.2 Environmental variables, TSS, sediment stable isotopes and C:N ratios

The sediment $\delta^{15}N$ and $\delta^{13}C$ composition of the Bight was found to be similar among Locations and Origin (marine *vs*. riverine) (1-way ANOSIM: Locations R = 0.607; p = 0.01; Origin R = 0.507; p = 0.01). The sediment isotope maps of the Bight (Fig. 3.4) suggest that overall there are localised differences close inshore to the estuaries and near the 100 m isobath, where signatures are stronger, while the remainder of the Bight appears to be more homogeneous.

Marine TSS and sediments formed isotopically distinct groups when C:N ratios were considered (Fig. 3.5), while estuarine TSS overlapped mainly with marine sediments (Fig. 3.5 A and B). There were significant similarities between some of the groups (ANOSIM; marine TSS vs. marine sediment $R = 0.791$; $p = 0.01$; riverine TSS vs. marine sediment; $R = 0.438$; $p = 0.01$). Differences between marine TSS and estuarine TSS (ANOSIM; $R = 0.183$; $p = 0.04$) were indicated by the low R value. To determine the importance of each TSS source to the sediment OM, a mixing model (MixSIR) analysis was completed. The MixSIR analysis indicated that both marine and riverine TSS made contributions to the sediment OM at most sites throughout the Bight (Fig. 3.6). Riverine TSS appears to have dominated the surface sediment composition throughout the centre of the Bight in both seasons, with riverine TSS accounting for more than 60 % of the OM origin in the central region of the Bight. The riverine TSS lost importance at the north and south edges of the Bight to marine TSS, but still played a major role dominating more than 40 % of OM. The central riverine TSS influence appears to have continued to the deepest sampling stations (~ 180 m).

Maps of the $\%C_{org}$ and %N of the sediment in the Bight indicated a plume of high levels of OM in the sediment protruding outwards from the Thukela River, with the levels of OM being greater in the wet season than the dry season (Fig. 3.7 A, B, D and E). C:N indicated that the Bight could contain OM from two origins; the northern end of the Bight had lower C:N ratios (~4), while the centre-southern region of the Bight had values >8 for both seasons (Fig. 3.7 C and F). Overall, the results appeared to indicate that the C and N in the central Bight were more labile, possibly hinting that younger OM originating from the estuaries was being deposited in this region and consumed during the dry months.

3.4.3 Isotope values and C:N ratios of demersal organisms

A total of 71 species were collected in both seasons, of which 25 and 12 species were exclusive to the wet and dry season respectively (for species and mean isotope values see Fig. 3.8). Organisms had significantly different $\delta^{13}C$ values among locations (ANOVA $df = 4$, $SS = 40.20$, $MS = 10.05$, $F = 17.1$, $p < 0.0001$). Further investigation

revealed that the $\delta^{13}C$ values of organisms collected on the Thukela Bank were significantly different to those from all other locations (Tukey's *post-hoc* $p < 0.01$). No significant differences in the $\delta^{15}N$ values of demersal organisms were found.

If the isotope values for organisms in the Thukela Bank area were separated from those of the stations in the Bight and the analysis repeated, the emerging pattern changed completely. The $\delta^{13}C$ or $\delta^{15}N$ values of demersal organisms of the same species were spatially homogeneous for the entire Bight (ANOVA, $p > 0.05$), with the exception of the Thukela bank, which was isotopically distinct. Within the Thukela Bank area neither $\delta^{13}C$ nor $\delta^{15}N$ values of the demersal organisms were significantly different among the suite of species, however.

Similarly, C:N ratios, an indicator of food quality, did not differ for the same species collected at different locations, or between different species collected at the same location, with the exception of four organisms, all of them teleosts, which had a much larger standard deviation (Fig. 3.8).

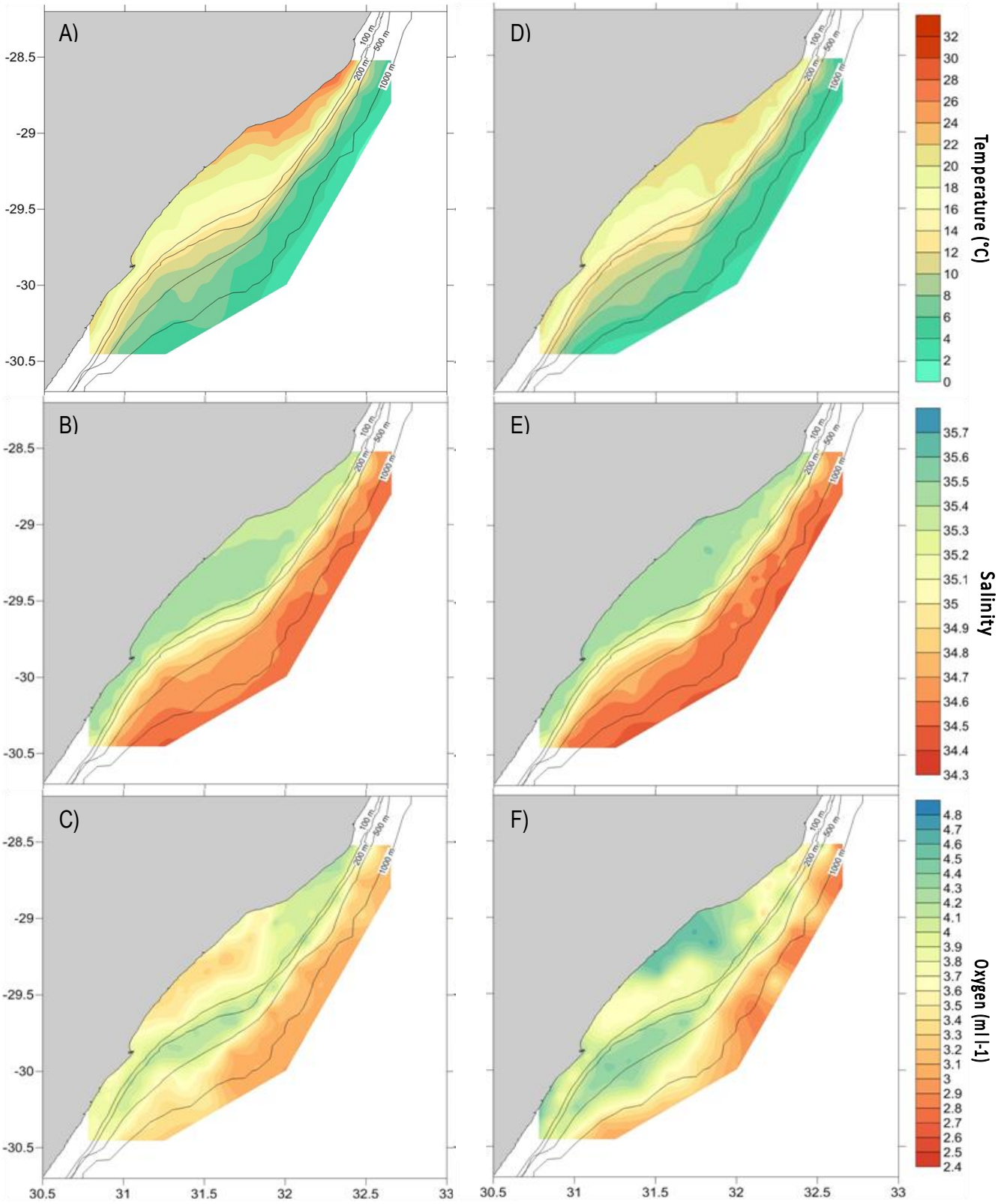

Figure 3.3. Bottom temperature, salinity and oxygen measurements for summer (A, B, C) and winter (D, E, F).

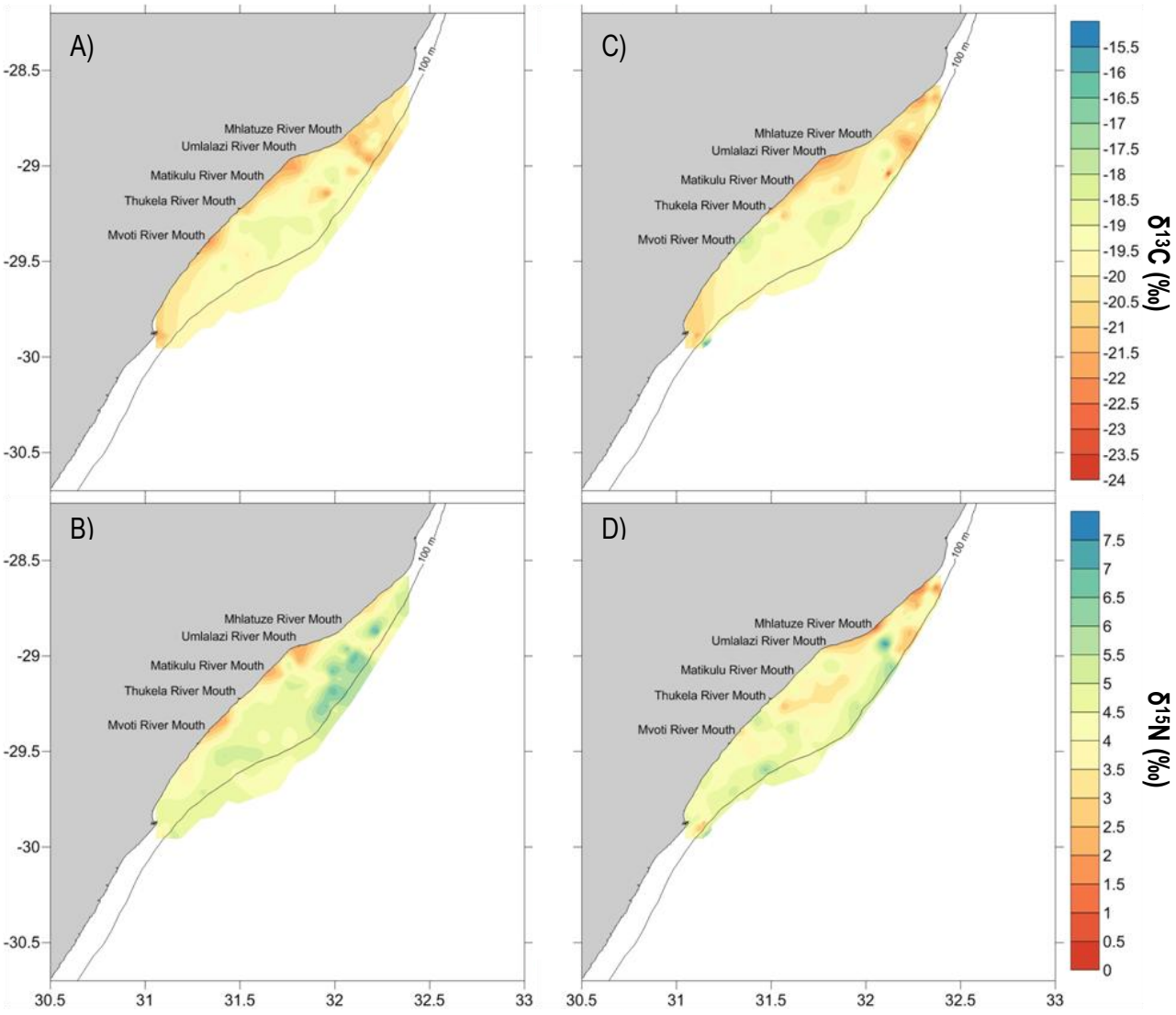

Figure 3.4. Surface sediment organic $\delta^{13}C$ and $\delta^{15}N$ values for summer (A, B) and winter (C, D).

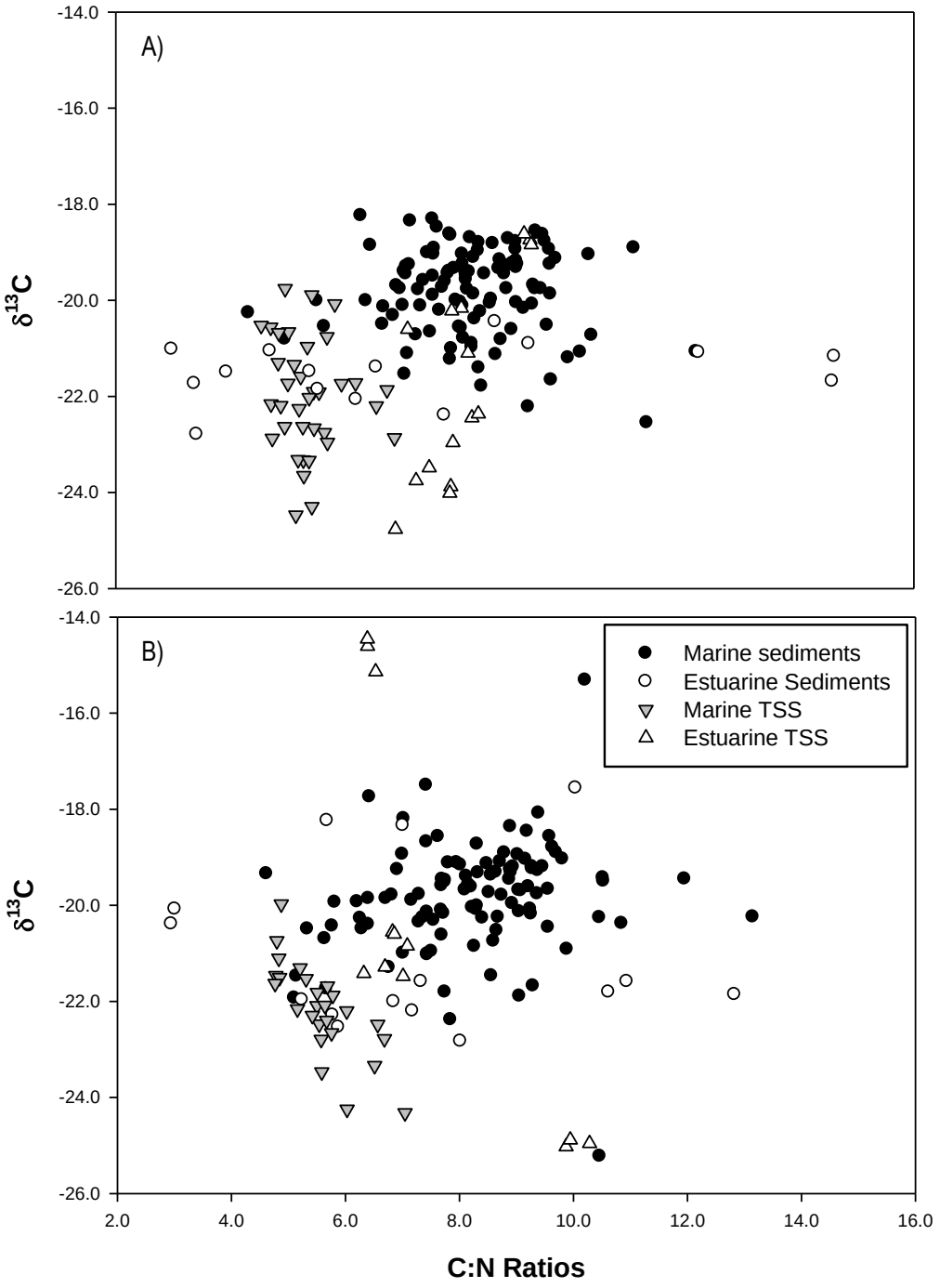

Figure 3.5. Carbon:Nitrogen ratios and $\delta^{13}C$ values for marine and estuarine TSS and sediments stations for A) summer and B) winter.

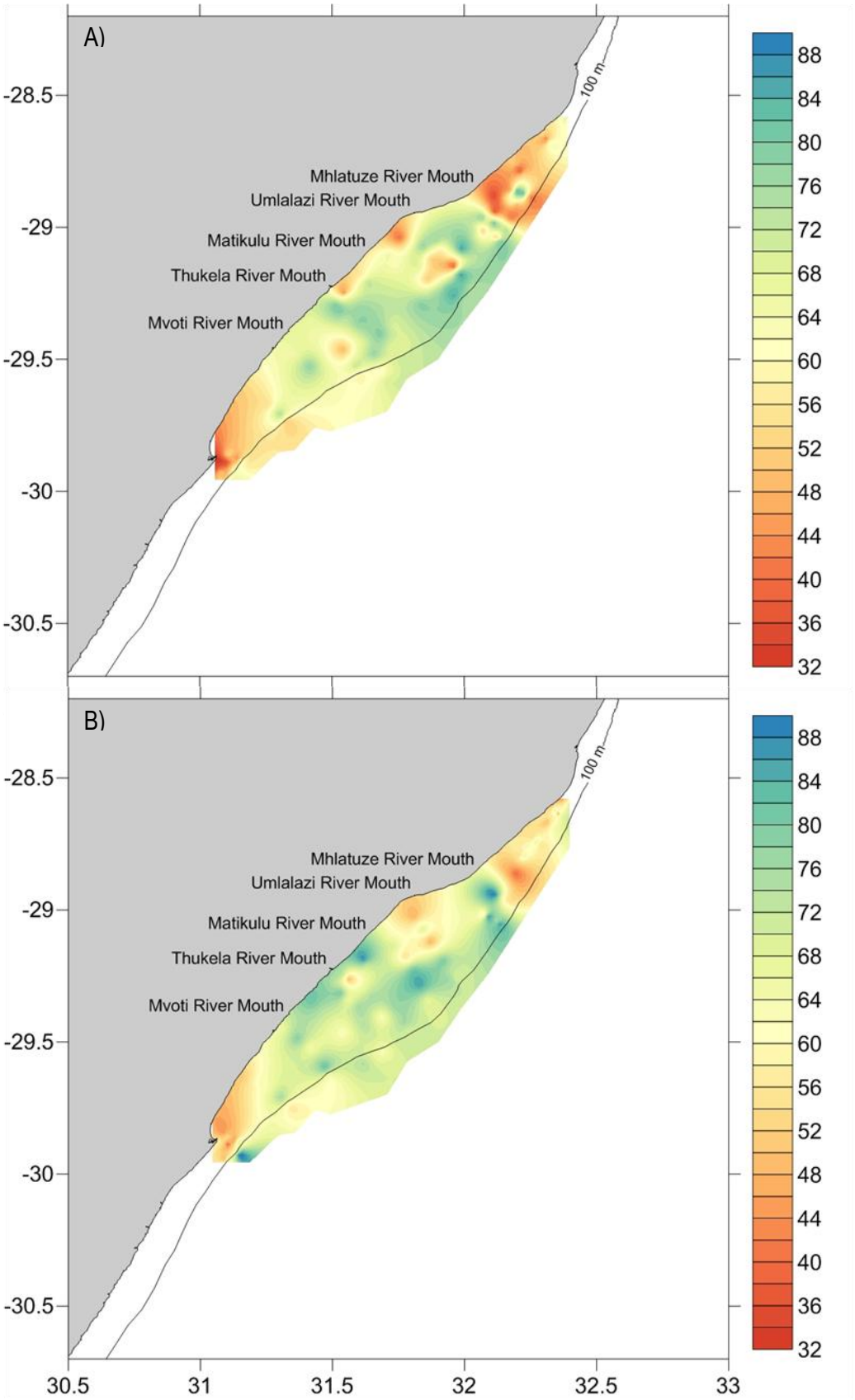

Figure 3.6. Percentage source of riverine TSS in the sedimentary organic matter for A) summer and B) winter as calculated with the mixing models. Only sampled river mouths are named.

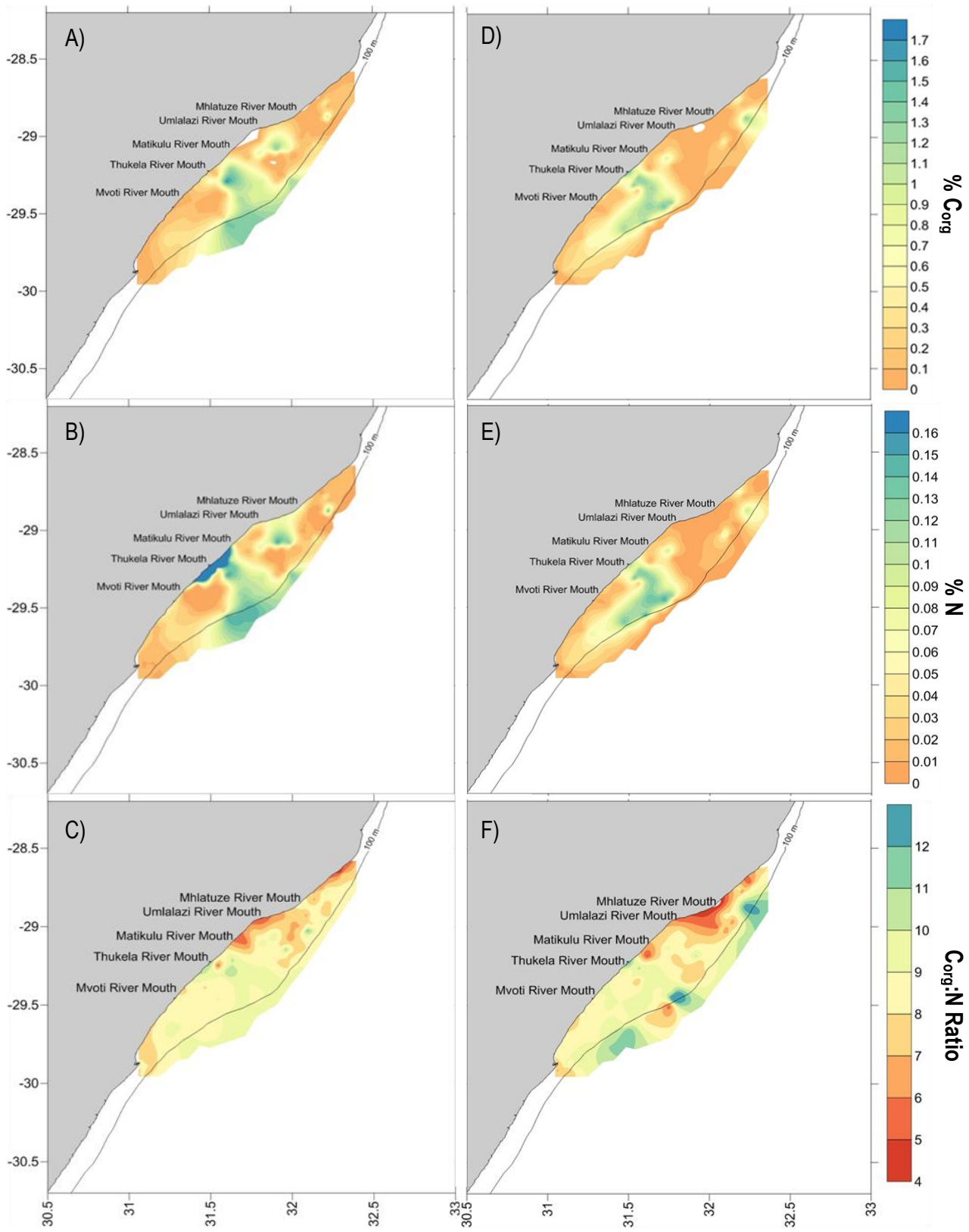

Figure 3.7. The Bight sediment maps showing %C, %N and C:N ratios for summer (A, B, C) and winter (D, E, F). Only sampled river mouths are named.

3.4.4 Trophic position and trophic linkages

A range of trophic positions were represented during both seasons, but the majority of organisms were between trophic positions 2 and 3 (Fig. 3.8). The organisms with the highest trophic positions were the Gastropod *Phalium craticulatum* (3.58 ± 0.33), while the lowest trophic positions were occupied by the Decapod *Plesionika martia* (1.87 ± 0.06). Cephalopods ranged in TP from 2.18 ± 0.14 (*Sepia acuminata*) to 2.98 ± 0.26 (*Veladona togata*), Decapods ranged from 3.50 ± 0.14 (*Chaceon macphersoni*) to the lowest TP recorded in this study (*Plesionika martia*), Teleosts ranged from 3.51 ± 0.04 (*Leiognathus equulus*) to 2.39 ± 0.02 (*Coelorinchus denticulatus*), Elasmobranch TPs were generally low ranging from 2.75 ± 0.30 (*Pliotrema warreni*) to 2.92 ± 0.30 (*Squalus megalops*). Other organisms collected included a Bivalve species (*Mactra aequisuleata*) with a TP of 2.41 ± 0.08 and Pennatulacea (*Actinoptilum molle*) with a TP of 2.29 ± 0.03.

A plausible food-web of all organisms collected for both seasons combined was constructed from the results of the mixing model analyses; the food-web matrix for "shallow" (up to 200 m) and "deep" (201 – 568 m) can be viewed in Fig. 3.9 and 3.10 respectively. Mixing models indicated that food-webs were strongly driven by the organic matter on the sediments at both shallow and deep sites. Sediment OM isotope data were used as proxy for macrobenthic infauna, which are highly dependent on this OM (Iken et al. 2010). Of the 45 species in the shallow food-web, 39 were offered the sediment OM as a possible food source in replacement of the macrobenthos, which were not sampled in this study. Of these organisms, 20.5 % had more than 40% of their diet derived from the OM in the sediments, and a further 64.1 % of organisms derived 20 – 40 % of their requirements from the OM in the sediments. For the deep food-web, OM in the sediments was given as one of the potential food sources for 26 of the 45 species, of which 36.2 % of species obtained more than 40 % of their diet from this source, while 29.9 % obtained 20 – 40 % of their diet from organisms that were directly dependant on the OM in the sediments. For the shallow food-web the Decapods *Penaeus indicus, Parapenaeopsis acclivirostris* and *Parapenaeus investigatoris* as well as the Bivalve *Mactra aequisulcata* and the Pennatulacean *Actinoptilum molle* appeared to be of great importance as a food source, as did some of the Cephalopods. Decapods such as

Metanephrops mozambicus, Munida incerta and *Haliporoides triarthrus* as well as some of the Cephalopods also appeared to be of great importance for organisms in the deep food-web.

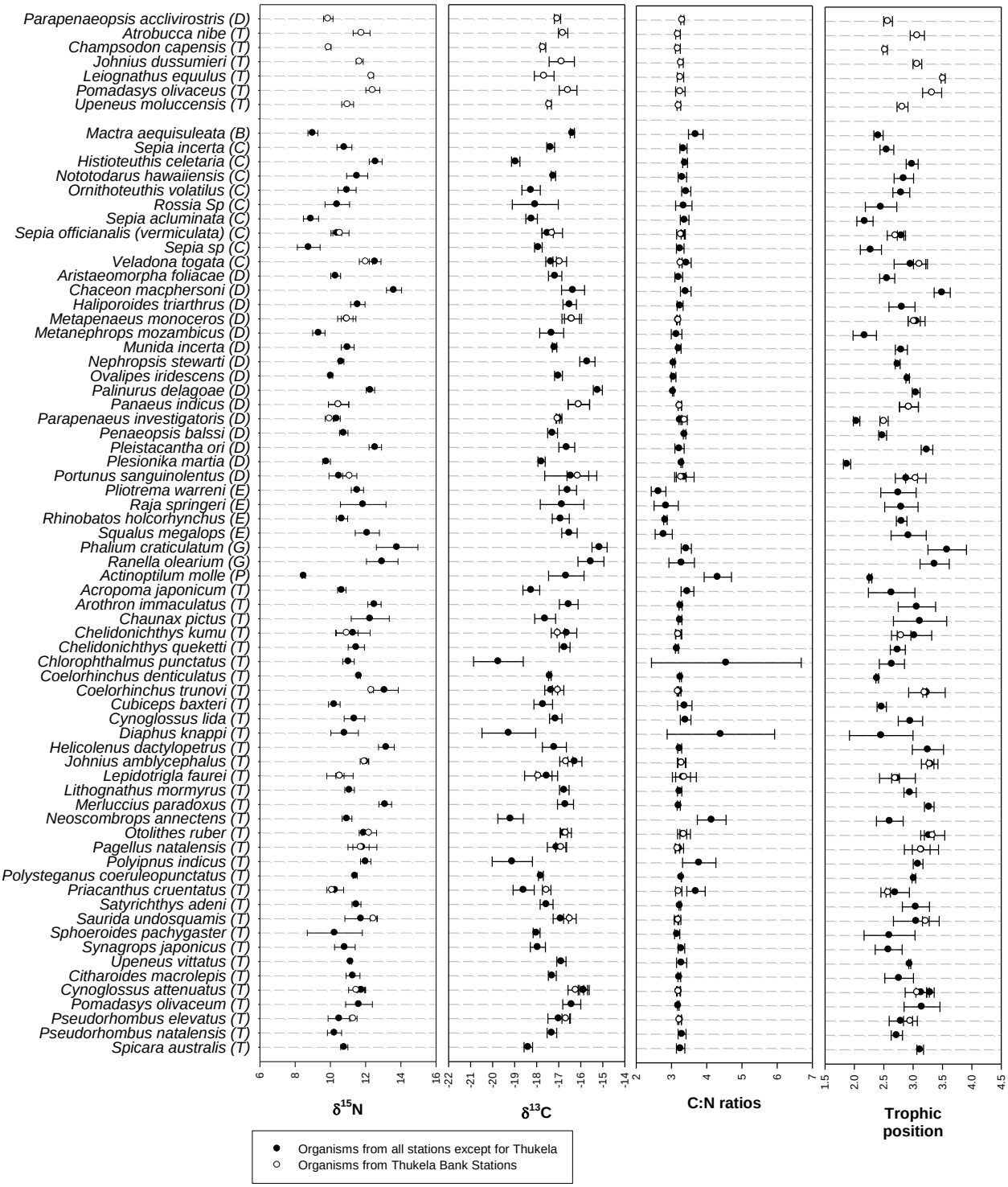

Figure 3.8. Mean (± SD) $\delta^{15}N$, $\delta^{13}C$ and TP for 1) Thukela Bank stations and 2) all other stations from the Bight. Letter in brackets indicates what group the animal belongs to. B = Bivalvia, C = Cephalopoda, D = Decapoda, E = Elasmobranchii, G = Gastropoda, P = Pennatulacea, T = Teleostei.

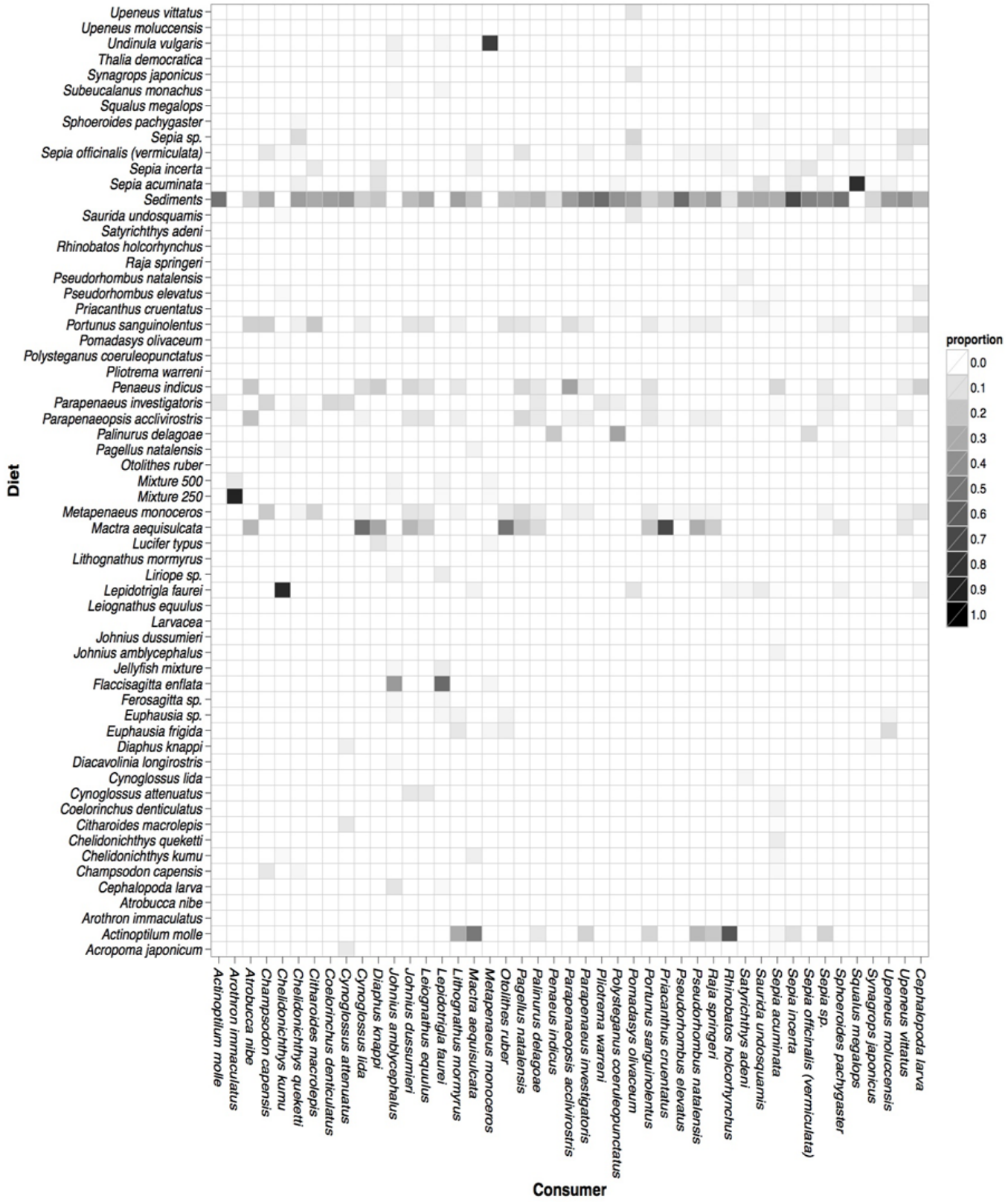

Figure 3.9. Food-web matrix showing mixing model results for shallow (up to 200 m) animals for the entire Bight. Refer to text for more information.

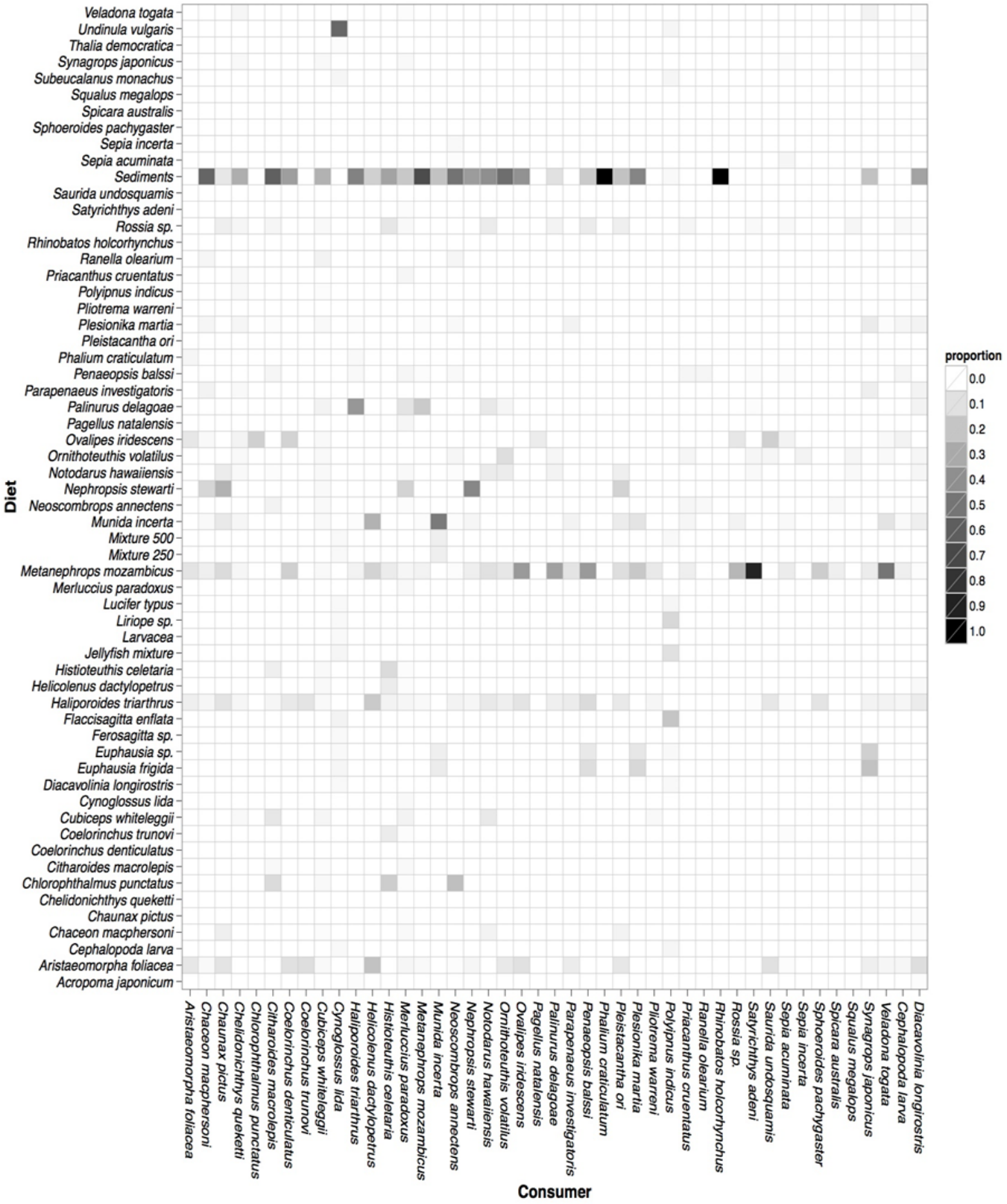

Figure 3.10. Food-web matrix showing mixing model results for deep (201 to 568 m) animals for the entire Bight. Refer to text for more information.

3.5 Discussion

This work is one of the most comprehensive stable isotope studies done on demersal organisms both on and off of the continental shelf. Furthermore, this study appears to be one of the best sampled sediment organic isotopic mapping studies of a large marine system, ~160 km along the coast and to a depth from ~20 m to ~180 m, and including the nearby estuaries.

3.5.1 Environmental variables, TSS, sediment stable isotopes and C:N ratios

The decreasing salinities and temperature with depth were in-line with previous works that found low salinities for the deeper waters close to the Bight (Pearce 1977, Roughan & Middleton 2002). Oxygen levels were higher in the dry season close to the Thukela River mouth, probably due to a decreased OM load from the nearby estuaries. Overall the bottom environmental variables did not appear to greatly change between these two seasons, which could suggest a reasonably stable benthic environment.

Since riverine TSS was found to be the dominant input of OM to the central Bight sediments according to the mixing model result. It was likely an important source of food for the demersal organisms in this low productivity environment; although its role was less important in the north and south of the Bight, its relative importance remained high (~40 %). This finding was further supported by the high $\%C_{org}$ and %N plume off the Thukela River and the small isotopic signature variability across the Bight for both seasons. Furthermore, it has been confirmed elsewhere that organic detrital material in the sediments can have a residence time of years in the deep-sea (Rowe et al. 1991). The lack of seasonal differences in sedimentary OM was likely due to the low productivity of the Bight and long residence time of the OM in the sediments, further suggesting that riverine OM input into the benthos was of great importance spatially and temporally. Finally, recent research on the primary productivity from an upwelling event in 2010 in the Bight, indicated that increased algal biomass and nutrient levels are flushed out of the Bight within days of its

occurrence (Dr Tarron Lammont, unpublished[6]). This suggests that there would be very little opportunity for any OM from these upwelling events to settle on the sediments of the Bight.

The range of riverine TSS $\delta^{13}C$ values (-14.73 ± 0.48 ‰ to -24.01 ± 1.42 ‰) corresponds to that expected from terrestrial runoff. The isotopic signature of OM contained in terrestrial runoff is known to be influenced by the type of vegetation present in the region (Huang et al. 2000, Knies & Martinez 2009). The vegetation in the province of KwaZulu-Natal is comprised of a range of woodlands, coastal forest, montane forest, thicket and grasslands (Fairbanks & Benn 2000). Furthermore, a major land use in KwaZulu-Natal is sugarcane (a C_4 plant) monoculture (Dominy et al. 2001). C_3 plant $\delta^{13}C$ values range between -22 to -33 ‰, while C_4 plants $\delta^{13}C$ values range between -9 to -16 ‰ (O'Leary 1988, Huang et al. 2000). The values obtained in this study for riverine TSS were within a range that could be explained by a mixing of the isotopic signatures of runoff from the two vegetation types. In addition C_4 plants have been confirmed as an important source of terrigenous OM for the marine environment in areas where these plants are present (Huang et al. 2000). Isotopic values for riverine phytoplankton, which range from -25 to -35 ‰ for $\delta^{13}C$ and around 5 ‰ for $\delta^{15}N$ (Owens 1987, Boutton 1991), fall outside the isotopic range found for riverine TSS, indicating that they probably had a negligible or minor influence. Also, their smaller size makes it less refractory, reducing the chances of accumulating on the sediments, as demonstrated by study of sedimentation of algae in lakes (Larocque et al. 1996). This highlights the importance of the terrestrial organic input from river outflow into a system like the Bight, and appears to indicate that rivers play a more fundamental role than previously thought (Lutjeharms 2006a), at least as far as the demersal system was concerned.

In addition to the influence of riverine TSS on marine sediments, the limited role played by marine TSS in the sediments probably results from marine phytoplankton particles, whose isotopic signatures range from -19.1 to -22 ‰ for $\delta^{13}C$ and 3 to 12 ‰

[6] Dr. Tarron Lammont (2012), Department of Environmental Affairs, South Africa

for $\delta^{15}N$, sinking to the seafloor (Gearing et al. 1984, Owens 1987). Because the marine sediment isotope values were not consistent with studies where C_3 plants are the dominant vegetation on the coast (Knies & Martinez 2009), I suggest that a combination of terrestrial C_3 and C_4 plant detritus with a small addition of marine phytoplankton outfall explains the $\delta^{13}C$ values seen in the marine sediments of the Bight, similar to results elsewhere (Goñi et al. 1998). In addition, and as mentioned above, recent results suggests that the window of opportunity for phytoplankton to settle within the Bight is small, highlighting once again the importance of other OM sources, such as of terrestrial input, in supporting the biology of the benthos (Dr Tarron Lammont, unpublished[7], Lamont and Barlow, *in prep*).

The C:N ratios further support the view of a central Bight dominated by riverine TSS input and the north by phytoplankton outfall. The marine TSS mirrored the typical C:N ratios for phytoplankton of 5 to 7 throughout the Bight, as phytoplankton tends to be rich in nitrogen (Bordovskiy 1965, Meyer 1994). In the central area of the Bight, this marine TSS clearly did not settle in the marine sediment which had C:N ratios of between 9 and 10, values which are just slightly lower than values for terrestrial C_3 plants (Andrews et al. 1998, Lamb et al. 2006), suggesting they originated in terrestrial runoff and riverine outflow. This is particularly evident in the winter season where C:N values were >9 for most of the central Bight, even below 100 m. Because winter is the dry season on the east coast of South Africa, this accumulation of terrestrial material must have occurred during the wet season. Ogrinc et al. (2005) found C:N ratios in sediment cores elsewhere with values similar to those found in this study and described them as a mixture of marine and terrestrial OM. Similarly, Bristow et al (2012) described the C:N values of 8.5 to 18.3 found for the OM of the Humber and Thames estuaries as having an origin other than phytoplankton.. Furthermore, it has been shown that although terrestrial plant material C:N ratios are traditionally regarded as being greater than 11 and geographically variable, they tend to be significantly decreased during bacterial degradation in the sediments and produce values similar to the values of 9 and 10 found in this study (Thornton & McManus

[7] Dr. Tarron Lammont (2012), Department of Environmental Affairs, South Africa

1994). Conversely, in the north of the Bight sediment C:N ratios of 4 to 7, were similar the marine TSS values, indicating that this area was dominated by of marine input in both seasons. C:N ratios, therefore support the evidence that terrestrial input plays an important role within the majority of the Bight. It also raises further questions for future research on the importance of bacteria in degrading the terrestrial material as food source for the benthic and demersal ecosystems of the Bight.

In addition, due to the greater levels of sedimentary $\%C_{org}$ and %N extending eastwards off the Thukela River along the sea floor, I suggest that most OM available in the benthos of the Bight to at least a depth of 200 m originates from the Thukela River and other estuaries in the vicinity, particularly during the wet season. The increase in the levels of %N and %C appears to correspond with the position of the Thukela Cone which leads to the Thukela canyon (Flemming & Hay 1988), as well as with a series of inner and outer shelf mud facies described by Bosman et al. (2007). Underwater canyons are considered a mechanism for exporting sediments and OM into the adjacent abyssal areas (Rowe & Staresinic 1979, Ramsay 1994). They have also been proven to be an important means for terrestrial organic material such as plant detritus to reach the deep-sea (Gage & Tyler 1991, Lawson et al. 1993). Terrestrial input plays a diminished role at the north and south ends of the Bight, where marine TSS appears to be more dominant. These two areas closely match the locales where i) the cyclonic eddy occurs south of the Bight (Heydorn et al. 1978, Schumann 1982, 1988b) and ii) where the upwelling occurs north of the Bight (Gill & Schumann 1979, Lutjeharms et al. 1989, Lutjeharms et al. 2000a, Lutjeharms & Machu 2000). As such, it was likely that these two upwelling-phenomena were dominating the sediment composition in these two areas.

3.5.2 Isotope values and C:N ratios of demersal organisms

Initially I anticipated finding more variability in the isotope values for such a wide variety of organisms occurring over a range of depths and locations and in two seasons. However, considering that the sediment OM environment showed limited variability in space and time in terms of isotope signatures, the limited variation in the

isotopic signatures of the demersal organisms between seasons and sites obtained here seems appropriate. There were, however, five teleost species (*Chlorophthalmus punctatus, Diaphus knappi, Neoscombrops annectens*, *Polyipnus indicus* and *Priacanthus cruentatus* – except for Thukela Bank individuals of the latter species) which had a distinct $\delta^{13}C$ value compared to the rest (Fig. 3.8). A detailed inspection revealed these to have a much larger C:N standard deviation than the other species, indicating that they fed on a wider range of food sources of variable quality, [but also that the quality of the food varies]. $\delta^{13}C$ values and the low C:N ratios for other species indicated that the food source were all originally from a similar source and of relatively good quality (Vanderklift & Ponsard 2003), with value close to 3.

The findings for Bight demersal system appear to disagree with the scenario of well-mixed riverine and marine food sources put forward elsewhere by Peters et al. (1978) and Schmidt et al. (2010). Instead they appear to agree with the scenario of a system with a well-defined source of food being introduced into it, as has been found by others elsewhere (Carlier et al. 2007, van Oevelen et al. 2009). This food source appeared to be riverine OM throughout the Bight, even in the north and south of the Bight where marine OM dominated the sediments, since no differences were found in the isotopic signatures of demersal organisms from the different locations throughout the Bight.

As mentioned earlier, the Bight has been described as an oligotrophic system (Bustamante et al. 1995) and more recently as a mesotrophic system (Barlow et al. 2008, Barlow et al. 2010). In the open ocean, benthic communities are reliant on organic particles, mainly phytoplankton, reaching the seafloor (Billett et al. 1983, Beaulieu 2002). However, oligotrophic systems are dominated by small planktonic organisms and any production from these cells is unlikely to reach the benthos in biologically significant amounts due to complex pelagic microbial food-web recycling it (Legendre & Michaud 1998, Calbet & Landry 2004). Duineveld et al. (2000) suggested that benthic organisms living in oligotrophic conditions would show little to no seasonal variation. This further supports the evidence gathered from the sediments OM of a stable benthic system dependant on riverine input.

I did, however, find what could potentially be a localised effect of riverine inputs on demersal organisms collected from two stations close to the Thukela River mouth. If this localised effect was due to the Thukela River the possibility exist of studying whether the animal's isotopic signatures in the Thukela Bank respond to isotopic changes from the OM entering through the river over time. If this was to be the case and isotopic seasonality occurs, it is probably explained by the marked wet and dry season in KwaZulu-Natal leading to changes in the quantity and origin of OM washed from the river. For research on seasonality of the Thukela River and the Thukela Bank refer to Chapter 4.

As mentioned in point 3.5.1, the TSS isotopic signature ranged within that of C_3 and C_4 plants; along with the TSS large plant material are likely to get washed into the sea. Studies have revealed that deep-sea invertebrates depend on terrestrial plant detritus being transported to the deep (Lawson et al. 1993, Young et al. 1993). Furthermore, deep-sea fish have been revealed to be opportunistic, feeding and scavenging on a wide range of OM and organisms (Kaehler et al. 2000, Drazen et al. 2001, Stowasser et al. 2009) including terrestrial plant material and phytodetritus (Jeffreys et al. 2010, Jeffreys et al. 2011). In an oligotrophic scenario, plant detritus derived from the nearby estuaries, might be one of the dominant factors controlling the food-web. The importance of this terrestrial detritus as a food source could extend as far as the nearby deep-sea food-web in the area adjacent to the continental shelf. The occurrence of a plume of %C and %N recorded off the Thukela River and reaching to a depth of ~180 m provides support for this suggestion (Fig. 3.6).

3.5.3 Trophic position and linkages

The majority of organisms from the Bight exhibited similar trophic positions (TP) and $\delta^{15}N$. Previous demersal studies have found that TP was not always related to the size of the organism, *i.e.* larger organisms did not always feed on and have a higher trophic position than smaller organisms (Jennings et al. 2001, Layman et al. 2005, Al-Habsi et al. 2008). An explanation for this is that predators feed at a wide variety of niches (Layman et al. 2005) and omnivory may be a common strategy (Polis & Strong 1996, McCann et al. 1998, Closs et al. 1999, Link 2002, Newsome et al. 2007, Al-Habsi et al.

2008). Because the Bight is an oligotrophic system, omnivory could be the second most important factor determining the food-web structure, after terrestrial detritus input. Thompson et al. (2007) found that moving up the food-web from primary consumers, the existence of a tangled web of omnivores becomes apparent in the marine environment. As shown in Appendix C, the majority of organisms collected in this study appear to be omnivorous, supporting the findings of Thompson et al. (2007) of omnivory being common in the marine environment. This further substantiates omnivory as an important strategy in the Bight and provides an additional explanation for the low $\delta^{15}N$ variability of demersal organisms. Furthermore, it has been shown that demersal fish and invertebrates could scavenge on nekton carcasses and fisheries discards (Jones et al. 1998, Witte 1999, Bozzano & Sardà 2002), *i.e.* on organisms theoretically higher-up the food chain, which would probably further reduce TP variability, owing to animals of different trophic levels scavenging on the same source of food. To complicate matters, tissue isotopic values can take a period of time to reach equilibrium with that of the diet (Martínez del Rio et al. 2009, Wolf et al. 2009), and consumers can change their diets repeatedly. As such, isotopic equilibrium in the tissue may not manifest, and instead of displaying either the current or the past diet isotopic signatures becomes a mixture of both diets (Hobson & Clark 1992, Gannes et al. 1997, Sweeting et al. 2005).

Furthermore, when examining organisms' seasonal isotopic changes, especially those of organisms that are longer lived, such as fish or crustaceans, isotopic fractionation of tissues, i.e. the period of time that the animal tissue takes to assimilate the isotopic signature of the food item, needs to be taken into account (Fry & Arnold 1982, Hesslein et al. 1993, Vanderklift & Ponsard 2003). Tissue isotopic values can take a period of time to reach equilibrium with that of the diet (Martínez del Rio et al. 2009, Wolf et al. 2009), and consumers will change their diets repeatedly. As such, isotopic equilibrium in the tissue may not manifest, and instead of showing either the current or the past diet becomes a mixture of both diets (Hobson & Clark 1992, Gannes et al. 1997, Sweeting et al. 2005).

Saying this, the food-web created in this study attempts to produce a visualisation (Fig. 3.9 and 3.10) of a possible predator-prey interaction scenario. It should be viewed as

preliminary guideline only, and its function is to aid the general understanding of the type of trophic interactions that seem likely in the Bight, and for which there is presently little information. The food-web demonstrates that there is a very complex system of trophic interactions; this is in spite of the fact that diets were trimmed to a suitable set of prey candidates for each predator. It supports the omnivory hypothesis, with a high level of interactions within and between each trophic level. It also suggests the possible importance of the macrobenthic organisms, underlying most higher trophic positions, which is in agreement with the findings of studies elsewhere (Snelgrove 1997, Gili & Coma 1998).

This latter conclusion was however, based on the assumption that the OM in the sediments was a lower-trophic level proxy for the macrobenthos (no macrobenthic samples were available). Mixing models indicated that several demersal species favoured the OM proxy, which suggests that they are indirectly dependent upon the OM. The previous section of this paper demonstrated OM in the Bight was predominantly of terrestrial origin. Because terrestrial OM is difficult for organisms to digest without the aid of bacterial degradation (McLeod & Wing 2009), it follows that some degradation and possibly incorporation through macrobenthos must occur prior to the demersal animals ingesting it. There is a need for further research to investigate how important the macrobenthic link from OM to demersal organisms actually is, and the role played by bacteria in the sediments of the Bight.

For the specific case of the Bight, the lack of clear distinctions in isotope values in either the sediments or in the demersal organisms, provides the insight that the benthic infauna, not analysed here, were likely to show a similar lack of clear isotopic distinction.

3.6 Conclusion

Our study sought to find the dominant organic matter (OM) source driving the system of the nutrient-poor KwaZulu-Natal Bight. I found that riverine TSS dominated as a source of OM in the marine sediments across most of the Bight, especially the central region, with oceanographic processes playing only a small role in shaping the demersal ecosystem. This was supported by $\delta^{13}C$, $\delta^{15}N$, $\%C_{org}$, %N and C:N ratio spatial patterns and the mixing model results, which all independently suggested that riverine/estuarine TSS was a very important source of OM for the demersal ecosystem. However, this dominance was not absolute, and marine OM did play a role, albeit it small, in the northern end of the Bight. This may be because, as recent studies have suggested, phytoplankton only have a small window of opportunity to settle within the Bight before being washed away. Therefore, bacteria likely play a very important role throughout the Bight in making the terrestrial OM available as a food source for the demersal ecosystem. I suggest that future studies should examine the role of bacteria in the sediments and their role in shaping the isotopic and C:N values of demersal ecosystems, as well as their role in the biology of the Bight as a whole.

Of great interest was the proposed likely dominance of the riverine OM role controlling the production and food-web structure of the benthic communities, from the shallow Thukela Bank to the deep-sea of the nearby ecosystem. Omnivory appeared to be a wide-spread strategy for demersal animals throughout the Bight. This was supported by the lack of clear δ15N enrichment between possible prey and predator and the low variability of trophic positions across a wide array of organisms. However, in order to fully understand the food-web and ecosystem processes of the benthic and demersal ecosystems of the Bight, or similar ecosystems elsewhere, it would be highly beneficial to understand: 1) the interactions occurring within and between macrobenthic communities, 2) between the demersal and macrobenthic communities, and 3) how these benthic and demersal communities interact with the pelagos. As Kaiser et al. (1999) pointed out, there is strong support for macrobenthic communities influencing assemblages of demersal organisms. Therefore, there is a need for future research to include bacterial degradation of OM and benthic infaunal

organisms in studies of demersal organisms to deepen our understanding of the processes occurring within the Bight and elsewhere, including the links between these assemblages.

3.7 Acknowledgments

I wish to acknowledge the African Coelacanth Ecosystem Programme (ACEP), the Thukela Bank Project and the National Research Foundation (NRF) of the South Africa Department of Science and Technology for their financial contribution towards this study. Dr Sven Kaehler from the IsoEnvironmental laboratory at Rhodes University for the running of and useful comments on the samples. Desmond Hayes and Chris Wilkinson of the Oceanographic Research Institute (ORI) for their assistance in the collection and processing of samples. I will also like to thank Sean Fennessy for identifying all the demersal species and allowing me to dissect them at ORI. Knud Sorenson, owner and crew of the Ocean Spray, for kindly making his trawler available and I will also like to thanks the FRV Algoa crew for their help while at sea. I also like to acknowledge Dr Jenny Huggett for allowing me to work with the samples in her lab. Last, but not least, I will like to acknowledge Rachel Cooper from the University of Cape Town for her editorial comments.

CHAPTER 4

Does riverine input control the food-wed on the near-shore Thukela Bank (KwaZulu-Natal, South Africa)? Evidence from seasonal stable isotope analysis

4 Does riverine input control the food-wed of the near-shore Thukela Bank (KwaZulu-Natal, South Africa)? Evidence from seasonal stable isotope analysis.

4.1 Abstract

The Thukela Bank, a mud bank on the continental shelf off the Thukela River in the KwaZulu-Natal (KZN) Bight, South Africa, supports two of the main fisheries on the East coast. Biological studies have suggested that the fisheries' catch on the Bank is affected by riverine input from the Thukela River, with catches increasing in response to increased fluvial runoff. However, a series of oceanographic studies suggests that the riverine input plays only a minor role on the biology of the Thukela Bank and the Bight as a whole. The aim of this study is to determine whether the Thukela River organic matter and the demersal animals have seasonal isotopic changes and whether these seasonal changes resemble each other. This is evaluated through the use of stable isotope techniques to determine whether the riverine organic matter input into the Bank has a strong seasonal signature and if the organisms stable isotope signatures match that of the river organic matter. To undertake this analysis, total suspended solids from the Thukela River and 11 demersal species from the Thukela Bank were collected over a period of three years (2008, 2009 and 2010) and isotopically analysed. Results indicated a strong seasonal effect was present for total suspended solids and animals from the Thukela Bank. This was especially true for animal $\delta^{13}C$ values, but less so for $\delta^{15}N$ values. I concluded that Thukela River organic matter was an important input to the food-web judged by the seasonal changes between the dry and wet seasons found for isotope values for both animals and total suspended solids.

4.2 Introduction

Rivers play an important role in the nearby neritic environment by shaping its ecology (Martin & Meybeck 1979, Billett et al. 1983, Milliman & Heade 1983, Milliman & Syvitski 1992, Beaulieu 2002, Gillanders & Kingsford 2002, Kristiansen & Hoell 2002). Terrigenous allochthonous material is one of the most important sources of nutrients for primary and secondary productivity in the neritic zone (Polis & Hurd 1996, Caddy 2000), and contributes to enhancing the overall productivity of these systems (Cloern 2001, Maslowski 2003).

A large number of studies have demonstrated that inshore fisheries, including those on the Thukela Bank, which is a subset of the study area examined as part of this thesis, are dependent on these inputs (Glaister 1978, Lamberth & Turpie 2003, Lamberth et al. 2009, Turpie & Lamberth 2010). The Thukela Bank is a shallow water bank formed by muddy sediment discharges of the Thukela River, extending north- and eastwards across the KwaZulu-Natal Bight (the KZN Bight, henceforth simply "the Bight") from the river's mouth (Fig. 4.1). The Thukela Bank fisheries are comprised of the commercial and recreational line fishery and South Africa's only prawn fishery (Fennessy & Groeneveld 1997, Lamberth et al. 2009). Turpie and Lamberth (2010) demonstrated that during periods of low freshwater input from the Thukela River, prawn catches were reduced by ~11 %, suggesting that riverine nutrient sources drive secondary productivity and hence influence fisheries yields. Lamberth et al. (2009) concluded that any major development reducing water flow could dramatically reduce line fishery catches on the Thukela Bank. Consequently if any future projects such as those suggested by the Department of Water Affairs and Forestry (DWAF 2004) should occur, then there is a need to understand how dependant animals in the Thukela Bank are to organic matter inputs from the Thukela River.

Despite this apparent food-web link between terrigenous allochthonous sources and productivity in the Thukela Bank region, oceanographers do not recognise riverine input into the Bight as playing a major role in affecting the region's ecosystem processes (Lutjeharms et al. 2000b, Lutjeharms 2006a, Hutchings et al. 2010). These authors accept that the most important hydrodynamic phenomenon dominating the

Bight in terms of nutrient input and associated productivity, and hence that of the Thukela Bank, is an upwelling cell that occurs intermittently, but persistently, in the northern part of the Bight where a higher concentration of nutrients can be found during these events (Carter & d'Aubrey 1988, Lutjeharms et al. 2000a, Meyer et al. 2002).

Nevertheless, the Thukela River accounts for more than 35% of the freshwater entering the entire KZN coastline (Begg 1978, Flemming & Hay 1988, Birch 1996, Whitfield & Harrison 2003, Bosman et al. 2007, Hutchings et al. 2010). The small estuarine area of the Thukela River means that most of the nutrients/organic matter in this outflow are exported to the nearby coast (Lamberth et al. 2009). Combined with the fact that the waters of the Bight have been described as oligotrophic (Bustamante et al. 1995), it raises the question of whether riverine input from the Thukela River and other estuaries within the region could be important for this environment. This is the basis of this chapter.

Chapter 3 demonstrated that riverine OM input dominates the trophic processes of the demersal ecosystem and to a certain extent also that of the pelagic ecosystem (Chapter 2) of the Bight. Addressing the question of whether there is a seasonal pattern in the relative importance of oceanic and riverine inputs will further support this finding. In order to investigate this, the focus of this chapter is on the animals from the region immediately adjacent to the Thukela River (*i.e.* the Thukela Bank), which would be the most likely to experience seasonal fluctuations in output of OM from the river.

The aim of this study is therefore to examine the OM from the Thukela River over several seasons using stable isotopes ($\delta^{13}C$ and $\delta^{15}N$) and whether these seasonal isotopic changes manifest in the animals of the nearby Thukela Bank and thereby resolve the seasonal importance of riverine input into the Bight. This will be achieved by testing the specific question of whether or not demersal organisms in the Thukela Bank show seasonal changes in their ^{13}C and ^{15}N abundance, similar to those of the Thukela River, and as a consequence that demersal animals in the Thukela Bank are dependent on the OM entering the Bight through the Thukela River on a seasonal or permanent basis. I hypothesise that estuarine nutrient and/or OM input, which occurs continuously, but with episodic and predominant flood events during the wet austral

summer season, i) has strong seasonal differences in its stable isotope composition and ii) that these seasonal changes in stable isotope signature can also be measured for the demersal organisms of the Thukela Bank. Stable isotopes have previously been used to successfully describe the origin of OM in aquatic systems and the food-webs associated with them (Mullin et al. 1984, Hobson et al. 1995, Schell et al. 1998, Lara et al. 2010, Pomerleau et al. 2011), and have also successfully been implemented in understanding seasonality within estuaries (Harmelin-Vivien et al. 2010, Maya et al. 2011).

4.3 Materials and methods

4.3.1 Study site

The Thukela Bank, a mud bunk off the Thukela River forming towards the North-East part of the Bight (For information of the Bight refer to Chapter 1) occupies an area of 300 km^2 and extends from 200 m to 16 km offshore (Fennessy & Groeneveld 1997). A series of fluvially induced processes occur within the Bight, which are dominated mainly by the Thukela River (M'Cormick et al. 1992, Bosman et al. 2007) with the greatest flow occurring from December to March, peaking around January-February (DWAF 2004). It has an annual flux of 3,865 × 10^6 m^3 and a total sediment input of 6.79 x 10^6 m^3 yr^{-1} into the Bight. The KZN region has a rainfall in the order of 1,000 to 1,200 mm y^{-1} (Day 1981), with very well defined wet and dry seasons. The height of the rainy season is January with a mean monthly precipitation of 118 mm, while August at the peak of the dry season has a mean monthly precipitation of 39 mm (Hunter 1988).

According to the Department of Environmental Affairs (DEA 2001) the Thukela catchment area is 29,101 km^2 with 75% described as natural; 15% of the area used for agriculture; 8% considered degraded and approximately 1 % of the catchment is consider urban. The vegetation surrounding most of the catchment is comprised of a range of woodlands, coastal forest, montane forest, thicket and grasslands (predominantly C_3 plants) (Fairbanks & Benn 2000), but sugarcane monoculture (a C_4 plant) is also present in the agriculturally-transformed catchment area (Dominy et al. 2001).

Sediment accumulating on the Thukela Bank is comprised of poorly-sorted sand close to the Thukela River mouth, with the majority of the bank, from the river mouth to the ~50 m isobaths, formed by mud settling out of the suspended fluvial load (Bosman et al. 2007). The Bank is home to a line fishery and South Africa's only prawn fishery (Fennessy & Groeneveld 1997, Lamberth et al. 2009). The prawn fishery and associated by-catch averages an annual catch of 703 t valued at over ZAR36 million (Turpie & Lamberth 2010). The line fishery has a commercial and recreational boat based line fishery component with a total catch landing of 291 t valued at ZAR28.9 million for the Thukela Bank, or 1,235 t with a value of ZAR76 million for the entire Bight (Lamberth et al. 2009).

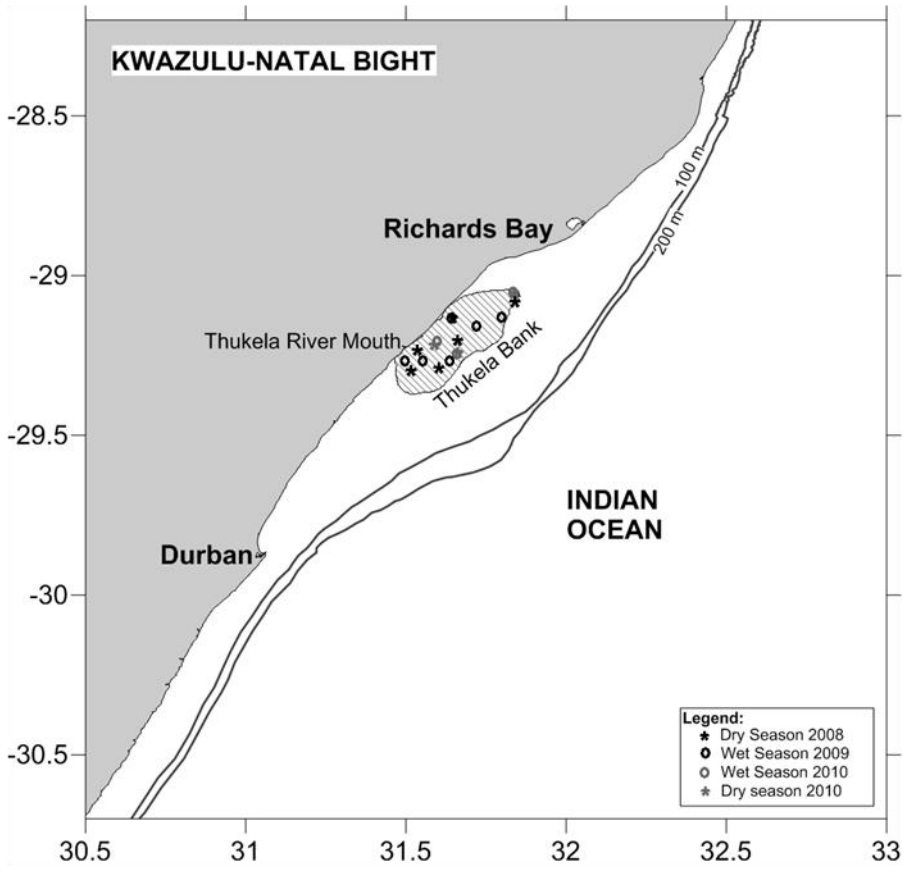

Figure 4.1. Map of the Bight showing the Thukela River mouth, Thukela bank and sampling locations.

4.3.2 *Sample collection*

Demersal organisms were collected on four occasions, twice during the dry season (August 2008 and 2010) and twice during the wet season (January 2009 and 2010), as part of a study on the biomass and community structure of demersal organisms from the Thukela Bank. Samples were collected from aboard the *Ocean Surf* during the 2008 dry season and the *Ocean Spray* on the other seasons. The trawl locations were chosen to match the extent of the prawn commercial fishing grounds on the Thukela Bank (Fig. 4.1). Organisms included in the isotopic studies were selected based on their availability within the trawls, and separated from the biomass study samples to avoid possible artefacts due to improper sample storage and preparation (Chapter 6: De Lecea et al. 2011b). A total of 11 species were collected, *viz*. 7 teleosts (*Atrobucca nibe, Cynoglossus attenuatus, C. lida, Johnius dorsalis* (*dussumieri*), *Otolithes ruber, Pomadasys olivaceum* and *Saurida undosquamis*) and four decapods (*Metapeaneus monoceros, Penaeus indicus, Portunus hastatoides* and *P. sanguinolentus*). Most of the organisms were possible to collect at each sampling event.

Total suspended solids (TSS) were collected for stable isotope analysis from the Thukela River mouth during a separate but concurrent study on the Thukela Bank. Samples were collected in the river mouth at outgoing low tide to ensure that estuarine and not marine TSS was collected. A total of three replicates were collected per sampling event.

4.3.3 *Sample preparation and stable isotope analysis*

Demersal organisms were partially defrosted prior to tissue sampling to keep leaching of cell contents to a minimum. Muscle was collected from the caudal peduncle on the same side of the body for teleosts. For decapods muscle tissue collection was more varied: for the Natantia the shell was removed from the abdomen from where tissue was sampled, while for the Brachyurans leg muscle tissue from inside the carapace was harvested. Great care was taken to ensure that non-muscular tissue (skin, bone, exoskeleton, intestine) was omitted from the samples.

A growing number of authors are questioning the necessity for lipid removal (Mintenbeck et al. 2008, Iken et al. 2010). Consequently I followed the suggestions of Boecklen et al. (2010) and did not perform any chemical lipid removal on the muscle tissue in order to avoid increasing uncertainties in $\delta^{15}N$ values. Preliminary results of a concurrent study on the effect of a variety of chemical lipid removal techniques on some of the same species used in this study reinforced this decision (De Charmoy et al. unpublished), since they demonstrated no significant effect of the different techniques on isotope values. Lipid removal models were thus unnecessary.

Muscle tissue samples were immediately placed in an air circulating oven for drying (60 °C for 48 hours), homogenised once dry and weighed into tin capsules (SANTI® Analytical, Teufen, Switzerland); ~1.00 mg dry mass was requisite to yield adequate $\delta^{15}N$ and $\delta^{13}C$ analysis.

For TSS water volumes of 500 ml were filtered through pre-combusted (4 hr at 450 ^{o}C) 40 mm diameter Whatman GF/F. TSS containing filters were frozen at -20 ^{o}C and stored for later isotopic analysis. Prior to analysis, the samples were acidified with a 2 % HCl solution to prevent $CaCO_3$ affecting $\delta^{13}C$ values, rinsed with Mill-Q water and oven dried at 65 °C.

The samples were analysed at IsoEnvironmental Isotope Facility at Rhodes University, Grahamstown, South Africa, by means of an ANCA SL Elemental Analyser coupled to a Europa Scientific 20-20 Isotope-Ratio Mass Spectrometer (Sercon Ltd. Crewe, UK). To maintain the quality of the results, each batch of 96 combustions contained 34 known standards; these were, 29 beet sugar and ammonium sulphate (in-house standards) and five certified protein standard casein (calibrated against IAEA-CH-6 and IAEA-N-1). The analytical precision of the instrument for muscle tissue was 0.09 ‰ for $^{15}N/^{14}N$ and 0.08 ‰ for $^{13}C/^{12}C$ and for TSS filters the precision was 0.07 ‰ for $^{15}N/^{14}N$ and 0.11 ‰ for $^{13}C/^{12}C$.

$^{13}C/^{12}C$ is expressed in terms of their value relative to that of Pee-Dee Belemnite (vPDB), while $^{15}N/^{14}N$ is conveyed in terms of their value relative to that of atmospheric N_2. Isotope ratios obtained from both instruments are given in the usual δ-notation (Epstein et al. 1953):

$$\delta\text{-value } (‰) = (R_{\text{sample}} / R_{\text{standard}} - 1) \times 1000$$

where R is the ratio of $^{15}N:^{14}N$ or $^{13}C:^{12}C$ in the sample (R_{sample}) and in the standard (R_{standard}), expressed relative to the international standard (Sulzman 2007).

4.3.4 Statistical analysis, trophic positions and mixing models

Data were tested for normality and homoscedasticity, transformed where appropriate and subsequently subjected to Welch *t*-test analysis to test whether TSS and animals $\delta^{13}C$ and $\delta^{15}N$ values had Seasonal (wet and dry) differences.

4.4 Results

4.4.1 Seasonal differences, wet vs. dry season

Isotopically, TSS differed statistically in $\delta^{13}C$ and $\delta^{5}N$, with samples collected during the wet season being ^{13}C-enriched and ^{15}N-depleted compared to those of the dry season (Fig. 4.2; Table 4.1).

Of the 11 demersal species collected for this study two species, *A. nibe* and *P. olivaceum*, displayed significant differences in $\delta^{15}N$ between seasons, with the value for TSS taken in the wet season enriched in ^{15}N relative to that of the dry season (Fig. 4.3 A; Table 4.1). On the other hand, eight of the 11 species were significantly enriched in ^{13}C during the dry season (Fig. 4.3 B; Table 4.2). Seven of these species were ^{13}C-depleted in the wet season compared to the dry season, unlike TSS, which were ^{13}C-enriched relative to those representing the dry season. *Cynoglassus lida* was the only species that had relatively enriched $\delta^{13}C$ signatures in the wet season, which could indicate a different food source.

Table 4.1. Welch *t*-test results comparing wet *vs.* dry for TSS and animals collected in the Thukela Bank. Refer to Figure 4.2 for TSS isotope values and 4.3 for animal's isotope values.

	Isotope tested	*t*	*df*	*p*		‰ diff. Means (wet – dry)
TSS	$\delta^{15}N$	2.75	19.47	0.01	**	-2.06
	$\delta^{13}C$	-4.82	10.10	0.00	***	3.31
Atrobucca nibe	$\delta^{15}N$	-2.97	9.80	0.01	**	0.34
	$\delta^{13}C$	-0.86	10.98	0.41		0.10
Cynoglossus attenuatus	$\delta^{15}N$	0.44	32.65	0.67		0.05
	$\delta^{13}C$	1.51	32.99	0.14		-0.16
Cynoglossus lida	$\delta^{15}N$	-1.11	12.78	0.29		0.16
	$\delta^{13}C$	-2.17	10.10	0.05		0.43
Johnius dorsalis	$\delta^{15}N$	2.04	33.67	0.06		-0.27
	$\delta^{13}C$	3.30	36.90	0.00	***	-0.42
Otolithes ruber	$\delta^{15}N$	0.38	44.23	0.70		-0.05
	$\delta^{13}C$	1.94	47.36	0.06		-0.24
Pomadasys olivaceum	$\delta^{15}N$	-2.21	30.55	0.03	*	0.31
	$\delta^{13}C$	3.59	34.74	0.00	***	-0.43
Saurida undosquamis	$\delta^{15}N$	-0.38	16.55	0.71		0.28
	$\delta^{13}C$	2.62	9.41	0.03	*	-0.57
Metapeaneus monoceros	$\delta^{15}N$	-0.51	16.73	0.62		0.07
	$\delta^{13}C$	4.66	31.85	0.00	***	-0.69
Penaeus indicus	$\delta^{15}N$	-0.97	28.92	0.34		0.13
	$\delta^{13}C$	3.19	9.58	0.01	**	-0.71
Portunus hastatoides	$\delta^{15}N$	-0.10	11.31	0.92		0.02
	$\delta^{13}C$	2.86	9.91	0.02	*	-0.47
Portunus sanguinolentus	$\delta^{15}N$	1.97	35.89	0.06		-0.23
	$\delta^{13}C$	4.59	30.81	0.00	***	-0.74

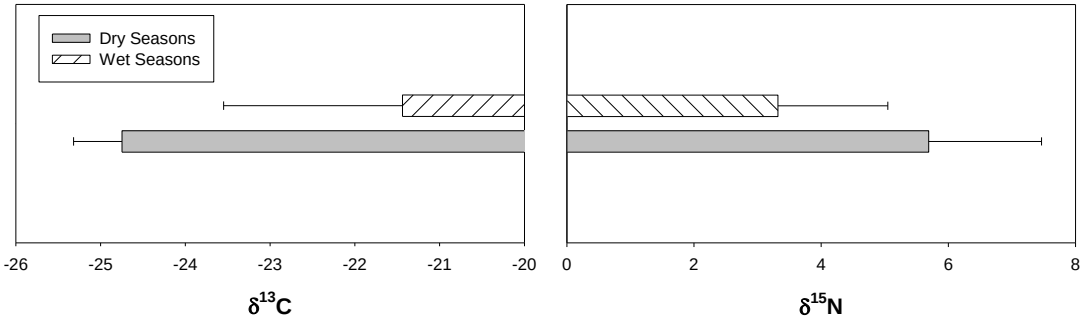

Figure 4.2. Mean (±SD) A) $\delta^{15}N$ and B) $\delta^{13}C$ (± SD) for TSS during dry and wet seasons pooled over all years for which data were available. For statistical results refer to Table 4.2.

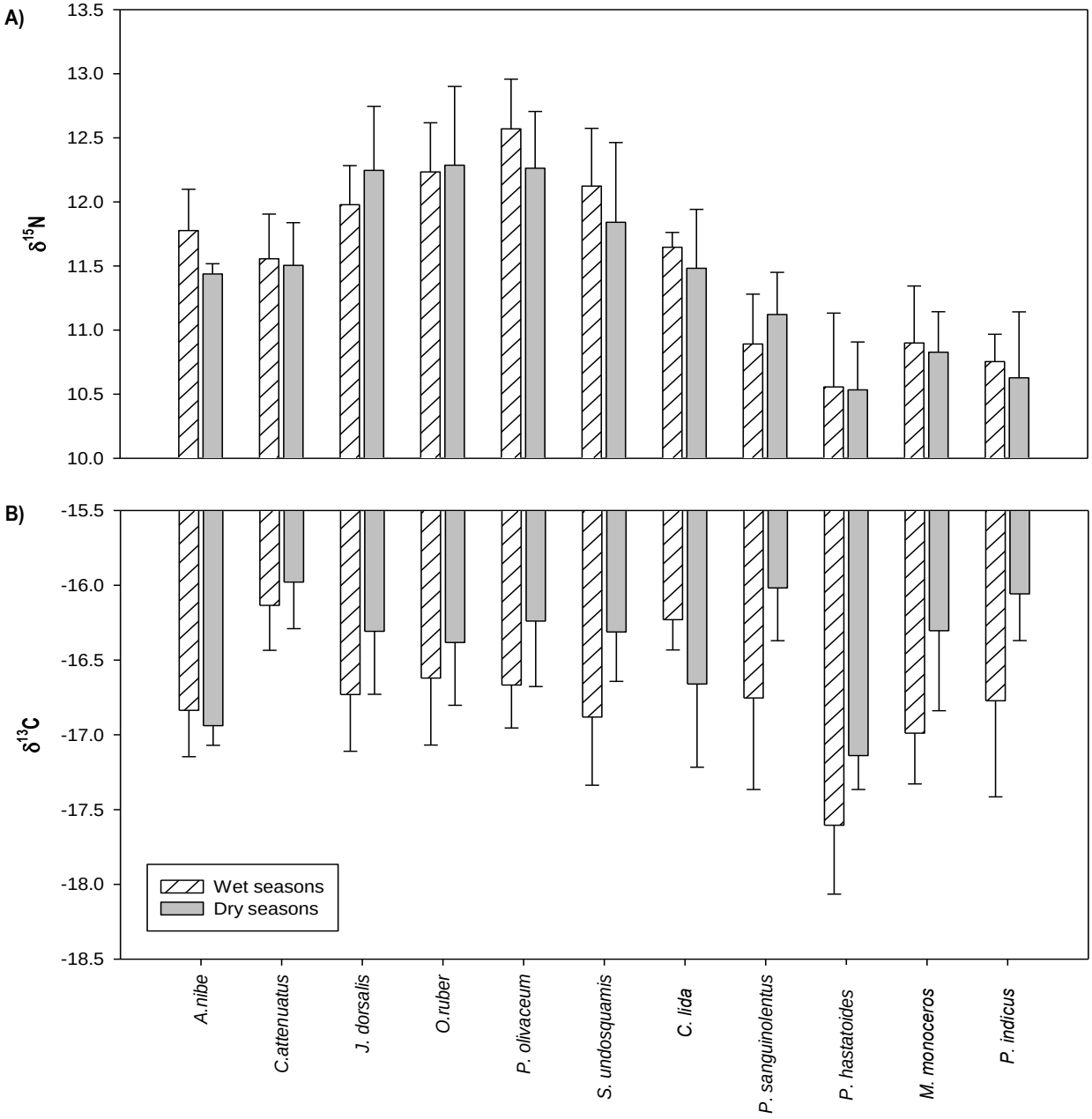

Figure 4.3. Mean (± SD) A) $\delta^{15}N$ and B) $\delta^{13}C$ of muscle tissue for all animals conbined over all years. For statistical results refer to Table 4.2.

4.4.2 Inter-annual variability

There was significant inter-annual variability in $\delta^{15}N$ and $\delta^{13}C$ isotopic values of TSS for both wet and dry seasons (Fig. 4.4 A-D; ANOVA, $p < 0.05$). However, no clear pattern emerged, and the wet season of 2010 had similar $\delta^{15}N$ and $\delta^{13}C$ values to those of the dry seasons (Fig. 4.4; Tukey's *post-hoc* tests).

Unlike in the seasonal comparison, few differences emerged inter-annually for species in either season (Fig. 4.5 and 4.6). *Saurida undosquamis* had significantly more enriched $\delta^{13}C$ values in the wet season of 2010 than in 2009. *Johnius dorsalis* had significantly different $\delta^{15}N$ values between dry seasons, with those in 2010 being relatively depleted, and, similarly, *Otolithes ruber, Pomadasys olivaceum* and *Cynoglassus lida* had significantly different $\delta^{13}C$ values between dry seasons, with values in 2010 relatively depleted to those of 2008.

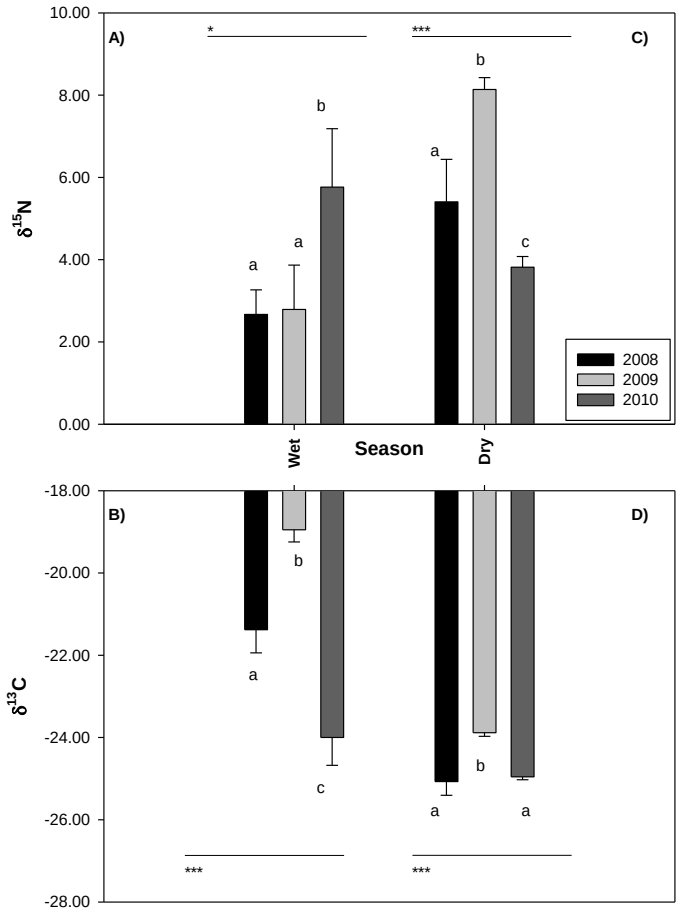

Figure 4.4. Inter-annual variability in the isotope values of the total suspended solids (TSS) from the Thukela mouth for wet (A and B) and dry (C and D) seasons for $\delta^{15}N$ (A and C) and $\delta^{13}C$ (B and D). Stars indicate significant differences (Two-way ANOVA; * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$). Letters indicate Tukey's post-hoc differences ($p < 0.05$).

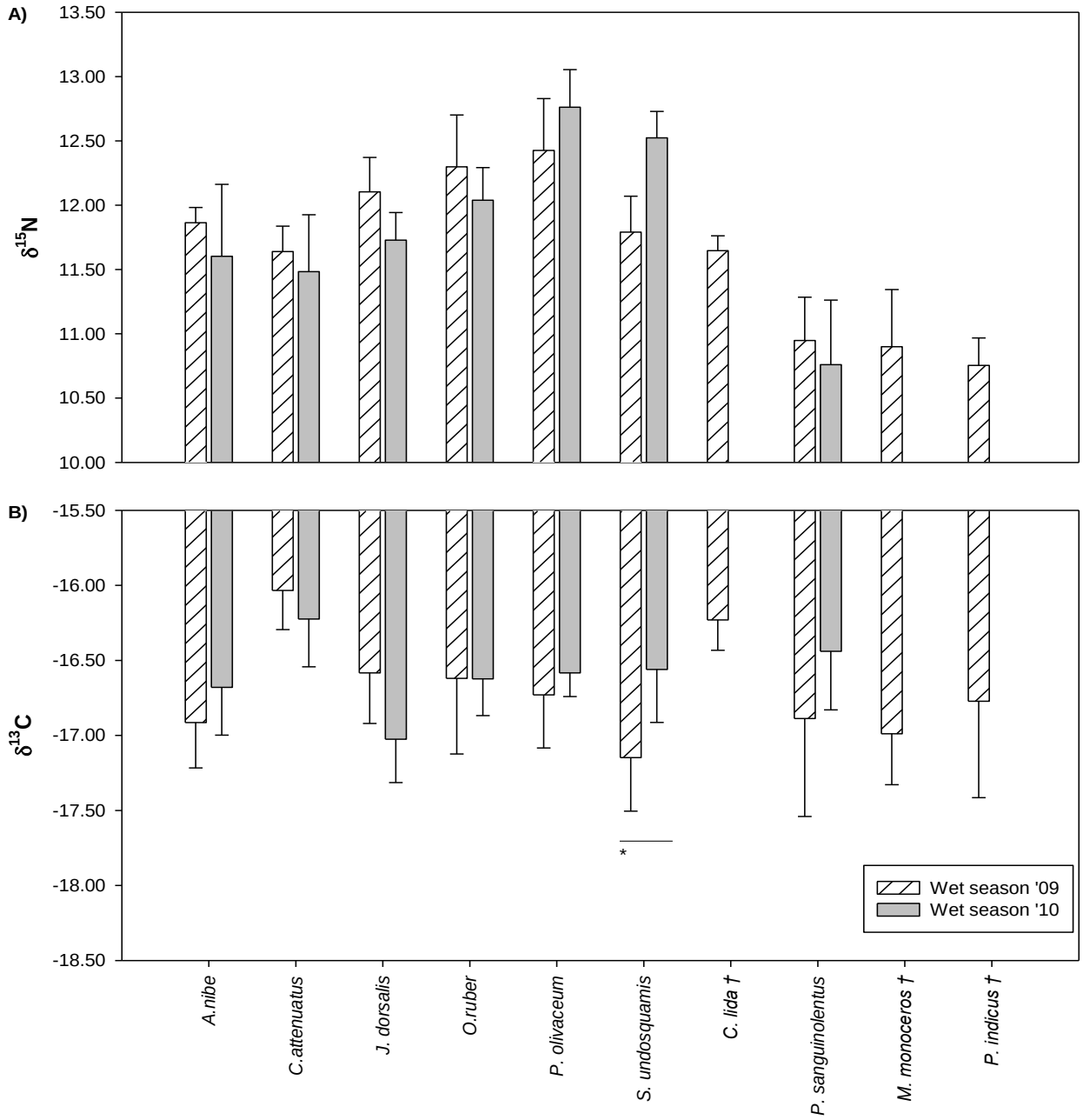

Figure 4.5. Muscle tissue inter-seasonal variances within wet seasons for A) $\delta^{15}N$ and B) $\delta^{13}C$. Start indicates significant differences between the two season (Welch T-test (* $p < 0.05$), no stars indicate no significant differences. † No animals of this species were collected in the wet season 2010.

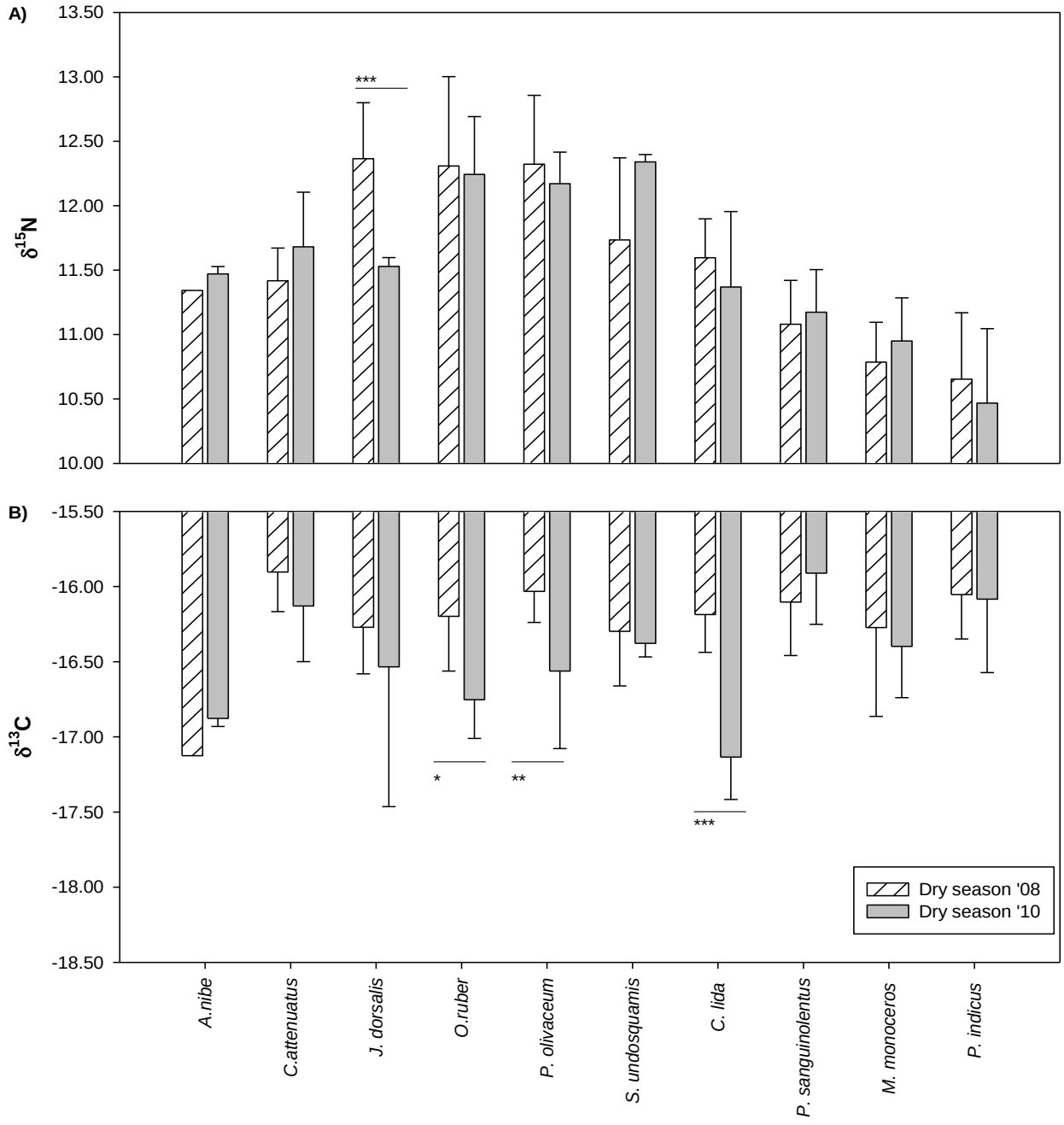

Figure 4.6. Muscle tissue inter-seasonal variances within dry seasons for A) $\delta^{15}N$ and B) $\delta^{13}C$. Start indicates significant differences between the two season (Welch T-test (* $p < 0.05$), no stars indicate no significant differences.

4.5 Discussion

The aim of this study was to clarify the role played by the Thukela River in introducing organic matter into the nearby Thukela Bank area. This study found that the seasonal change in animal isotopic response was opposite to the changes in TSS isotopic signature, *i.e.* the differences seen for TSS in the wet and dry season (wet season ^{13}C-enriched compared to dry season), were the opposite for animals (dry season ^{13}C-enriched compared to wet season). This could have been due to tissue turnover rates, and could indicate that TSS from the Thukela played an important role in the demersal food-web of the Thukela Bank. The reasoning behind this could be that riverine input has a well-marked wet and dry season (Day 1981), while upwelling events are not seasonal in nature (Hutchings et al. 2010).

Strong seasonality in the isotopic signatures of TSS collected from the Thukela River mouth was found, with that of the dry season having significantly enriched ^{15}N and significantly depleted ^{13}C values compared to those of the wet season. Seasonality in estuarine isotopic values are known to be common due to the highly dynamic environment (Simier et al. 2004, Banaru et al. 2007, Faye et al. 2011); this also applies to tropical and subtropical estuaries where wet and dry seasons are well defined (Maya et al. 2011, Olin et al. 2011). The relatively depleted TSS ^{13}C values in both seasons was likely due to the presence of C_3 plant detritus from the Thukela River catchment where the main vegetation types are woodlands, coastal forest, montane forest, thicket and grasslands (Fairbanks & Benn 2000), which account for 75 % of the catchment area (DEA 2001). The $\delta^{13}C$ of C_3 plants range from -22 to -33 ‰, while that of C_4 plants range from -9 to -16 ‰ (O'Leary 1988, Huang et al. 2000), while both C_3 and C_4 plants have $\delta^{15}N$ ranging from -7 to 7 ‰ (Kelly 2000).

It is well known that $\delta^{13}C$ values are a useful and strong indicator of the origin of animals' food sources (Rubenstein & Hobson 2004). In this study mean demersal faunal $\delta^{13}C$ values in the wet and dry seasons were opposite to those of the TSS from different seasons, with the majority of species having had more enriched $\delta^{13}C$ in the dry season. The only known, well defined seasonal inputs into the Bight are riverine in origin, marked by a well defined dry and wet season (Day 1981). As already mentioned, the

seasonal changes in the isotopic signatures of animals and TSS were in opposite directions. Because the upwelling cell is well known not to be seasonal in occurrence (Hutchings et al. 2010), two possible scenarios could explain the results obtained here; 1) there was another source of seasonal OM into the Thukela Bank which was not accounted for, or; 2) a time-lag occurred in the animal tissue assimilating the isotopic signature of the OM released by the Thukela River.

Time-lags in animal tissue assimilating the isotopic signature of its food are common due to the period of time that the animal tissue takes to reach equilibrium with that of the diet (Fry & Arnold 1982, Hesslein et al. 1993, Vanderklift & Ponsard 2003, Martínez del Rio et al. 2009, Wolf et al. 2009). In addition, upwelling processes in the Bight are sporadic and unsystematic (Lutjeharms et al. 1989). As such, if animals did not depend on the OM from the river then their isotopic signatures would not have had such a well-defined seasonal change. Furthermore, TSS was only sampled at one point in time during each season; OM with similar isotopic signatures could have kept entering the system for the remainder of the wet or dry seasons. Overall there were some congruence between seasonal changes in TSS and animal isotope values if isotopic incorporation times are taken into account.

It has been established that the time required for an animal's tissue $\delta^{15}N$ values to reach equilibrium with that of the diet may differ to that of $\delta^{13}C$ (Olive et al. 2003). Furthermore, it has been demonstrated that isotopic equilibrium between predator muscle tissue and that of the diet in the wild is limited (Sweeting et al. 2005). It appears that ^{13}C from the diet was assimilated faster in the muscle tissue than ^{15}N, as $\delta^{13}C$ values showed a clear seasonality for most species, while this was not the case for $\delta^{15}N$ values. This could be due to nitrogen isotopic values varying due to a number of reasons, including different forms of nitrogen excretion (Minagawa & Wada 1984, Ponsard & Averbuch 1999), isotopic fractionation (Fry & Arnold 1982, Hesslein et al. 1993, Vanderklift & Ponsard 2003) or due to physiological or metabolical processes (Olive et al. 2003). If ^{15}N took longer to be assimilated by the muscle tissue than ^{13}C, consumers could have changed their diet several times prior to ^{15}N being assimilated in their bodies. In these instances $\delta^{15}N$ isotopic equilibrium in the tissue may not

manifest, and instead of showing either the current or the past diet becomes a mixture of both diets (Hobson & Clark 1992, Gannes et al. 1997, Sweeting et al. 2005).

Omnivory could also complicate the interpretation of $\delta^{15}N$ data, as animals could reach an equilibrium resembling a mixture of prey items at different trophic levels, and as such seasonal changes in their isotopic signatures could be masked by dietary shifts. This implies that the seasonal importance of TSS would also be masked when seen from the point of view of $\delta^{15}N$. Thompson et al. (2007) found that omnivory is common in marine systems, and that higher up a food-web (from primary consumers) the more apparent the existence of a tangled web of omnivores becomes. In this study the majority of organisms are from upper trophic positions and are omnivorous (for diet information on some of the animals see: George 1974, Bingel & Avsar 1988, Fischer et al. 1990, Van der Elst & Adkin 1991, Rajaguru 1992, Sukumaran & Neelakatan 1997, Fennessy 2000).

Finally, Chapter 3 agrees with the findings in Chapter 4 on the importance of the riverine input into the Bight, by assessing the role that the Thukela River plays on the food-web of the Thukela Bank demersal organisms over time.

4.6 Conclusion

This study shows strong evidence of isotopic seasonality in the TSS collected from the Thukela River mouth and on the $\delta^{13}C$ collected from the organisms on the Thukela Bank. This study agrees with those that suggest that organisms from the Thukela Bank are dependent on the riverine runoff, due to their dependence on OM which enters the system through the Thukela River (Lamberth & Turpie 2003, Lamberth et al. 2009, Turpie & Lamberth 2010), but does this providing isotopic evidence. I found that the isotopic differences seen for TSS in the wet and dry season (wet season ^{13}C-enriched compared to dry season), are the opposite for animals (dry season ^{13}C-enriched compared to wet season). The discrepancy between the different seasonal enrichment in $\delta^{13}C$ was consigned to the time it takes for tissue to acquire the isotopic signature from the diet.

Finally, if the Thukela River plays an important role as an input to the food-webs as suggested by this study (Chapter 3 and 4) and to fisheries as suggested by previous studies (Lamberth et al. 2009, Turpie & Lamberth 2010), then further consideration should be given to the freshwater reserves planned for the Thukela catchment area (DWAF 2004), and their potential effects on the two important fisheries of the East Coast of South Africa and to the biology of the entire Bight.

4.7 Acknowledgments

I wish to acknowledge the African Coelacanth Ecosystem Programme (ACEP), the Thukela Bank Project and the National Research Foundation (NRF) of the South Africa Department of Science and Technology for their financial contribution towards this study, Dr Sven Kaehler from the IsoEnvironmental laboratory at Rhodes University for the running of and useful comments on the samples. The crew of the *Ocean Surf* and *Ocean Spray*, for kindly making his trawler available, and to Rachel Cooper from the University of Cape Town for her editorial comments.

METHODOLOGICAL STUDIES

CHAPTER 5

The effects of preservation methods, dyes and acidification on the isotopic values ($\delta^{15}N$ and $\delta^{13}C$) of two zooplankton species from the KwaZulu-Natal Bight, South Africa

5 The effects of preservation methods, dyes and acidification on the isotopic values ($\delta^{15}N$ and $\delta^{13}C$) of two zooplankton species from the KwaZulu-Natal Bight, South Africa.

5.1 Abstract

Stable isotopes are an important tool for ecosystem trophic linkage studies. It is ideal to use fresh samples for isotopic analysis, but in many cases organisms must be preserved and analysed later. In some cases dyes must be used to help distinguish organisms from detritus. Since preservatives and dyes are carbon-based their addition could influence isotopic readings. This study aims to improve understanding of the effects of sample storage method, dye addition and the effects of acidification on $\delta^{15}N$ and $\delta^{13}C$ of zooplankton (*Euphasia frigida* and *Undinula vulgaris*). Zooplankton was collected and preserved by freezing, the addition of 5% formalin, 70% ethanol, or 5% formalin with added Phloxine B or Rose Bengal and stored for 1 month before processing. Samples in 5% formalin and 70% ethanol were also kept and processed after 3 and 9 months to study changes over time. Formalin caused the largest enrichment for $\delta^{13}C$ and a slight enrichment for $\delta^{15}N$, while ethanol produced a slight depletion for $\delta^{13}C$, while $\delta^{15}N$ effects varied depending on the species. In formalin, dyes depleted $\delta^{13}C$ values, but had variable effects on $\delta^{15}N$, relative to formalin alone. Acidification had no significant effect on $\delta^{15}N$ or $\delta^{13}C$ for either species. Long term storage showed that the effects of the preservatives were species-dependent. Although effects on $\delta^{15}N$ varied, a relative enrichment in carbon of samples occurred with time. This can have important consequences for the understanding of the organic flow within a food web and for trophic studies.

5.2 Introduction

Stable isotopes have become important tools in ecological studies concerned with nutrient flows and trophic linkages (Fry 1991, Angradi 1994, Vander Zanden et al. 1999, Kaehler & Pakhomov 2001). Ideally samples should be dried and processed for isotopic analysis instantaneously upon collection (Ponsard & Amlou 1999), but since the processing relies on access to clean laboratory facilities, field samples – especially those sought in remote environments – often cannot be processed immediately (Hobson et al. 1997, Kaehler & Pakhomov 2001, Carabel et al. 2009). Instead most samples are preserved and stored, and only processed after a period of time (Ponsard & Amlou 1999, Kaehler & Pakhomov 2001, Carabel et al. 2009). A number of studies have examined the effects of different preservation methods on stable isotope ratios. Preservation methods that have been examined include freezing (Bosley & Wainright 1999, Kaehler & Pakhomov 2001, Edwards et al. 2002, Bugoni et al. 2008), instant oven drying (Mullin et al. 1984, Kaehler & Pakhomov 2001), freeze-drying (Hobson et al. 1997) and preservation in formalin (Kaehler & Pakhomov 2001, Edwards et al. 2002, Kelly et al. 2006), ethanol (Kaehler & Pakhomov 2001, Kelly et al. 2006, Bugoni et al. 2008), formalin-ethanol (Bosley & Wainright 1999, Carabel et al. 2009), a saturated sodium chloride solution (Fábián 1998, Ponsard & Amlou 1999, Bugoni et al. 2008) and ethylene glycol (Ponsard & Amlou 1999).

Most preservation and storage techniques appear to have an effect on the isotope value of the tissues that are being preserved. All of the techniques mentioned earlier, except for immediate oven drying, have been reported to alter the $^{15}N/^{14}N$ and $^{13}C/^{12}C$ ratios. Of course it cannot be known if even immediate oven drying alters isotope ratios, because fresh samples cannot be analysed. The reason for this alteration in isotopic changes is not fully understood, but some of the reasons given are the extraction of lipids (Syväranta et al. 2008), exchange of light and heavy isotopes between the organism and the preservative (Hobson et al. 1997, Edwards et al. 2002), or the hydrolysis of proteins during preservation (Arrington & Winemiller 2002, Sarakinos et al. 2002). For example, it has been reported that formalin preservation depletes the ^{13}C of samples, while ethanol preservation either enriches or slightly depletes the ^{13}C content (Kaehler & Pakhomov 2001). Some studies have also tried to ascertain a

correction factor for preserved samples, but found that it is not possible or that it has to be species specific (Bosley & Wainright 1999, Sarakinos et al. 2002, Carabel et al. 2009). Bosley and Wainright (1999) reported that the only method of preservation and storage that should be used in order not to affect stable isotope ratios, until further investigation, should be freezing if drying is not possible. However in the case of zooplankton, where samples are generally comprised of mixtures of species, it is to be expected that the freezing process would lyse cells, leading to the possible exchange of isotopes with other organisms contained within the sample.

The first aim of this research is to examine the effect of different preservation methods on two species of zooplankton. The preservation methods chosen for the study are 70% ethanol, 5% formalin, freezing and thawing and refreezing and rethawing. Refreezing/rethawing was examined because some times during transportation from the field to the laboratory thawing can take place.

In the study of marine organisms, dye is frequently added to samples to distinguish the desired organisms from inorganic or detrital material, and to aid the microscopic visualisation of the organism. Dyes tend to be carbon based and it is possible that they could affect the isotopic ratio of the preserved sample. The second aim was therefore to determine the effect of dyes (Rose Bengal and Phloxine B) added to one of the preservation methods on the isotopic composition of the preserved samples.

When studying small organisms such as zooplankton, there is a need to remove the outer shell via acidification to avoid erroneous $\delta^{13}C$ isotopic readings. Studies looking at the effect of acidification have been done on macrofauna, algae, molluscs, panaeid shrimp, marine algae and seagrass (Bunn et al. 1995, Bosley & Wainright 1999, Ng et al. 2007, Kolasinski et al. 2008). It has been shown by different authors that $\delta^{15}N$ is affected by the acidification process (Schubert & Nielsen 2000, Kolasinski et al. 2008). Studies have also shown that poorer results for organic $\delta^{13}C$ are produced when samples are acidified, but these remain within the limits of what are considered acceptable analytical error (Kolasinski et al. 2008). The third aim was to examine the effect of inorganic carbon removal through acidification and its interaction with the preservation method.

The fourth aim of this research was to examine the effect that long term storage of samples in 70% ethanol and 5% formalin has on two species of zooplankton over a period of 9 months.

5.3 Materials and Methods

5.3.1 Sample collection and study species

Zooplankton samples were collected while on board of the *FRV Algoa* during October 2009. The samples were collected in the KwaZulu-Natal Bight (29°36.826 S; 31°24.777 E) at a depth of 60 m using a double oblique bongo net (200 µm and 300 µm mesh), which was towed for 1 hour at a speed of 1 knot in a westward direction. The cod ends were emptied into a bucket and samples were well mixed to homogenise the species composition. The sample was split using a zooplankton splitter and the resulting samples stored in honey jars under different preservation methods as outlined below. The same two species were used throughout the four experiments, *viz*. *Euphasia frigida* larval stage and *Undinula vulgaris*. Both of these organisms are crustaceans and as such have a chitin exoskeleton which might affect the organic carbon readings.

5.3.2 Sample preservation and experimental layout

Freezing was used as a reference as the samples were collected at sea and there was little possibility to sort them while the ship was sailing. I assumed that the isotope signature of frozen samples would be the closest to instantly dried samples, in accordance with Bosley and Wainright [4]. Experiment 1 tested the effect of different preservation methods. All of the samples, except the reference, were analysed after one month. The reference samples were analysed within 2 weeks of collection and after thawing once, these samples were refrozen, kept in the freezer for two days and rethawed at room temperature. The different treatments used were therefore, i) frozen at sea immediately after collection, thawed and processed (reference); ii) freezing and

thawing for a second time (F2); iii) preservation in 70% ethanol (EtOH); and 4) preservation in 5% formalin (FM).

Experiment 2 looked at the effects of dyes in samples preserved in 5% formalin, the dyes used were i) 0.05 g of Phloxine B ($C_{20}H_2Br_4Cl_4Na_2O_5$) (FMP) and ii) 0.05 g of Rose Bengal ($C_{20}H_2Cl_4I_4Na_2O_5$) (FMRB). Five replicates were used for each treatment. Samples for experiment 1 and 2 were bathed in a 2% HCl to remove the exoskeleton. Seawater collected from the same region as the sample was used for the frozen samples and to dilute the ethanol and formalin, it was filtered using GF/F glass fibre filters with 0.7 µm nominal porosity. Samples were stored at ambient temperature in the dark in PVC bottles. Three replicates of pure Phloxine B and Rose Bengal were also sent for analysis for comparative purposes.

Experiment 3 examined the effect of acid-washed versus non-acid-washed on the $\delta^{13}C$ and $\delta^{15}N$ values. For this a sub-sample was collected from experiment 1 and left untreated (non-acid washed). This was done to compare the effects that acidification has on the overall results for carbon and nitrogen. The same nomenclature as experiment 1 and 2 was used for the non-acid-washed samples, but NA was added after the name, *i.e.* i) Reference-NA, ii) EtOH-NA, iii) FM-NA, iv) FMP-NA and v) FMRB-NA.

Experiment 4 tested the effect of preservatives on the isotope ratios of the samples over time. Sub-samples were taken from only the EtOH and FM treatments at 1, 3 and 9 months with time 0 being the same as experiment 1 reference. The medium that the organisms were preserved in was kept and the same jars re-used to avoid any possible contamination. Samples collected for experiment 4 were bathed in a 2% HCl to remove the chitin exoskeleton.

Processing of samples subsequent to preservation and storage involved rinsing all the samples with Milli-Q water prior to drying. Those samples bathed in 2% HCl, were rinsed with 1 extra wash with 100 ml of Milli-Q water.

All samples were dried for 48 hours at 65°C, after which they were homogenised and weighed into tin capsules (SANTI® Analytical, Teufen, Switzerland). For isotope analysis 0.5 mg dry mass is needed to yield sufficient nitrogen and carbon for analysis.

Where possible 0.6 mg dry weight was collected from the samples, but in the case of insufficient zooplankton individuals approximately 0.3 - 0.4 mg were collected per sample. The samples were sent to the University of Cape Town and were combusted in a Flash EA 1112 series elemental analyzer (Thermo Finnigan, Milan, Italy). The gases were passed to a Delta Plus XP IRMS (isotope ratio mass spectrometer) (Thermo electron, Bremen, Germany), via a Conflo III gas control unit (Thermo Finnigan, Bremen, Germany). Merck Gel - a proteinaceous gel produced by Merck (Darmstadt, Germany) was used as standard and was calibrated against IAEA (International Atomic Energy Agency, Vienna, Austria) standards. Nitrogen is expressed in terms of its value relative to atmospheric nitrogen, while carbon is expressed in terms of its value relative to Pee-Dee Belemnite. The analytical precision of the instrument was 0.20 ‰.

5.3.3 Data analysis

Isotopes ratios were expressed in the usual δ-notation that signifies differences in isotopic ratios ($^{15}N/^{14}N$ or $^{13}C/^{12}C$) between unknown samples (R_{sample}) and that of a known reference standard ($R_{standard}$) (Hoefs 2004, Sulzman 2007) in per mil (‰) units. The δ–value for each isotope is calculated as (Epstein et al. 1953):

$$\delta(‰) = (R_{sample} / R_{standard} - 1) \times 1000$$

No data transformation was needed because data were homogeneous in their variance (Levene's test $p < 0.05$). A factorial two-way ANOVA was used to determine the combined effect of treatment and acidification on the results. One-way ANOVA was used to examine the effects of storage length of preservatives on isotopic values of species and treatment. A two-way ANOVA (time*preservative) was used to compare the effects of FM versus EtOH over time on isotopic ratios of samples for each species. Tukey's HSD *post-hoc* test was used to determine specific differences among means. (R 2.11.1 software, R Foundation for Statistical Computing, Vienna, Austria)

5.4 Results

5.4.1 Experiment 1: The Effects of Different Preservation Methods

Preservation method had a significant effect on $\delta^{13}C$ and $\delta^{15}N$ values in both species (Table 5.1; Fig. 5.1 C, D, E, F).

The mean $\delta^{13}C$ values for *Euphasia frigida* ranged from -19.02 ± 0.27 ‰ when preserved in FM to -21.54 ± 0.70 ‰ in the reference, while the mean values for $\delta^{15}N$ ranged from 4.76 ± 0.27 ‰ after a double freeze-thaw cycle (F2) to 5.76 ± 0.30 ‰ in 5 % formalin (FM). Treatment had a significant effect on *E. frigida* $\delta^{13}C$ values (Table 5.1) and a *post-hoc* test revealed that all treatments were significantly different from the reference in both the acid-washed and the non-acid-washed samples (Fig. 5.1 C). The FM treatment caused the most dramatic difference of +2.52 ‰ and was significantly different to all treatments in the acid-washed results (Fig. 5.1 C). The $\delta^{13}C$ values in FM and EtOH, were significantly different from each other in the acid-washed samples, but were not significantly different in the non-acid-washed treatments.

The $\delta^{15}N$ values determined for *E. frigida* differed significantly among treatments (Table 5.1; Fig. 5.1 D). The reference was significantly different only from F2, while the latter was significantly different from all treatments for the acid-washed results (Fig. 5.1 D). No other treatments were significantly different from each other (Fig. 5.1 D). As for the non-acid-washed results, neither the preservation treatments nor the reference were different from each other.

Undinula vulgaris mean $\delta^{13}C$ values ranged from -19.65 ± 0.17 ‰ in FM to -20.51 ± 0.09 ‰ in the reference, while the mean values for $\delta^{15}N$ varied from 4.65 ± 0.45 ‰ in the reference to 6.75 ± 0.31 ‰ in 5 % formalin (FM). Although preservation treatment had a significant effect on $\delta^{13}C$ values in *U. vulgaris* (Table 5.1; Fig. 5.1 E), there was considerably less variability among $\delta^{13}C$ values for the acid-washed samples, for different preservation methods than was found for *E. frigida* (Fig. 5.1 C). Only FM had a significant effect compared to the reference (Fig. 5.1 E), while F2 was significantly different from FM (Fig. 5.1 E). The non-acid-washed samples were not significantly different among any of the treatments or between the treatments and the reference.

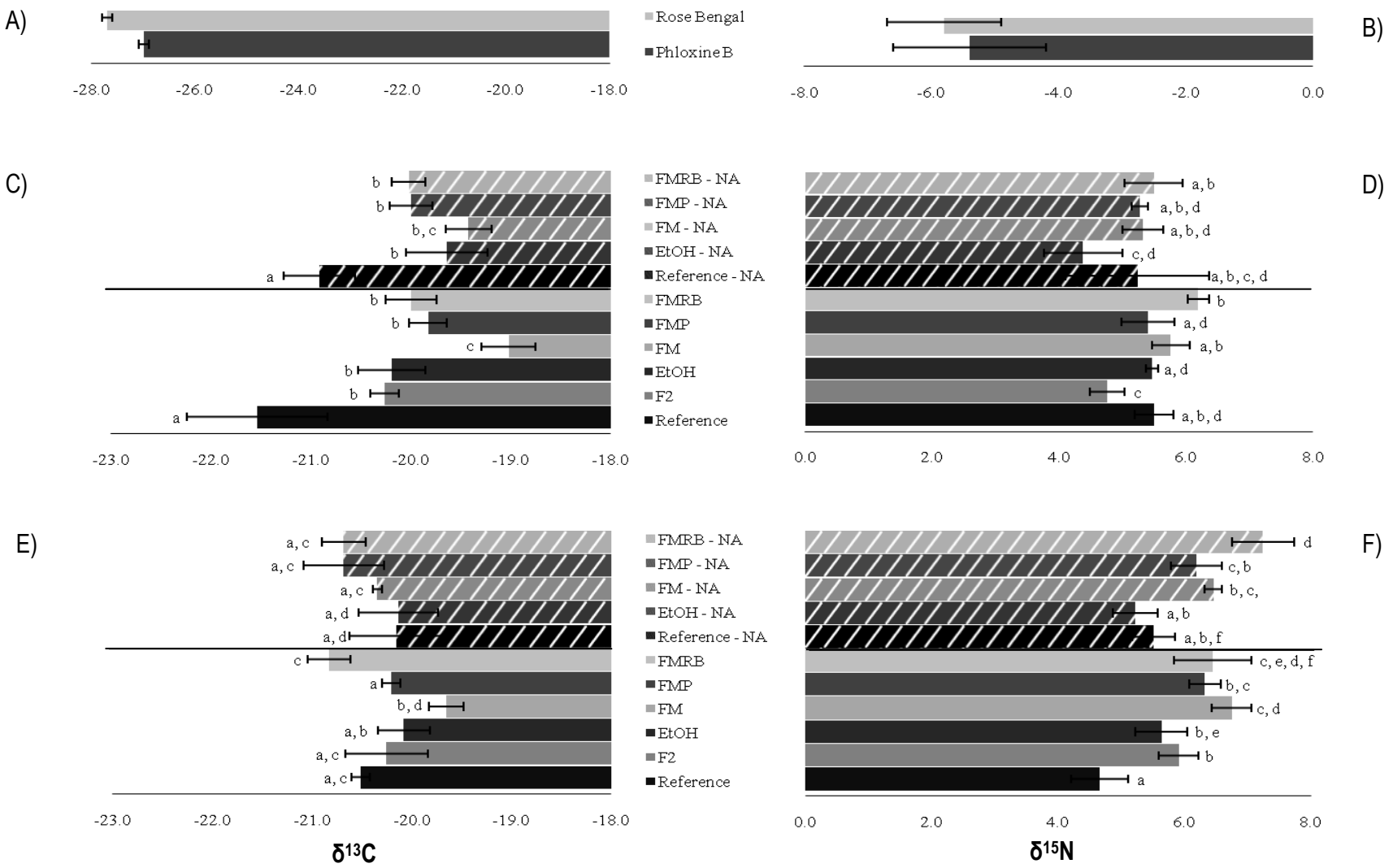

Figure 5.1. Shows the carbon $\delta^{13}C$ and $\delta^{15}N$ mean values (± SD) for pure Rose Bengal and Phloxyne B (A and B), *Euphasia frigida* (C and D) and *Undinula vulgaris* (E and F). Letters show the significant difference between the treatments ($p < 0.05$). Treatment name followed by NA indicates non-acid washed samples. Note the different X-axis values for Rose Bengal and Phloxyne B for both $\delta^{13}C$ and $\delta^{15}N$.

Conversely, preservation method as compared with the reference had more of an effect on $\delta^{15}N$ values in *U. vulgaris* than *E. frigida* for acid-washed samples. Overall preservation treatments were significantly different in terms of $\delta^{15}N$ values (Table 5.1; Fig. 5.1 F), with the reference samples $\delta^{15}N$ values being isotopically lighter compared to all the treatments (Fig. 5.1 F). Nitrogen δ-values differed between F2 and FM as well as EtOH differing with respect to FM and F2 (Fig. 5.1 F). The non-acid-washed results were also more variable than those of *E. frigida*, however, there were no significant differences between the treatments or the treatments and the reference.

Table 5.1. Shows the results for a two-way factorial ANOVA that acidification, treatments and the combined effect of acidification and treatment have on the $\delta^{13}C$ and $\delta^{15}N$ values for *E. frigida* and *U. vulgaris*.

	Comparison	*df*	*F*	*p*
E. frigida – $\delta^{13}C$	Acidification	1	0.09	0.76
	Treatment	4	42.47	*** > 0.001
	Treatment*Acidification	4	4.33	** > 0.01
E. frigida – $\delta^{15}N$	Acidification	1	7.50	** > 0.01
	Treatment	4	6.76	** > 0.01
	Treatment*Acidification	4	1.24	0.31
U. vulgaris – $\delta^{13}C$	Acidification	1	1.97	0.17
	Treatment	4	6.15	*** > 0.001
	Treatment*Acidification	4	2.48	0.12
U. vulgaris – $\delta^{15}N$	Acidification	1	0.09	0.76
	Treatment	4	26.12	*** > 0.001
	Treatment*Acidification	4	4.84	** > 0.01

5.4.2 Experiment 2: The Effects of Dyes

For comparative purposes, Rose Bengal and Phloxine B were analysed as pure substances (Fig. 5.1 A and B). Both were isotopically depleted of heavy isotopes for nitrogen and carbon relative to the standards.

The ANOVA results were the same as experiment 1 as dyes were run as one more treatment (Table 5.1). In *E. frigida* $\delta^{13}C$ values were significantly more enriched in FMRB and FMP than in the reference (Fig. 5.1 C) for acidified and non-acidified samples. However, dyed samples FMRB and FMP were only significantly depleted relative to FM in the acidified samples (Fig. 5.1 C). The FMRB-NA and FMP-NA $\delta^{13}C$ values were not significantly different from each other (Table 5.1; Fig. 5.1 C), while

they showed a significant difference in the acidified results (Fig. 5.1 C). Dyes had no effect on $\delta^{15}N$ of *E. frigida*; FMRB and FMP were not significantly different from either FM or the reference (Fig. 5.1 D) and FMRB and FMP were only significantly different to each other for acidified samples (Fig. 5.1 D). In the non-acid-washed samples only EtOH-NA significantly differed from FMRB-NA (Fig. 5.1 D).

Similar results were found for $\delta^{13}C$ values of non-acid-washed *U. vulgaris*, with FMRB-NA and FMP-NA not significantly differing from FM-NA or the reference-NA (Fig. 5.1 E). While acid-washed FMRB and FMP were significantly depleted relative to FM and FMRB was depleted relative to FMP, but neither were different from the reference (Fig. 5.1 E). *Undinula vulgaris* $\delta^{15}N$ values were significantly enriched in FMRB-NA relative to all non-acid-washed treatments (Fig. 5.1 E), while FMP and FMRB were both significantly different from the reference in the acidified samples (Fig. 5.1 E).

5.4.3 Experiment 3: The Effect of Acidification

For *Euphasia frigida* $\delta^{13}C$ values, acidification had no significant effect, while treatment and the combined effect of treatment and acidification had a significant interactive effect on $\delta^{13}C$ values (Table 5.1). However, no treatment was different from its acidified counterpart nor did the acidified and non-acidified references differ (Fig. 5.1 C).

Conversely, for $\delta^{15}N$ results in *E. frigida* there was a significant difference between acidified and non-acidified samples (Table 5.1), but the interactive effect of treatment and acid-washing was not significant. Tukey's *post-hoc* test showed that there were no significant differences between acid-washed and non-acid-washed counterparts (Fig. 5.1 D).

Acid-washing did not result in any significant difference in $\delta^{13}C$ values in *U. vulgaris* (Table 5.1), but the interactive effect of acid-washing and preservation treatment was significant (Table 5.1). Investigation by a Tukey's *post-hoc* test revealed that the only difference between acid and non-acid-washed samples was for treatment FM (Fig. 5.1 E).

Similarly, for *U. vulgaris* $\delta^{15}N$ values there were no significant differences between the acid and non-acid washed samples. Preservation treatment and acidification had a significant interactive effect on $\delta^{15}N$ values (Table 5.1), but when each acidified preservation treatment was compared against its non-acidified counterpart with Tukey's *post-hoc*, no significant differences were found.

5.4.4 Experiment 4: The Effect of Storage Length

Turning to the effect of storage time on measured isotope ratios in the two zooplankton species, for all cases length of storage had a significant effect on isotopic signatures. In all treatments $\delta^{13}C$ values of samples reached a peak after 3 months, but equilibrium with the storage medium was apparently not yet reached after 9 months in storage (Figs. 5.2 A and 5.2 B). This was true for all $\delta^{13}C$ and $\delta^{15}N$ values measured in samples preserved in FM and EtOH.

Storage time had a significant effect on the $\delta^{13}C$ values of *E. frigida* for samples preserved in both EtOH (ANOVA $df = 3$, $f = 17.51$, $p < 0.01$) and FM (ANOVA $df = 3$, $f = 66.87$, $p < 0.01$). Samples stored in EtOH for 3 and 9 months had $\delta^{13}C$ values significantly different from the reference sample (frozen, time zero), while samples stored for 1 month were only significantly different from 3 months and those stored for 9 months were not different from 1 or 3 months (Fig. 5.2 B; Table 5.2). Storage time also had a significant effect for *E. frigida* stored in FM, with samples stored for 1, 3 and 9 months being different to the reference (ANOVA $df = 3$, $f = 66.87$, $p < 0.01$); $\delta^{13}C$ values at 3 months were significantly different from those at 1 and 9 months (Table 5.2). There were no significant differences between the isotope ratios after 1 month and 9 months. When comparing EtOH against FM over time there was a significant difference in $\delta^{13}C$ for *E. frigida* (ANOVA $df = 1$, $f = 33.44$, $p < 0.01$).However, when time periods were considered individually $\delta^{13}C$ values only differed significantly between EtOH and FM at 1 month (Tukey's *post-hoc,* $q = 1.18$, $p < 0.01$).

Conversely, $\delta^{15}N$ for *E. frigida* preserved in EtOH did not change significantly over time (Fig. 5.2 A). The $\delta^{15}N$ values of *E. frigida* in FM did change significantly with time (ANOVA $df = 3$, $f = 9.3$, $p < 0.01$), with the major difference with respect to the reference

(+0.53‰) found after 3 months (Table 5.2). The reference $\delta^{15}N$ values were not significantly different from either 1 or 9 months storage in FM. When comparing time period, $\delta^{15}N$ values measured at 1 month were not significantly different from those at 3 months in FM, but were significantly different from those at 9 months and values were significantly different between 3 and 9 months (Table 5.2). A comparison of $\delta^{15}N$ values obtained from EtOH against those from FM for 1, 3 and 9 months, revealed both time and treatment to have significant effects as individual factors (ANOVA $df = 3$, $f = 6.07$, $p < 0.01$ and $df = 1$, $f = 30.33$, $p < 0.01$ respectively), but interactively they produced a marginally non-significant effect on $\delta^{15}N$ values (ANOVA $df = 2$, $f = 3.03$, $p = 0.07$). When time periods were considered individually, FM and EtOH only produced significantly different $\delta^{15}N$ values at 3 months (Tukey's *post-hoc*, $q = 1.1$, $p < 0.01$) with an average difference of +0.31 ‰.

Preservation in EtOH had no significant effect on *Undinula vulgaris* $\delta^{13}C$ values over time (Fig. 5.2 B), while preservation in FM did (ANOVA $df = 3$, $f = 55.73$, $p < 0.001$). A Tukey's *post-hoc* revealed carbon isotope ratios of the reference to be significantly different from those in FM storage for 1, 3 and 9 months (Fig. 5.2 B; Table 5.3). Differences existed between $\delta^{13}C$ values stored in FM for different lengths of time; 1 month was significantly different from both 3 and 9 months and 3 months was significantly different to 9 months (Table 5.3). There were no significant differences between $\delta^{13}C$ values of samples stored in EtOH as opposed to FM at any time period for *U. vulgaris*.

Long term storage in both EtOH and FM had a significant effect on $\delta^{15}N$ for *Undinula vulgaris* over time (Fig. 2 A, ANOVA; EtOH $df = 3$, $F = 8.42$, $p < 0.01$, FM $df = 3$, $f = 59.7$, $p < 0.001$). There were no significant differences in the *U. vulgaris* $\delta^{15}N$ values between 1, 3 and 9 months storage in either EtOH or FM, with the exception of 3 months storage being different from 9 months in FM. However, storage in both preservatives and for all periods of time produced $\delta^{15}N$ values that were significantly different to the reference – time zero – values (Fig. 5.2 A; Table 5.3), with a mean enrichment after 1 month of +1.26 ‰ for EtOH and +2.10 ‰ for FM compared to the reference. A comparison between EtOH and FM over time revealed that time and preservative had a significant interactive effect on *U. vulgaris* $\delta^{15}N$ values (ANOVA $df = 2$, $f = 4.32$, p

<0.05). Specifically, those samples stored for 1 and 3 months were significantly different, with samples stored in FM being more enriched than those in EtOH (Tukey's *post-hoc,* 1 month $df = 0.84$, $p < 0.01$ and 3 months $df = 1.35$, $p < 0.01$), but after 9 months the difference was not apparent.

Table 5.2. Tukey's *post-hoc* test results for *E. frigida* comparing the reference (time 0) and the three storage periods (1, 3 and 9 months). Values are shown in figure 5.2.

		Time	1	3	9
EtOH	$\delta^{13}C$	Reference	$q = 1.34$, $p < 0.05$ *	$q = 2.50$, $p < 0.01$ **	$q = 1.90$, $p < 0.01$ **
		1		$q = 1.16$, $p < 0.05$	$q = 0.59$, $p = 0.48$
		3			$q = 0.57$, $p = 0.41$
FM	$\delta^{15}N$	Reference	$q = 0.26$, $p = 0.42$	$q = 0.54$, $p < 0.05$ *	$q = 0.23$, $p = 0.53$
		1		$q = 0.27$, $p = 0.32$	$q = 0.49$, $p < 0.05$ *
		3			$q = 0.77$, $p < 0.01$ **
	$\delta^{13}C$	Reference	$q = 2.45$, $p < 0.01$ **	$q = 2.52$, $p < 0.01$ **	$q = 3.31$, $p < 0.01$ **
		1		$q = 0.78$, $p < 0.01$ **	$q = 0.86$, $p = 0.98$
		3			$q = 0.86$, $p < 0.01$ **

Table 5.3. Tukey's *post-hoc* test results for *U. vulgaris* comparing the reference (time 0) and the three storage periods (1, 3 and 9 months). Values are shown in figure 5.2.

		Time	1	3	9
EtOH	$\delta^{15}N$	Reference	$q = 1.25$, $p < 0.01$ **	$q = 1.11$, $p < 0.01$ **	$q = 1.21$, $p < 0.01$ **
		1		$q = 0.14$, $p = 0.93$	$q = 0.04$, $p = 0.99$
		3			$q = 0.10$, $p = 0.97$
FM	$\delta^{15}N$	Reference	$q = 2.09$, $p < 0.01$ **	$q = 2.45$, $p < 0.01$ **	$q = 1.82$, $p < 0.01$ **
		1		$q = 0.36$, $p = 0.17$	$q = 0.27$, $p = 0.39$
		3			$q = 0.64$, $p < 0.01$ **
	$\delta^{13}C$	Reference	$q = 0.86$, $p < 0.01$ **	$q = 1.28$, $p < 0.01$ **	$q = 0.36$, $p < 0.05$ *
		1		$q = 0.42$, $p < 0.01$ **	$q = 0.50$, $p < 0.01$ **
		3			$q = 0.92$, $p < 0.01$ **

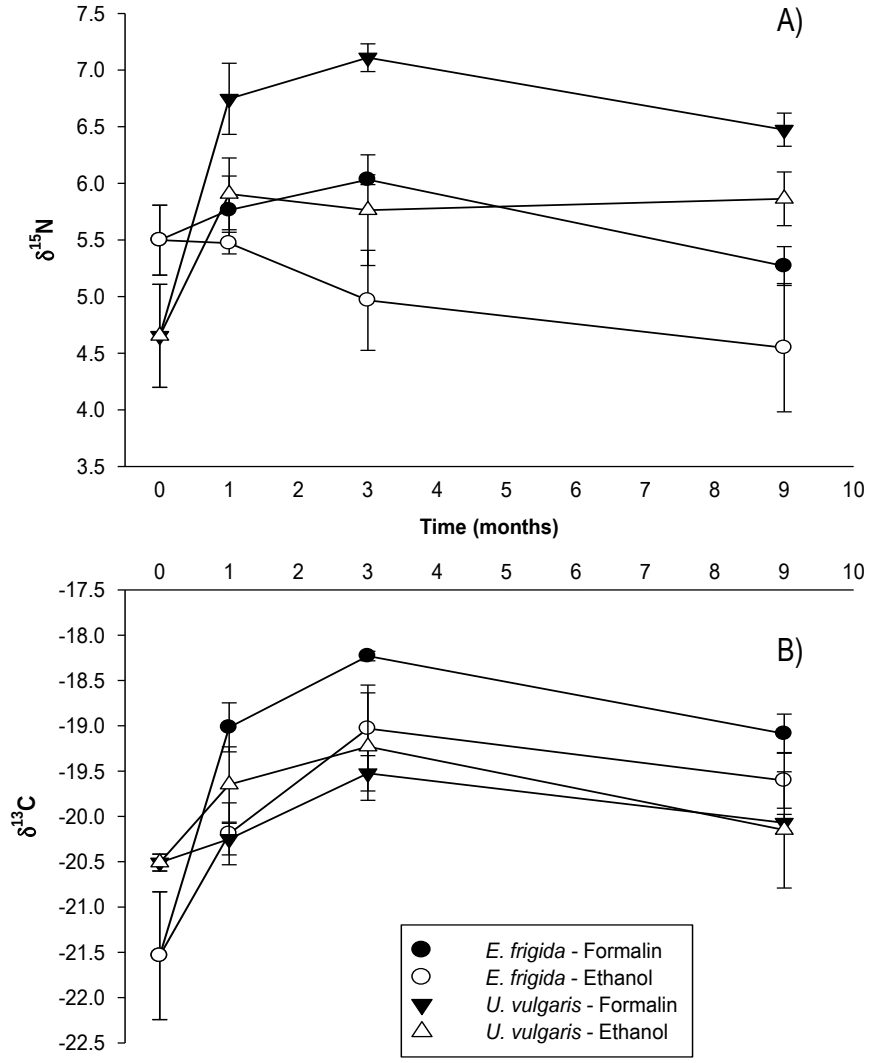

Figure 5.2. Mean (± SD) $\delta^{15}N$ (A) and $\delta^{13}C$ (B) values for *E. frigida* and *U. vulgaris* as influenced by storage time. The reference sample is taken at a time of 0 days after collection. The treatment samples were analysed at 1, 3 and 9 months. See Table 5.2 for Tukey's *post-hoc* results for *E. frigida* and Table 5.3 for Tukey's post-hoc results for *U. vulgaris.* Five samples were analysed for each period of time and each preservation method.

5.5 Discussion

In animals $\delta^{13}C$ and $\delta^{15}N$ tend to be enriched by around + 1 ‰ and + 0.3 to 3.4 ‰, respectively, compared to that from the diet (Ng et al. 2007). Consequently, even a small change in these values due to preservation in a carbon-based medium could affect the interpretations I make regarding the relation between prey and consumer organisms.

In this study, preservatives and dyes were shown to have a clear effect on $\delta^{13}C$ and $\delta^{15}N$ of two species of zooplankton, *Euphasia frigida* and *Undinula vulgaris*. This work also demonstrates that the effect of 70% ethanol and 5% formalin on the preserved samples was time, preservative and species dependent. Both dyes exhibited variable effects on the sample $\delta^{13}C$ and $\delta^{15}N$ values, with Phloxine B generally producing more depleted $\delta^{15}N$ and $\delta^{13}C$ than Rose Bengal. The only observable pattern was for $\delta^{13}C$, where both dyes added to formalin caused depletion relative to formalin alone. It may be of interest to conduct further studies to examine the effects of dyes on frozen samples.

In experiment one and two, it was expected that both ethanol and formalin would have an effect, along with the dyes which are also carbon-based and lacking in nitrogen, on the $\delta^{13}C$ measurements. In experiment one the effect of thawing and refreezing on zooplankton isotope ratios was examined, since it was sometimes difficult to maintain the samples completely frozen while transporting them from the collection point in the field to the laboratory for analysis or even due to the malfunctioning of the freezing facilities. If the samples thaw, there was a risk that the $\delta^{15}N$ and $\delta^{13}C$ could be affected through protein denaturation or cells lysis during the freezing and thawing process. Changes in $\delta^{15}N$ and $\delta^{13}C$ were observed for both species, but the changes did not follow any consistent pattern.

The effects of thawing and freezing for a second time on *E. frigida* are considerable with it resulting in a difference in $\delta^{13}C$ of 1.27 ‰ compared to the reference. Samples stored in ethanol, formalin with Phloxine B and formalin with Rose Bengal are very similar to those samples subjected to a double freeze-thaw cycle, with none of them being significantly different from each other. The $\delta^{13}C$ measurements are preservative, dye

and species dependent. The *E. frigida* reference sample was depleted in ^{13}C relative to all the treatments with the greatest difference observed in the formalin preserved samples (difference of the means of -2.52 ‰). On the other hand, ethanol creates a smaller difference compared to the reference with a deviation of -0.07 ‰ for $\delta^{15}N$ and +1.35 ‰ for $\delta^{13}C$. These are considerable errors, and they are well outside the accepted enrichment between a predator and its prey (~1.0 ‰) (Fry & Arnold 1982, Minagawa & Wada 1984).

Euphasia frigida showed an average difference of the means +0.74 ‰ for $\delta^{15}N$ between twice frozen/thawed samples as compared to the reference samples which were frozen/thawed once only. Conversely, $\delta^{15}N$ measured in *U. vulgaris* shows the opposite trend with the reference sample being depleted relative to the twice frozen/thawed sample, with an average difference of -0.98 ‰. Through observations of these two species under a compound microscope after the initial thawing, it was noticed that *U. vulgaris* contained more lipid droplets than *E. frigida*. Pinnegar and Polunin (1999) suggested that lipid removal, in the case of rainbow trout (*Oncorhynchus mykiss*) tissue, led to enrichment of the tissue $\delta^{15}N$ values. A different study by Sotiropoulos et al. (2004) also found that $\delta^{15}N$ in fish tissue became enriched after lipid extraction, and it was suggested by the authors that this could be due to the secondary release of proteins from the tissue. It was likely that the changes in $\delta^{15}N$ in this study may have resulted from a similar breakdown of zooplankton tissue due to the repeated freezing and thawing. Indeed a breakdown of tissue was observed when sorting samples under a compound microscope. It was possible that any degradation of the tissue could have led to lipid-release resulting in the enrichment of tissue in fattier *U. vulgaris* after being frozen for a second time. This tissue breakdown and lipid loss might also explain the differences observed for *U. vulgaris* between the reference and any of the preservatives or preservative and dye treatments.

For *U. vulgaris* it can be seen that none of the treatments, except formalin preservation, caused as great a difference from the reference as they did in *E. frigida*. In the case of formalin preservation, enrichment in ^{13}C, similar to that seen for *E. frigida*, by +0.86 ‰ differences in the mean relative to the reference sample was observed. The biggest discrepancy for this species was between formalin with Rose Bengal and formalin only,

with the latter having a difference of the means of +1.18 ‰ more enriched relative to those in formalin. Ethanol on the other hand only caused a difference of the means of +0.26 ‰. As with *E. frigida*, this was outside of what will be acceptable for natural variation.

As can be seen from the results (Figs. 1 A and B) both dyes were depleted in ^{13}C, and as such it was expected that they would cause the tissue to become depleted when they were incorporated into the tissue during sample staining. The results for both species indicate that both dyes had a strong effect, which in most cases resulted in the preserved and dyed treatments differing greatly from the reference values. As seen in Fig. 5.1 (F, D), formalin without dyes enriched the $\delta^{15}N$ values relative to the reference in both species. The addition of Phloxine B to formalin depleted the samples in ^{15}N relative to that in formalin-only treated samples, but formalin with Phloxine B remained enriched relative to the reference values. The effect of adding Rose Bengal to formalin appears to be species dependent. *E. frigida* in formalin with Rose Bengal (Fig. 5.1 D) showed a slight enrichment compared to values in formalin alone, while the mean $\delta^{15}N$ value of *U. vulgaris* preserved in formalin with added Rose Bengal was slightly depleted relative to values of samples stored in formalin alone. This could perhaps indicate that the $\delta^{15}N$-depleted Phloxine B dye actually depletes the final $\delta^{15}N$ values of samples stored in formalin. However, $\delta^{15}N$ values did not follow a clear pattern in samples stained with Rose Bengal despite this dye also being $\delta^{15}N$-depleted.

Formalin with Rose Bengal caused the largest enrichment effect on *E. frigida* with an average difference of +0.70 ‰ relative to the reference, while ethanol and formalin with Phloxine B were not different from the reference. *Undinula vulgaris* does not follow the same pattern, at least not for the reference which was depleted in $\delta^{15}N$ compared to all the other treatments with an average difference of all means of -1.56 ‰. Formalin causes the biggest enrichment compared to the reference with differences of the means of +2.09 ‰. Ethanol was once again the treatment with the smallest difference to the reference of +0.98 ‰ of the means. The implication was that the effect of preservation method or dye addition can under some circumstances be quite large. Isotopic shifts forced by preservation methods could rival the magnitude of isotopic change that occurs in $\delta^{13}C$ and $\delta^{15}N$ during trophic flows of C and N from one trophic

position to the next. Not accounting for the effects of preservation medium could erroneously allow one to conclude that the study organism is in a lower or higher trophic position than it is in reality. Both, formalin and ethanol, are within the acceptable ranges mentioned earlier for nitrogen, however, the smaller error produced by the ethanol should be taken into consideration when selecting a preservation method.

In experiment three, acidification was tested on the same two species of planktonic crustaceans, *E. frigida* and *U. vulgaris*. Acidification was tested on the samples preserved for 1 month to see whether it had any effect on the $\delta^{15}N$ values. This study, as with previous studies, indicates that acidification does not have an overall significant effect on the $\delta^{15}N$, with acidified samples having a difference of the mean averaging 0.09 ‰ more enriched values than non-acidified samples (Bosley & Wainright 1999, Pinnegar & Polunin 1999, Kolasinski et al. 2008).

Acid-washing had no significant effect on reference $\delta^{13}C$ measurements, with non-acid-washed samples being enriched by +0.21 ‰ on average relative to acid-washed samples. Only formalin preserved samples differ between the acidified and non-acidified counterpart for *U. vulgaris*. These results agree with the changes obtained by Bunn *et al*. (1995) of +0.3 ‰, but disagree on the fact that in this study variability was not found to increase in the acid-washed samples as reported by the same authors.

However, this study also found that even if there were no significant differences between acidified and non-acidified samples for either $\delta^{15}N$ or $\delta^{13}C$, non-acidified treatments tend to differ less between each other than their acidified counterparts differ between themselves. Overall I believe that acidification is not necessary for planktonic crustaceans as the exoskeleton is formed mainly of chitin with some $CaCO_3$ (Boßelmann et al. 2007), as such, acidification will remove only the $CaCO_3$ leaving the chitin behind. Therefore, I believe that acidification will only create more uncertainty in the final results. There is a need to find a method that will remove both $CaCO_3$ and chitin with the least impact possible on the final results.

Returning to the results of experiment one, it was found that the effects of preservatives and/or dyes on the $\delta^{15}N$ value of an organism are not solely due to the

dye or preservative of interest altering the $\delta^{15}N$ values, but that these effects are also species-dependent. This was supported by the species-dependent results obtained in the time series for ethanol and formalin in experiment four, where *U. vulgaris was* shown to be enriched in ^{15}N by both of these preservatives with time as compared to the reference samples. *E. frigida* on the other hand showed a slight enrichment in $\delta^{15}N$ when stored in formalin for 3 months, with differences between the means of + 0.53 ‰; however, by 9 months it becomes depleted relative to the reference with differences between the means of -0.20 ‰ (Fig. 5.2). When preserved in ethanol *E. frigida* showed a depletion pattern from the first month to 9 months after being placed in storage. At 9 months the $\delta^{15}N$ value of *E. frigida* averaged 4.55 ± 0.57 ‰, which was much lower than that of the reference samples (5.50 ± 0.31 ‰) and lower than the reference value of *U. vulgaris* (4.65 ± 0.45 ‰), and much lower than this species in the same preservation method after 9 months (5.86 ± 0.24 ‰). A recent study showed that ^{15}N in Asiatic clam (*Corbicula fluminea*) increased in different preservation methods compared against the control, but after the initial change values remain constant over time (Syväranta et al. 2011). This was unlike the findings presented here where after the initial enrichment a depletion occurred by 9 months.

In the case of $\delta^{15}N$ in experiment four, formalin and ethanol do not produce clear isotopic patterns over the 9 month period. Of significance was the fact that long-term storage in both preservatives resulted in *U. vulgaris* $\delta^{15}N$ values becoming more enriched than those of *E. frigida* after 1 month, remaining as such for the rest of the study. This suggests that relative positions on a food web can become distorted for organisms stored over lengthy periods of time.

Other studies have similarly found variable effects of the same preservatives for different species or groups, suggesting that preservative effects are species-dependent as indicated in the results of this study. Barrow et al. (2008) carried out a literature review of studies on the effects of preservatives and reported that of 11 studies using ethanol none showed any significant effect on $\delta^{15}N$, while of 29 studies on the effects of formalin, three showed some enrichment, six a depletion and twenty show no difference. Bosley and Wainright (1999) also demonstrated a slight increase of 0.5 – 1.4 ‰ in the $\delta^{15}N$ values of two marine organisms preserved in formalin. A more recent

study by Fanelli et al. (2010) on deep sea macrofauna showed that after 12 months none of the five species preserved in formalin had any significant differences in $\delta^{15}N$ whereas only one of the organisms showed some $\delta^{15}N$ depletion after 12 months preservation in ethanol. In addition to the effects of preservatives being species-dependent, it may also be possible that bottles of various materials are used as storage vessels in different studies and that this could have an effect on the isotopic signature, especially for samples stored over long periods of times. The two types of preservatives used here may also interact differently with containers; for example ethanol or formalin in a glass container or a particular type of plastic.

The sample preservation times of 1, 3 and 9 months have an informative outcome with *E. frigida* and *U. vulgaris*, both reaching an enrichment peak at three months in either EtOH or FM and becoming depleted again by 9 months. The most dramatic change was for *E. frigida* where samples in ethanol and formalin became enriched having a difference of the means compared against the reference of +1.3 ‰ and +2.5 ‰, respectively, after 1 month. This pattern continued after 3 months having a mean difference compared to the mean for reference for $\delta^{13}C$ of +2.5 ‰ for ethanol and +3.3 ‰ for formalin, relative to the reference samples. By 9 months *E. frigida* values were expected to either continue being enriched or reach a plateau of enrichment. Instead the results indicate that depletion occurred somewhere between 3 and 9 months, with values returning to those similar to month 1. It could be argued that this was due to external contamination. However, this pattern was again observed for *U. vulgaris* and it also occurs in both treatments, as such, removing any speculation about possible contamination. No obvious or conclusive explanation could be deduced for these patterns. One possibility was that the material of the storage container, in this case PVC honey jars, might have reacted with the preservation chemicals in such a way as to cause the observed outcome.

It was also surprising that both preservatives displayed a similar pattern. For instance, ethanol is a solvent and as such one would expect lipids to be removed over time causing the tissue to become enriched, as demonstrated by Kaehler and Pakhomov (2001), and as observed here for the first three months. In this study, formalin caused carbon isotope values to become enriched. This was in contrast to the general trend of

formalin depleting carbon-values highlighted by the literature review of Barrow *et al*. (2008). However, a recent study completed on zooplankton shows a small 1.1 ‰ enrichment (Feuchtmayr & Grey 2003), seeming to agree with the initial enrichment observed in this study. It has been suggested that the main reason for this could be that formalin binds to animal tissue, as such giving a value closer to that of formalin (Kaehler & Pakhomov 2001, Sarakinos et al. 2002).

In general, ethanol and formalin had similar effects on the $\delta^{13}C$ values of both species over time. *Euphasia frigida* appears to be more susceptible than *U. vulgaris*. For both species an anomalous enrichment-depletion pattern occurs over time, which was proposed to be the result of storage containers.

5.6 Conclusion

This study clearly shows that preservatives and dyes have an effect on the $\delta^{13}C$ and to a lesser extent on $\delta^{15}N$. Formalin produced the largest difference compared to the reference for either $\delta^{15}N$ or $\delta^{13}C$ in either species. As for the dyes the results were variable and more studies are needed in order to understand their full effect on the final results. The study also found that the effect of bathing samples in 2% HCl had no significant effect on results, in this respect I believe that acidification of zooplankton could be avoided as it will not remove the chitin proportion of the exoskeleton. The study also suggests that samples that are preserved for too long may ultimately not give very reliable results as both organisms were not affected the same way. This is especially important for trophic linkage studies as the organisms $\delta^{15}N$ and $\delta^{13}C$ values changed considerably from the reference. The carbon isotope ratios became enriched for both species over time, but not to the same extent with *Undinula vulgaris* becoming more enriched than *Euphasia frigida*. This study also suggests that the materials of the storage containers may possibly interact with chemicals in preservatives to alter isotopic signatures of the preservative and ultimately the organism within the container. It is suggested that studies on the effect of storage in containers of different materials on isotopic signatures are needed. In addition, it should be considered that samples stored for different lengths of time may not be comparable. It is therefore

suggested that lengthy storage in preservatives should be avoided and where unavoidable the variable effects of time should be borne in mind. Preservation and staining considerations may have more important for trophic linkage studies than previously thought.

5.7 Acknowledgements

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CHAPTER 6

The effects of freeze-thaw periods and drying methods on isotopic and elemental carbon and nitrogen in marine organisms, raising questions on sample preparation

6 The effects of freeze-thaw periods and drying methods on isotopic and elemental carbon and nitrogen in marine organisms, raising questions on sample preparation.

6.1 Abstract

Stable isotopes are an increasingly important tool in trophic linkage ecological studies. In studies of large marine animals, isotopic sampling is often given secondary priority to sampling for diversity and biomass aspects. Consequently, isotopic samples are frequently collected subsequent to repeated freezing and thawing of animals, and results of these studies are often based on the assumption that this does not affect isotopic values. My study tested this assumption and examined the difference between oven- and freeze-drying on isotopic values and elemental carbon-to-nitrogen (C:N) ratios. Values for $\delta^{15}N$ and $\delta^{13}C$, percentage nitrogen and carbon, and the C:N ratios were determined from tissues of six marine species, including invertebrates and fish, as i) fresh samples, ii) samples thawed once and iii) samples thawed twice. Drying method, thawing treatment and their interaction did significantly affect $\delta^{15}N$ and $\delta^{13}C$ isotope values for all species. Oven-dried samples had slightly more enriched $\delta^{13}C$ and $\delta^{15}N$ values than freeze-dried samples, although not significant in most instances. For most species, oven-drying produced lower percentage carbon and nitrogen values than freeze-drying for samples that had been thawed once, but C:N ratio was unaffected by drying method. Repeated freezing and thawing did not affect isotope values, but did decrease percentage carbon and nitrogen for both desiccation methods. I recommend drying samples from fresh wherever possible, and a careful choice of desiccation method in light of the fact that most lipid-models are based on oven-dried samples and oven-drying could cause enrichment of ^{15}N or ^{13}C through evaporation of volatile compounds richer in lighter-isotopes such as some lipids. Finally, I recommend that further studies on the specific effects of freezing and desiccation on elasmobranchs is needed. Overall I recommend the use of freeze-drying when possible and to use the samples from freshly caught organisms.

6.2 Introduction

Stable isotopes have become an important tool in ecological studies concerned with nutrient flows and trophic linkages (Fry 1991, Angradi 1994, Vander Zanden et al. 1999, Kaehler & Pakhomov 2001). The ratio $^{15}N/^{14}N$ differentiates the trophic positions of organisms, with higher-level consumers reflecting nitrogen isotope ratios with substantially heavier $\delta^{15}N$-values (enriched by up to +3.4‰) compared to their dietary items (Ponsard & Amlou 1999). On the other hand, $^{13}C/^{12}C$ is used to track the flow of organic matter through organisms in a food web, as consumers reflect only slightly enriched $\delta^{13}C$-values (+0.5 to +1‰) compared to that of their diet (Fry 1991, Edwards et al. 2002). To assure the most precise isotopic results, samples should be dried and processed for analysis immediately upon collection (Ponsard & Amlou 1999), but since processing relies on access to clean laboratory facilities, field samples – especially those obtained in remote environments – often cannot be processed in the field (Hobson et al. 1997, Kaehler & Pakhomov 2001, Carabel et al. 2009). For this reason, most samples are preserved, stored and processed after a period of time (Ponsard & Amlou 1999, Kaehler & Pakhomov 2001, Carabel et al. 2009, De Lecea et al. 2011a (Chapter 5)).

Isotopic δ-values of freshly prepared samples have been shown to differ from those obtained following various approaches to preservation and fixation, which themselves differ among each other. Post-collection treatments include instant oven-drying (Mullin et al. 1984, Kaehler & Pakhomov 2001), freeze-drying (Hobson et al. 1997), fixation in formalin (Kaehler & Pakhomov 2001, Edwards et al. 2002, Kelly et al. 2006, De Lecea et al. 2011a (Chapter 5)), ethanol preservation (Kaehler & Pakhomov 2001, Kelly et al. 2006, Bugoni et al. 2008, De Lecea et al. 2011a (Chapter 5)) and storage in a formalin-ethanol mixture (Bosley & Wainright 1999, Carabel et al. 2009). Several studies have examined the comparative effects of freezing and preservation or fixation methods on stable isotopes signals (Bosley & Wainright 1999, Edwards et al. 2002, Bugoni et al. 2008), or have studied the effect of long-term frozen-storage on the isotope ratios obtained from different organisms (Kaehler & Pakhomov 2001). Bosley and Wainright (1999) showed that freezing was the only method that did not alter δ-values when compared with other preservation or fixation methods. However, to the best of our knowledge, no studies have considered the effect of repeated freezing and

thawing on stable isotope ratios obtained from marine organisms. I consider the effect of multiple thawing and freezing events on isotopic measurements to be an important issue, since such events frequently arise due to tissue samples for isotopic analysis being collected secondarily from specimen animals that have already been used for species identification, biomass measurements, chemical assay studies, or other studies.

When tissue is frozen ice crystals grow, and this causes the osmotic removal of water, alteration of proteins, loss of cell integrity, and changes in lipid content may occur (Shenouda 1980, Thyholt & Isaksson 1997). This process not only has the potential to affect the isotope values, but could also have an effect on elemental carbon and nitrogen composition, and consequently could affect the carbon to nitrogen (C:N) ratio of samples. Numerous studies use C:N ratios for applications such as lipid extraction models (Sweeting et al. 2006, Bodin et al. 2007, Logan et al. 2008, Tarroux et al. 2010), to assess trophic position (Alamaru et al. 2009) and diet studies (Pearson et al. 2003, Hussey et al. 2010). Elemental carbon and nitrogen values and C:N ratios are also used in environmental modelling, where the stoichiometric proportion of the elements plays a central role (Sterner & George 2000). Consequently, changes in these values due to freezing and thawing could affect the validity of these applications.

There is also a need to understand whether oven-drying or freeze-drying produce isotopic, elemental, and C:N differences depending on whether the samples were oven- or freeze-dried from fresh, once-thawed, or twice-thawed. Any variable effects due to desiccation method could be significant for all isotopic studies, because all samples used for isotopic analysis, whether fresh or preserved, have to be desiccated by oven-drying or freeze-drying prior to isotope ratio assessment. Kaehler and Pakhomov (2001) showed that oven-drying did not alter the $\delta^{15}N$ or $\delta^{13}C$ values. However, a pertinent question to ask is whether oven-dried or freeze-dried samples differ greatly from each other. Furthermore, freezing changes muscle integrity (as discussed above), and if multiple freezing and thawing occurs, it is expected that muscle tissue, water content, and lipid structure will behave differently between drying methods.

In this light, this study sets out to test the hypotheses that multiple freezing and thawing events and different means of drying a) affect the isotopic ratios of $\delta^{15}N$ and $\delta^{13}C$, b) decrease the percentage content of carbon and nitrogen due to leaching of

compounds such as lipids and/or sugars, and c) decrease the C:N ratios of muscle tissue due to carbon loss in the leaching. Finally, I tested the hypothesis that d) the treatment effects of multiple freezing and thawing and the method of drying behave in a consistent manner across multiple species representing a range of habitats and foraging modes.

To test these hypotheses, six marine species were chosen, *viz*. two crustaceans, *Haliporoides triarthrus* and *Penaeus japonicus*, the mollusc, *Veladona togata*, two teleost fish, *Chaunax pictus* and *Otolithes ruber*, and one elasmobranch, *Squalus megalops*. Differences in their physiology and anatomy could conceivably lead to different effects of desiccation and freezing on nitrogen and carbon isotope signals and elemental values of muscle tissue. For example, *S. megalops*, like other elasmobranches and unlike the teleosts, have muscle tissue that contains urea for osmotic balance (Fisk et al. 2002), which could alter the $\delta^{15}N$ values in response to the drying method and thawing (Fisk et al. 2002, Estrada et al. 2003). Similarly, *Veladona togata*, being a cephalopod, has a musculature with densely packed muscle fibres creating a 'muscular-hydrostat' (Kier & Thompson 2003), dissimilar to the musculature of the other five species.

The overarching aim is therefore to evaluate whether or not some of the well-established methods that are considered routine for ecological isotope studies, whether terrestrial or marine, produce an unbiased reflection of the true isotope ratio and elemental composition of carbon and nitrogen in muscle tissue.

6.3 Material and methods

6.3.1 Sample collection and study species

During a research expedition off the east coast of South Africa (KwaZulu-Natal province) on the industrial crustacean trawler *Ocean Spray*, six different species of marine animals were collected to study the effects of freezing and thawing on their isotope ratios, the elemental carbon and nitrogen values of muscle tissue, and their corresponding C:N ratios. The organisms collected were *Haliporoides triarthrus* (29^{o}

58.990 S; 31° 14.360 E; depth – 425 m), *Penaeus japonicus* (28° 44.050 S; 32° 14.700 E; depth – 31 m), *Veladona togata* (29° 38.990 S; 31° 46.600 E; depth – 446 m), *Chaunax pictus* (29° 10.325 S; 32° 05.371 E ; depth – 293 m), *Otolithes ruber* (29° 12.973 S; 31° 35.344 E; depth – 29 m) and *Squalus megalops* (28° 42.380 S; 32° 07.555 E; depth – 108 m).

6.3.2 Sample preservation and experimental layout

For each species, five individuals were set aside as replicates – these were of similar size to avoid possible diet differences or other ontogenetic-related effects. Once the animals were brought onto the deck, small pieces of muscle tissue were collected immediately from each of the replicate individuals for all species and set aside in Eppendorf microcentrifuge tubes as unprocessed samples (the *fresh* treatment); these were immediately oven dried at 60 °C for 48 hours. For *V. togata* the muscle was collected from the back of the head, and for the elasmobranch and teleosts it was taken from the caudal peduncle on the same side of the fish at all times. For the two species of euphausid the shell was removed from the abdomen from where tissue was collected. Great care was taken to ensure that non-muscle tissue (skin, bone, exoskeleton, intestine) was excluded from the samples. The remnants of the animals were placed individually into zip-seal packets and immediately frozen for the ensuing freezing and thawing treatments. Individual storage in the manner just described prevented the contamination by liquids leaching from other organisms during freezing and thawing.

In addition to taking samples from the animals when freshly caught, tissue was also taken after being frozen/thawed once and frozen/thawed twice (freeze/thaw cycle treatment with levels *fresh*, *thawed × 1, thawed × 2*). The duration from when the samples were frozen immediately after collection to the first thawing was seven days, and this was followed by another five days until the second thawing. Thawing involved placing the zip-seal packets at ambient air temperature and leaving the samples to defrost for a few hours until they felt thawed to the touch. For the thawed × 1 and thawed × 2 treated samples, two cubes of ~0.5 cm^2 muscle tissue were collected from each individual sample and placed in separate Eppendorf microcentrifuge tubes. One of the tubes was then immediately transferred to an air circulating oven for drying

(60 °C for 48 hours), while the other tube was placed in a freezer (-20 °C) for pre-freezing for 15 hours before placing into a freeze-dryer for desiccation by sublimation of water. The drying method treatment is therefore another factor in the experimental procedure, with levels *oven-drying* and *freeze-drying*.

Once dried, all samples were homogenised and weighed into tin capsules (SANTI® Analytical, Teufen, Switzerland). For isotope analysis ~1.00 mg dry mass was required to yield sufficient nitrogen and carbon for analysis. The samples were analysed by Dr Sven Kaehler at IsoEnvironmental Isotope Facility at Rhodes University, Grahamstown, South Africa, using an ANCA SL Elemental Analyser coupled to a Europa Scientific 20-20 IRMS (Isotope-Ratio Mass Spectrometry) (Sercon Ltd. Crewe, UK). To ensure the quality of the results, each batch of 96 combustions contained 34 known standards; these were, 29 beet sugar and ammonium sulphate (in-house standards) and five certified protein standard casein (calibrated against IAEA-CH-6 and IAEA-N-1). Nitrogen isotope ratios are expressed in terms of their value relative to atmospheric di-nitrogen, while carbon isotope ratios are expressed in terms of its value relative to Pee-Dee Belemnite (vPDB). The analytical precision of the instrument was 0.20 ‰ for $^{15}N:^{14}N$ and 0.12 ‰ for $^{13}C:^{12}C$.

Isotope values used here are expressed in the usual δ-notation (Epstein et al. 1953):

$$\delta(‰) = (R_{sample}/R_{standard} - 1) \times 1000$$

Where R is the ratio of heavy to light isotopes, $^{15}N/^{14}N$ or $^{13}C/^{12}C$, in the sample (R_{sample}) and in the standard ($R_{standard}$), expressed relative to the international standard (Sulzman 2007).

6.3.3 Data analysis

All data were homoscedastic and no data transformation was necessary. I *a priori* suspected isotopic differences among species, but the hypothesis about inter-species differences in itself was not interesting. I nevertheless retained species as a factor in the ensuing statistical analysis, because I was interested in whether or not the two applied treatments, drying method and freeze/thaw cycle, interacted with species, thus

allowing us to test the null hypothesis that the treatments do not vary with species (*i.e.* the effect direction of the different drying methods and freeze/thaw cycles is consistent across species). To this end, I applied a linear mixed model (LME) with species, drying method (levels *oven-drying* and *freeze-drying*) and freeze/thaw cycle (levels *fresh*, *thawed × 1*, *thawed × 2*) as fixed effects, and repeatedly measured replicates undergoing the freeze/thaw cycle treatments as random effects, to determine the main effects of species, desiccation method, and freeze/thaw cycle, as well as the interactive effect of the factors on isotopic δ-values, C and N elemental values, and C:N ratio. In order to arrive at the best-fit model I optimised the Akaike's Information Criterion (AIC) through stepwise addition of new model terms to the LME. Data were summarised after removing inter-subject variability as per the method of Morey, (Morey 2008) and plotted as mean ± 95% confidence intervals (95%-CI); significant differences are inferred from non-overlapping error bars (whiskers). All statistical analyses were done in R 2.13.1 (R Development Core Team, 2010).(R Development Core Team 2010) LME models were fitted using the *nlme* package (Pinheiro & Bates 2000) and model selection facilitated with the help of the *AICcmodavg* package.

6.4 Results

6.4.1 Effects on isotopic δ-values

For $\delta^{15}N$ an LME model with all three factors interacting was selected (Table 6.1). As expected, $\delta^{15}N$ varies strongly with species ($p < 0.0001$), reflecting the different ecological trophic positions of the animals sampled here. In all six species $\delta^{15}N$ values are significantly affected by drying method (Table 6.2), with oven dried samples generally being more enriched compared with their freeze-dried counterparts (Fig. 6.1). In some instances the freeze/thaw cycle also affected the measured $^{15}N:^{14}N$ ratios, particularly for the oven-dried samples of *C. pictus*, *V. togata*, and *H. triarthrus*, and the freeze-dried samples of *H. triathrus*. *Veladona togata* has the greatest variability between individuals shown by the highest variance. This species, along with *H. triarthrus* also has the greatest enrichment for $\delta^{15}N$ oven-dried values after being thawed twice. The

isotopic response was inconsistent, generally becoming more enriched with increasing number of times frozen and thawed, but on occasion also becoming depleted, *S. megalops* (Fig. 6.1). This was confirmed by the significant interaction terms noted in Table 6.2 for $\delta^{15}N$, with freeze/thaw interacting strongly with drying method and species ($p < 0.005$).

Variation among the $\delta^{13}C$ data was best explained by an LME model incorporating all three factors as additive effects only (Table 6.1). For $\delta^{13}C$, ecological influences are also readily observed as evidenced by the significant differences in ^{13}C enrichment between species ($p < 0.0001$; Table 6.2 and Fig. 6.2). As with $\delta^{15}N$, $\delta^{13}C$ also varies significantly between the other main effects of drying method ($p < 0.0001$) and freeze/thaw cycle ($p < 0.005$) (Table 6.2). Since the model does not include any interactive effects the two treatments behave in an easy-to-explain manner, but significant effects are only observed in *S. megalops;* here thawed × 1 samples that had been oven-dried are more enriched with respect to their freeze-dried paired samples and compared to their oven-dried-from-fresh counterparts ($p < 0.0001$ in both cases; Table 6.2 and Fig. 6.2). None of the treatments were significant in the remaining five species.

Table 6.1. Model selection among a set of linear mixed models applied to $\delta^{15}N$, $\delta^{13}C$, % N, % C and C:N data resulting from experiments outlined in the paper. AIC – Akaike's Information Criterion (smaller is 'better'); ΔAIC – change in AIC from one model to the next; AIC_{wt} – Akaike weights, *i.e.* the model probabilities that indicate the level of support given by the model. In the "model term(s)" column, "random" indicates a baseline model with only random effects and no fixed effects. In all cases, the model with the smallest AIC was selected; the expanded statistics are provided in Tables 6.2 and 6.3.

model term(s)	AIC	ΔAIC	AIC_{wt}	Log-likelihood
$\delta^{15}N$				
species x drying x freeze/thaw	134.3575	0.0000	1	-18.6681
species + drying + freeze/thaw	191.2999	56.9423	0	-83.8642
species	270.5984	136.2409	0	-126.8782
drying	288.9405	154.5830	0	-140.3560
freeze/thaw	339.2833	204.9257	0	-164.4692
random	349.8207	215.4632	0	-171.8422
$\delta^{13}C$				
species + drying + freeze/thaw	104.8131	0.0000	0.9587	-40.6208
species x drying x freeze/thaw	111.1035	6.2904	0.0413	-7.0411
species	132.7587	27.9457	0	-57.9583
drying	156.2264	51.4133	0	-73.9989
freeze/thaw	167.2500	62.4369	0	-78.4526
random	174.9917	70.1786	0	-84.4277
%N				
species x drying x freeze/thaw	533.5491	0.0000	1	-218.2639
species + drying + freeze/thaw	602.7976	69.2485	0	-289.6131

Table 6.1. Continuation.

freeze/thaw	669.6757	136.1266	0	-329.6654
species	698.9181	165.3690	0	-341.0380
drying	749.6392	216.0901	0	-370.7053
random	751.1211	217.5720	0	-372.4924
%C				
species x drying x freeze/thaw	954.3783	0.0000	1	-428.6785
species + drying + freeze/thaw	1030.9446	76.5663	0	-503.6866
freeze/thaw	1053.0346	98.6563	0	-521.3449
species	1116.9862	162.6078	0	-550.0720
random	1128.6520	174.2736	0	-561.2578
drying	1128.7124	174.3341	0	-560.2419
C:N				
species	-226.0063	0.0000	0.5847	121.4242
species + drying + freeze/thaw	-225.3216	0.6847	0.4152	124.4465
species x drying x freeze/thaw	-205.0939	20.9123	0	151.0576
random	-139.2572	86.7491	0	72.6968
freeze/thaw	-139.2185	86.7878	0	74.7817
drying	-138.9881	87.0182	0	73.6083

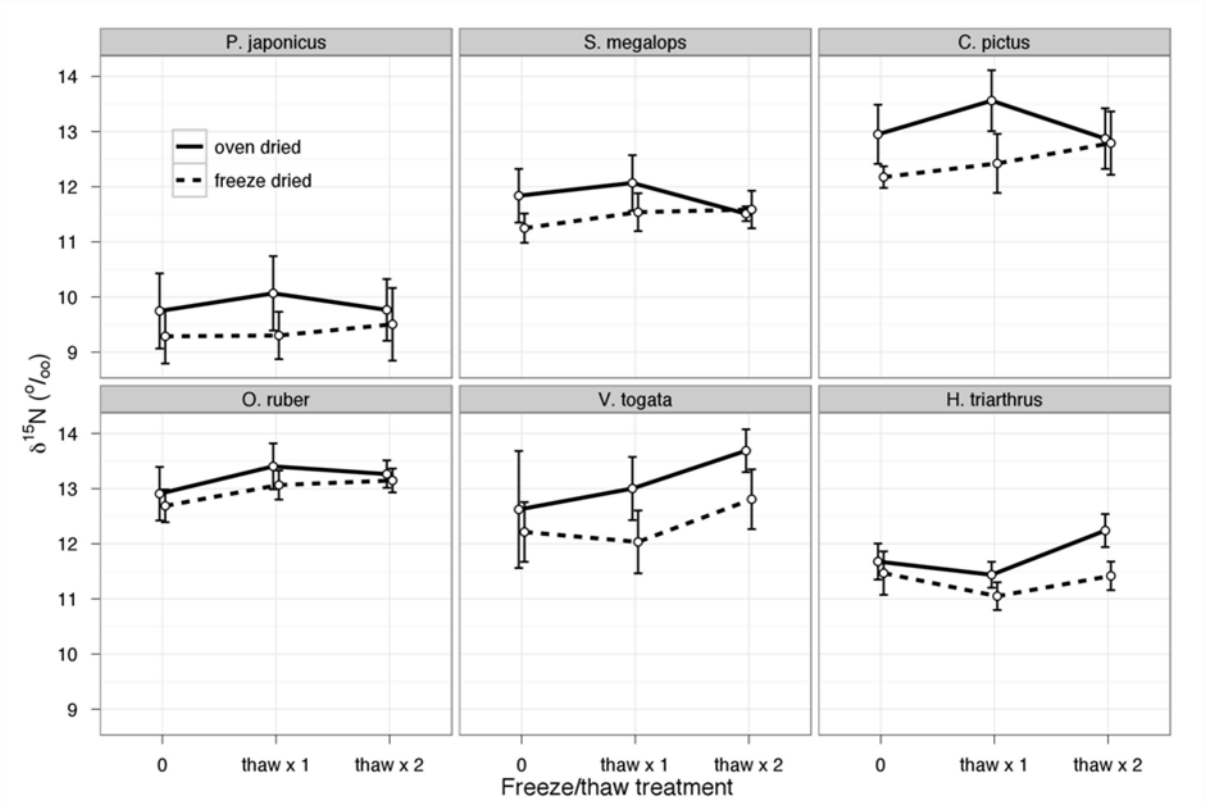

Figure 6.1. Muscle tissue $\delta^{15}N$ values under various treatment regimes and desiccation methods. Error bars indicate the 95% confidence intervals around the means.

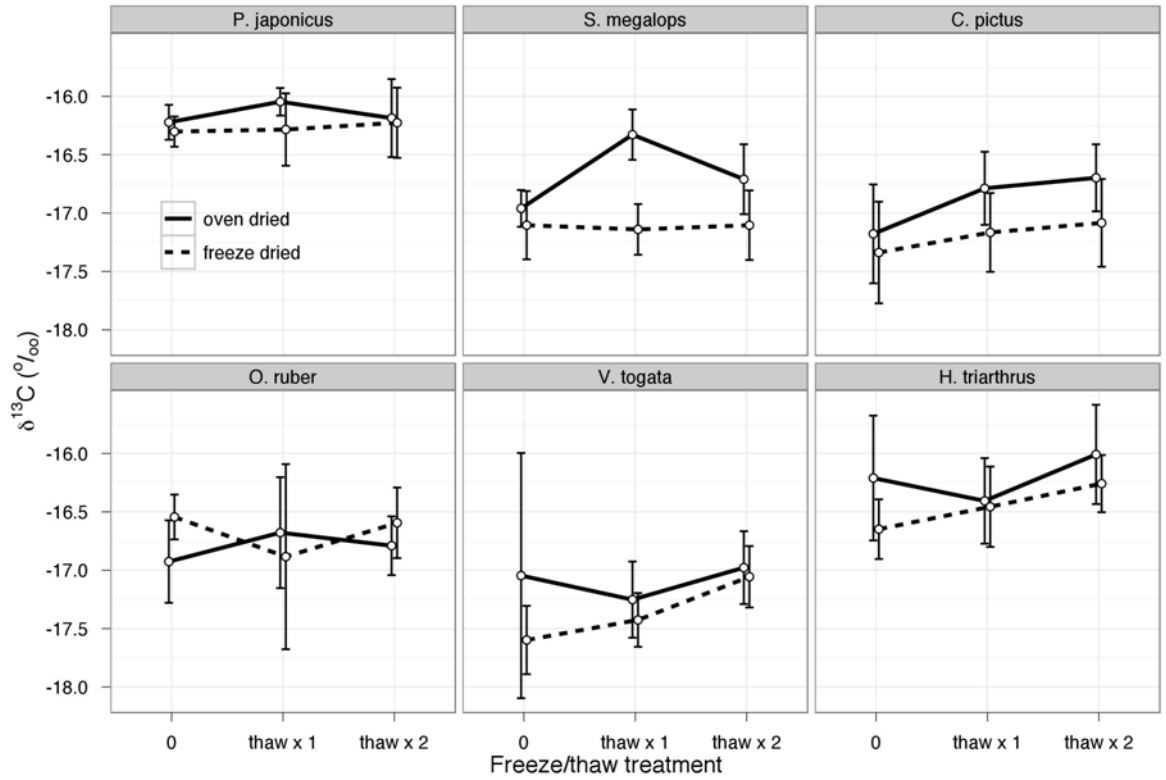

Figure 6.2. Muscle tissue $\delta^{13}C$ values under various treatment regimes and desiccation methods. Error bars indicate the 95% confidence intervals around the means.

Table 6.2. Mixed effects model of freeze/thaw cycle treatment, drying method and their interactive effects on the $\delta^{15}N$ and $\delta^{13}C$ values of six marine species. Asterisks (*) indicate significant treatment effects; na – model terms or interactions not included in model.

	$\delta^{15}N$			$\delta^{13}C$		
	df	*F*-value	*p*-value	*df*	*F*-value	*p*-value
species	5	91.38	<0.0001 *	5	27.58	<0.0001 *
drying	1	174.13	<0.0001 *	1	23.31	<0.0001 *
freeze/thaw	2	23.78	<0.0001 *	2	6.88	0.0014 *
species x drying	5	4.55	0.0008 *	na		
species x freeze/thaw	10	8.86	<0.0001 *	na		
drying x freeze/thaw	2	7.34	0.0010 *	na		
species x drying x freeze/thaw	10	4.02	0.0001 *	na		

6.4.2 *Effects on elemental values*

Elemental composition of N and C was best described by full LME models with all three factors interacting (Table 6.1). The response of %N and %C to the drying and freeze/thaw treatments was indistinguishable, with both showing significant effects between species and levels of the drying and freeze/thaw treatments, as well as for all possible interaction terms (refer to Table 6.3 for all statistical data). The significant interaction terms, as before, make it difficult to see consistent changes in the response variables, with the exception of the pattern of response to the freeze/thaw cycle treatment that differs noticeably between oven- and freeze-dried samples, across most species. The %N and %C of oven dried samples generally remained unchanged from fresh samples (no freezing and thawing) to samples that had been frozen and thawed once only, but then change significantly after the second iteration of the cycle (Figs. 6.3 and 6.4). Freeze-dried samples, on the other hand, changed markedly due to being frozen once only, but then remain unchanged after a second cycle had been completed (Figs. 6.3 and 6.4). In all cases, the change, whether it occurred after being frozen once (*i.e.* freeze-dried samples) or twice (*i.e.* oven dried) is in the direction of decreasing elemental concentration of N and C. *Veladona togata* and *H. triarthrus* are the exception, *V. togata* has little change in the concentration of N or C after being thawed once or twice. *Haliporoides triarthrus* shows an increase of N and C after being thawed once, followed by a decrease.

LME model selection for C:N was not parsimonious, with marginally better support for a simple model incorporating species only over one including all factors fully interacting (Table 6.1). For simplicity – due to the fact that is has a better Akaike weight (Table 6.1) and because of support of the 95%-CI structure in Fig. 6.5 – I favoured the species-only model here. Although C:N ratios differ between species ($p < 0.0001$; Table 6.3), changes were small and insignificant across all species regardless of the treatments imposed on the samples (Fig. 6.5).

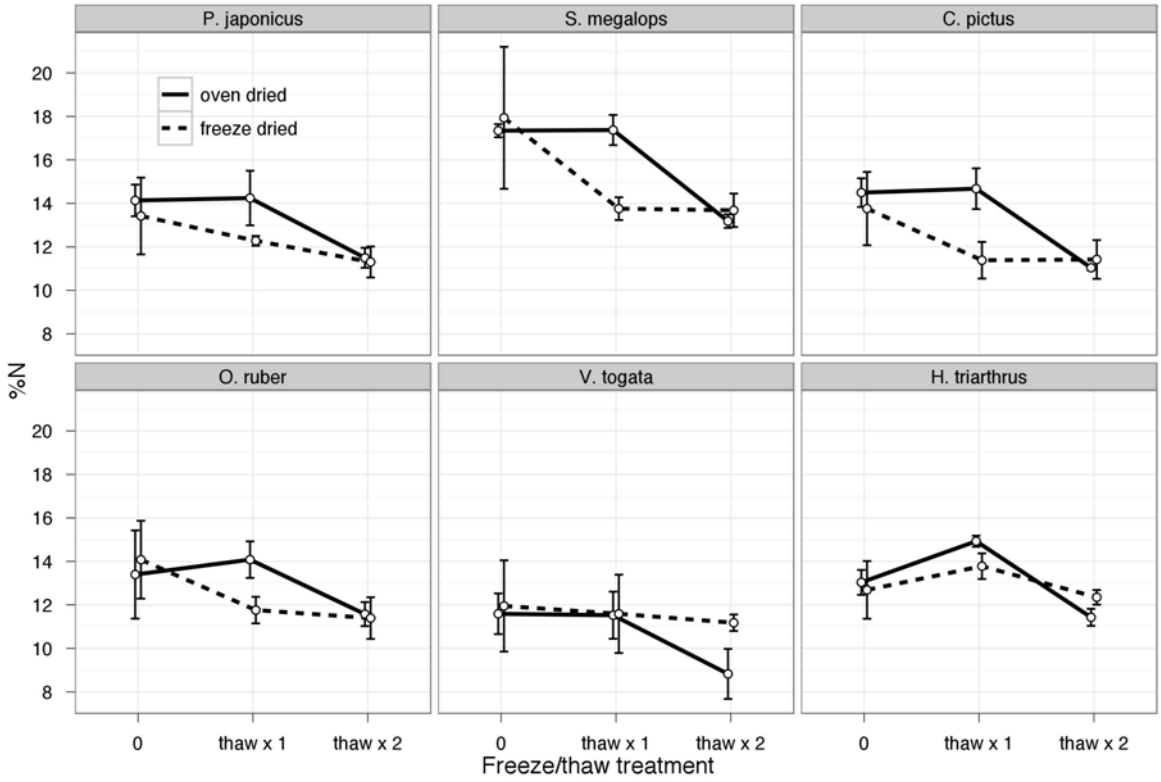

Figure 6.4. Muscle tissue % nitrogen values under various treatment regimes and desiccation methods. Error bars indicate the 95% confidence intervals around the means.

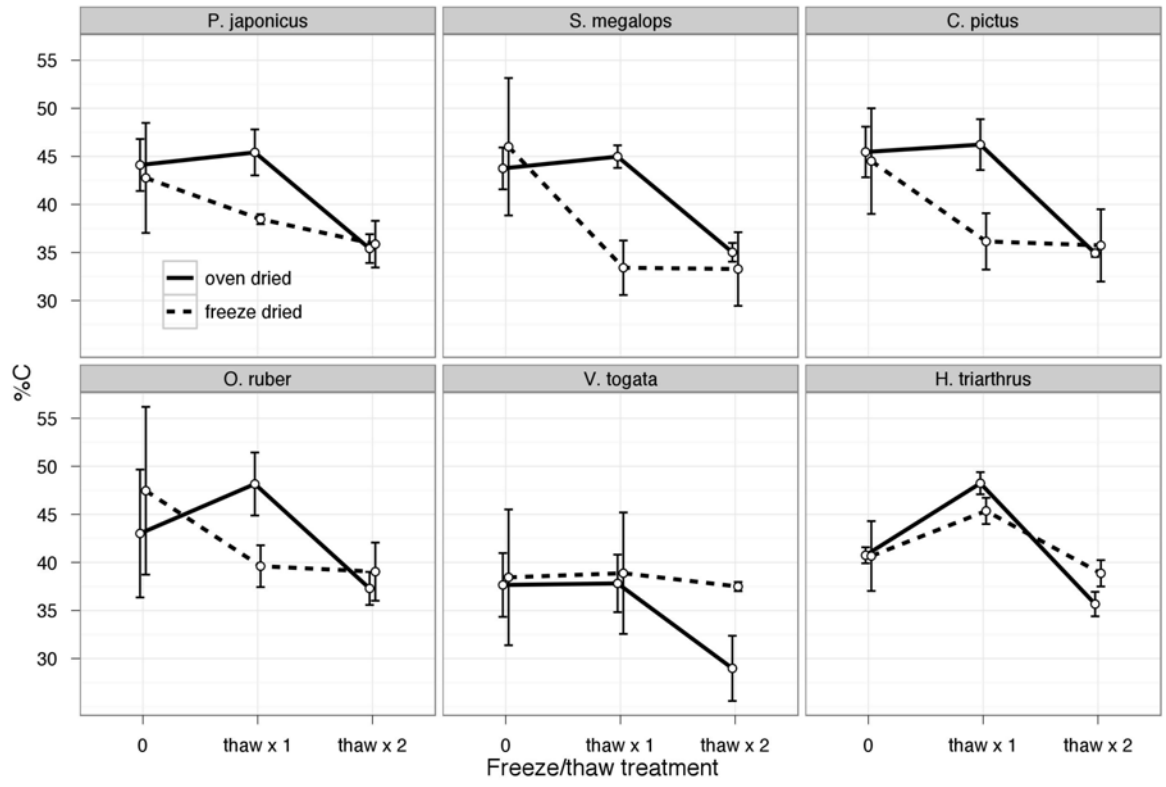

Figure 6.3. Muscle tissue % carbon values under various treatment regimes and desiccation methods. Error bars indicate the 95% confidence intervals around the means.

Table 6.3. Mixed effects model of freeze/thaw cycle treatment, drying method and their interactive effects on the elemental carbon and nitrogen content and C:N ratio of six marine species; na – not applicable as the model terms were not included in the analysis. Asterisks (*) indicate significant treatment effects; na – model terms or interactions not included in model.

	%N			%C			C:N		
	df	*F*-value	*p*-value	*df*	*F*-value	*p*-value	*df*	*F*-value	*p*-value
species	5	61.976	<0.0001 *	5	11.578	<0.0001 *	5	143.56	<0.0001 *
drying	1	13.046	0.0004 *	1	7.394	0.0075 *	na		
freeze/thaw	2	120.529	<0.0001 *	2	113.363	<0.0001 *	na		
species x drying	5	5.585	0.0001 *	5	6.699	<0.0001 *	na		
species x freeze/thaw	10	5.705	<0.0001 *	10	5.495	<0.0001 *	na		
drying x freeze/thaw	2	36.529	<0.0001 *	2	39.949	<0.0001 *	na		
species x drying x freeze/thaw	10	2.195	0.0224 *	10	2.724	0.0047 *	na		

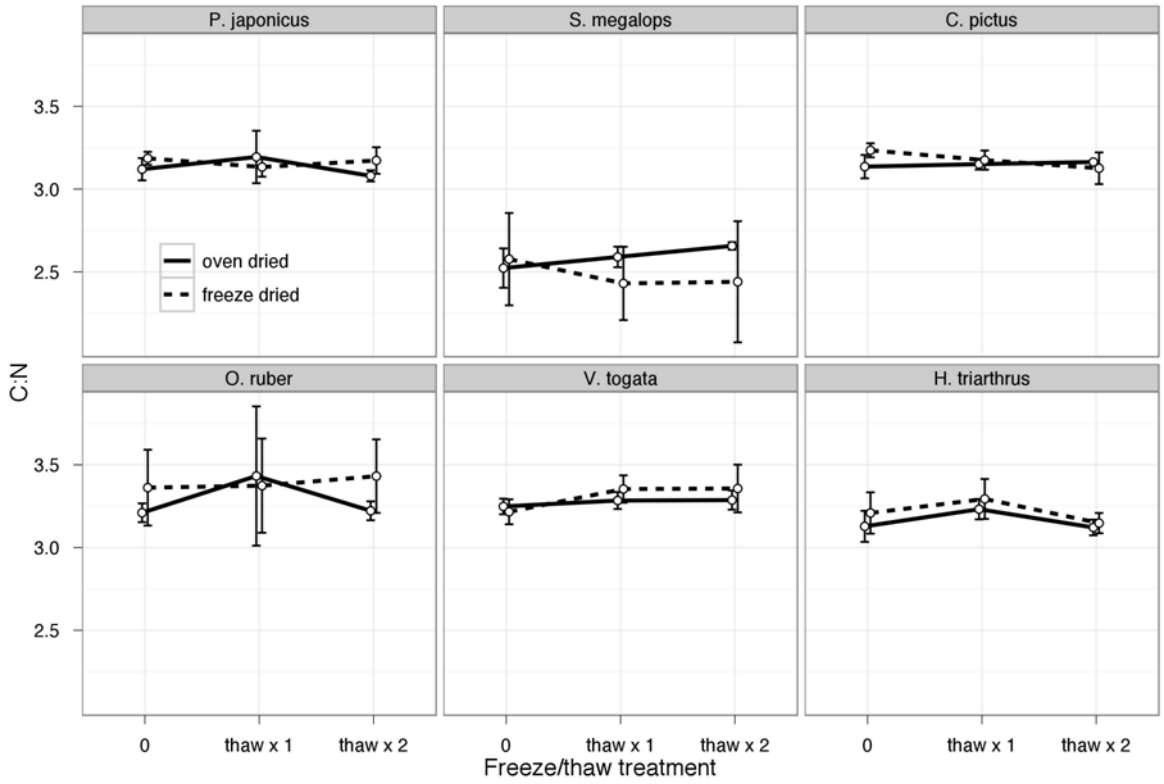

Figure 6.5. Muscle tissue C:N ratios under various treatment regimes and desiccation methods. Error bars indicate the 95% confidence intervals around the means.

6.5 Discussion

The aim of this study was to establish whether freeze/thaw cycle and drying method affected the isotopic ratios of several marine species, their elemental composition, and C:N ratio. The issue raised by this study should not merely be considered for marine organisms, but instead raises the question as to how any biological sample that requires preservation and desiccation, could be affected by routine 'standard' methods.

I found that the use of different drying methods consistently results in significantly different $\delta^{15}N$ and $\delta^{13}C$ values. I also found that oven-drying changes elemental carbon and nitrogen content in samples that had been thawed once, compared to freeze-drying which in most cases only affects these values after samples had been thawed a second time. When looking at patterns of isotope ratios or elemental content in the figures, consistent responses are not always similar when comparing shallow- with deep-water organisms, or invertebrates with vertebrates, indicating that different muscle tissues might behave differently depending on species. I found that there was no consistent trend in isotope signatures with treatment across species, but for elemental carbon and nitrogen values, the majority of species, with the exception of *H. triarthrus* and *V. togata*, demonstrated a similar trend with treatment type (explained below).

6.5.1 Stable isotope ratios

In most cases $\delta^{15}N$ and $\delta^{13}C$ values of oven-dried samples were qualitatively enriched compared to freeze-dried samples. The choice of drying method used could, therefore, alter $\delta^{15}N$ or $\delta^{13}C$ values of some samples, and I therefore recommend that one drying method is to be used per study – freeze dried samples should not be compared with oven dried samples. The slight enrichment observed for oven-dried $\delta^{15}N$ or $\delta^{13}C$ values could result from the heat produced by the oven removing the more volatile lipid compounds, particularly those lipids rich in light carbon isotope, ^{12}C, and the evaporation of aqueous liquid containing dissolved substances rich in ^{15}N. Similarly substances rich in light isotopes of carbon or nitrogen may leach out and evaporate more easily from muscle tissue during the repeated thawing and freezing process of

thawed × 1 and × 2 treatments; this may also explain why in the majority of instances oven- and freeze-dried sample isotopic values do not differ to the same extent in samples that had been thawed for a second time, unlike the fresh samples, where no freezing and thawing or potential leaching had taken place. Enrichment of $\delta^{13}C$ values has been discussed in the lipid removal literature (Pinnegar & Polunin 1999, Post et al. 2007b), while various authors have discussed the enrichment of muscle $\delta^{15}N$ values because of the lipid removal process (Pinnegar & Polunin 1999, Sotiropoulos et al. 2004, Murry et al. 2006); I speculate that these processes may explain some of the patterns seen in this study.

In the case of *S. megalops* the changes in $\delta^{15}N$ could be due to urea leaching from the tissue. Urea is present at high levels in shark tissues for osmoregulation purposes (Fisk et al. 2002). This compound, which is isotopically light (Fisk et al. 2002), may break down into gaseous ammonia during the oven-drying process which is subsequently lost from the tissue, leaving behind shark muscle tissue with more enriched $\delta^{15}N$ values. For all species, including *S. megalops*, the changes in isotope values could be due to the leaching of amino acids from the tissues. Pinnegar and Polunin (1999) suggested that the main reason for tissue $\delta^{15}N$ differences was due to amino acids. What I might have been seeing here was a pool of enriched amino acids left in the tissue, while on the other hand a loss of ^{15}N-depleted nitrogen by-products occurs such as ammonia, urea and uric acid (Macko et al. 1986, Macko et al. 1987, Wolf et al. 2009). If what I suggest was correct, it could be possible that ^{15}N-depleted amino acids were being lost from the muscle tissue creating the observed increase noted in my study.

Looking at the effect of the freeze/thaw cycle in more detail, it was evident that this treatment has different effects on isotopic values in oven- versus freeze-dried samples. For oven-dried samples I found that a single freeze/thaw event followed by oven-drying had a significant effect on $\delta^{13}C$ or $\delta^{15}N$ values in muscle tissue, agreeing with a recent study by Syväranta et al. (2011) and disagreeing with results by Sweeting et al. (2004). Thawing for a second time enriched the $\delta^{15}N$ of *V. togata* and *H. triarthrus*. *Veladona togata* is the only mollusc included in the study and it is plausible that tissues of this invertebrate responded differently to the various treatments compared to the other taxa studied. Feuchmayr and Grey (2003) suggested that enrichment of $\delta^{15}N$

values could be due to leaching following mechanical destruction of the cells while the samples are being prepared, or during thawing. The $\delta^{13}C$ values of *S. megalops* changes significantly after having been thawed once; but then remained similar with a second freezing and thawing. The reason behind this is presently not understood, but I speculated that isotopically light urea (Fisk et al. 2002) leaches from the elasmobranch tissue during initial freezing and thawing. However, this warrants further study as this could simply be an unusual situation or it could be a pattern that generally affects elasmobranch stable isotopic signals.

For freeze-dried samples, thawing treatment differences were smaller than their oven-dried counterparts for both $\delta^{15}N$ and $\delta^{13}C$. This outcome was in agreement with the those of Bosley and Wainright (1999) who found δ-isotopic changes for juvenile winter flounder (*Pleuronectes americanus*) and mud shrimp (*Crangon septemspinosa*) after freeze-drying.

Our results showed that the combined effect of thawing treatment and oven-drying was an important factor affecting the outcome of isotopic studies. It is plausible that single thawing of samples prior to desiccation could change the integrity of muscle tissue, and as such when this freeze-thaw treatment is followed by oven-drying desiccation, which also alters the integrity of the tissue, the variability of the isotope values increases.

6.5.2 Elemental carbon and nitrogen concentrations

Percentage tissue nitrogen and carbon differed significantly depending on drying method. In most cases the greatest differences appeared in samples that had been thawed once. Freeze-drying results in water removal from muscle tissue by the process of sublimation. In contrast, during oven-drying water was instead removed by evaporation, which could also remove some of the more volatile lipid compounds, thereby creating a change in the muscle elemental composition. Freeze-drying therefore minimises the chemical changes in the muscle tissue making this method advantageous over oven-drying (Rovero et al. 1991).

Drying method and freeze/thaw cycle treatment had a significant interactive effect on percentage nitrogen and carbon for all species. As such, samples from the same individual that have been subjected to the same thawing treatment will produce different isotopic values when desiccated through oven-drying than through freeze-drying. For oven-drying, samples of *C. pictus, S. megalops, O. ruber* and *P. japonicus* all showed the same pattern, *viz.* a sudden decreased in percentage nitrogen and carbon after being thawed once. This could be due to the leaching of amino acids and proteins such as casein, which contain carbon an nitrogen (Parsons et al. 1977). Elemental concentrations of carbon and nitrogen in *V. togata* and *H. triarthrus* were the only ones that did not followed the general patterns. *Haliporoides triarthrus* was the only species in which results obtained for both oven- and freeze-dried showed the same pattern.

Because nitrogen and carbon values were affected similarly by the various treatments, carbon-to-nitrogen (C:N) ratios were largely unaffected by either drying method or thawing treatment. Similarly, in a previous study on the Asiatic clam (*Corbicula fluminea*), Syväranta et al. (2011) found no difference in the C:N ratio after freezing. As mentioned in the introduction, % carbon, % nitrogen and C:N are used for environmental modelling (Sterner & George 2000). My findings potentially showed that models could underestimate the carbon and nitrogen sources in organisms leading to erroneous results. The changes observed in C:N ratios due to thawing treatments and desiccation methods, are also of importance, C:N ratios are used for lipid extraction models (Sweeting et al. 2006, Bodin et al. 2007, Logan et al. 2008, Tarroux et al. 2010), trophic position (Alamaru et al. 2009), and diet studies (Pearson et al. 2003, Hussey et al. 2010). As such changes in the ratios could eventually lead to misinterpretation on the trophic position of organisms. Caution should be implemented when dealing with % carbon, % nitrogen and C:N ratios from samples not prepared from fresh tissue.

Our study indicates that there was an important interaction between drying method and thawing treatment. These effects are seen most clearly for percentage nitrogen and carbon, but seemed less important for the isotope values of most species, only significantly affecting *S. megalops*.

6.6 Conclusion

A slight isotopic enrichment for both $\delta^{15}N$ or $\delta^{13}C$ values of oven-dried samples over their freeze-dried counterparts is seen for most species, possibly caused by the removal of volatile, isotopically lighter compounds from the tissue during oven-drying. This provides some impetus for a cautionary approach when considering how to desiccate samples, since alterations in isotopic values could have far-reaching consequences for isotopic applications. Although the number of freeze-thaw events could potentially affect isotopic ratios, I found very little evidence in this regard, apart from $\delta^{15}N$ changes for *V. togata* and $\delta^{13}C$ for *S. megalops*. Desiccation method is therefore likely to be more important than the number of times a sample is frozen and defrosted, except possibly for elasmobranchs. This is important to note, since all tissue samples used in isotopic analysis, even if prepared from fresh, have to be desiccated by some means.

On the other hand, thawing treatment appears to be quite important for percentage carbon and nitrogen values of muscle tissue. Although not significant for all species, percentage carbon and nitrogen changes from fresh samples to those that have been frozen and thawed once; this could be very important for trophic linkage studies should animals be sampled after a varying number of thawing occasions. The drying method also appears to have important effects on percentage carbon and nitrogen, especially when considered interactively with thawing treatment. This could have important consequences for lipid removal models, trophic position studies as well as environmental modelling.

In conclusion, I recommend drying samples from fresh wherever possible to avoid changes in percentage composition of carbon and nitrogen. Additionally, I recommend that the merits and disadvantages of different drying methods are carefully considered; current lipid-models may be based on oven-dried samples and, for large sample batches, oven-drying is more convenient, but could produce losses of volatile compounds richer in lighter-isotopes. I also recommend that further studies on the effect of the specific treatment on elasmobranchs is needed.

Finally, I suggest that in cases where freeze-drying is possible, this method should be chosen over oven-drying. Samples should also be desiccated from freshly caught

organisms and to avoid freezing if oven-drying is the preferred, or only available, desiccation method.

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CHAPTER 7

General Conclusions

7 General conclusions

As has been mentioned throughout the thesis the KwaZulu-Natal Bight ("the Bight") has been of great interest to oceanographic research, and is now of increasing biological interest, as geographically it induces a perturbation on the otherwise persistent north-south flow of the Agulhas Current along the shelf edge. This perturbation causes the development of upwelling cells, counter-currents and eddies on the shelf. These oceanographic processes have until now been thought of as the main drivers of the Bight ecosystem. However, on the whole, since the multi-disciplinary research published in 1988 entitled "*Coastal ocean studies off Natal, South Africa*" (Schumann 1988a), the research in the area has been sparse and inconsistent and until ACEP II, the multidisciplinary project of which research reported in this thesis forms part, little research had been done on the role played by rivers in the overall productivity of this oligotrophic coastline. Some recent research has, however, shown that fluvial processes may play an important role in controlling South Africa's only prawn and a line fishery, which in terms of economics is the most important fishery on the east coast of South Africa. Research provided here provides a far deeper understanding of these processes within the Bight, and supports the notion that oceanographic processes are not the only drivers of the functioning of the system.

The objectives of this thesis fall within those of ACEP II: describing the drivers of the Bight biology and understanding the ecosystem function of the region. There are a few shortcomings of the study, but regardless this thesis has helped clarify the role of riverine input in the Bight ecosystems.

7.1 Shortcomings of the study

As with any good study, it is fundamental to recognise its shortcomings and limitations. Some of the shortcomings of this particular study were due to resources being limited; nevertheless within these constrains there are some more fundamental challenges that need consideration before the full implications of the findings are accepted.

Firstly, in the zooplankton study (Chapter 2), I decided to separate the organisms into size fractions and to focus most of my efforts on separating and identifying those organisms larger than 1,000 μm to the highest taxonomic resolution possible. For the zooplankton smaller than 1,000 μm I decided not to separate them into taxa but instead processed them by size fraction, because identifying and sorting such small organisms into taxa is extremely time-consuming and requires a high level of expertise. I unfortunately did not have the time to develop such expertise within the scope of my Ph.D., nor the financial resources to pay a specialised professional. By analysing the smaller size fractions a better picture of the functioning of the Bight could have been shaped and additionally a comprehensive food-web could have been created.

A further practical recommendation or suggestions for improvement in terms of the zooplankton samples (Chapter 2) emerged from the simultaneous preservative studies carried out in Chapter 5. Zooplankton were preserved in formalin and, as I explained in Chapter 5, formalin has an effect on the sample stable isotopes signatures. Ideally in future studies, zooplankton should be frozen or instantly dried. Regrettably for my study and mainly due to logistics, the experimental results for preservatives (Chapter 5) were not obtained until after the wet season samples for Chapter 2 had been collected; at that stage I decided that the dry season samples should be preserved in the same way to standardise data across sampling seasons. One of my recommendations from the preservative study for other authors is that they retain one method of sample preparation and preservation throughout their study to ensure samples are comparable and that errors introduced by preservatives are at least universal in their samples.

In the demersal study of Chapter 3, I attempted to produce a comprehensive study of the demersal ecosystem, its main drivers and trophic linkages. There were, however, some caveats for the various abiotic and biotic components sampled within the demersal system. First of all, the sediment maps, although useful and insightful could only be produced to a maximum depth of ~180 m, the maximum sediment sample depth, while some of the demersal organisms were obtained from greater depths (>560 m in both seasons). This was unavoidable due to the high Agulhas Current speeds closer to the continental margin affecting the operation of the VanVeen grab at greater depths. Additionally macrobenthos, one of ACEP's foci, would ideally have been

included in this study. They were not included for three reasons. Firstly, macrobenthos samples were preserved in formalin, unlike the demersal organisms or the sediment samples, which were frozen, making it difficult to compare the isotopic signatures; secondly, due to the period of time it takes the macrobenthic team to sort these samples, there were only fauna from a very small selection of stations and from one season available to work with. Thirdly, due to lengthy processing times, macrobenthic samples would not all have been preserved in formalin for the same period of time, leading to further uncertainties being introduced into the isotopic signatures. All things considered I believe that including the macrofauna would have increased the number of questions rather than answers, and I therefore came to the difficult decision of omitting this important group from the study. This is a gap that mars the full understanding of the demersal/benthic ecosystem.

There were historical and resource limitations to the scale of the seasonality study (Chapter 4). I would like to have looked at the seasonality of the entire Bight. But with samples collected in other studies (prior to the Ph.D.) being limited to the Thukela Bank, and with my own resources being limited, I decided to examine only this area. In my opinion, this was not a major shortcoming, as the Thukela Bank is an area large enough to provide insights into seasonal changes in the Bight functioning. Furthermore, I assumed that if there was any seasonality in the Bight linked to the Thukela River runoff that this area would be the most likely to show it. The biggest issue in this study was replication, while I had three replicates for each season for total suspended solids; I only had two replicates for each season for organisms. The effects of seasonality are clearly observable, but further replication would have been ideal to strengthen the argument.

One possible way for authors of similar studies to improve upon the general methodology applied in Chapters 2 to 4 would be to minimize the use of preservatives. This was not possible with the facilities available on board of the research vessel in this instance and is often a logistical limitation in sample collection for many other isotope studies. The methodological chapters (Chapter 5 and 6) were conceived against this backdrop with the aim of informing how preservation and sample treatment could have affected outcomes. These studies highlight the importance of careful

consideration for sample handling. It is important to be aware of these limitations and to at least apply a consistent method and duration of preservation, where preservation is indeed necessary.

Despite the afore-mentioned shortcomings, this thesis provides new and insightful knowledge on the ecosystem functioning of the Bight. The thesis also provides important insights on biological sample handling for isotopic studies.

7.2 Synopsis of findings: Ecosystem studies

Within the Bight there are a series of oceanographic processes which are considered to be of great importance to the biology of the Bight. These are; i) an upwelling cell near Richards Bay, caused by the increase in width of the continental shelf and ii) a cyclonic lee eddy that forms in the south of the Bight, causing water to become trapped in the Bight and deeper water to flow onto the continental shelf. Additionally, there are a series of rivers depositing their load into the Bight, with the Thukela River, the third largest river in southern Africa, being the most important. Oceanographers have argued that the most important process controlling the production and hence biology of the Bight was the intermittent upwelling cell where high nutrient levels can be measured, while more recent fisheries studies have demonstrated some importance of local rivers such as the Thukela River. In this light I attempted to identify the origin of nutrients and organic matter (OM) entering the Bight and subsequently to identify which phenomena were important drivers of the biology of the Bight. Finally, I sought to provide preliminary insights into the food-webs of the Bight, which are poorly understood.

It is important to note at the outset that this study did not find evidence of upwelling occurring in either of the sampling seasons. Although unforeseen, this allowed me to investigate what role the riverine influence has when the upwelling is not happening. My study provided empirical evidence that the riverine OM plays an important role in periods of flooding or as a fall-back nutrient source when the upwelling is not occurring as was supposed in earlier studies (Meyer et al. 2002, Hutchings et al. 2010).

In the pelagic environment in Chapter 2 I sought to test the hypotheses that i) oceanic and fluvial sources differ in terms of isotope ratios of their N- and C-bearing materials and ii) that a spatial gradient across the Bight exists in terms of the dominant nutrient source driving zooplankton production. I found that there were isotopic differences between fluvial and oceanic total suspended solids (TSS). According to TSS $\delta^{13}C$ signatures, TSS of Thukela riverine origin dominated only the Richards Bay area in the wet season and the Thukela Mouth and Mid Shelf areas in the dry season. The riverine dominance of the northern region of the Bight indicated that upwelling was not occurring at the time of sampling, also showing the occurrence of a northwards flowing current. The Mid Shelf station was the most productive station during the wet season, with a prevailing diatom (*Thalassionema itzschiodes)* bloom, which a different ACEP team hypothesised was the result of water from an upwelling event being transported south. Therefore the most important source of primary C as a biological driver in the pelagic environment was the Mid Shelf TSS, which most likely originated in an old upwelling event that was transported south. Hence, the Bight was pelagic ecosystem was marine-driven in the two seasons sampled in 2010.

I did not find a clear spatial gradient in terms of the dominant nutrient source driving zooplankton production. I did, however, find that the zooplankton in the Bight were being transported by currents of the region, and that the suggested path they took matched closely the water currents described in the literature for the Bight. Most of the zooplankton were found to have been feeding in the Mid Shelf prior to their being transported elsewhere, which strongly supports the importance of the Mid Shelf station on the productivity of the entire Bight in the wet and dry seasons.

Moving into the demersal ecosystem (Chapter 3), I sought to determine whether oceanographic or fluvial processes were important drivers of the system, whether demersal animals fed on the benthos and to get an idea of the general demersal community trophic pathways. I found that the estuaries of the Bight did play an important role as an OM source in both seasons, as hypothesised. According to Bayesian mixing models, the majority of OM present in surface sediments from the Bight's continental shelf originated from estuaries. Towards either the farthest northern or southern edges of the Bight, close to the upwelling and eddy areas, the riverine

input remained significant, but OM of marine origin played a stronger role (Fig. 3.6.). This agrees with my hypothesis that isotopic signatures of the sediment OM do vary slightly spatially in line with the location of oceanographic and fluvial processes, but the spatial isotopic variation was small overall.

The riverine influence was strong in both the wet and dry season in terms of $\%C_{org}$, but, as demonstrated, the greatest levels of OM occurred in the wet season, with levels dropping but still noticeable in the dry season. Relatively high levels of $\%C_{org}$ were seen as a plume extending from the Thukela River out over the continental shelf in both seasons. This once again highlights the importance of riverine OM in the Bight.

The demersal animals collected at the same time as the sediments showed little isotopic variability regardless of location, depth, functional group, feeding mode, species, or other factors. This was mainly attributed to the omnivorous nature of the majority of organisms and the low variability of the sediment OM isotopic signatures. When constructing the food-web, OM from sediments was used to substitute macrobenthos, (which were probably the direct dietary item accessed by the demersal fauna) in the mixing models, my study found that a large number of organisms collected were feeding on the OM from the sediments (via macrofauna), as hypothesised. As the OM from the Bight was found to be of riverine origin as mentioned earlier, this strengthened the importance that the riverine input plays in the ecosystem of the Bight.

Following from the finding that the rivers play such a major role in the demersal ecosystem of the Bight, the question of seasonality was raised (Chapter 4). That is, does the estuarine OM play a major role as a food source in both seasons in different years? In other words would organisms use a different source of food during the well-defined dry and wet seasons? I found that the OM $\delta^{13}C$ signature derived from the Thukela River TSS changed seasonally, as expected, in the three years sampled, with the wet season having more enriched $\delta^{13}C$ values than the dry season, and *vice versa* for $\delta^{15}N$. Seasonal differences were also found for animals, with wet season $\delta^{13}C$ values being more depleted than those of dry season. The animal's ^{13}C-enrichment occurring on the dry instead of the wet season was attributed to tissue isotopic incorporation times. Seasonality was not evident for $\delta^{15}N$, which was attributed to a series of factors, including nitrogen excretion and omnivory behaviour.

As mentioned throughout the thesis, the Bight is considered to be an oligotrophic environment (Bustamante et al. 1995). Based on this premise, I hypothesised at the beginning that riverine influence has been greatly underestimated and plays a more important role than oceanographers have suggested and as already discussed, this was the case. This is not to say that the oceanographers were incorrect in their findings, but rather that the focus of their research is on what drives primary productivity in the oceans, resulting in their focus on inorganic nutrients. On the other hand, scientists working in estuarine or coastal biology focus more on particulate organic matter (POM), a food supply that has been recognised as important in many studies, while oceanographers do not account for it. As such, there is an inconsistency between biologists and oceanographers in their consideration of what drives the food supply of an ecosystem and there is a need for both groups to consider a variety of sources.

7.3 Synopsis of findings: Methodological studies

Chapter 5 and 6 were designed to inform how preservation/fixation and sample treatment could have affected the interpretation of data and outcomes on ecological studies. These two methodological studies valuably increase the current knowledge on sample handling.

Chapter 5 examined how the stable isotopes signature of two zooplankton species could be affected by i) different preservation methods, ii) dyes, iii) acidification and iv) long term storage (in preservatives). The findings clearly showed that preservatives and dyes have an effect on the $\delta^{13}C$ and $\delta^{15}N$ values, to a lesser extent on the latter, with storage in formalin for 1 month producing the greatest effect on isotopic signatures. Dyes have an effect on the final stable isotope signature, but the results are variable, while acidification of samples in 2% HCl had no effect on the final results. Finally, the effect on stable isotopes from preservation of samples over a long period of time (1, 3 and 9 months) is different for each preservative and each species. The findings, therefore, suggest that samples should be dried or frozen in the field and in cases where this is not possible it is advisable that research be conducted as to how each species stable isotopes signature might be affected. I also call into question the

need for zooplankton sample acidification as this process only removes calcium carbonate but will not remove the chitin portion of the exoskeleton. Furthermore acidification increased the uncertainties in the $\delta^{15}N$ values. On the basis of these findings I also recommended that the use of samples that have been preserved for long periods of time for stable isotopes research should be considered carefully, as samples preserved for different periods of time will not be comparable.

Chapter 6 investigated the effects of repeated freezing and thawing and of two different desiccation methods (oven- *vs.* freeze-dried) on the stable isotopes signature and % carbon (C), % nitrogen (N) and carbon:nitrogen (C:N) ratio of muscle tissue. Oven-dried samples have more enriched ^{15}N and ^{13}C values than their freeze-dried counterparts. This was considered to be caused by the removal of volatile, isotopically lighter compounds from the tissue during oven-drying. For stable isotopes signatures, desiccation method rather than repeated freezing and thawing plays a more important role. On the other hand, both thawing treatments and desiccation methods plays an important role on %C and %N. Based on these studies I recommend that the merits and disadvantages of different drying methods are carefully considered, as they have different effects on the %N and %C, which could consequently affect the final results of lipid removal models.

7.4 Recommendations for future research

The Bight has proven to be more intricate than originally thought, at least from the point of view of stable isotope. Thanks to ACEP II, momentum was provided to allow for this oceanographically and biologically important area to be studied in depth. It also provided the platform for multi-disciplinary research to better understand the ecosystem functions. However, ACEP II has by no means answered all the questions in detail, for instance, upwelling did not occur in either of the two seasons sampled, and as such its overall importance could not be assessed. Also, ACEP II has created further research questions for the future; therefore my first recommendation is for another multi-disciplinary study to benefit from the momentum provided by ACEP II.

Additional recommendations arise from this thesis. While trying to understand the zooplankton food-web functioning (Chapter 2), I realised that in the literature there is a lack of in-depth studies on zooplankton food-webs. In the majority of stable isotope studies zooplankton were only analysed at the broad taxonomic level, without taking into consideration that within these groups there might be different trophic positions or feeding guilds. Furthermore, studies that use zooplankton size fractions as samples, as I did, can be erroneous, since size fractions could include primary or secondary consumers as well as eggs; the later would have the parents stable isotope signature. Overall there is a need for stable isotope zooplankton studies to follow a standard protocol in order for them to become comparable. There appears to be an important gap in the literature with respect to zooplankton tissue isotopic fractionation times. Isotopic fractionation times are of great importance in the study of other organisms (e.g. birds, mice, fish, etc.); I believe that this kind of data would also be of great use and importance in zooplankton isotopic studies, especially in cases such as mine where links to the underlaying hydrodynamics are inferred.

I found that the biology of deep-sea animals on the east coast of South Africa, such as those studied in Chapter 3, was very poorly studied at best. Gut content studies would have greatly add to creating the food web presented in Chapter 3. However, gut content studies were unrealistic during the period of time of a Ph.D., but I took the opportunity to collected whole stomachs of fish during this thesis and preserved them for future research. Otolith bones from some of the fish were also collected should extra funding become available in the future to study the life history of these fish. Trophic research is also needed for the macrobenthos of the Bight as this would provide an important link between the sedimentary organic matter and the demersal organisms. These samples should become available in the future once the macrobenthos team has finished processing them and it will help increase the understanding of the Bight ecosystem functioning.

Extraction of sufficient quantities of organic matter from sediment samples (Chapter 3) proved to be difficult, time consuming and expensive, because the samples had low OM levels and the instruments can only analyse small sample sizes at one time, which will contain insufficient amount of carbon and nitrogen. Consequently, samples had to

be analysed more than once in order to obtain useful data. This calls for a need to develop a method by which OM can be concentrated from the sediments and subsequently analysed.

7.5 Summary

In conclusion, in this thesis I demonstrated that the demersal ecosystems, and to a lesser extent the pelagic ecosystem, of the Bight were dominated by estuarine organic matter input and that at least in the absence of upwelling, the organic matter in the runoff from land that enters the Bight via rivers is an important food source. Given the importance of this estuarine organic matter in both seasons and its prevalence in the Bight sediments, it seems likely that it may still be a significant food source when upwelling does occur or at the very least that it represents an alternative food source for primary production in the absence of upwelling. Similar research is warranted for comparative periods when upwelling is occurring. Thus, as is becoming the trend in ecological research, and with climate change in mind, there is a need for long term monitoring to fully understand the role of estuarine input in the biology of the Bight.

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APPENDICES

Appendix A: Zooplankton wet season mixing model results showing percentage diet contribution from TSS from different locales

		Durban Eddy		Thukela Mouth		Middle Shelf		RB South		RB North	
Location	**Species/Taxon**	**Surface**	***Fmax***	**Surface**	***Fmax***	**Surface**	***Fmax***	**Surface**	***Fmax***	**Surface**	***Fmax***
Durban Eddy											
	Cephalopoda larva	0.08 (0.01 - 0.32)	0.05 (0.00 - 0.19)	0.09 (0.01 - 0.31)	0.07 (0.01 - 0.31)	**0.17 (0.01 - 0.42)**	**0.15 (0.01 - 0.44)**	0.04 (0.00 - 0.14)	0.04 (0.00 - 0.15)	0.05 (0.00 - 0.18)	0.05 (0.00 - 0.19)
	Ferosagitta sp.	0.05 (0.00 - 0.24)	0.03 (0.00 - 0.12)	0.05 (0.01 - 0.23)	0.05 (0.00 - 0.30)	**0.38 (0.03 - 0.57)**	**0.18 (0.01 - 0.60)**	0.02 (0.00 - 0.09)	0.02 (0.00 - 0.11)	0.03 (0.00 - 0.13)	0.03 (0.00 - 0.12)
	Flaccisagitta enflata	0.08 (0.01 - 0.37)	0.03 (0.00 - 0.12)	0.04 (0.00 - 0.17)	0.05 (0.00 - 0.29)	**0.27 (0.02 - 0.47)**	**0.25 (0.04 - 0.51)**	0.03 (0.00 - 0.10)	0.03 (0.00 - 0.12)	0.04 (0.00 - 0.17)	0.03 (0.00 - 0.13)
	Panaeus sp.	0.07 (0.01 - 0.27)	0.08 (0.01 - 0.31)	0.05 (0.00 - 0.17)	0.07 (0.01 - 0.28)	0.05 (0.00 - 0.18)	0.04 (0.00 - 0.16)	**0.13 (0.01 - 0.37)**	**0.12 (0.01 - 0.36)**	0.09 (0.01 - 0.32)	0.08 (0.01 - 0.31)
	Subeucalanus monachus	0.06 (0.00 - 0.57)	0.02 (0.00 - 0.09)	0.02 (0.00 - 0.10)	0.03 (0.00 - 0.12)	0.02 (0.00 - 0.12)	**0.22 (0.01 - 0.38)**	0.02 (0.00 - 0.10)	0.02 (0.00 - 0.11)	**0.45 (0.06 - 0.58)**	0.03 (0.00 - 0.10)
	Ostracoda	0.06 (0.01 - 0.26)	0.08 (0.01 - 0.30)	0.05 (0.00 - 0.17)	0.06 (0.01 - 0.25)	0.04 (0.00 - 0.16)	0.05 (0.00 - 0.17)	**0.13 (0.01 - 0.38)**	**0.12 (0.01 - 0.37)**	**0.11 (0.01 - 0.35)**	0.09 (0.01 - 0.33)
	Undinula vulgaris	**0.16 (0.01 - 0.57)**	0.03 (0.00 - 0.11)	0.03 (0.00 - 0.12)	0.03 (0.00 - 0.13)	0.03 (0.00 - 0.14)	**0.11 (0.01 - 0.35)**	0.03 (0.00 - 0.12)	0.03 (0.00 - 0.15)	**0.38 (0.02 - 0.59)**	0.03 (0.00 - 0.14)
	Lucifer typus	0.07 (0.01 - 0.28)	0.05 (0.00 - 0.21)	0.04 (0.00 - 0.16)	0.04 (0.00 - 0.16)	0.03 (0.00 - 0.16)	**0.10 (0.01 - 0.27)**	0.06 (0.01 - 0.24)	0.07 (0.01 - 0.30)	**0.30 (0.03 - 0.50)**	0.07 (0.01 - 0.33)
	Euphausia sp.	0.04 (0.01 - 0.14)	0.01 (0.00 - 0.10)	0.01 (0.00 - 0.04)	0.01 (0.00 - 0.07)	0.02 (0.00 - 0.08)	**0.24 (0.14 - 0.34)**	0.01 (0.00 - 0.05)	0.02 (0.00 - 0.13)	**0.54 (0.42 - 0.63)**	0.01 (0.01 - 0.08)
	Mixture 500	0.05 (0.00 - 0.17)	0.03 (0.00 - 0.13)	0.03 (0.00 - 0.10)	0.02 (0.00 - 0.10)	0.02 (0.00 - 0.08)	0.08 (0.01 - 0.19)	0.04 (0.00 - 0.15)	0.06 (0.00 - 0.09)	**0.53 (0.40 - 0.64)**	0.05 (0.00 - 0.19)
	Mixture 250	0.03 (0.00 - 0.12)	0.03 (0.00 - 0.10)	0.02 (0.00 - 0.09)	0.02 (0.00 - 0.08)	0.02 (0.00 - 0.07)	0.04 (0.00 - 0.12)	0.04 (0.00 - 0.13)	0.05 (0.00 - 0.16)	**0.64 (0.53 - 0.74)**	0.04 (0.01 - 0.15)
	Euphausia frigida	0.02 (0.00 - 0.15)	0.02 (0.00 - 0.07)	0.02 (0.00 - 0.07)	0.01 (0.00 - 0.07)	0.02 (0.00 - 0.08)	0.04 (0.00 - 0.15)	0.02 (0.00 - 0.01)	0.02 (0.00 - 0.09)	**0.71 (0.60 - 0.79)**	0.03 (0.00 - 0.11)
	Larvacea	0.04 (0.00 - 0.17)	0.04 (0.00 - 0.20)	0.03 (0.00 - 0.10)	0.04 (0.00 - 0.17)	0.02 (0.00 - 0.09)	0.03 (0.00 - 0.10)	**0.30 (0.01 - 0.62)**	**0.13 (0.01 - 0.60)**	0.07 (0.01 - 0.51)	0.05 (0.01 - 0.24)
	Creseis sp.	0.06 (0.00 - 0.44)	0.04 (0.00 - 0.30)	0.03 (0.00 - 0.14)	0.04 (0.00 - 0.25)	0.03 (0.00 - 0.13)	0.05 (0.00 - 0.21)	0.06 (0.00 - 0.40)	0.06 (0.00 - 0.48)	**0.21 (0.01 - 0.58)**	0.05 (0.00 - 0.39)
	Cephalopoda larva	0.04 (0.00 - 0.23)	0.05 (0.00 - 0.27)	0.03 (0.00 - 0.12)	0.04 (0.00 - 0.17)	0.03 (0.00 - 0.11)	0.03 (0.00 - 0.13)	**0.20 (0.01 - 0.56)**	**0.11 (0.01 - 0.54)**	**0.10 (0.07 - 0.53)**	0.05 (0.00 - 0.30)
	Thalia democratica	0.02 (0.00 - 0.05)	0.01 (0.00 - 0.07)	0.02 (0.00 - 0.05)	0.03 (0.00 - 0.06)	0.02 (0.00 - 0.07)	0.03 (0.00 - 0.22)	0.01 (0.00 - 0.11)	0.02 (0.00 - 0.07)	**0.72 (0.03 - 0.80)**	0.02 (0.00 - 0.09)
Thukela Mouth											
	Ostracoda	0.07 (0.01 - 0.29)	0.06 (0.00 - 0.23)	**0.12 (0.01 - 0.40)**	0.08 (0.01 - 0.30)	**0.15 (0.01 - 0.40)**	**0.12 (0.01 - 0.37)**	0.04 (0.00 - 0.16)	0.04 (0.00 - 0.16)	0.05 (0.00 - 0.18)	0.05 (0.00 - 0.21)
	Flaccisagitta enflata	0.02 (0.00 - 0.11)	0.02 (0.00 - 0.08)	0.03 (0.00 - 0.13)	0.02 (0.00 - 0.08)	**0.64 (0.14 - 0.77)**	**0.11 (0.00 - 0.67)**	0.01 (0.00 - 0.06)	0.02 (0.00 - 0.07)	0.02 (0.00 - 0.09)	0.02 (0.00 - 0.09)
	Scyphozoa	0.07 (0.01 - 0.28)	0.04 (0.00 - 0.16)	**0.11 (0.01 - 0.35)**	0.06 (0.01 - 0.26)	**0.18 (0.02 - 0.42)**	**0.22 (0.02 - 0.48)**	0.03 (0.00 - 0.12)	0.03 (0.00 - 0.13)	0.04 (0.00 - 0.16)	0.04 (0.00 - 0.16)
	Liriope sp.	0.05 (0.00 - 0.27)	0.03 (0.00 - 0.12)	0.05 (0.00 - 0.14)	0.04 (0.00 - 0.32)	**0.46 (0.02 - 0.66)**	**0.16 (0.01 - 0.70)**	0.02 (0.00 - 0.08)	0.03 (0.00 - 0.11)	0.03 (0.00 - 0.12)	0.03 (0.00 - 0.10)
	Lucifer typus	0.08 (0.01 - 0.38)	0.04 (0.00 - 0.16)	0.06 (0.01 - 0.25)	0.05 (0.00 - 0.28)	0.08 (0.01 - 0.33)	**0.30 (0.03 - 0.53)**	0.04 (0.00 - 0.13)	0.04 (0.00 - 0.15)	0.06 (0.00 - 0.23)	0.05 (0.00 - 0.18)
	Mixture 500	0.05 (0.00 - 0.24)	0.04 (0.00 - 0.12)	0.05 (0.00 - 0.20)	0.04 (0.00 - 0.14)	0.04 (0.01 - 0.15)	**0.49 (0.28 - 0.61)**	0.03 (0.00 - 0.12)	0.03 (0.00 - 0.13)	0.06 (0.00 - 0.22)	0.04 (0.00 - 0.16)
	Undinula vulgaris	0.08 (0.01 - 0.53)	0.03 (0.00 - 0.12)	0.04 (0.00 - 0.17)	0.43 (0.00 - 0.42)	0.05 (0.00 - 0.36)	**0.40 (0.02 - 0.65)**	0.03 (0.00 - 0.11)	0.03 (0.00 - 0.13)	0.05 (0.00 - 0.20)	0.04 (0.00 - 0.14)
	Mixture 250	0.05 (0.00 - 0.25)	0.03 (0.00 - 0.15)	0.04 (0.00 - 0.15)	0.03 (0.00 - 0.13)	0.04 (0.00 - 0.13)	**0.51 (0.35 - 0.64)**	0.03 (0.00 - 0.13)	0.04 (0.00 - 0.14)	0.08 (0.01 - 0.21)	0.03 (0.00 - 0.15)
	Creseis sp.	**0.16 (0.00 - 0.64)**	0.03 (0.00 - 0.14)	0.04 (0.00 - 0.14)	0.07 (0.00 - 0.59)	0.05 (0.00 - 0.49)	0.08 (0.00 - 0.64)	0.03 (0.00 - 0.11)	0.03 (0.00 - 0.14)	0.04 (0.00 - 0.17)	0.03 (0.00 - 0.13)
	Thalia democratica	0.05 (0.00 - 0.45)	0.03 (0.00 - 0.16)	0.03 (0.00 - 0.12)	0.03 (0.00 - 0.12)	0.03 (0.00 - 0.12)	0.03 (0.00 - 0.11)	0.03 (0.00 - 0.11)	0.03 (0.00 - 0.13)	**0.54 (0.12 - 0.69)**	0.03 (0.00 - 0.14)
	Diacavolinia longirostris	**0.12 (0.01 - 0.49)**	0.04 (0.00 - 0.18)	0.05 (0.00 - 0.21)	0.06 (0.00 - 0.40)	0.05 (0.00 - 0.27)	**0.13 (0.01 - 0.50)**	0.04 (0.00 - 0.17)	0.05 (0.00 - 0.20)	0.08 (0.01 - 0.39)	0.05 (0.00 - 0.21)

Appendix A: Continuation.

		Durban Eddy		Thukela Mouth		Middle Shelf		RB South		RB North	
Location	**Species/Taxon**	**Surface**	***Fmax***	**Surface**	***Fmax***	**Surface**	***Fmax***	**Surface**	***Fmax***	**Surface**	***Fmax***
Middle Shelf											
	Flaccisagitta enflata	0.05 (0.00 - 0.25)	0.03 (0.00 - 0.12)	0.06 (0.00 - 0.22)	0.04 (0.00 - 0.26)	**0.42 (0.03 - 0.61)**	**0.13 (0.01 - 0.60)**	0.02 (0.00 - 0.09)	0.02 (0.00 - 0.09)	0.03 (0.00 - 0.12)	0.03 (0.00 - 0.12)
	Squilla sp. larval stage	0.07 (0.01 - 0.34)	0.04 (0.00 - 0.17)	0.09 (0.01 - 0.33)	0.07 (0.00 - 0.33)	**0.21 (0.02 - 0.47)**	**0.15 (0.01 - 0.47)**	0.03 (0.00 - 0.13)	0.04 (0.00 - 0.14)	0.04 (0.00 - 0.16)	0.04 (0.00 - 0.17)
	Siriella sp.	0.08 (0.01 - 0.29)	0.07 (0.01 - 0.25)	**0.12 (0.01 - 0.38)**	0.08 (0.01 - 0.30)	**0.13 (0.01 - 0.36)**	**0.10 (0.01 - 0.33)**	0.05 (0.00 - 0.18)	0.05 (0.00 - 0.19)	0.05 (0.00 - 0.20)	0.06 (0.01 - 0.23)
	Liriope tetraphylla	0.08 (0.01 - 0.32)	0.05 (0.00 - 0.20)	**0.10 (0.01 - 0.37)**	0.07 (0.01 - 0.31)	**0.17 (0.01 - 0.42)**	**0.15 (0.01 - 0.42)**	0.04 (0.00 - 0.14)	0.04 (0.00 - 0.15)	0.05 (0.00 - 0.18)	0.05 (0.00 - 0.19)
	Leptostraca larva	0.08 (0.01 - 0.46)	0.04 (0.00 - 0.15)	0.05 (0.00 - 0.21)	0.06 (0.00 - 0.43)	**0.24 (0.01 - 0.55)**	**0.13 (0.01 - 0.57)**	0.03 (0.00 - 0.12)	0.03 (0.00 - 0.12)	0.04 (0.00 - 0.15)	0.04 (0.00 - 0.15)
	Lucifer typus	0.09 (0.01 - 0.43)	0.04 (0.00 - 0.15)	0.06 (0.00 - 0.23)	0.05 (0.00 - 0.30)	0.07 (0.01 - 0.32)	**0.33 (0.03 - 0.56)**	0.03 (0.00 - 0.13)	0.04 (0.00 - 0.15)	0.06 (0.01 - 0.24)	0.04 (0.00 - 0.18)
	Subeucalanus monachus	**0.10 (0.01 - 0.50)**	0.04 (0.00 - 0.16)	0.05 (0.00 - 0.22)	0.07 (0.00 - 0.44)	0.08 (0.01 - 0.42)	**0.17 (0.01 - 0.55)**	0.04 (0.00 - 0.14)	0.04 (0.00 - 0.15)	0.05 (0.00 - 0.23)	0.04 (0.00 - 0.23)
	Mixture 500	0.04 (0.00 - 0.53)	0.03 (0.00 - 0.10)	0.03 (0.00 - 0.10)	0.03 (0.00 - 0.10)	0.03 (0.00 - 0.12)	**0.60 (0.10 - 0.71)**	0.03 (0.00 - 0.10)	0.03 (0.00 - 0.12)	0.06 (0.01 - 0.20)	0.03 (0.00 - 0.12)
	Cephalopoda larva	**0.11 (0.01 - 0.49)**	0.04 (0.00 - 0.17)	0.05 (0.00 - 0.22)	0.06 (0.00 - 0.42)	0.07 (0.00 - 0.38)	**0.16 (0.01 - 0.53)**	0.04 (0.00 - 0.15)	0.04 (0.00 - 0.17)	0.06 (0.00 - 0.27)	0.04 (0.00 - 0.19)
	Mixture 250	0.08 (0.01 - 0.45)	0.03 (0.00 - 0.15)	0.03 (0.00 - 0.14)	0.03 (0.00 - 0.17)	0.03 (0.00 - 0.14)	**0.21 (0.02 - 0.46)**	0.04 (0.00 - 0.16)	0.04 (0.00 - 0.21)	**0.28 (0.02 - 0.51)**	0.04 (0.00 - 0.21)
	Undinula vulgaris	0.02 (0.00 - 0.74)	0.02 (0.00 - 0.05)	0.02 (0.00 - 0.06)	0.02 (0.00 - 0.07)	0.01 (0.00 - 0.05)	**0.80 (0.04 - 0.85)**	0.02 (0.00 - 0.05)	0.01 (0.00 - 0.05)	0.03 (0.00 - 0.13)	0.02 (0.00 - 0.09)
	Larvacea	0.05 (0.00 - 0.25)	0.07 (0.01 - 0.30)	0.04 (0.00 - 0.14)	0.05 (0.00 - 0.23)	0.04 (0.00 - 0.13)	0.04 (0.00 - 0.14)	**0.16 (0.01 - 0.45)**	**0.13 (0.01 - 0.43)**	**0.11 (0.01 - 0.41)**	0.08 (0.01 - 0.33)
	Euphausia frigida	**0.14 (0.01 - 0.59)**	0.03 (0.00 - 0.14)	0.03 (0.00 - 0.14)	0.03 (0.00 - 0.35)	0.03 (0.00 - 0.14)	0.09 (0.01 - 0.53)	0.04 (0.00 - 0.16)	0.04 (0.00 - 0.18)	**0.14 (0.01 - 0.59)**	0.04 (0.00 - 0.18)
RB South											
	Ferosagitta sp.	**0.10 (0.01 - 0.33)**	0.05 (0.00 - 0.20)	0.08 (0.01 - 0.27)	0.07 (0.01 - 0.28)	**0.11 (0.01 - 0.31)**	**0.17 (0.02 - 0.39)**	0.04 (0.00 - 0.16)	0.05 (0.00 - 0.18)	0.07 (0.01 - 0.24)	0.06 (0.01 - 0.22)
	Mysida	0.08 (0.01 - 0.29)	0.07 (0.01 - 0.26)	0.08 (0.01 - 0.30)	0.07 (0.01 - 0.26)	0.08 (0.01 - 0.26)	**0.11 (0.01 - 0.32)**	0.06 (0.01 - 0.22)	0.07 (0.01 - 0.24)	0.08 (0.01 - 0.28)	0.07 (0.01 - 0.27)
	Flaccisagitta enflata	0.05 (0.00 - 0.26)	0.02 (0.00 - 0.13)	0.04 (0.00 - 0.19)	0.03 (0.00 - 0.19)	0.07 (0.01 - 0.47)	**0.53 (0.04 - 0.72)**	0.02 (0.00 - 0.08)	0.03 (0.00 - 0.11)	0.02 (0.00 - 0.11)	0.03 (0.00 - 0.10)
	Pterotrachea sp.	**0.13 (0.01 - 0.57)**	0.04 (0.00 - 0.13)	0.05 (0.01 - 0.17)	0.07 (0.00 - 0.51)	0.06 (0.00 - 0.43)	**0.12 (0.01 - 0.59)**	0.03 (0.00 - 0.12)	0.03 (0.00 - 0.15)	0.05 (0.00 - 0.26)	0.04 (0.00 - 0.17)
	Undinula vulgaris	0.08 (0.01 - 0.34)	0.06 (0.00 - 0.28)	0.05 (0.00 - 0.23)	0.06 (0.00 - 0.28)	0.05 (0.00 - 0.19)	0.09 (0.01 - 0.31)	0.07 (0.01 - 0.27)	0.07 (0.01 - 0.31)	**0.12 (0.01 - 0.40)**	0.07 (0.01 - 0.32)
	Subeucalanus monachus	0.05 (0.00 - 0.25)	0.03 (0.00 - 0.18)	0.03 (0.00 - 0.11)	0.03 (0.00 - 0.12)	0.02 (0.00 - 0.10)	0.04 (0.00 - 0.13)	0.05 (0.00 - 0.27)	0.05 (0.00 - 0.52)	**0.50 (0.03 - 0.66)**	0.05 (0.00 - 0.20)
	Mixture 500	0.04 (0.00 - 0.21)	0.04 (0.00 - 0.18)	0.03 (0.00 - 0.11)	0.03 (0.00 - 0.13)	0.03 (0.00 - 0.11)	0.04 (0.00 - 0.16)	0.07 (0.01 - 0.42)	0.07 (0.00 - 0.47)	**0.39 (0.03 - 0.61)**	0.05 (0.00 - 0.34)
	Lucifer typus	0.05 (0.00 - 0.29)	0.05 (0.00 - 0.31)	0.03 (0.00 - 0.14)	0.04 (0.00 - 0.19)	0.03 (0.00 - 0.12)	0.04 (0.00 - 0.16)	**0.11 (0.01 - 0.50)**	0.09 (0.01 - 0.50)	**0.14 (0.01 - 0.55)**	0.06 (0.00 - 0.37)
	Mixture 250	0.02 (0.00 - 0.09)	0.03 (0.00 - 0.10)	0.02 (0.00 - 0.11)	0.02 (0.00 - 0.07)	0.02 (0.00 - 0.06)	0.02 (0.00 - 0.06)	0.04 (0.00 - 0.09)	0.04 (0.01 - 0.08)	**0.74 (0.63 - 0.80)**	0.02 (0.00 - 0.08)
	Euphausia frigida	0.05 (0.00 - 0.56)	0.04 (0.00 - 0.18)	0.03 (0.00 - 0.11)	0.03 (0.00 - 0.48)	0.02 (0.00 - 0.11)	0.03 (0.00 - 0.16)	0.04 (0.00 - 0.23)	0.05 (0.00 - 0.53)	**0.48 (0.01 - 0.69)**	0.04 (0.00 - 0.22)
	Annelida	0.03 (0.00 - 0.25)	0.04 (0.00 - 0.22)	0.03 (0.00 - 0.13)	0.03 (0.00 - 0.13)	0.02 (0.00 - 0.07)	0.03 (0.00 - 0.11)	**0.10 (0.00 - 0.62)**	0.07 (0.01 - 0.60)	**0.17 (0.01 - 0.67)**	0.05 (0.00 - 0.22)
RB North											
	Ferosagitta sp.	0.07 (0.00 - 0.52)	0.03 (0.00 - 0.11)	0.04 (0.00 - 0.17)	0.04 (0.00 - 0.38)	0.07 (0.00 - 0.48)	**0.43 (0.03 - 0.68)**	0.03 (0.00 - 0.10)	0.03 (0.00 - 0.11)	0.04 (0.00 - 0.14)	0.03 (0.00 - 0.11)
	Flaccisagitta enflata	**0.36 (0.01 - 0.60)**	0.03 (0.00 - 0.13)	0.04 (0.00 - 0.16)	0.07 (0.00 - 0.54)	0.05 (0.00 - 0.44)	0.08 (0.01 - 0.44)	0.03 (0.00 - 0.11)	0.03 (0.00 - 0.14)	0.05 (0.00 - 0.19)	0.04 (0.00 - 0.14)
	Subeucalanus monachus	**0.10 (0.01 - 0.39)**	0.05 (0.00 - 0.20)	0.06 (0.01 - 0.26)	0.07 (0.01 - 0.35)	0.08 (0.01 - 0.32)	**0.16 (0.01 - 0.46)**	0.04 (0.00 - 0.17)	0.05 (0.00 - 0.19)	0.07 (0.01 - 0.29)	0.05 (0.00 - 0.22)
	Euphausia sp.	**0.11 (0.01 - 0.45)**	0.05 (0.00 - 0.21)	0.05 (0.00 - 0.23)	0.06 (0.00 - 0.37)	0.05 (0.00 - 0.24)	**0.12 (0.01 - 0.44)**	0.04 (0.00 - 0.19)	0.05 (0.00 - 0.24)	**0.10 (0.01 - 0.41)**	0.05 (0.00 - 0.25)
	Mixture 500	0.04 (0.00 - 0.35)	0.03 (0.00 - 0.11)	0.03 (0.00 - 0.10)	0.03 (0.00 - 0.15)	0.02 (0.00 - 0.10)	0.05 (0.00 - 0.20)	0.03 (0.00 - 0.11)	0.03 (0.00 - 0.16)	**0.56 (0.25 - 0.69)**	0.05 (0.00 - 0.15)
	Copepoda	**0.16 (0.01 - 0.60)**	0.03 (0.00 - 0.13)	0.03 (0.00 - 0.12)	0.03 (0.00 - 0.46)	0.03 (0.00 - 0.12)	0.05 (0.00 - 0.23)	0.03 (0.00 - 0.15)	0.04 (0.00 - 0.19)	**0.31 (0.01 - 0.66)**	0.04 (0.00 - 0.15)
	Mixture 250	0.03 (0.00 - 0.76)	0.02 (0.00 - 0.08)	0.02 (0.00 - 0.08)	0.02 (0.00 - 0.04)	0.02 (0.00 - 0.10)	0.03 (0.00 - 0.08)	0.02 (0.00 - 0.09)	0.02 (0.00 - 0.07)	**0.75 (0.02 - 0.85)**	0.01 (0.00 - 0.06)

Appendix B: Zooplankton dry season mixing model results showing percentage diet contribution from TSS from different locales

		Durban Eddy		Thukela Mouth		Middle Shelf		RB South	RB North	
Location	**Species/Taxon**	**Surface**	***Fmax***	**Surface**	***Fmax***	**Surface**	***Fmax***	**Surface and *Fmax***	**Surface**	***Fmax***
Durban Eddy										
	Flaccisagitta enflata	0.02 (0.00 - 0.10)	0.03 (0.00 - 0.10)	0.01 (0.00 - 0.05)	0.03 (0.00 - 0.10)	0.02 (0.00 - 0.08)	0.03 (0.00 - 0.13)	0.09 (0.01 - 0.27)	0.02 (0.00 - 0.08)	**0.67 (0.54 - 0.80)**
	Heterpoda	0.04 (0.00 - 0.18)	0.04 (0.00 - 0.15)	0.03 (0.00 - 0.11)	0.04 (0.00 - 0.15)	**0.45 (0.01 - 0.64)**	0.04 (0.00 - 0.17)	0.06 (0.01 - 0.24)	0.04 (0.00 - 0.15)	**0.10 (0.01 -0.66)**
	Euphausia mixture	0.04 (0.00 - 0.21)	0.04 (0.00 - 0.16)	0.02 (0.00 - 0.09)	0.05 (0.00 - 0.18)	**0.10 (0.01 - 0.50)**	0.05 (0.00 - 0.21)	**0.12 (0.01 - 0.42)**	0.04 (0.00 - 0.15)	**0.37 (0.02 - 0.61)**
	Euphausia sp.	0.03 (0.00 - 0.14)	0.03 (0.00 - 0.13)	0.02 (0.00 - 0.09)	0.04 (0.00 - 0.14)	**0.39 (0.00 - 0.65)**	0.04 (0.00 - 0.15)	0.07 (0.01 - 0.26)	0.03 (0.00 - 0.12)	**0.23 (0.01 - 0.73)**
	Subeucalanus monachus	0.03 (0.00 - 0.11)	0.02 (0.00 - 0.10)	0.02 (0.00 - 0.06)	0.03 (0.00 - 0.12)	0.04 (0.00 - 0.61)	0.04 (0.00 - 0.14)	**0.10 (0.01 - 0.32)**	0.02 (0.00 - 0.09)	**0.60 (0.02 - 0.77)**
	Mixture 500	0.03 (0.00 - 0.12)	0.02 (0.00 - 0.09)	0.02 (0.00 - 0.07)	0.03 (0.00 - 0.12)	0.02 (0.00 - 0.29)	0.03 (0.00 - 0.12)	**0.21 (0.02 - 0.81)**	0.02 (0.00 - 0.12)	**0.50 (0.04 - 0.69)**
	Undinula vulgaris	0.03 (0.00 - 0.14)	0.03 (0.00 - 0.10)	0.01 (0.00 - 0.07)	0.03 (0.00 - 0.12)	0.02 (0.00 - 0.43)	0.03 (0.00 - 0.14)	**0.16 (0.02 - 0.40)**	0.02 (0.00 - 0.10)	**0.57 (0.13 - 0.71)**
	Scyphozoa	0.07 (0.01 - 0.37)	0.05 (0.00 - 0.19)	0.04 (0.00 - 0.16)	0.06 (0.00 - 0.21)	**0.27 (0.01 - 0.50)**	0.06 (0.00 - 0.23)	0.09 (0.01 - 0.32)	0.05 (0.00 - 0.19)	**0.13 (0.01 - 0.51)**
	Mixture 250	0.03 (0.00 - 0.14)	0.02 (0.00 - 0.07)	0.01 (0.00 - 0.07)	0.03 (0.00 - 0.10)	0.02 (0.00 - 0.17)	0.03 (0.00 - 0.11)	**0.67 (0.19 - 0.83)**	0.02 (0.00 - 0.08)	0.09 (0.00 - 0.54)
	Creseis sp.	0.06 (0.00 - 0.53)	0.04 (0.00 - 0.14)	0.03 (0.00 - 0.15)	0.04 (0.00 - 0.19)	**0.42 (0.10 - 0.64)**	0.04 (0.00 - 0.16)	0.06 (0.01 - 0.24)	0.03 (0.00 - 0.14)	0.07 (0.00 - 0.65)
Thukela Mouth										
	Flaccisagitta enflata	0.02 (0.00 - 0.08)	0.03 (0.00 - 0.09)	0.01 (0.00 - 0.05)	0.02 (0.00 - 0.07)	**0.63 (0.00 - 0.81)**	0.03 (0.00 - 0.09)	0.04 (0.00 - 0.15)	0.02 (0.00 - 0.08)	0.10 (0.01 - 0.85)
	Undinula vulgaris	0.02 (0.00 - 0.08)	0.02 (0.00 - 0.09)	0.01 (0.00 - 0.06)	0.02 (0.00 - 0.08)	0.02 (0.00 - 0.75)	0.02 (0.00 - 0.09)	0.05 (0.00 - 0.14)	0.02 (0.00 - 0.08)	**0.74 (0.09 - 0.86)**
	Subeucalanus monachus	0.03 (0.00 - 0.12)	0.03 (0.00 - 0.13)	0.02 (0.00 - 0.09)	0.04 (0.00 - 0.14)	**0.60 (0.02 - 0.74)**	0.04 (0.00 - 0.14)	0.05 (0.00 - 0.18)	0.04 (0.00 - 0.12)	0.06 (0.00 - 0.71)
	Mixture 500	0.02 (0.00 - 0.10)	0.02 (0.00 - 0.11)	0.02 (0.00 - 0.06)	0.03 (0.00 - 0.10)	**0.64 (0.01 - 0.78**	0.03 (0.00 - 0.11)	0.05 (0.01 - 0.18)	0.02 (0.00 - 0.10)	0.07 (0.01 - 0.81)
	Mixture 250	0.03 (0.00 - 0.11)	0.03 (0.00 - 0.10)	0.02 (0.00 - 0.06)	0.03 (0.00 - 0.12)	**0.64 (0.01 - 0.80)**	0.03 (0.00 - 0.12)	0.05 (0.00 - 0.18)	0.02 (0.00 - 0.10)	0.06 (0.00 - 0.78)
Middle Shelf										
	Flaccisagitta enflata	0.05 (0.00 - 0.22)	0.04 (0.00 - 0.17)	0.03 (0.00 - 0.13)	0.05 (0.00 - 0.18)	**0.45 (0.03 - 0.64)**	0.05 (0.00 - 0.18)	0.06 (0.00 - 0.23)	0.04 (0.00 - 0.16)	**0.07 (0.01 - 0.58)**
	Undinula vulgaris	0.04 (0.00 - 0.16)	0.04 (0.00 - 0.13)	0.02 (0.00 - 0.10)	0.04 (0.00 - 0.16)	**0.57 (0.04 - 0.71)**	0.04 (0.00 - 0.14)	0.05 (0.01 - 0.18)	0.03 (0.00 - 0.13)	**0.05 (0.01 - 0.66)**
	Euphausiid mixture	0.05 (0.00 - 0.22)	0.04 (0.00 - 0.17)	0.03 (0.00 - 0.13)	0.04 (0.00 - 0.17)	**0.44 (0.03 - 0.63)**	0.05 (0.00 - 0.19)	0.06 (0.00 - 0.23)	0.04 (0.00 - 0.16)	**0.07 (0.01 - 0.58)**
	Mix 500	0.04 (0.00 - 0.16)	0.04 (0.00 - 0.14)	0.03 (0.00 - 0.12)	0.04 (0.00 - 0.15)	**0.53 (0.04 - 0.69)**	0.04 (0.00 - 0.16)	0.06 (0.00 - 0.22)	0.04 (0.00 - 0.14)	**0.05 (0.00 - 0.62)**
	Mix 250	0.04 (0.00 - 0.18)	0.04 (0.00 - 0.15)	0.03 (0.00 - 0.14)	0.04 (0.00 - 0.15)	**0.52 (0.04 - 0.68)**	0.04 (0.00 - 0.17)	0.06 (0.01 - 0.22)	0.06 (0.00 - 0.62)	**0.06 (0.00 - 0.22)**
RB South										
	Flaccisagitta enflata	0.02 (0.00 - 0.10)	0.03 (0.00 - 0.10)	0.02 (0.00 - 0.07)	0.03 (0.00 - 0.12)	0.03 (0.00 - 0.69)	0.03 (0.00 - 0.12)	0.04 (0.00 - 0.17)	0.02 (0.00 - 0.10)	**0.69 (0.02 - 0.83)**
	Undinula vulgaris	0.04 (0.00 - 0.17)	0.04 (0.00 - 0.14)	0.03 (0.00 - 0.11)	0.04 (0.00 - 0.16)	**0.42 (0.01 - 0.62)**	0.05 (0.00 - 0.18)	0.08 (0.01 - 0.29)	0.03 (0.00 - 0.14)	**0.12 (0.01 - 0.66)**
	Euphasia sp.	0.08 (0.01 - 0.35)	0.06 (0.00 - 0.22)	0.04 (0.00 - 0.17)	0.07 (0.01 - 0.25)	**0.13 (0.01 - 0.40)**	0.07 (0.01 - 0.29)	**0.12 (0.01 - 0.44)**	0.06 (0.00 - 0.22)	**0.14 (0.01 - 0.43)**
	Mix 500	0.04 (0.00 - 0.17)	0.03 (0.00 - 0.12)	0.02 (0.00 - 0.07)	0.03 (0.00 - 0.14)	0.03 (0.00 - 0.33)	0.04 (0.00 - 0.15)	**0.44 (0.03 - 0.78)**	0.03 (0.00 - 0.11)	**0.22 (0.01 - 0.59)**
	Subeucalanus monachus	0.04 (0.00 - 0.19)	0.04 (0.00 - 0.14)	0.03 (0.00 - 0.11)	0.04 (0.00 - 0.16)	**0.41 (0.01 - 0.61)**	0.04 (0.00 - 0.18)	0.09 (0.01 - 0.30)	0.04 (0.00 - 0.14)	**0.11 (0.01 - 0.66)**
	Euphasia frigida	0.07 (0.01 - 0.47)	0.05 (0.00 - 0.18)	0.04 (0.00 - 0.17)	0.05 (0.00 - 0.21)	**0.30 (0.00 - 0.54)**	0.05 (0.00 - 0.21)	0.08 (0.00 - 0.31)	0.04 (0.00 - 0.18)	**0.10 (0.01 - 0.54)**
	Mix 250	0.04 (0.00 - 0.17)	0.02 (0.00 - 0.10)	0.02 (0.00 - 0.07)	0.03 (0.00 - 0.11)	0.02 (0.00 - 0.09)	0.03 (0.00 - 0.12)	**0.71 (0.36 - 0.81)**	0.02 (0.00 - 0.09)	0.04 (0.00 - 0.17)
RB North										
	Flaccisagitta enflata	0.05 (0.00 - 0.34)	0.04 (0.00 - 0.18)	0.03 (0.00 - 0.12)	0.05 (0.00 - 0.20)	**0.33 (0.01 - 0.59)**	0.05 (0.00 - 0.20)	0.06 (0.01 - 0.25)	0.04 (0.00 - 0.16)	**0.15 (0.01 - 0.61)**
	Ostracod	0.06 (0.00 - 0.37)	0.05 (0.00 - 0.19)	0.03 (0.00 - 0.13)	0.05 (0.00 - 0.22)	**0.26 (0.01 - 0.53)**	0.05 (0.00 - 0.23)	0.07 (0.01 - 0.30)	0.05 (0.00 - 0.18)	**0.16 (0.01 - 0.56)**
	Subeucalanus monachus	0.04 (0.00 - 0.14)	0.03 (0.00 - 0.12)	0.02 (0.00 - 0.09)	0.04 (0.00 - 0.14)	**0.12 (0.00 - 0.60)**	0.04 (0.00 - 0.16)	**0.11 (0.01 - 0.33)**	0.03 (0.00 - 0.11)	**0.45 (0.01 - 0.71)**
	mix 500	0.04 (0.00 - 0.33)	0.02 (0.00 - 0.10)	0.02 (0.00 - 0.08)	0.03 (0.00 - 0.11)	0.04 (0.00 - 0.42)	0.03 (0.00 - 0.13)	**0.44 (0.03 - 0.78)**	0.03 (0.00 - 0.09)	**0.16 (0.01 - 0.62)**
	mix 250	0.16 (0.01 - 0.57)	0.03 (0.00 - 0.13)	0.03 (0.00 - 0.13)	0.04 (0.00 - 0.19)	0.06 (0.00 - 0.47)	0.04 (0.00 - 0.17)	**0.15 (0.01 - 0.59)**	0.03 (0.00 - 0.13)	0.09 (0.01 - 0.57)

Appendix C: Supporting data relevant to Chapter 3. The table indicates whether animals were collected in the summer (S) or winter (W) season as well as the number of animals collected in total, the depth that they were collected from, the depth recorded in the literature and a diet description. For animals where diet could not be found in the literature, the diet of a close relative has been described; in those instances the animal in question is mentioned.

Season collected	Name (No. Of individuals sampled)	Description	General Prey Items	Collection depth (m ± SD)	Literature depth (m)	Reference
S+W	*Acropoma japonicum* (*n* = 9)	Teleost	Foramoniferans; Sponges; Coelenterates; Bivalves; Gastropods; Cephalopods; Polychaetes; Crustaceans; Echinoderms; Osteichthyes	302.97 ± 92.69	1 – 500*	Rainer (1992)
S	*Actinoptilum molle* (*n* = 3)	Pennatulacea	Based on the diet of deep-water corals. Detrital and suspended matter.	34.75	12 - 333	Roberts et al (2006)
S+W	*Aristaeomorpha foliacea* (*n* = 21)	Decapod	Crustecean and Osteichthyes, cephalopod important in some areas	446.49 ± 116.75	120 - 1300	Bello and Pipitone (2002)
S	*Arothron immaculatus* (*n* = 6)	Teleost	Benthic algae; Benthic invertebrates; Detritus	117.96 ± 11.02	1-17*	Masuda and Allen (1993)
S+W	*Atrobucca nibe* (*n* = 9)	Teleost	Crustacea: Mysidacea, Natantia, Anomura; Cephalopoda; Osteichthyes	56.08 ± 15.55	45 – 200*	Fennessy (2000)
S+W	*Chaceon macphersoni* (*n* = 9)	Decapod	Based on *Chaceon notialis*. Osteichthyes; Gastropods; Nematods; Polychaeta, Ophiuroidea	434.04 ± 10.55		Domingos et al (2007)
S	*Champsodon capensis* (*n* = 3)	Teleost	Osteichthyes	69.49	64 – 552*	Zhang et al (2005)
S+W	*Chaunax pictus* (*n* = 18)	Teleost	N/A	352.65 ± 125.55	200 – 1000*	
S+W	*Chelidonichthys kumu* (*n* = 21)	Teleost	Great varaiety of crustaceans; Echinoid; Pelecypods; Osteichthyes; Cephalopods	135.20 ± 87.11	1 – 200*	Godfriaux (1970)
W	*Chelidonichthys queketti* (*n* = 3)	Teleost	Polychaetes; Wide variety of crustaceans; Bivalves; Gastropods; Cephalopods; Osteichthyes	118.87	0 – 150*	Meyer and Smale (1991b)
S+W	*Chlorophthalmus punctatus* (*n* = 33)	Teleost	Osteichthyes such as myctophids and phosichthyids; Natantia and other organisms.	454.37 ± 73.02	280 – 450*	Karuppasamy et al (2008)
S+W	*Citharoides macrolepis* (*n* = 17)	Teleost	Based on *Citharoides macrolepidotus*. Benthic invertebrates	291.21 ± 113.54	182 – 200*	Hensley (2001)

Appendix C: Continuation

Season collected	Name (No. Of individuals sampled)	Description	General Prey Items	Collection depth (m ± SD)	Literature depth (m)	Reference
S	*Coelorinchus denticulatus* (*n* = 3)	Teleost	Based on *Coelorinchus fasciatu.* Polychaete; Echinoderms; wide variety of crustaceans; Cephalopods; Osteichthyes	250.55	64 – 335*	Meyer and Smale (1991b)
S+W	*Coelorinchus trunovi* (*n* = 38)	Teleost	Based on *Coelorinchus fasciatu.* Polychaete; Echinoderms; wide variety of crustaceans; Cephalopods; Osteichthyes	464.91 ± 77.86	421 – 552*	Meyer and Smale (1991b)
W	*Cubiceps baxteri* (*n* = 6)	Teleost	Zooplankton, specially salps.	418.80 ± 10.02	1 – 100*	Gorelova et al (1994)
S+W	*Cynoglossus attenuatus* (*n* = 18)	Teleost	Benthic invertebrates	31.70 ± 6.76		Fischer et al (1990)
S	*Cynoglossus lida* (*n* = 6)	Teleost	Zoobenthos, diatoms, prawns (lucifer) cuttlefish, Osteichthyes, benthic algae, amphipods, crabs, isopods, starfish, bivalves, gastropods, polychaetes, fish egg, copepods, jellyfish	232.26 ± 2.00	24 – 27*	Rajaguru (1992)
S	*Diaphus knappi* (*n* = 6)	Teleost	Based on *Diaphus taaningi* and *D. theta*. Zooplankton: fish larval stages; planktonic invertebrates, planktonic copepods and other planktonic crustaceans; Zoobenthos: benthic crustaceans, polychaetes	349.31 ± 108.18	24 – 27*	Baird et al (1975); Moku et al (2000)
S+W	*Haliporoides triarthrus* (*n* = 45)	Decapod	Based on *Haliporoides sibogae*. Foramoniferans; Sponges; Coelenterates; Bivalves; Gastropods; Cephalopods; Polychaetes; Crustaceans; Echinoderms; Osteichthyes	441.84 ± 10.02	360 – 460^{+}	Rainer (1992)
S+W	*Helicolenus dactylopterus* (*n* = 15)	Teleost	Primariley benthic crustaceans, polychates found in smaller size animals	520.90 ± 44.49		Macpherson (1985)
S	*Histioteuthis celetaria* (*n* = 3)	Cephalopod	Based on *Histioteuthis reversa* and *H. bornnelli*. Crustacea: Natantia; Osteichthyes; Cephalopoda	528.52		Quetglas et al (2010)
S+W	*Johnius amblycephalus* (*n* = 15)	Teleost	Polychaeta; Crustacea: Mysidacea, Stomatopoda, Natantia, Anomura, Brachyuran; Cephalopoda; Pelecypoda; Gastropoda; Asteroidea; Osteichthyes	34.20 ± 6.09	1 – 40*	Fennessy (2000)
S+W	*Johnius dussumieri* (*n* = 9)	Teleost	Polychaeta; Crustacea: Copepoda, Ostracoda, Mysidacea, Stomatopoda, Natantia, Anomura, Brachyuran; Cephalopoda; Pelecypoda; Osteichthyes	40.23 ± 8.23	1 – 40*	Fennessy (2000)
W	*Leiognathus equulus* (*n* = 3)	Teleost	Great variety of zooplankton and phytoplankton species. Pelagic copepod dominant, some benthic animals also found	31.09	10 – 110*	Tiews et al (1968)
S+W	*Lepidotrigla faurei* (*n* = 33)	Teleost	Based on *Lepidotrigla cavillone*. Crustaceans: Euphausiids, mysids, decapod (natantia and reptantia), amphipoda,	126.10 ± 147.67	50 – 175*	Terrats et al (2000)

Appendix C: Continuation

Season collected	Name (No. Of individuals sampled)	Description	General Prey Items	Collection depth (m ± SD)	Literature depth (m)	Reference
S+W	*Lithognathus mormyrus* (*n* = 3)	Teleost	Osteichthyes; Echinoidea; Gastropods; Decapods; Amphipods; Other crustaceans; Bivalves; Sedentaria; Errantia	34.29 ± 0.50	0 - 150 (usually 10 - 20)*	Kallianiotis et al (2005)
S	*Mactra aequisulcata* (*n* = 3)	Bivalve	N/A	32.92		
S+W	*Merluccius paradoxus* (*n* = 11)	Teleost	Osteichthyes; Crustaceans; Cephalopods and cannibalism	491.78 ± 74.34	200-850*	Pillar and Wilkinson (1995)
S+W	*Metanephrops mozambicus* (*n* = 27)	Decapod	Based on *Metanephrops andamanicus, M. australiensis and M. Boschmai*. Osteichthyes, crustacean and cephalopod (squid). In very small amounts other preys were bivalves, gastropods and foraminiferans	434.64 ± 37.33	187 - 842	Wassenberg and Hill (1989)
W	*Metapenaeus monoceros* (*n* = 6)	Decapod	Small crustaceans: Copepods, mysids, tanaidacea, amphipods, decapod larvae; Vegetable matter; Diatoms; Polychaetes; Detritus	38.86 ± 7.51	10 - 30^{+}	George (1974)
S	*Munida incerta* (*n* = 3)	Decapod	Based on *Munida sarsi*. Selective deposit feeder. Has been recorded preying on krill.	448.06		Garm and Høeg (2000); Hudson and Wigham (2003)
S+W	*Neoscombrops annectens* (*n* = 15)	Teleost	N/A	362.10 ± 73.42	100 – 550*	
W	*Nephropsis stewarti* (*n* = 3)	Decapod	N/A	563.27	500 – 750^{+}	
W	*Nototodarus hawaiiensis* (*n* = 3)	Cephalopod	Based on *Nototodarus gouldi*. Crustaceans; Osteichthyes and Cephalopos.	427.94		O'Sullivan and Cullen (1983)
S	*Ornithoteuthis volatilus* (*n* = 9)	Cephalopod	Based on *Ornithoteuthis antillarum*. Epi- and mesopelagic species of mesa- and macroplanktonic and micronektonic crustaceans, nemertines, chaetognaths, heteropods, molluscs, cephalopod (squid) and Osteichthyes.	332.23 ± 83.21		Arkhipkin et al (1998)
S+W	*Otolithes ruber* (*n* = 15)	Teleost	Crustacea: Natantias, Brachyurans, Mysidacea, Stomatopda, Anomura; Osteichthyes; Cephalopoda; Polychaeta; Pelecypoda; Vegetation; Prawns; Acetes; Squilla; Apogonid Fishes and Juvenile Sciaenids	37.49 ± 6.98	10 – 40*	Fennessy (2000)
W	*Ovalipes iridescens* (*n* = 3)	Decapod	Based on *Ovalipes stephensoni*. Amphipods; Isopods; Gastropods; Bivalves; Polychaetes; Ornithoteuthis; Decapods, Corals; Echinoderms	232.26		Haefner (1985)

Appendix C: Continuation

Season collected	Name (No. Of individuals sampled)	Description	General Prey Items	Collection depth (m ± SD)	Literature depth (m)	Reference
S+W	*Pagellus natalensis* (*n* = 45)	Teleost	Benthic crustaceans and invertebrates, non-annelid worms	102.29 ± 73.55	? – 150*	Van der Elst and Adkin (1991)
W	*Palinurus delagoae* (*n* = 2)	Decapod	Based on *Palinurus elephas*. Crustacea: Isopod, Decapods, other; Gastropods; Bivalves; Polyplacophora; Scaphopods; Cephalopoda; Brachiopoda; Echinoidea; Ostrichthyes; Chondrichthyes; Algae; Polychaet; Ascidians	427.94		Goñi et al (2001)
W	*Penaeus indicus* (*n* = 3)	Decapod	N/A	32.00	1 – 90†	
S	*Parapenaeopsis acclivirostris* (*n* = 3)	Decapod	Based on *Parapenaeus longirostris*.Osteichthyes; Cephalopods; Crustaceans; Polychaetes; Foraminiferans	29.26		Sobrino et al. (2005)
S	*Parapenaeus investigatoris* (*n* = 6)	Decapod	Based on *Parapenaeus longirostris*.Osteichthyes; Cephalopods; Crustaceans; Polychaetes; Foraminiferans	163.68 ± 95.16		Sobrino et al. (2005)
S	*Penaeopsis balssi* (*n* = 3)	Decapod	N/A	405.99		
S	*Phalium craticulatum* (*n* = 3)	Gastropod	N/A	568.76		
S	*Pleistacantha ori* (*n* = 3)	Decapod	N/A	234.09		
S	*Plesionika martia* (*n* = 3)	Decapod	Consist mainly of bentho-pelagic crustaceans - eupahusiids	407.82		Cartes (1993)
S+W	*Pliotrema warren* (*n* = 15)	Elasmobranch	Cephalopods; Osteichthyes; Crustaceans: shrimp/prawn, mysids	254.21 ± 100.45	60 – 430*	Compagno et al (1989)
S	*Polyipnus indicus* (*n* = 6)	Teleost	N/A	487.36 ± 67.09	50 – 500*	
S	*Polysteganus coeruleopunctatus* (*n* = 3)	Teleost	Based on *Polysteganus undulosus*. Pelagic fishes, small reef fish; Cephalopods and Crustaceans.	107.90	? – 100*	Garrat (1996)
S+W	*Pomadasys olivaceus* (*n* = 18)	Teleost	Benthic crustaceans and invertebrates, non-annelid worms	52.12 ± 20.56		Van der Elst and Adkin (1991)
S+W	*Portunus sanguinolentus* (*n* = 20)	Decapod	Predator of slow moving benthic macro-invertebrates. Preference for crustaceans and molluscs. Females preferred Osteichthyes in addition to crustaceans. Although fish remains are important it is unlikely that *P. sanguinolentus* can actively hunt healthy fish.	32.28 ± 6.14	10 - 30; females up to 80 m	Sukumaran and Neelakantan (1997)

Appendix C: Continuation

Season collected	Name (No. Of individuals sampled)	Description	General Prey Items	Collection depth (m ± SD)	Literature depth (m)	Reference
S+W	*Priacanthus cruentatus* (*n* = 15)	Teleost	Crustaceans: pistol or snapping shrimp, swimming crabs, isopods, stomatopods; Cephalopods: octopus; Osteichthyes; Polychaetes	200.07 ± 67.60	3 - 300 (usually 3 - 35 m)*	Hiatt and Strasburg (1960)
S+W	*Pseudorhombus elevates* (*n* = 18)	Teleost	Unspecified zoobenthos	56.69 ± 31.69	7 – 200*	Fischer et al (1990)
S	*Pseudorhombus natalensis* (*n* = 6)	Teleost	Unspecified zoobenthos	132.59 ± 107.18	60 – 260*	Fischer et al (1990)
S	*Raja (dipturus) springeri* (*n* = 9)	Elasmobranch	Osteichthyes; Cephalopods: squid/cuttlefish; Crustaceans	310.89 ± 144.02	88 – 740*	Compagno et al (1989); Ebert et al (1991)
S	*Ranella olearium* (*n* = 3)	Gastropod	N/A	336.50		
S	*Rhinobatos holcorhynchus* (*n* = 3)	Elasmobranch	N/A	230.43	75 – 253*	
S+W	*Rossia Sp* (*n* = 6)	Cephalopod	Based on *Rossia macrosoma*. Crustaceans; Osteichthyes and molluscs	416.97 ± 12.02		Mangold-Wirz (1963)
S+W	*Satyrichthys adeni* (*n* = 12)	Teleost	N/A	232.59 ± 1.60	58 – 295*	
S+W	*Saurida undosquamis* (*n* = 32)	Teleost	Osteichthyes; Crustaceans: shrimp/prawn, panaeus spp, stolephorus sp, crabs; Cephalopods: octopus, squid/cuttlefish; Other mollusks; Fish egg and larvae	123.07 ± 78.70	1 – 350*	Bingel and Avsar (1988)
S+W	*Sepia acuminate* (*n* = 5)	Cephalopod	Most cuttlefish feed mostly on crustaceans and Osteichthyes.	234.09		Castro and Guerra (1990)
W	*Sepia incerta* (*n* = 3)	Cephalopod	Most cuttlefish feed mostly on crustaceans and Osteichthyes.	118.87	90 - 345+	Castro and Guerra (1990)
S+W	*Sepia officinalis (vermiculata)* (*n* = 9)	Cephalopod	Polychaetes; Crustacea; Cephalopods; Osteichthyes. Cannibalism is common.	57.91 ± 17.37	? - 200†	Castro and Guerra (1990)
S	*Sepia sp* (*n* = 3)	Cephalopod	Most cuttlefish feed mostly on crustaceans and Osteichthyes.	27.43		Castro and Guerra (1990)
S	*Sphoeroides pachygaster* (*n* = 3)	Teleost	Cephalopods	234.09	50 – 250*	Scheneider (1990)
W	*Spicara australis* (*n* = 3)	Teleost	Based on *Spicara axillaris*. Crustacea: Copepoda, Amphipoda, Mysids, Euphausiids, Bivalves	232.26	80 – 400*	Meyer and Smale (1991a)
S+W	*Squalus megalops* (*n* = 18)	Elasmobranch	Crustacea; Cephalopoda; Polychaeta; Osteichthyes	247.20 ± 84.74	80 – 300*	Ebert et al (1992)

Appendix C: Continuation

Season collected	Name (No. Of individuals sampled)	Description	General Prey Items	Collection depth (m ± SD)	Literature depth (m)	Reference
S+W	*Synagrops japonicus* (*n* = 21)	Teleost	Echinoderms: starfish; Osteichthyes; Euphausiids; Cephalopods	386.92 ± 108.97	100 – 800*	Yamamura et al (1998)
W	*Upeneus moluccensis* (*n* = 3)	Teleost	Crustaceans most important food item: Decapoda, copepoda; Other items include Polychaeta; Bivalvia; Gastropoda; Cephalopoda; Echinodermata; Osteichthyes	69.49	10 – 120*	Kaya et al (1999)
S	*Upeneus vittatus* (*n* = 3)	Teleost	Mainly Osteichthyes and crustaceans; Other benthic invertebrates	27.43	5 – 100*	Prabha and Manjulatha (2008)
S+W	*Veladona togata* (*n* = 26)	Cephalopod	Based on *Octopus vulgaris*. Crustaceans: Large variety of large and small crustaceans; Molluscs: Bivalves; Limpets; Octopus, other; Polychaetes	414.01 ± 95.78	400 - 600	Smith (2003); Silva et al (2009)

*Depth data obtained from www.FishBase.com database.
+Depth data obtained from www.SeaLifeBase.com database.
†Data depth obtained from www.FAO.org/fishery/ database.

Appendix C: References

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