Trophodynamics on Mid-Ocean Ridges: spatial patterns in macro-consumers of the Mid-Atlantic and East Scotia Ridges

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A Thesis submitted to Newcastle University for the Degree of Doctor of Philosophy

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Submitted:

October 30th 2012

Breton Fisherman's Prayer

Dear God,

Be good to me,

The sea is so wide,

And my boat is so small.

Abstract

The global mid-ocean ridge (MOR) system is ~60 000 km long and accounts for 9% of the seafloor. Deep-sea organisms living on MOR have two potential energy sources; chemosynthesis and the downward flux of photosynthetic organic matter. This study examines the trophodynamics of benthic fauna collected from non-vent sites north and south of the Charlie-Gibb Fracture Zone (CGFZ) on the Mid-Atlantic Ridge (MAR) and hydrothermal vents fields (E2 and E9) on the East Scotia Ridge (ESR) using stable isotopes of carbon ($\delta^{13}C$), nitrogen ($\delta^{15}N$) and sulphur ($\delta^{34}S$).

$\delta^{13}C$ and $\delta^{34}S$ values revealed the MAR benthos was sustained by photosynthetic primary production and no chemosynthetic food source was detected. $\delta^{15}N$ values of benthic invertebrates were lower than the surficial sediments at the southern site but this did not occur at the northern site. Benthic invertebrates appeared to comprise a separate food chain to bentho-pelagic fishes and crustaceans but size-based trends in $\delta^{13}C$ and $\delta^{15}N$ revealed at certain life history stages bentho-pelagic fishes may consume benthic fauna. Size-based trends in $\delta^{13}C$ and $\delta^{15}N$ trends varied spatially and temporally in some bentho-pelagic fishes, which suggested differences in feeding plasticity among the species.

Spatial differences among sites were observed in $\delta^{13}C$, $\delta^{15}N$ and $\delta^{34}S$ of the ESR vent fauna. These were thought to reflect differences in the vent fluid chemistry, vent derived carbon fixation pathways and incorporation of photosynthetic organic matter into the vent system depending on the species and the magnitude of the difference among sites. Size and sex were important determinants of intra-population variability in stable isotope values of three species of vent fauna but this was not consistent among sites.

The present study revealed the importance of undertaking a tri-isotope approach to deep-sea trophic studies in order to elucidate production sources and at different sizes deep-sea organisms can link different trophic pathways.

Acknowledgements

I embarked on this PhD knowing it would be an adventure: exploring underwater water mountain ranges, working in frontal regions in the North Atlantic Ocean, research surveys to the Antarctic and using scientific equipment at the forefront of UK deep-water research. I think I should start by acknowledging Ben Boorman of National Oceanography Centre, Southampton (NOCS) for his expertise in trawling on the Mid-Atlantic Ridge and Dave Turner and his ROV team (NOCS) for their skill in ROV operations. Without these dedicated people I would not have collected the samples for this PhD.

I would like to thank my supervisors Dr Ben Wigham and Prof. Nicolas Polunin for all their help and support throughout the PhD. It was not the most-straight forward of PhD's due to postponement of research cruises, delays in sample return from the Antarctic and an unsuccessful foray into the world of lipids. Their guidance and encouragement has been invaluable, I have learnt so much and enjoyed working with them.

I would like to thank members, past and present, within the research group at Newcastle University, especially Dr Benjamin Kurten for advice on stable isotopes and Dr Aileen Mill for advice on stable isotopes and statistics. A big thank you has to be given to Dr Chris Sweeting for his advice, interesting discussions on all aspects of isotope methodology and ecology, help with writing grant proposals and reading through various pieces of work. Further people who need my thanks out with the research group are Dr Kate Broughton and Dr Gordon Port for using their micro-balance and Dr Martin Edwards for freeze drying samples for me.

This PhD was involved in the ECOMAR and ChEsSo NERC funded consortium projects and there is a long list of who have helped me in the last four years. Firstly, within the ECOMAR I would like to thank Prof. Monty Priede (University of Aberdeen) for inviting me on two ECOMAR cruises to the Mid-Atlantic Ridge. I was assisted with sample collection on these trips by Dr Ben Wigham, Andrew Oliphant and Gary Robinson (both Newcastle University) along with various other scientists who were sorting trawls and carrying cores. Special mentions go to Dr Nikki Cousins (University of Aberdeen) for co-ordinating the deep-sea fish identifications and providing advice on their ecology, Claudia Alt for co-ordinating the invertebrate identifications from the trawl samples, and Dr Tracy Schimmield and Dr Richard Abell for providing sediment trap samples. Secondly, within the ChEsSo project I would like to the thank Prof. Alex Rogers (University of Oxford) and Prof. Paul Tyler (University of Southampton) as PIs on the two ChEsSo cruises, Dr Katrin Linse (British Antarctic Survey) for co-ordinating the sample sorting and identifications, Dr Katrin Zwirglmaier (formally of BAS now Technical University Munich, Germany) for always keeping me up-to-date with progress on the identification of epi- and endo-symbionts of the invertebrates, and Leigh Marsh (University of Southampton) for providing information on the spatial distribution of vent organisms at each site. Finally, a thank you to all those I sailed with on the R.R.S *James Cook* including the Captains, crew, chiefs and scientists who made each trip memorable.

The stable isotope analysis was undertaken at Iso-Analytical (Cheshire, UK) by Steve Brookes and his team and at SUERC (East Kilbride) by Rona McGill. I visited SUERC on numerous occasions and was always made to feel welcome. Thanks you to Rona McGill who taught me some of the ins and outs of isotope ratio mass spectrometry.

I would like to thank Dr Andrew Jackson (Trinity College, Dublin) for his statistical assistance in Chapter 5. This involved help with writing the code for the R statistical programme and taking the time to run through interpretation the results.

I'm grateful to Dr Odd Askell Bergstad and Dr Tone Falkenhaug (Institute of Marine Research, Norway) and Dr Tracey Sutton (VIMS) for their support through the MAR-ECO network as well as MAR-ECO for providing funds to attend workshops and the Deep-Sea Biology Symposium in Iceland.

The work was funded by the Natural Environment Research Council and consisted of a studentship NE/F010664/1; sample analysis via Life Sciences Mass Spectrometry Facility grants EK127-10/08, EK150-12/09 and LSMFBRIS043_04/10_R_09/10; ECOMAR consortium grant NR/C512961/1; and ChEsSo consortium grant NE/DO1249X/1.

I'm very grateful to my parents and sister for all the love and support they have given me over the years. Finally, I would like to thank Charlotte. Thank you for your patience, support, encouragement, cups of tea, cooked meals and constantly being there for me whether I was at sea, home or when I thought the PhD was crumbling around me. I could never have completed this without your love.

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$\%C_{org}$	percentage organic carbon
%TN	percentage total nitrogen
a	semi-major axis of the standard ellipse
ACC	Antarctic Circumpolar Current
ANCOVA	analysis of covariance
ANOVA	analysis of variance
ASPF	Arctic Sub-polar Front
b	semi-minor axis of the standard ellipse
CBB	Calvin-Benson-Bassham cycle
CGFZ	Charlie-Gibb Fracture Zone
ChEsSo	Chemosynthetically-driven Ecosystems South of the Polar Front: Biogeography and Ecology
CIR	Central Indian Ridge
CSAA-SIA	compound-specific amino acid stable isotope analysis
ΔTPc	shift in intra-population trophic position indicated by carbon
ΔTPn	shift in intra-population trophic position indicated by nitrogen
E	eccentricity
ECOMAR	Ecosystem of the Mid-Atlantic Ridge at the Sub-Polar Front and Charlie Gibbs Fracture Zone
EPR	East Pacific Rise
ESR	East Scotia Ridge
GAR	Galapagos Rift
GOR	Gorda Ridge
JDF	Juan de Fuca Ridge

MAP	Mariana Arc
MAR	Mid-Atlantic Ridge
MOR	mid-ocean ridge
MUFA	monounsaturated fatty acid
OTSB	semi-balloon otter trawl
P	predator
P/S	predator/ scavenger
PAFL	pre-anal fin length
PNG	Papua New Guinea
POC	particulate organic carbon
POM	particulate organic matter
PUFAs	polyunsaturated fatty acids
ROV	remotely operated vechile
rTCA	reductive tricarboxylic acid cycle
RuBisCO	ribulose-1,5-biphosphate carboxylase/oxygenase
SB	Southern Antarctic Circumpolar Boundary
SDF	surface deposit feeder
SEA_B	Bayesian standard ellipse area
SEA_C	corrected standard ellipse area
SF	suspension feeder
SIA	stable isotope analysis
SL	standard length
SSDF	subsurface deposit feeder
TN	total nitrogen
Tukey's HSD	Tukey’s honest significant differences
UNESCO	United Nations Eductional, Scientific and Cultural Organisation

$\Delta^{13}C$	trophic discrimination of carbon
$\Delta^{15}N$	trophic discrimination of nitrogen
$\Delta^{34}S$	trophic discrimination of sulphur
$\Delta Lmax$	proportional maximum length range
$\delta^{13}C_{norm}$	lipid-normalised $\delta^{13}C$
θ	the angle in degrees between the semi-major axis of the corrected standard ellipse area and the x-axis

Chapter 1: Introduction

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1.1. The deep-sea ecosystem

The oceans cover 362 million km^2 of the Earth's surface, with approximately 90% of the volume classified as the deep sea (Ramirez-Llodra et al. 2010). The deep sea forms the largest ecosystem on Earth but only 5% has been explored by either remote instrumentation or active sampling (Ramirez-Llodra et al. 2010). The deep sea plays a significant role in global elemental cycles. It provides an important carbon sink via sedimentation to the seafloor, storage as living biomass and the drawdown of atmospheric CO_2 during deep water formation (Sabine et al. 2004, Buesseler et al. 2007). Conversely, the deep sea provides nutrients to the euphotic zone that are required for primary production through upwelling and deep-water mixing (Dugdale & Wilkerson 1998, Korb et al. 2005, Rixen et al. 2005). Deep-sea organisms facilitate many parts of the element cycling through alteration of inorganic or organic matter. Understanding the role of deep-sea organisms in recycling, remineralising or removal of elements at the various deep-sea habitats is important for identifying their role in biogeochemical cycling.

The four dimensional nature of the oceans means there are essentially two realms within which deep-sea organisms live. The deep-pelagic realm starts beyond the shelf break and below the euphotic zone at a depth of 200 m: the point where usable sunlight for photosynthesis does not penetrate (Gage & Tyler 1992). This descends through a series of zones (meso-, bathy- and abysso-pelagic), with concurrent changes in pressure, temperature and light (Gage & Tyler 1992, Thistle 2003). The depth of these zones varies among the oceans because of the interaction of water masses with different physical properties (e.g. temperature, salinity). They are also blurred in terms of biological constituents as deep-pelagic fauna vertically migrate or are associated with

submergence of water masses at fronts and oceanic features (Angel 2003, Collins et al. 2012). The deep-sea floor starts at the edge of continental slope and descends onto and across the abyssal plains. It is broken up by a series of habitats including canyons, seamounts, coral mounds, hydrothermal vents, cold seeps, trenches and mid-ocean ridges (MOR). These benthic habitats interact with the pelagic realm through abiotic and biotic processes.

The deep-sea floor has two striking features; these are the world-wide MOR system and the circum-oceanic system of deep trenches (Juteau & Maury 1999). MOR are topographically complex systems that have proved logistically very difficult to sample. However, with the advancement of modern technology, these habitats are becoming increasingly accessible to study. The purpose of this investigation is to examine spatial patterns in trophodynamics at two sections of MOR with contrasting energy inputs; non-vent sections of the Mid-Atlantic Ridge (MAR), North Atlantic, and hydrothermal vents of East Scotia Ridge (ESR), Southern Ocean. The following introduction provides the background information on food web theory, techniques for investigating trophodynamics with an emphasis on stable isotope analysis, an overview of the existing knowledge on food sources sustaining benthic organisms living in non-vent and vent areas of the deep sea, their response to food availability and patterns of species distributions, and the role of body size in trophodynamics. Finally, an overview of MOR habitats and an introduction to the study areas will be presented, which will lead into an outline of the subsequent chapters and objectives of the thesis.

1.2. Food chains and webs: a fundamental ecosystem process

An ecosystem can be described as a natural functioning unit that comprises both abiotic and biotic components. In order for an ecosystem to operate it needs energy and a series of processes that transfer it. A food chain is the simplest depiction of energy transfer

through an ecosystem. Autotrophic organisms can harness energy by using inorganic species through photosynthesis or chemosynthesis, to produce organic compounds. Autotrophs are referred to as primary producers and are the basal organisms of the ecosystem. Energy stored in organic compounds of primary producers can be utilised by heterotrophic organisms and also by the autotrophs themselves. Heterotrophs gain the organic compounds they need for metabolic processes, somatic growth and development by consuming autotrophs and are called primary consumers, herbivores or grazers. In turn heterotrophic organisms from higher trophic positions can consume other heterotrophs through predation until a top predator is reached. Thus energy is transferred through a series of linear steps or trophic positions resulting in a food chain. Top predators can be a food source either for parasites or, once they die, provide energy for decomposers and scavengers. At each step metabolic waste products are excreted that can act as a food source (i.e. detritus, dissolved organic matter) or provide the nutrients for primary production.

Ecosystems contain a number of diverse communities and habitats, with numerous trophic interactions and often with organisms utilising different energy sources (Jennings & Mackinson 2003). Compiling all these food chains or trophic pathways together results in a food web. The total biomass of organisms decreases at each trophic position as nutritional energy is lost either as heat or through respiration. This is because not all of the autotrophic or heterotrophic production can be assimilated by the consumer at the higher trophic position (Jennings et al. 2001a). Understanding the structure of food webs is an important question in ecology as these components offer structure to communities and play important roles in biogeochemical fluxes. Species are often arranged into functionally similar groups so that energy flux and transfer efficiencies can be measured (Jennings et al. 2002c, Polunin & Pinnegar 2002, Rooney et al. 2006, Van Oevelen et al. 2011).

Trying to produce complete food webs is fraught with difficulties because they are extremely complex (Post 2002b), have high degrees of spatial and temporal variation (Vander Zanden et al. 2000), are time consuming to construct and are often subjective in their scope and resolution (Post 2002b). A great deal of research has been directed at developing hypotheses to describe the structure and function of food webs. These studies suggest the number of trophic positions and energy flow through the food web can be limited by productivity (Pimm 1982), space (Post et al. 2000), the stability of the various food chains within the food web (Post 2002a, Rooney et al. 2006) or its ecological efficiency in transferring energy to higher trophic positions (Jennings & Warr 2003). However, no single universal factor influences the structure and function of food webs (Post 2002b). Instead a pragmatic approach dictates that the biological processes behind these hypotheses interact and will vary in importance depending on different ecosystems (Post 2002b).

1.3. Investigating trophodynamics

Trophodynamic studies investigate the trophic relationships among organisms in a community (Lindeman 1942) and attempt to identify fluxes of nutrients and energy between them (Polunin & Pinnegar 2002). The deep sea is a logistically and technically demanding place to undertake trophic studies. Technological advances in camera systems have provided insight into the behaviour of many species around baited experiments (Priede et al. 1994, King et al. 2006) and have allowed observations of deposit feeders consuming phytodetritus (Bett et al. 2001). However, sampling individuals in order to understand energy flow and elucidate trophic interactions is problematic. Many deep-sea organisms are fragile and are damaged during collection by abrasive sampling equipment or through the effects of hydrostatic pressure. This often leads to stomach contents being regurgitated by the time individuals arrive on deck

(Mauchline & Gordon 1984a). When stomach content analysis can be undertaken there are a series of problems that make assessing the importance of dietary items difficult. Poor taxonomic knowledge, highly digested prey items and low sample sizes all potentially undermine the conclusions based on assessing diet.

A complementary approach to stomach content analysis is to use biochemical techniques (Michener & Kaufman 2007, Iverson 2009). These have advantages over conventional prey identification and behavioural studies because they represent the organic matter that has been assimilated from the diet and incorporated into the consumers' tissues (Sargent et al. 1987, Hesslein et al. 1993). Many higher marine consumers must acquire specific biochemical compounds from their diet as they cannot biosynthesise *de novo* (Pond et al. 1997, Nichols 2003). Specific biomarkers such as fatty acids (Pranal et al. 1996, Howell et al. 2003), sterols (Ginger et al. 2000, Phleger et al. 2005) and carotenoid and chlorophyll pigments (Negre-Sadargues et al. 2000, Wigham et al. 2003), provide high-resolution information on the ultimate source of primary production and transfer through the food web. For example, different species of phytoplankton, types of bacteria and copepods can biosynthesise specific lipids, which make lipid analysis a valuable tool in identifying potential prey items and energy sources (Sargent et al. 1987, Pond et al. 1998, Iverson 2009).

1.3.1. Stable isotope analysis

Stable isotope analysis is a complementary approach to the biochemical techniques outlined above and is now one of the main ways to investigate trophic interactions (Layman et al. 2011). Stable isotopes are atoms with the same number of protons and electrons but contain one or more extra neutrons within the nucleus resulting in a different atomic mass. They are energetically stable, do not decay and are not radioactive. The stable isotope composition of a sample is reported relative to an

international standard and is expressed in parts per mill (‰) deviation from the standard (Equation 1):

$$\delta^{H}X\ (‰) = [(R_{sample}/ R_{standard} -1)] * 1000 \text{ (Eq. 1)}$$

where ^{H}X is the heavier isotope (e.g. ^{13}C, ^{15}N), R is the ratio of the heavy to light isotope (e.g. $^{13}C/^{12}C$, $^{15}N/^{14}N$), R_{sample} is the ratio of the sample and $R_{standard}$ is the standard. A positive δ-value indicates the sample has more of the heavy isotope than the standard while a negative value indicates the sample has less of the heavy isotope than the standard. The large difference between abundant and rare (e.g. ^{14}N = 99.635 and ^{15}N = 0.365) as well as large differences in atomic mass makes them useful for ecological studies.

Isotopic fractionation is key to using stable isotopes in ecological studies and is the basis for natural variation in the heavy and light isotopes in biological material (Owens 1987). Isotopic fractionation can be physical or chemical (Owens 1987, Hoefs 2004, Fry 2006). Physical fractionation (e.g. diffusion) results in the lighter isotope moving faster through a medium than the heavier isotope. Chemical fractionation occurs during the making or breaking of bonds as a result of equilibrium isotope effects or kinetic isotope effects (Fry 2006). Equilibrium isotope effects (e.g. atmospheric CO_2 dissolving in water) are exchange reactions and the heavy isotope accumulates where the bonds are strongest. Kinetic isotope effects (e.g. photosynthesis or chemosynthesis) are irreversible reactions that result in the accumulation of heavier isotopes in the substrate and lighter isotopes in the product (Hoefs 2004). Equilibrium and kinetic isotope effects refer to the reactions of substrates into products at a chemical level. However, within an organism's tissues there are multiple fractionation reactions involved with assimilation, metabolism and excretion of waste products, which determine the tissue's isotopic value (del Rio et al. 2009). In ecological studies it is more appropriate to refer to fractionation

as trophic discrimination (or trophic shift) which describes the difference in isotopic value between the tissue of the consumer and its food source (Equation 2) (del Rio et al. 2009)

$$\Delta^{H}X = \delta_{tissue} - \delta_{food} \text{ (Eq. 2)}$$

where ^{H}X represents the heavier isotope (e.g. ^{13}C, ^{15}N), δ_{tissue} is the value of the consumer's tissue and δ_{food} is the food source consumed.

Stable isotopes differ in their level of trophic discrimination as they pass into, through and out of consumers making them amenable for use in ecological and environment studies (Michener & Kaufman 2007). The main stable isotopes used in ecological studies are carbon ($^{13}C/^{12}C$ expressed as $\delta^{13}C$), nitrogen ($^{15}N/^{14}N$ expressed as $\delta^{15}N$) and sulphur ($^{34}S/^{32}S$ expressed as $\delta^{34}S$) but oxygen ($^{18}O:^{16}O$ expressed as $\delta^{18}O$) and hydrogen ($^{2}H/^{1}H$ expressed as δD) are also used. Stable isotopes record the assimilated diet over time, which is influenced by what an individual consumes and the habitat in which it lives (Newsome et al. 2007). The stable isotope chosen to address ecological questions depends on the objectives of the investigation. $\delta^{13}C$, $\delta^{15}N$ and $\delta^{34}S$ values are primarily used in trophic studies (Michener & Kaufman 2007) because unique information can be gained which identifies energy sources ($\delta^{13}C$ and $\delta^{34}S$) and relative trophic position ($\delta^{15}N$) (Vander Zanden & Rasmussen 1999, Vander Zanden et al. 1999, Iken et al. 2001, Polunin et al. 2001). $\delta^{18}O$ and δD are commonly used to examine large scale spatial movements of organisms (Hobson 2007, Shephard et al. 2007) and ambient, short- and long-term changes in climatic or environmental conditions (Fricke et al. 1995, Buick & Ivany 2004, Chauvaud et al. 2005).

Stable isotopes of carbon show very little trophic discrimination ($\Delta^{13}C$) between food source and consumer, typically ranging between 0 and 1.5‰ per trophic position (DeNiro & Epstein 1978, Tieszen et al. 1983, Peterson & Fry 1987, Caut et al. 2009). A

step-wise enrichment of ^{13}C in the consumer relative to the food source is a result of a series of processes: including respiration which produces ^{13}C depleted CO_2 (DeNiro & Epstein 1978); preferential absorption of ^{13}C enriched compounds during digestion and assimilation (Rau et al. 1983); and isotopic discrimination during synthesis of new tissue and different biochemical compounds (e.g. lipids and protein) (Tieszen et al. 1983, Post et al. 2007, Wolf et al. 2009). Post (2002b) compiled a comprehensive review of $\Delta^{13}C$ and calculated a mean $\Delta^{13}C$ of 0.39 ± s.d. 1.3‰. There is still some debate as to whether the mean is an accurate value to use across multiple trophic positions as $\Delta^{13}C$ can vary for a variety of reasons, not least among taxa (Caut et al. 2009, del Rio et al. 2009).

The fidelity in the $\delta^{13}C$ values between consumer and food source allow carbon to be used to identify the energy source sustaining the base of the food web: where primary producers have distinct $\delta^{13}C$ values, it can elucidate the relative importance of these sources to the consumer (Michener & Kaufman 2007). In one of the most pioneering uses of stable isotopes in ecological studies, $\delta^{13}C$ values of hydrothermal vent fauna were found to be ^{13}C depleted relative to phytoplankton and non-vent deep-sea fauna (Rau & Hedges 1979), which led to the hypothesis that vent fauna were sustained by chemosynthetic primary production. Their use in identifying energy sources has also been successful in other marine habitats. For example, macroalgae, seagrasses, marsh plants, phytoplankton and benthic algae can be identified by their different $\delta^{13}C$ values (Jennings et al. 1997, Kaehler et al. 2000, Bouillon et al. 2002, Petursdottir et al. 2008, Attrill et al. 2009).

Stable sulphur isotopes are also used to identify the energy source (Peterson et al. 1986, Michener & Kaufman 2007, Erickson et al. 2009). Large differences in the $\delta^{34}S$ values of inorganic sulphur sources result in primary producers having distinctive $\delta^{34}S$ values.

The stable sulphur isotope trophic discrimination ($\Delta^{34}S$) between consumer and food source during feeding, assimilation and metabolism is between -1 and 2‰ with the mean trophic shift of 0.2‰ (Peterson & Fry 1987, Michener & Kaufman 2007). Oceanic phytoplankton use seawater sulphate with $\delta^{34}S$ values of ~20‰ resulting in $\delta^{34}S$ values of ~18‰ (Thode 1991, Connolly et al. 2004). Marine consumers dependent on photosynthetic primary production therefore have $\delta^{34}S$ values between 16 and 19‰ (Fry 1988). In contrast, $\delta^{34}S$ values of organic compounds mediated by microorganisms can vary widely depending on the type of chemical reaction (e.g. disproportionation reactions), the class of microorganism undertaking the reaction (e.g. archaea or sulphate reducing bacteria) and in the inorganic substrate (e.g. sulphate or sulphide) (Orphan et al. 2001, Jorgensen 2006). This results in consumers of chemoautotrophic bacteria at reducing habitats having $\delta^{34}S$ values between -32 and 14‰ (MacAvoy et al. 2005, Cordes et al. 2010). When $\delta^{34}S$ and $\delta^{13}C$ are used in conjunction, they have the potential to identify different energy sources where $\delta^{13}C$ values of primary producers are similar (Peterson et al. 1986, Connolly et al. 2004, Erickson et al. 2009).

Stable nitrogen isotopes undergo a greater trophic discrimination between food source and consumer than carbon and sulphur (Minagawa & Wada 1984, Peterson & Fry 1987, Caut et al. 2009). Stable nitrogen trophic discrimination ($\Delta^{15}N$) occurs at many stages during cellular nitrogen metabolism (Macko et al. 1987) and results in the consumer being ^{15}N enriched relative to the food source on average by 3.4‰ (Minagawa & Wada 1984). However, 3.4‰ is the mean of a series of $\Delta^{15}N$ values that range from 1.3 to 5.3‰ (Minagawa & Wada 1984, Post 2002b, Vanderklift & Ponsard 2003, Caut et al. 2009). Although influenced by the inorganic nitrogen source, $\delta^{15}N$ values give an indication of the relative trophic position of an organism relative to a baseline organism (Vander Zanden et al. 1997, Post 2002b, Michener & Kaufman 2007). Increasing $\delta^{15}N$ values at successive trophic positions are believed to be a result of isotopic discrimination

processes during amino acid synthesis (Macko et al. 1987) and the resultant loss of the lighter ^{14}N in nitrogenous waste products (DeNiro & Epstein 1981, Minagawa & Wada 1984, Gannes et al. 1998). $\Delta^{15}N$ may vary with type and quality of food (Vander Zanden & Rasmussen 2001, Mill et al. 2007, del Rio et al. 2009), among taxa (Minagawa & Wada 1984, Vanderklift & Ponsard 2003), among tissue types and organs within an organism (Pinnegar & Polunin 1999), with nutritional stress (Ambrose & DeNiro 1986, Hobson et al. 1993, Adams & Sterner 2000) and with temperature (Power et al. 2003).

1.3.2. Isotopic turnover rates of tissue

The isotopic values of primary producers and consumers are rarely stable through time and can change depending on various factors, including amongst other things, nutrient availability, temperature and life history stage (Ostrom et al. 1997, Jennings et al. 2001b, Fanelli & Cartes 2010). The rate at which spatial and temporal changes in the isotopic values of primary producers or shifts in diet are incorporated into the consumer's tissues is important in trying to understand trophic interactions. Isotopic changes at the base of the food web or a new diet do not manifest as instant changes in the isotopic values of consumer's tissues because it takes time for the individual to assimilate the new isotopic values of the food source (Sweeting et al. 2005, Fry 2006).

The rate at which the new isotopic values of prey or primary producers are assimilated into the consumer's tissue depends on the balance between somatic growth and the metabolic breakdown and maintenance of tissue (Hobson & Clark 1992, Hesslein et al. 1993, Fry 2006). The importance of these processes varies depending on body size, developmental stage and growth rate. Laboratory experiments indicate that young fast growing individuals incorporate new isotopic values quickly because they are synthesising tissue through somatic growth and metabolic turnover (Fry & Arnold 1982,

Hesslein et al. 1993, Bosley et al. 2002). Older or slow-growing individuals will take longer because the synthesis of new tissue is likely to be dominated by the metabolic breakdown and maintenance of existing tissue (Fry 2006). Furthermore, isotopic turnover times vary among different tissue types. For example, metabolically-active tissue like liver or the hepatopancreas may have faster turnover rates than muscle (Bodin et al. 2007a, Guelinckx et al. 2007).

1.4. Productivity, food sources and their influence on the deep sea

The deep sea is sustained by two contrasting sources of primary production. The majority of the deep-sea organisms are sustained by photosynthetic primary production in the euphotic zone. Photosynthesis is undertaken by phytoplankton, which fix carbon dioxide into energy-rich organic compounds using energy from sunlight. Deep-sea organisms are then dependent on this material sinking or being advected into deeper water. On average, only 0.5 to 2% of the net primary production in the surface waters reaches the sea floor at depths below 2000 m (Buesseler et al. 2007) via a process known as bentho-pelagic coupling. The benthic environment of the deep sea is therefore considered food-limited and nearly entirely heterotrophic (Gage 2003). Exceptions to this are the chemosynthetic habitats where reduced inorganic compounds are discharged from the seafloor at hydrothermal vents, cold seeps or decomposing cetacean carcasses (Smith & Baco 2003, Tunnicliffe et al. 2003). Here, microorganisms perform a similar role to phytoplankton as primary producers but instead of light they use chemical energy via chemosynthesis to fix carbon into organic matter (Karl 1995). The continuous supply of chemical energy at chemosynthetic environments results in a rich and abundant but localised food source.

1.4.1. Food inputs and the response of non-vent deep-sea fauna

Food supply to the deep sea varies with distance from the continental shelf edge, with depth and closely follows the patterns of overlying surface primary productivity (Gage & Tyler 1992, Lampitt & Antia 1997). Organic material produced in the euphotic zone is transported into deeper waters in various different forms including: aggregating remains of small phytoplankton and faecal pellets; downslope turbidity currents and slumps, which may include terrigenous and shelf sea organic matter; large plant detritus; large and small animal carcasses; and vertically interconnecting food chains (Gage 2003). These mechanisms represent different organic matter pathways to the deep sea and transport organic matter at different speeds and on arrival will be available to different trophic guilds (Gage 2003).

The downward flux of phytodetritus arriving at the sea-floor is an important food source entering the deep sea. Evidence that the surface waters are connected to the deep sea through seasonal pulses of organic matter became apparent in the 1970s and 1980s, as a green flocculent layer was photographed settling on the sea-floor (Deuser & Ross 1980, Billett et al. 1983). The organic matter flux is not just composed of phytoplankton, but contains amorphous organic material, dead zooplankton, bacteria, crustacean eggs and faecal pellets (Madin 1982, Billett et al. 1983, Pfannkuche & Lochte 1993). The importance of this food source is reflected in the dominance of deposit feeding invertebrates in deep sea communities in terms of abundance and biomass (Billett et al. 1983, Gage 2003). Benthic deposit feeders have developed various morphological adaptations and behaviours in order to exploit this food source (Billett 1991, Roberts & Moore 1997). Resource partitioning among deposit feeders is further apparent because of the different biochemical fractions of the organic matter pool assimilated into their tissues (Howell et al. 2003, Wigham et al. 2003).

The benthic invertebrate community responds to the arrival of organic matter to the sea floor both functionally and numerically depending on body size. The nature of the response varies in different oceanographic settings (Gooday 2002). Bacteria and foraminifera show increased metabolic activity after the arrival of organic matter, followed by increase in their abundance (Pfannkuche & Lochte 1993, Pfannkuche & Soltwedel 1998, Gooday & Hughes 2002, Grove et al. 2006). This is reflected by the excretion of enzymes (Pfannkuche 1993) and an increase in the sediment community oxygen consumption (Smith et al. 2002). The response by meiofauna and macrofauna to organic matter is harder to detect and has varied depending on the study site. Some investigations suggest no detectable link, (Pfannkuche 1993) while others suggest that it affects growth and recruitment (Vanreusel et al. 2001) and increased abundances (De Bovee et al. 1990). Within the largest size class of deep-sea organisms, the megafauna, reproduction and growth in some species are linked to the seasonal pulse of phytodetritus which presumably allows larvae and consumers to take advantage of higher food availability (Young et al. 1992, Tyler et al. 1993, Campos-Creasey et al. 1994, Kemp et al. 2008).

A far less predictable energy source entering the benthic environment is marine carrion, which varies in size from fish to large marine mammals (Smith 1985, Jones et al. 1998, Kemp et al. 2006). These provide localised high energy food sources for scavenging fauna including fish, sharks and some invertebrates (Priede et al. 1991, Kemp et al. 2006, King et al. 2006). Deep-sea fishes and amphipods are often the first taxonomic groups to arrive and begin to consume marine carrion. Depending on the depth there may be a succession of different species of fish arriving at the food source, which is related to their local abundance and swimming speeds (Priede et al. 1994). Scavenging fauna break-down the food source and then distribute it over wider areas through excretion and defecation making it available to deposit feeders (Priede et al. 1991).

Large food falls like those of cetaceans can undergo a series of defined successional stages (Smith & Baco 2003). Amphipods and fish initially consume the flesh (Smith & Baco 2003, Kemp et al. 2006). Once the majority of flesh is removed, other scavenging crustaceans and polychaetes consume what is left as well as feed on the organic-rich sediment below the carcass (Smith & Baco 2003). Throughout this process, non-scavenging fauna can be attracted to the food source in order to prey on those feeding on the remains (Kemp et al. 2006). The final stage is when the lipid within the bones is broken down by anaerobic bacteria which release sulphides resulting in a chemosynthetic habitat (Smith & Baco 2003).

The deep scattering layer plays an important role in cycling organic matter in the interior of the ocean and transporting it down through the water column. Diel vertically migrating zooplankton and fishes actively transport organic matter in their stomachs over large depth ranges and may defaecate at greater depths (Angel 1997). During these vertical migrations zooplankton increase the rate of carbon flux by approximately 10% (Ducklow et al. 2001). Furthermore, during their migrations zooplankton and fishes may come into contact with mid-water predators or come within the range of bentho-pelagic predators. The later often happens when migrating organisms impinge on slopes of continental margins or mid-ocean ridges or get trapped over seamounts (Mauchline & Gordon 1991, Rogers 1994, Sutton et al. 2008). Many species of benthopelagic fish on ocean margins have diets dominated by mid-water fish or crustaceans (Pearcy & Ambler 1974, Mauchline & Gordon 1984a, b). This results in a series of overlapping vertical food chains that transport organic matter through the water column to the sea-floor.

1.4.2. Influence of food quantity and quality on benthic abundance & biomass

A large proportion of the variation in abundance, biomass and diversity of organisms with increasing depth and among oceans appears to be related to food supply in the deep sea (Johnson et al. 2007, Rex & Etter 2010). The measurement of particulate organic carbon (POC) flux to the seafloor can act as a proxy for the quantity of food available to deep-sea benthic organisms. Estimated POC fluxes, using sediment traps and satellite remote sensing, indicate that there is a latitudinal relationship between the quantity of food arriving at the seafloor and benthic abundance and biomass (Rex & Etter 2010, Wei et al. 2010). For example, under the equatorial Pacific macro- and megafauna abundances are positively correlated with POC flux (Smith et al. 1997), whereas in the North Atlantic abyssal benthic biomass is lower under oligotrophic compared to eutrophic surface waters (Thurston et al. 1998). Vertical trends in biomass are also apparent within the benthic and pelagic realm. All major size classes of benthic organisms (bacteria, meio-, macro- and megafauna) decrease in biomass with depth (Rex & Etter 2010). In the open ocean there is also an exponential decrease in biomass with depth (Herring 2002), although at the MAR, pelagic biomass peaks in the bathypelagic zone between 1500 and 2300 m (Sutton et al. 2008).

Temporal variability in the abundance and composition of abyssal communities has been attributed to the changes in the quantity and quality of the downward flux of POC from the euphotic zone (Billett et al. 2001, Ruhl & Smith 2004, Bailey et al. 2006). In the North Atlantic there was an overall increase in the abundance of meio-, macro and megafauna as a result of increases in food supply (Billett et al. 2001, Kalogeropoulou et al. 2010, Soto et al. 2010). One species of holothurians, *Amperima rosea*, increased in abundance by three orders of magnitude (Billett et al. 2001) and its abundance has fluctuated since (Billett et al. 2010). In the northeast Pacific, temporal fluctuations in

the abundance of holothurians were linked changes in POC flux (Ruhl & Smith 2004). However, not all megafaunal species increased in abundance during periods of high food input; some species decreased (Ruhl & Smith 2004, Billett et al. 2010). Variability in selectivity and assimilation of different biochemical fractions of the organic matter from the sediment may provide some deposit feeders with a competitive advantage under certain food supply conditions (Wigham et al. 2003). Overall, this suggests that spatial and temporal changes in abundance, biomass and diversity are driven partly by bottom-up processes resulting from differential responses among species to resource variability (Ruhl et al. 2008, Bailey et al. 2009).

1.4.3. Hydrothermal vents and their food sources

Hydrothermal vents are a very different habitat to the rest of the deep sea in terms of environmental conditions and the energy sources that sustain life. The discovery of hydrothermal vents in the late 1970s, and the dense biological communities thriving around hot fluids discharging from the seafloor changed the view that primary production was absent in the deep sea (Corliss et al. 1979, Karl et al. 1980). The fluid emitted from seafloor vents is modified by a combination of temperature-related seawater-rock interactions in the oceanic crust, magmatic degassing and subseafloor biological processes (German & Von Damm 2003). As seawater percolates down through the oceanic crust and is heated through magmatic or tectonic processes, sulphate and magnesium are stripped out of the seawater and replaced by reduced inorganic compounds including H_2S, CH_4, and H_2 and certain metals including iron and manganese (German & Von Damm 2003, Kelley et al. 2005). The heated fluids begin to rise up through conduits and are emitted from the seafloor via chimneys and diffuse flow areas. The resultant vent fluids have elevated temperatures, differing pH and are enriched in reduced inorganic compounds relative to seawater (Van Dover 2000).

Microbes oxidise the reduced inorganic compounds in vent fluids and utilise the energy released to fix CO_2 or other single carbon compounds (e.g. CO, CH_4) into cellular material (Karl 1995). In turn they provide the base of the hydrothermal vent food web.

The continuous supply of reduced inorganic compounds in the vent fluid provides an abundant source of energy for microbial primary production (Campbell & Cary 2004, Campbell et al. 2006, Dubilier et al. 2008). The constant supply of energy available for chemosynthesis and the specialised host-symbiont relationships account for the higher biomass observed at hydrothermal vents compared to the surrounding deep sea (Van Dover 2000, Tunnicliffe et al. 2003). However, the extreme environmental conditions at hydrothermal vents excludes many deep-sea fauna from exploiting the high biomass and productivity found at these habitats (Tunnicliffe et al. 2003). Instead, hydrothermal vent organisms have developed specific physiological adaptations to cope with the high temperatures and toxic conditions (Childress & Fisher 1992, Pranal et al. 1995, Henry et al. 2008). The result is that hydrothermal vents are often dominated by one or two species and diversity is far lower than the surrounding deep sea (Van Dover 2000, Tunnicliffe et al. 2003, German et al. 2011).

There are multiple food sources at hydrothermal vents, of which vent fauna take advantage through endo- and episymbiotic relationships and by consuming free-living microorganisms from surfaces or in suspension. Sulphur and methane oxidation appear to be the two principal energy acquisition pathways, which microorganisms use to drive carbon fixation (Conway et al. 1994, Karl 1995, Tunnicliffe et al. 2003). The most pertinent carbon fixation pathways to hydrothermal vent fauna are thought to be the Calvin-Benson-Bassham (CBB) and reductive tricarboxylic acid (rTCA) cycles (Desbruyeres et al. 1994, Robinson & Cavanaugh 1995, Campbell et al. 2003, Markert et al. 2007, Hugler & Sievert 2011). The CBB cycle is characterised by the enzyme

ribulose-1,5-biphosphate carboxylase/oxygenase (RuBisCO). Four forms of the RuBisCo enzyme are known but it is forms I and II that appear to be important in carbon fixation at hydrothermal vents (Robinson et al. 2003, Nakagawa & Takai 2008, Berg 2011). The rTCA cycle is a reversal of the Kreb's cycle (Nakagawa & Takai 2008, Hugler & Sievert 2011). Since the key enzymes in this carbon fixation process are oxygen sensitive (Berg 2011), this carbon fixation pathway may be restricted to the hottest areas of the hydrothermal vent, which are either anaerobic or have very low oxygen concentrations (Hugler & Sievert 2011). Photosynthetic primary production is a further food source that may be incorporated into the vent habitat because some species are dependent on it at certain stages or their life cycle (Pond et al. 2000, Stevens et al. 2008).

1.4.4. Global variation in hydrothermal vent habitats and communities

Hydrothermal venting has been discovered in every ocean from high latitudes to the equator (German & Von Damm 2003, Tunnicliffe et al. 2003, Pedersen et al. 2010). The geological setting, spreading rate and type of venting can have profound influences on vent fluid chemistry (German & Von Damm 2003). Sediment-hosted systems (e.g. Guaymas Basin on the East Pacific Rise or Middle Valley on the Juan de Fuca Ridge) where sediment is either intercalated or lies over the basalt have higher pH and greater contributions of thermogenic and biogenic methane than basalt-hosted MOR vents (German & Von Damm 2003). Off-axis venting is known to occur on the Mid-Atlantic Ridge at the Lost City vent field (Kelley et al. 2001). Tectonic rather than magmatic or volcanic activity results in vent fluids that are alkaline and are enriched in CH_4 and H_2 (Kelley et al. 2005). At back-arc spreading centres newly created crust is a complex mixture of melted oceanic crust and pelagic sediments from the subduction zone and magma from beneath the ridge (Reeves et al. 2011). The resultant fluids are highly

acidic, with elevated concentrations of CO_2 and other reduced compounds (Gamo et al. 2006, Reeves et al. 2011).

Hydrothermal vent fauna also vary geographically but the processes leading to global scale patterns in biogeography are strongly debated (Van Dover et al. 2002, Tunnicliffe et al. 2003). Six major hydrothermal vent biogeographical provinces are identified, which are characterised by the presence or absence of rimicarid shrimps, siboglinid and alvinellid polychaetes, provannid gastropods and bathymodiolid mussels (Desbruyeres et al. 1994, Van Dover et al. 2001, Tunnicliffe et al. 2003, Bachraty et al. 2009). Vicariance, seafloor spreading and larval dispersal potentially play important roles in the differences among the provinces (Tunnicliffe & Fowler 1996, Van Dover et al. 2002). The ecological and functional role of the dominant fauna also differ (Van Dover et al. 2002), which may have implications for food web structure. For example, the vent shrimp *Rimicaris exoculata* and siboglinid polychaete *Riftia pachyptila* are the dominant species occupying the hottest areas close to vent orifices on the MAR and East Pacific Rise, respectively (Tunnicliffe et al. 2003). They have similar $\delta^{13}C$ values indicating they potentially use a similar energy source (Fisher et al. 1994, Colaco et al. 2002) but their functional role is very different: *R. exoculata* forms dense swarms over the chimneys while the long tubes formed by *R. pachyptila* during growth add habitat complexity and greater surface area. The discovery of hydrothermal vent sites consisting of new species assemblages and different vent fluid chemistries continues to reveal novel trophic interactions (Limen & Juniper 2006, Petersen et al. 2011).

1.5. The role of body size in trophodynamics

Understanding the interaction of body size and productivity within an ecosystem and how organisms divide this into niche space are important questions in ecology. Body size is an important ecological driver of an organism's energy requirements and life

history strategies (Brown et al. 2004, Atkinson & Hirst 2007) as well as playing a vital role in structuring inter- and intra-specific interactions (France et al. 1998, Jennings et al. 2001b, Jennings et al. 2002b). Evidence that body size plays an important role in structuring aquatic communities became apparent when Sheldon et al. (1972) examined the relationship of body size, abundance and mass in phytoplankton. Within the deep sea, investigations into body-size trends have been directed towards understanding bathymetric trends in body size. At the community level, there is an overall decrease in mean size with depth, which is hypothesised to be the result of decreased food availability (Thiel 1975, Rex & Etter 2010). However, contrasting body-size trends among and within taxa occur as some species increase in biomass while others decrease with depth (Soltwedel et al. 1996, Olabarria & Thurston 2003, Collins et al. 2005, Rex & Etter 2010). This indicates there is no single factor that can account for these patterns and ecological factors within physiological constraints may determine patterns of body size (Collins et al. 2005).

Investigations into body-size trends in the deep sea have tended to focus on specific taxa's responses to food availability (Soltwedel et al. 1996) or spatial segregation of life history stages (Polloni et al. 1979, Macpherson & Duarte 1991, Priede et al. 1994). The role of intra-specific or intrapopulation trophodynamics has only been explored on limited occasions (Levesque et al. 2003, Stowasser et al. 2009). One of the most important interactions affected by body size is consumer-resource interactions (Cohen et al. 1993, Brose et al. 2006). In shallow marine systems body size can determine habitat utilisation, ingestion rate and potential size range of prey an individual can consume (Scharf et al. 2000, Lukoschek & McCormick 2001, Hatase et al. 2002). When consumers are classified by size instead of species, there is a near continuous increase in trophic position with size (France et al. 1998, Jennings et al. 2001b, Jennings et al. 2002b); much of this is driven by increases in intrapopulation trophic position (Jennings

et al. 2002b). This indicates that body size can exert strong structuring forces on marine communities.

Stable isotope analysis provides a powerful tool for describing certain aspects of intrapopulation variability in trophodynamics (Jennings et al. 2008, Layman et al. 2011). The variability around the mean has been suggested as a measure of trophic niche width (Bearhop et al. 2004). However, $\delta^{13}C$ and $\delta^{15}N$ often show systematic shifts with body size (Jennings et al. 2002b, Galvan et al. 2010, Greenwood et al. 2010). For example, Jennings et al. (2002a) found the $\delta^{15}N$ values of 18 out of 31 species of North Sea fish were a function of body size. In many cases the addition of size into the analysis can provide greater insight into the process that drives isotopic variability (Figure 1.1). Mean $\delta^{15}N$ and variance of example data may be similar among species but the systematic shift in $\delta^{15}N$ with size is very different (Figure 1.1). The $\delta^{15}N$-size trends, therefore provide additional information that coupled with data on habitat use, ontogenetic behaviour, responses to environmental variables or diet, provide a greater understanding of a species' trophic role within an ecosystem which may not be apparent from mean values and variance alone.

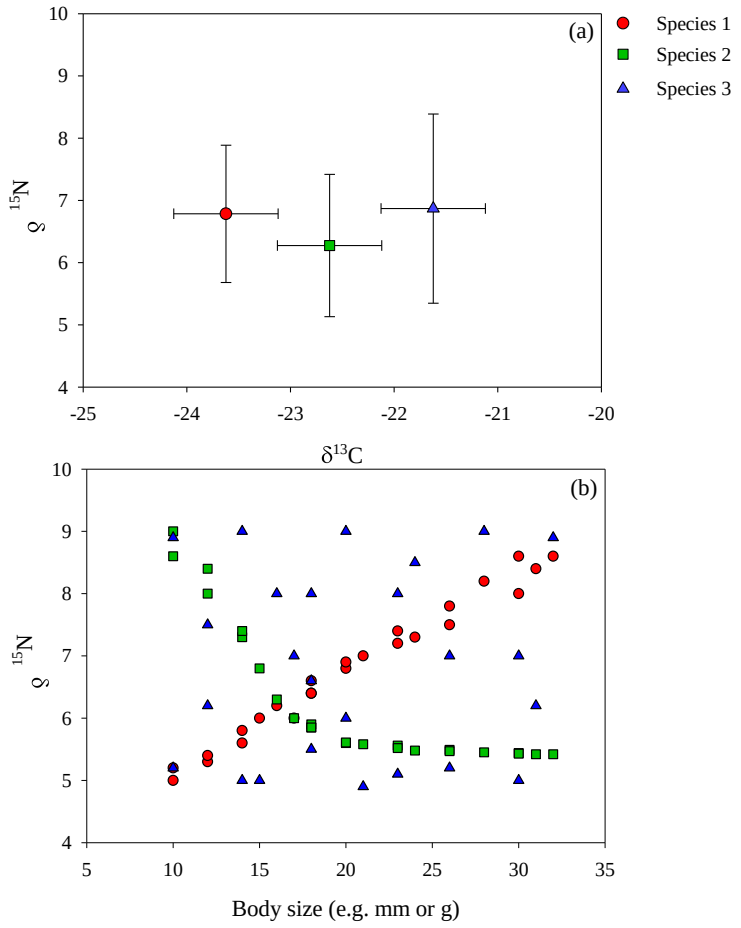

Fig 1.1: Hypothetical example of the potential differences in trophodynamics among three species, which may not be apparent when examining mean and variance of $\delta^{13}C$ and $\delta^{15}N$ (a) without the inclusion of body size as either biomass (e.g. g) or length (e.g. mm) (b). These values are for illustration only and are not the result of analysis of real species.

1.6. Mid-ocean ridges, study sites and thesis objectives

1.6.1. Mid-ocean ridges

MOR and trenches reflect the mobility of the ocean floor and are the areas where new oceanic crust forms or is being destroyed, respectively. MOR form a continuous system that runs through three major oceans, is approximately 60 000 km long (Juteau & Maury 1999) and accounts for approximately 9% of the ocean floor (Ramirez-Llodra et al. 2010). The spreading rate of MOR varies among the different oceans with fast-spreading ridges found in the Pacific Ocean (e.g. East Pacific Rise) and slow-spreading

ridge occur in the Atlantic Ocean (Mid-Atlantic Ridge). MOR rise from the sea floor to depths comparable to the continental slopes and therefore potentially interact with meso- and bathypelagic zones (Sutton et al. 2008). Further, ridges can develop as back-arc spreading centres in association with island arc systems and oceanic trenches. Oceanic crust subducted at trenches is melted and then rises up behind the island arc forming a small spreading ridge (Juteau & Maury 1999, Tunnicliffe et al. 2003) but all-in-tense purposes are similar to MOR (Juteau & Maury 1999).

MOR systems have complex topography and geology. This results in a remarkable diversity of habitats including: vast areas of bare rock cliff faces; axial valleys and fracture zones that extend to depths of 4000 m; seamounts which reach into the upper 1000 m; sediment filled bowls and plains; and hydrothermal vents (Van Dover 1995, Felley et al. 2008, Yesson et al. 2012). The presence of MOR and the occurrence of transform faults and ridge segmentation along the axis affects the distribution and dispersal of pelagic and benthic organisms (Bergstad et al. 2008, Sutton et al. 2008, Matabos et al. 2011). Primary production sustaining MOR biological communities also differs depending on the habitat. The majority of the MOR is thought to be heterotrophic (Bergstad & Godo 2003), and like the rest of the deep sea depends on the downward flux of organic matter from the surface waters produced via photosynthesis (Billett et al. 1983). However, as MOR are areas of crustal formation they also contain hydrothermal vents, which are fuelled by chemoautotrophic primary production (Tunnicliffe et al. 2003); food is unlikely to be limiting and biomass can be higher than in surrounding benthic habitats (German et al. 2011). Such contrasting habitats result in differences in elemental cycling and the degree of trophic connectivity in the wider deep sea.

This PhD focuses on two sections of the global ridge system in the Atlantic Ocean where trophodynamics and community structure have not previously been investigated, a northern section of the MAR between 48^{o} and 52^{o}N as well as the ESR in the Atlantic sector of the Southern Ocean (Figure 1.2). The energy sustaining these benthic communities varies. The MAR locations contain no known hydrothermal vent sources. Therefore, they are hypothesised to represent ridge segments sustained by photosynthetic primary production from the surface waters, but may well be distinct from similar depths at ocean margins because of an absence of terrigenous input of sediments and organic matter. The ESR locations are sites of hydrothermal venting, which will be sustained by a mix of photosynthetic and chemosynthetic primary production. Although the two ridge sections are disparate in terms of locality and sources of production, they provide a unique opportunity to investigate deep-sea trophodynamics at scales of tens of metres to hundreds of kilometres. An advantage of selecting these locations for spatial analysis, is that they are all at a similar depth (~2700 m), which potentially removes confounding issues of depth (Polunin et al. 2001, Bergmann et al. 2009). The main aim of this thesis is, therefore, to try and understand spatial differences in trophodynamics from species to assemblage levels in benthic macro-consumers of both invertebrates and fishes.

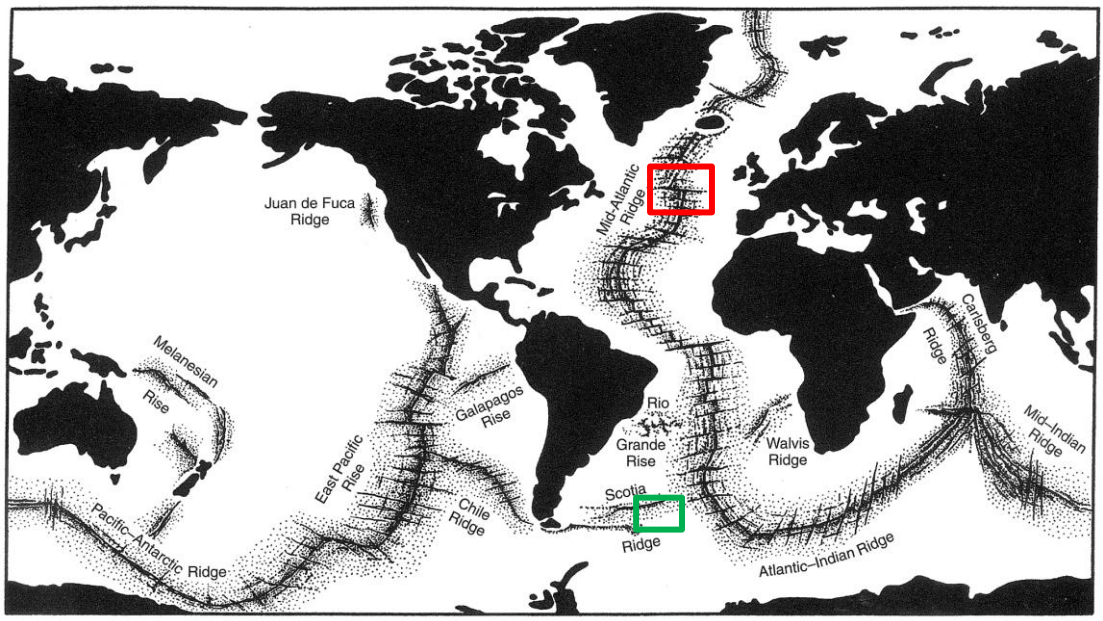

Fig. 1.2: Map of the worldwide oceanic ridge system adapted from Garrison (1993), indicating the study area on the Mid-Atlantic Ridge (red box) and the East Scotia Ridge (green box).

1.6.2. Mid-Atlantic Ridge

The Atlantic Ocean is divided in two by the MAR, which creates new oceanic crust at a rate of 20 mm yr^{-1} and is characterised as a slow-spreading ridge. It originates at the junction of the Gakkel Ridge northeast of Greenland and descends southwards. The ridge system bisects the continental shelf surrounding Iceland and this northern section is known as the Reykjanes Ridge. This stretches south westwards to 52^{o}N where there is a large fracture zone called the Charlie-Gibbs Fracture Zone (CGFZ), which offsets the ridge by 5^{o}E. Past the facture zone the ridge carries on to the Azores archipelago where it breaks the surface. The width of the ridge is wider north of the CGFZ where it is characterised by a series of sediment filled terraced valleys (Felley et al. 2008). South of the fracture zone the ridge system becomes narrower with a series of peaks, rough terrain and narrow valleys (Felley et al. 2008). Beyond the Azores, the MAR continues toward the Bouvet Triple Junction at the interface between the Atlantic and Southern Oceans resulting in a continuous 10 000 km underwater mountain range.

The hydrology and productivity over the northern section of the MAR is very complex and the ridge system plays an important role in North Atlantic circulation by influencing water flowing along it as well as crossing it (Soiland et al. 2008, Read et al. 2010). Deep water flow is influenced by the MAR (Read et al. 2010). This originates in the Iceland Basin and flows down the eastern axis of the ridge, with the majority of the water crossing the ridge in a westward direction at the CGFZ before heading northwards along the western axis (Soiland et al. 2008). The North Atlantic Current dominates the water flow in the upper 2000 m (Read et al. 2010). The majority of water crossing the MAR between 48^{o}N and 52^{o}N is in the form of two persistent branches of the eastward flowing NAC (Soiland et al. 2008). These positions correspond to the CGFZ where a large frontal feature, the Arctic Sub-polar Front (ASPF), lies to the south. The front splits the North Atlantic into two water masses which are cold, seasonally productive waters to the north and warmer less productive water to the south (Longhurst 2006). However, eddies and warm core rings associated with the ASPF and hydrographic interactions with the ridge produce a complex pattern of primary productivity that is not consistent with a simple north-south divide at the ASPF (G. Tilstone & A. Dale pers. comms.).

1.6.3. East Scotia Ridge

The Scotia Sea is situated in the Atlantic sector of the Southern Ocean. It is enclosed by a series of the underwater banks, ridges and islands that form a chain starting at the tip of South America and finishing at the Antarctic Peninsula. At the eastern boundary of the Scotia Sea, lie the South Sandwich Islands and South Sandwich Trench, which forms a complex chain of islands, volcanoes and a subduction zone. The ESR lies west of the South Sandwich Islands, separating the Scotia and Sandwich plates. It is spreading at an intermediate rate of between 65 and 70 mm yr^{-1}, creating a back-arc

basin (Livermore et al. 1997, Larter et al. 2003). The ESR is ~500 km long and consists of nine second order ridge segments (E1 to E9), separated by a series of non-transform discontinuities (Livermore 2003). The spreading axis at segments E3 to E8 is characterised by deep rift valleys. However, E2 and E9 segments have smooth axial volcanic highs which are similar in morphology to fast-spreading ridges including the East Pacific Rise (Livermore et al. 1997). An axial magma chamber seismic reflector is situated underneath the central part of the E2 segment (Livermore et al. 1997). At E9, seismic reflection profiles reveal a series of small pockets of magma underneath the axial volcanic ridge (Bruguier & Livermore 2001). Hydrothermal plumes are located over these ridge segments (German et al. 2000), which support dense life (Rogers et al. 2012).

The hydrology and productivity in the Scotia Sea is influenced by the surface Antarctic Circumpolar Current (ACC) and various topographic features (Murphy et al. 2007). The ACC is characterised by a series of four frontal zones that are at their narrowest in the Drake Passage but spread out as they enter the Scotia Sea (Naveria Garabato et al. 2002). Starting from the northern most front, they are the sub-Antarctic Front, Polar Front, the Southern Antarctic Circumpolar Front and the Southern Antarctic Circumpolar Boundary (SB). The SB is diverted northwards as it comes into contact with the ESR (Naveria Garabato et al. 2002). The Southern Ocean is dominated by high nutrient low chlorophyll conditions but the Scotia Sea represents some the most productive waters in the Antarctic (Murphy et al. 2007). However, there is high spatial variability in primary productivity and this reflects the large-scale variation in nutrients and physical conditions across the region (Korb et al. 2005, Murphy et al. 2007). Enhanced primary productivity mainly occurs downstream of South Georgia and the South Sandwich Islands and potentially over the ESR with lower productivity in the central regions of the Scotia Sea (Korb et al. 2005).

1.6.4. Thesis outline and objectives

Chapter 2 aims to elucidate the trophic pathways and energy sources sustaining the MAR benthos at two sites north and south of the CGFZ. The chapter explores spatial and temporal differences in $\delta^{13}C$ and $\delta^{15}N$ values of potential food sources, benthic and bentho-pelagic fishes and invertebrates. It utilises $\delta^{34}S$ to identify whether there is any influence of chemosynthetic primary production at these sites. Finally, it examines whether there are any isotopic differences in trophic guilds that may help to identify if there is more than one trophic pathway.

Chapter 3 examines whether intra-population variability in stable isotope values of four species of deep-sea fish collected from the MAR can be described by size. Size-based stable isotope trends are ubiquitous among shallow water fish but the prevalence of these trends has not been investigated in the deep sea. Spatial and temporal patterns in size-based stable isotope trends will also be investigated to see whether there is any evidence for feeding plasticity among the species.

Chapter 4 investigates the trophic interactions at the newly discovered hydrothermal vent sites on the ESR. It examines spatial differences in $\delta^{13}C$, $\delta^{15}N$ and $\delta^{34}S$ values of vent fauna among venting sites with contrasting vent fluid chemistries. Vent fauna will also be compared to non-vent deep-sea fauna from the surrounding area to see whether photosynthetic primary production is potentially augmenting the diet of animals at the hydrothermal vent.

Chapter 5 focuses on the variability in $\delta^{13}C$ and $\delta^{15}N$ of three key macro-consumers on the ESR and whether this can be explained as a function of size. The chapter uses a new multivariate statistical test to compare isotopic variability among sites. This is followed by conventional univariate statistics to see whether techniques are complementary.

Chapter 6 presents a synthesis of the main findings of the thesis, addresses potential limitations of the study and places the thesis is a wider context.

Chapter 2: Elucidating trophic pathways in benthic deep-sea assemblages of the Mid-Atlantic Ridge north and south of the Charlie-Gibbs Fracture Zone

Chapter 2: Elucidating trophic pathways in benthic deep-sea assemblages of the Mid-Atlantic Ridge north and south of the Charlie-Gibbs Fracture Zone

2.1. Introduction

The mid-ocean ridge (MOR) system is a conspicuous topographic feature in the deep sea, approximately 60 000 km long and is potentially distinct from similar depths on ocean margins because of the absence of major terrigenous input (Juteau & Maury 1999). Dissecting the world's ocean seafloor, they are regions where new oceanic crust forms and consequently contain large expanses of hard substrate, coupled with sediment-filled terraces, creating a complex topography and hydrology. The Atlantic Ocean seafloor is divided in two by the Mid-Atlantic Ridge (MAR), which originates at the junction of the Gakkel Ridge northeast of Greenland and descends southwards, bisecting Iceland and the Azores, and continues toward the Bouvet Triple Junction at the interface between the Atlantic and Southern Oceans. The complex topography of the MAR means the benthos has remained largely unexplored, the exception being isolated areas of hydrothermal venting that are sustained by chemosynthesis (Gebruk et al. 1997). However, hydrothermal vent communities are not representative of the ridge system as a whole (Copley et al. 1996, Bergstad et al. 2008) and the influence of chemosynthetic primary production along the ridge may only be small (Bergstad & Godo 2003). Consequently an understanding of the diversity, biomass and trophodynamics of the non-hydrothermal vent areas on the MAR is severely lacking.

Energy input sustaining benthic consumers on the MAR is expected to vary dramatically depending on whether the food source is chemosynthetic or photosynthetic in origin, and thus has implications for understanding the role MOR fauna play in carbon cycling. Primary production at hydrothermal vents is supported by

chemoautotrophic bacteria which utilise reduced volatile compounds such as hydrogen sulphide as an electron donor to fix carbon from inorganic into organic compounds and thus provide nutrition for consumers (Van Dover 2000). Away from hydrothermal vents, the link between primary producers and consumers in the deep sea is very different. The main food source for non-vent benthic deep-sea fauna is photosynthetically derived organic matter from the surface waters, which sinks to the seafloor in the form of phytodetritus (Billett et al. 1983), consisting of a mix of dead phyto- and zooplankton, faecal pellets and bacteria (Gooday 2002). Bacteria and benthic invertebrates ingest, process and redistribute organic matter by incorporating it into biomass (Smith et al. 2002, Witte et al. 2003, Drazen et al. 2008a) forming a food chain largely dependent on phytodetritus. Further food sources arrive at the sea-floor as discrete food falls of marine carrion (Smith 1985, Tyler et al. 1993), bathypelagic fauna impinging on topographic features or vertically interconnecting food chains through the meso- and bathypelagic realms (Angel 1997). Mobile benthic and bentho-pelagic predators and scavengers consume these food sources and form the end point of a bathypelagic food chain.

Understanding energy flow and trophic ecology within the deep sea is important as this may influence community structure and biomass (Wei et al. 2010, Wolff et al. 2011) but it also provides information for building functional food web and carbon cycling models (Wei et al. 2010). Deep-sea predator/ scavenger food chains become increasingly linear with depth, suggesting stronger trophic connections and channelling of energy flow (Polunin et al. 2001). The inclusion of deposit feeders in trophodynamic studies adds further complexity, as this may now include multiple trophic pathways that are less distinct and non-linear (Iken et al. 2001, Mincks et al. 2008). Bathypelagic fishes associated with the ridge crest are elevated in biomass between 1500-2300 m relative to the open-ocean above the adjacent abyssal plains, which may result in an enhanced

pelagic food source and strong trophic connections to bentho-pelagic fishes (Sutton et al. 2008). The MAR is also hypothesised to be the largest area of benthic lower bathyal habitat in the North Atlantic, defined over a depth range of 800-3500 m by UNESCO (2009), over 90% of which is covered in sediment (I G Priede pers. comm.). These two factors have potential implications for the transportation to and the processing of organic matter by the benthic community, whether reflected as a linear food web or as separate phytodetrital and predator/ scavenger food chains.

Investigating the different trophic roles of deep-sea organisms in food webs is challenging in terms of sample collection and method of examination. Low sample sizes, damaged fauna and gut eversion, all make assessing deep-sea trophodynamics difficult. Stable isotope analysis (SIA) of carbon ($^{13}C/^{12}C$ expressed as $\delta^{13}C$), nitrogen ($^{15}N/^{14}N$ expressed as $\delta^{15}N$) and sulphur ($^{34}S/^{32}S$ expressed as $\delta^{34}S$) measures material naturally incorporated into the organism's tissue, providing a temporally and spatially integrated analysis of the assimilated diet (Hesslein et al. 1993). Consumers show a trophic discrimination (or trophic shift) in the heavier isotope (^{13}C, ^{15}N and ^{34}S) compared to their food source as a result of isotopic discrimination occurring during a series of metabolic reactions. Trophic discrimination results in consumers being between 0 to1.5‰ enriched in ^{13}C and ^{34}S and 2.3 to 5‰ enriched in ^{15}N relative to their food source (Peterson & Fry 1987, Post 2002b). Small trophic discriminations in stable carbon and sulphur isotopes allow them to be used in identifying energy sources and when these are isotopically distinct multiple sources can be elucidated. In the case of $\delta^{34}S$, it can distinguish between chemosynthetic and photosynthetic primary production because photosynthetic primary production uses seawater sulphate (~19‰) to produce organic sulphur compounds (Thode 1991, Connolly et al. 2004) while chemosynthetic primary production at hydrothermal vents uses sulphides that can range between -2.8 to 10‰ (Shanks 2001, Reeves et al. 2011). The larger trophic discrimination in stable nitrogen

isotopes helps to indicate the relative trophic position of an organism from a basal resource (Post 2002b).

This study provides the first insight into the trophodynamics of a non-vent benthic MOR assemblage. The main aim of this study was to explore the potential trophic pathways supporting the benthic assemblages at two stations of the MAR. These stations lie north and south of the Charlie-Gibbs Fracture Zone (CGFZ), which defines the position of the Arctic Sub-Polar Front, and are therefore situated under different oceanographic regimes with contrasting surface primary production (Longhurst 2006, Martinez-Vicenta et al. 2012). They also underlie surface waters experiencing decreasing surface primary productivity as a result of increasing sea surface temperatures (Tilstone et al. 2009). It is hypothesised that within the MAR benthic community there will be two distinct trophic pathways: one based on the downward flux of phytodetritus utilised by benthic deposit feeders, and a second that utilises the elevated bathyal biomass present in association with the ridge (Sutton et al. 2008) and scavenging. The investigation examined (1) stable carbon and nitrogen isotope values of sediment and particulate organic matter as potential food sources, (2) spatial and temporal differences in stable isotope values of potential food sources and benthic and bentho-pelagic consumers, (3) stable sulphur isotope values of benthic and bentho-pelagic consumers to indentify whether there is an input of chemosynthetic organic matter, (4) isotopic differences in feeding guilds, and (5) whether there is more than one food chain evident within the MAR benthic assemblage.

2.2. Methods

2.2.1. Study sites

Two stations on the eastern axis of the MAR north and south of the CGFZ were sampled from onboard R.R.S *James Cook* during summers 2007 (13 July to 18 August)

and 2009 (1 August to 9 September 2009) (Figure 2.1). They were chosen as part of Natural Environment Research Council consortium grant ECOMAR. The overarching aim of the ECOMAR project was to determine the local and regional impact of the MAR as a physical structure on the ecology of the North Atlantic Basin in terms of production, biomass and biodiversity from the surface to the deep-sea benthos. The stations are remote from any islands and seamounts, with no known hydrothermal activity and are regarded as "typical" ridge segments. These stations are located ~690 km apart at depths between 2400 m and 2750 m.

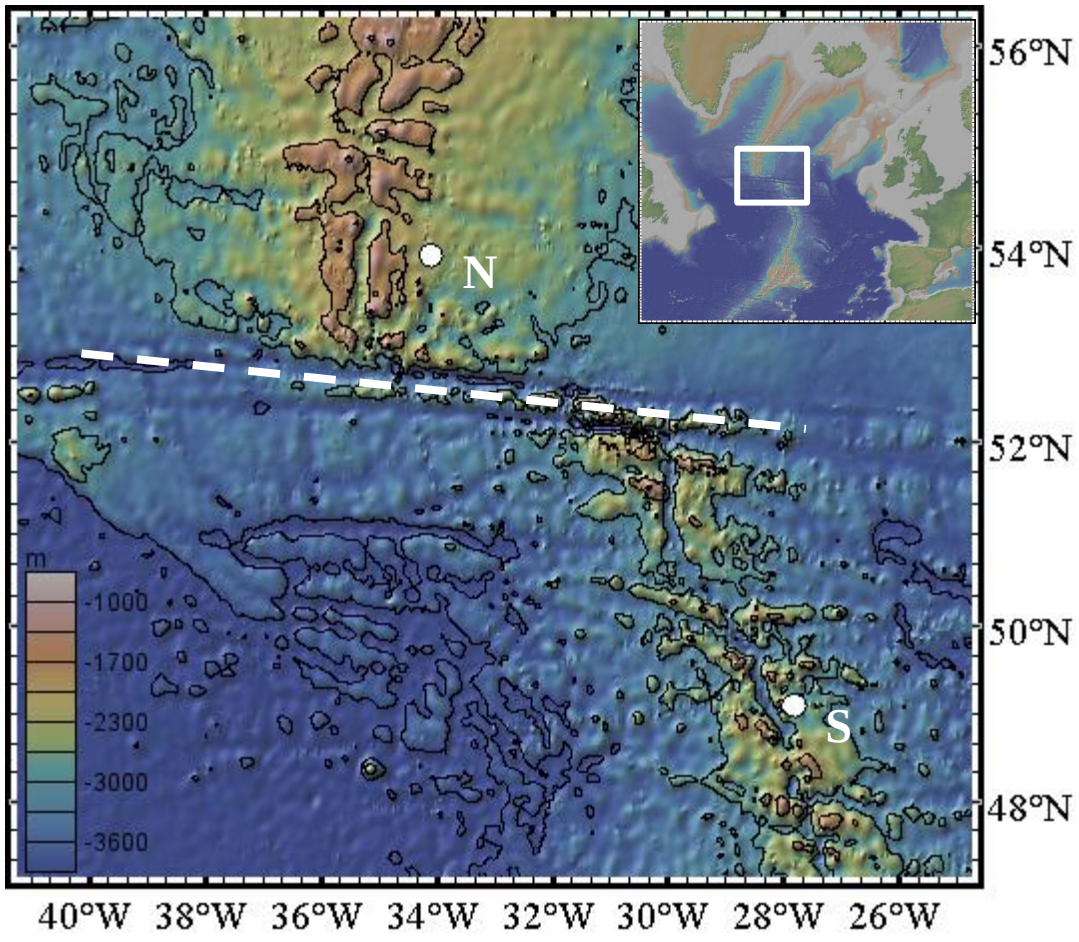

Fig. 2.1: Bathymetric map of the Mid-Atlantic Ridge indicating the sampling stations (white circles) north (N) and south (S) of the Charlie-Gibbs Fracture Zone (dashed white line). Insert represents the position of the study area in relation to the rest of the North Atlantic Ocean.

2.2.2. Sample collection and preparation

A swath bathymetry survey of the stations was undertaken during the 2007 research survey to assess the bottom topography to find suitable ground for the sediment trap mooring and benthic sampling (Table 2.1). Benthic fauna were collected using a single warp semi-balloon otter trawl (OTSB) with a wing end spread of 8.6 m and a headrope height of 1.5 m. Further details of the net configuration can be found in Merrett & Marshall (1981). Scavenging amphipods were sampled using a free-fall, baited amphipod trap. Catches were sorted on board to the lowest possible taxonomic resolution. Abundant organisms were selected for trophic analysis in 2007 and a broader range of samples was selected in 2009 to gain a better understanding of the wider community. Muscular tissue or body wall were dissected and frozen at -80^{o}C in glass vials. In order to obtain enough tissue mass for ophiuroids, amphipods and irregular sea urchins, arms, whole individuals and gonads, respectively, were dissected. No trawl samples were collected from the northern station in 2009 due to problems with the trawling gear.

Sediment cores were collected in 2007 and 2009 using a Bowers-Connolly megacorer fitted with eight 100 mm diameter coring tubes (Gage & Bett 2005). Three undisturbed cores with intact sediment layers were selected for sampling. Surface (0 to 5 mm) and subsurface sediments (5 to 10 mm) were collected and stored at -80^{o}C in glass vials for carbon and nitrogen SIA. Particulate organic matter (POM) was collected in sediment traps at 100 m above the seafloor at monthly intervals. Trap contents were preserved in formalin. Mobile organisms (e.g. pelagic copepods) were removed and each bottle was split into a series of aliquots for analysis. Sediment traps were not deployed prior to sample collection in 2007 and because no faunal samples were collected from the northern station in 2009, only samples from March to July from the southern station

Table 2.1: Stations, dates, positions, depths and gear used to collect samples from the Mid-Atlantic Ridge in the summers of 2007 and 2009.

Station	Sampling gear	Deployment date	Depth (m)	Start position		End position	
				Latitude (N)	Longitude (W)	Latitude (N)	Longitude (W)
Southeast	OTSB*	21/07/2007	2700	49°14.68'N	27°42.31'W	49°03.43'N	27°53.86'W
Southeast	OTSB	22/07/2007	2718	48°54.59'N	27°50.00'W	49°15.85'N	27°50.00'W
Southeast	Megacorer	23/07/2009	2763	49°05.42'N	27°50.24'W		
Northeast	OTSB	10/08/2007	2405	54°06.33'N	33°58.27'W	53°47.47'N	34°02.89'W
Northeast	OTSB	11/08/2007	2410	54°05.68'N	33°58.54'W	53°46.94'N	34°03.02'W
Northeast	OTSB	12/08/2007	2404	54°05.68'N	33°58.54'W	53°47.71'N	34°02.83'W
Northeast	Amphipod trap	09/08/2007		54°04.08'N	34°09.43'W		
Northeast	Megacorer	09/08/2007	2500	54°00.65'N	34°10.42'W		
Southeast	Sediment trap mooring	31/07/2008	2503	49°02.60'N	27°43.48'W		
Southeast	OTSB	10/08/2009	2700	48°58.73'N	27°51.01'W	49°11.14'N	27°49.17'W
Southeast	OTSB	10/08/2009	2700	48°58.05'N	27°51.06'W	49°14.36'N	27°49.29'W
Southeast	OTSB	18/08/2009	2700	48°57.34'N	27°49.83'W	49°13.55'N	27°50.92'W
Southeast	Megacorer	07/08/2009	2720	49°05.39'N	27°50.24'W		
Southeast	Megacorer	08/08/2009	2720	49°05.40'N	27°50.22'W		
Southeast	Megacorer	08/08/2009	2720	49°05.40'N	27°50.22'W		

*OTSB: Semi-balloon otter trawl

were analysed. Formalin preservation could not be avoided because the traps were deployed in a remote location for twelve month periods. The $\delta^{13}C$ and $\delta^{15}N$ values are reported with caution because formalin fixation can have a variable effect on stable isotope values (Bosley & Wainright 1999, Kaehler & Pakhomov 2001, Bickenll et al. 2011). Sediments were freeze dried while POM was dried in an oven at 50^{o}C until constant weight prior to homogenisation.

Faunal tissue was freeze dried and ground using a pestle and mortar and separated into aliquots for carbon, nitrogen and sulphur SIA. Aliquots for carbon SIA were lipid extracted because lipids are depleted in ^{13}C relative to protein and variation in lipid content between species can confound food web analysis when the $\delta^{13}C$ values of source material are similar (Post et al. 2007). The samples were immersed in a 2:1 chloroform:methanol mix 20 times their sample volume, agitated for 30 minutes and then centrifuged at 3400 rpm for 10 minutes. The procedure was repeated up to three times or until the supernatant became clear after centrifuging. Samples were dried to constant weight at 50^{o}C in an oven for 48 hours. Aliquots of fauna, POM and sediment were tested for carbonates prior to analysis with 0.1 N HCl. If the sample effervesced this indicated carbonates were present and it was subsequently acidified by further addition of acid until the effervescence ceased. Samples were redried at 50^{o}C for 48 hours. If the sample did not effervesce no acidification was carried out.

Carbon, nitrogen and sulphur aliquots were weighed into separate tin capsules with the catalyst vanadium pentoxide added to the sulphur samples. Dual stable carbon and nitrogen isotope ratios were measured by continuous-flow isotope ratio mass spectrometry using a Costech Elemental Analyser interfaced with either a Thermo Finnigan Delta Plus XP Mass Spectrometer or a Thermo Finnigan Delta V Plus Mass Spectrometer (Natural Environment Research Council, Life Sciences Mass

Spectrometry Facility, SUERC, East Kilbride, United Kingdom). Two laboratory standards were analysed every ten samples in each analytical sequence for linearity and drift corrections: paired alanine standards, of differing $\delta^{13}C$ and $\delta^{15}N$, and an internal laboratory gelatin standard. Sulphur SIA was conducted by Iso-Analytical (Crewe, United Kingdom) using a SERCON Elemental Analyser coupled to a Europa Scientific 20-20 mass spectrometer. Laboratory standards of barium sulphate (two sets of differing $\delta^{34}S$) and silver sulphide were used for calibration and drift correction. An internal standard of whale baleen was used for quality control (n = 7, 16.87‰ ± s.d. 0.23). Laboratory standards are traceable to international standards v-PDB (Pee Dee Belemnite), AIR (atmospheric nitrogen), NBS-127 (barium sulphate), IAEA-S-1 (silver sulphide) and IAEA-SO-5 (barium sulphate). An external standard of freeze dried and ground white fish muscle (*Antimora rostrata*) was also analysed ($\delta^{13}C$, n = 30, -18.89‰ ± s.d. 0.07; $\delta^{15}N$, n = 30, 13.34‰ ± s.d. 0.14; $\delta^{34}S$, n = 8, 19.03‰ ± s.d. 0.73).

2.2.3. Statistical analyses

Data were assessed for normality and variance using Shapiro-Wilk and Fligner-Killen or an F-test. Either Pearson's product momentum (r) or Spearman rank correlation (r_s), depending on normality, was undertaken to assess the association between isotopic variables. Fauna were split into hypothesised trophic guilds after consulting published literature on morphology (e.g. Jangoux 1982, Billett 1991, Roberts & Moore 1997), behaviour (e.g. Collins et al. 2005, Kemp et al. 2006) and diet (e.g. Mauchline & Gordon 1984a, Gartner et al. 1997, Howell et al. 2003). Statistical differences in trophic guilds, monthly differences in POM and spatial and temporal differences in surface sediments were performed by one-way ANOVA or a two sample t-test, when data were normally distributed with homogeneous variances. Tukey's honest significant differences (HSD) *post-hoc* analysis was used to identify significant differences among

groups. Welch's ANOVA or t-test was used when data had unequal variances but were normally distributed. Welch's tests use adjusted degrees of freedom to protect against Type I errors when variances are unequal (Quinn & Keough 2002). *Post-hoc* analysis in these circumstances was undertaken by a series of two sample t-tests with Bonferroni adjusted alpha level of 0.005 (0.05/10) per test.

2.3. Results

2.3.1. Potential food sources

There were spatial differences in the surface (0 to 5 mm: t-test, $t = -11.60$, $p < 0.05$) and subsurface sediment (5 to 10 mm: t-test, $t = -19.34$, $p < 0.001$) layers, with the northern station depleted in ^{13}C compared to southern station in 2007 (Figure 2.2, Table 2.2). Temporal differences in the surface sediment layer of the southern station were evident (t-test, $t = -7.91$, $p < 0.05$), with the surface sediments collected in 2007 being ^{13}C depleted compared to 2009 (Figure 2.2, Table 2.2). But there were no difference in $\delta^{13}C$ of the subsurface sediment layer at the southern station between 2007 and 2009. Differences in the surface and subsurface layers at each station were also observed in 2007. The subsurface sediment layer was enriched in ^{13}C compared to the surface sediment layer at the southern (t-test, $t = -11.07$, $p < 0.001$) and northern (t-test, $t = -5.50$, $p < 0.01$) stations (Figure 2.2, Table 2.2). There were no differences between the surface and subsurface layers in $\delta^{13}C$ at the southern station in 2009.

The surface sediment layer had a greater spread in $\delta^{15}N$ at the southern (-0.74 to 23.14‰) compared to northern station (3.19 to 6.81‰) in 2007 (F-test, $F = 0.02$, $p < 0.05$). But mean $\delta^{15}N$ values of the surface sediment layer did not differ between the two stations ($p = 0.91$) (Figure 2.2, Table 2.2). In the subsurface sediment layer, the spread of $\delta^{15}N$ values were greater at the northern station (0.39 to 11.34‰) compared to the southern (7.00 to 7.83‰) (F-test, $F = 177.31$, $p < 0.01$). Although there was still no

between-station difference in $\delta^{15}N$ ($p = 0.76$) (Figure 2.2, Table 2.2). Temporal differences at the southern stations were observed in the subsurface sediment layer with a greater spread of $\delta^{15}N$ values observed in 2009 (F-test, $F = 0.035$, $p < 0.05$), but there was no difference in the mean values ($p = 0.26$). There were differences in the spread of $\delta^{15}N$ between the surface and subsurface sediment layers at the southern station in 2007 (F-test, $F = 108.84$, $p < 0.01$) but there was no difference in the mean $\delta^{15}N$ values ($p = 0.99$). The surface and subsurface sediment layers did not differ in $\delta^{15}N$ at the northern station in 2007 ($p = 0.79$) nor the southern station in 2009 ($p = 0.32$).

Sediment trap POM $\delta^{13}C$ differed between months (ANOVA, $F_{4, 10} = 3198.2$, $p < 0.001$), with all five months being different (Tukey's HSD, $p < 0.05$). Samples became increasingly enriched in ^{13}C from March to July (-24.47 to -21.69‰) but were consistently depleted in ^{13}C relative to the surface sediment layer (Table 2.2). $\delta^{15}N$ varied between months (ANOVA, $F_{4, 10} = 28.39$, $p < 0.001$) with values ranging from 0.98‰ (April) to 2.69‰ (July) and no consistent temporal pattern (Table 2.2).

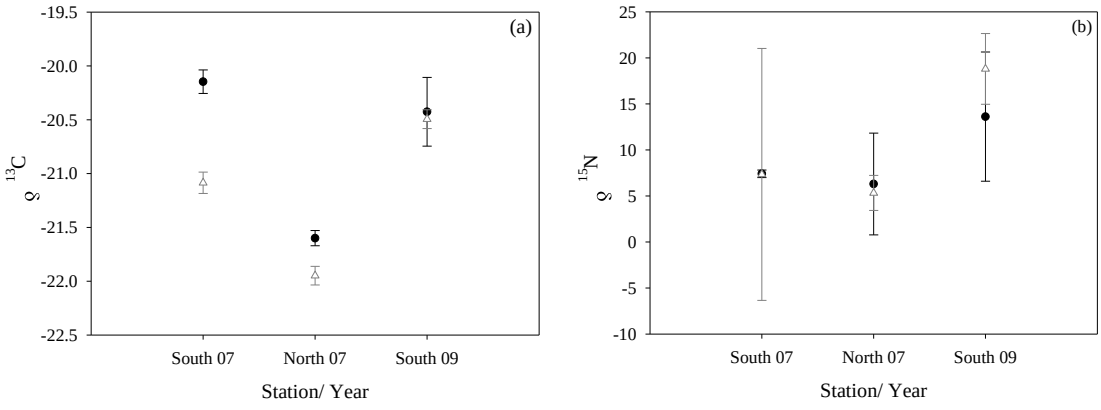

Fig. 2.2: Mean (± s.d.) $\delta^{13}C$ (a) and $\delta^{15}N$ (b) of sediment, sampled at 0-5 mm (△) and 5-10 mm (●) collected from the Mid-Atlantic Ridge at the same southern station in 2007 and 2009 and at the northern station in 2007.

Table 2.2: Mean percentage organic carbon (%C_{org}), percentage total nitrogen (%TN), $\delta^{13}C$ and $\delta^{15}N$ values (‰) of surficial sediments and particulate organic matter collected from two locations on the Mid-Atlantic Ridge. Standard deviations are in parentheses.

Potential food source	Station	Month/ Year	N	%C_{org}	%TN	$\delta^{13}C$	$\delta^{15}N$
Surface sediment (0-5mm)	South	2007	3	3.10 (1.85)	0.18 (0.12)	-21.09 (0.10)	7.34 (13.68)
	South	2009	3	2.37 (0.24)	0.13 (0.02)	-20.49 (0.09)	18.80 (3.84)
	North	2007	3	2.38 (0.10)	0.17 (0.03)	-21.95 (0.09)	5.33(1.89)
Sub-surface sediment (5-10mm)	South	2007	3	2.54 (0.26)	0.09 (0.01)	-20.15 (0.27)	7.41 (0.41)
	South	2009	3	2.08 (0.30)	0.10 (0.01)	-20.43 (0.32)	13.62 (7.01)
	North	2007	3	1.63 (0.10)	0.13 (0.01)	-21.60 (0.07)	6.30 (5.52)
Sediment trap	South	MAR 2009	3	17.25 (0.21)	0.33 (0.03)	-24.47 (0.02)	1.85 (0.31)
	South	APR 2009	3	10.80 (0.03)	0.39 (0.01)	-23.49 (0.04)	0.98 (0.15)
	South	MAY 2009	3	4.84 (0.16)	0.32 (0.01)	-22.76 (0.04)	1.00 (0.30)
	South	JUN 2009	3	5.66 (0.10)	0.27 (0.02)	-22.07 (0.02)	1.80 (0.09)
	South	JUL 2009	3	7.94 (0.17)	0.33 (0.02)	-21.69 (0.04)	2.69 (0.21)

2.3.2. Spatial and temporal differences in trophic assemblages and fauna

Mean $\delta^{13}C$ was greater at the southern station compared to the northern station in 2007 (t-test, $t = -4.86$, $p < 0.001$). $\delta^{13}C$ values were lowest in the decapod *Acanthephyra* sp. (-18.43‰ ± s.d. 0.42) and the fish *Bathylagus euryops* (-18.83‰ s.d. ± 0.26) and highest in the asteroids *Plutonaster* sp. (-14.55‰ ± s.d. 0.53) and *Bathybiaster vexillifer* (-15.88‰ ± s.d. 0.18), at the south and north stations respectively (Figure 2.3 a & b; Table 2.3 & 2.4). Mean $\delta^{13}C$ ranged between -18.93‰ ± s.d. 0.26 in the lophogastrid *Gnathophausia zoea* and -14.34‰ ± s.d. 0.31 in *Plutonaster* sp. at the southern station in 2009 (Figure 2.4; Table 2.5).

Mean $\delta^{15}N$ did not differ between the southern and northern stations in 2007 ($p = 0.63$). $\delta^{15}N$ was lowest in the holothurians *Staurocucumis abyssorum* (7.92‰ ± s.d. 0.28) and *Peniagone azorica* (7.78‰ ± s.d. 0.37) whereas the brisingid *Freyella* sp. (14.02‰ ± s.d. 0.08) and sipunculan *Sipunculus norvegicus* (16.18‰ ± s.d. 0.34) had the highest values, at the south and north stations, respectively (Figure 2.3 a & b; Table 2.3 & 2.4). Mean $\delta^{15}N$ ranged between 7.73‰ ± s.d. 0.15 and 15.78‰ ± s.d. 1.43 at the southern

station in 2009 in the holothurians *S. abyssorum* and *Molpadia musculus* (Figure 2.4, Table 2.5).

Mean $\delta^{34}S$ did not differ between the southern and northern stations of the MAR in 2007 ($p = 0.47$). The grenadier *Coryphaenoides armatus* (18.01‰ ± s.d. 1.33) and decapod *Glyphocrangon sculpta* (16.92‰ ± s.d. 1.64) had the lowest $\delta^{34}S$ values while the asteroid *Hymenaster membranaceus* (21.10‰ ± s.d. 0.42) and echinoid *Urechinus naresianus* (21.92‰ ± s.d. 0.08) had the highest $\delta^{34}S$ values at the southern and northern stations, respectively (Figure 2.3 c & d, Table 2.3 & 2.4).

Spatial and temporal variability in $\delta^{13}C$ and $\delta^{15}N$ were examined for fauna, which were the same between stations. Consistently higher $\delta^{13}C$ values at the southern station compared to the northern (t-test, $p < 0.05$) were observed in the holothurian *Benthothuria funebris*, decapod *Glyphocrangon sculpta* and *Stereomastis nana* and fishes *Coryphaenoides armatus, Coryphaenoides brevibarbis* and *Halosauropsis macrochir*. Spatial differences in $\delta^{15}N$ were observed in three species. *G. sculpta* had lower $\delta^{15}N$ at the southern station compared to the north while both *C. brevibarbis* and *S. nana* had higher $\delta^{15}N$ at the southern station compare to the north (t-test, $p < 0.05$). There was only one sample of *S. nana* collected at the southern station in 2007, therefore this test should be treated with caution. There were temporal decreases in $\delta^{13}C$ between 2007 and 2009 at the southern station for *B. funebris* and *C. brevibarbis* (t-test, $p < 0.05$) while $\delta^{13}C$ increased between 2007 and 2009 in the anthozoan *Anthomastus agaricus*. There were no temporal differences in $\delta^{15}N$ values at the southern station.

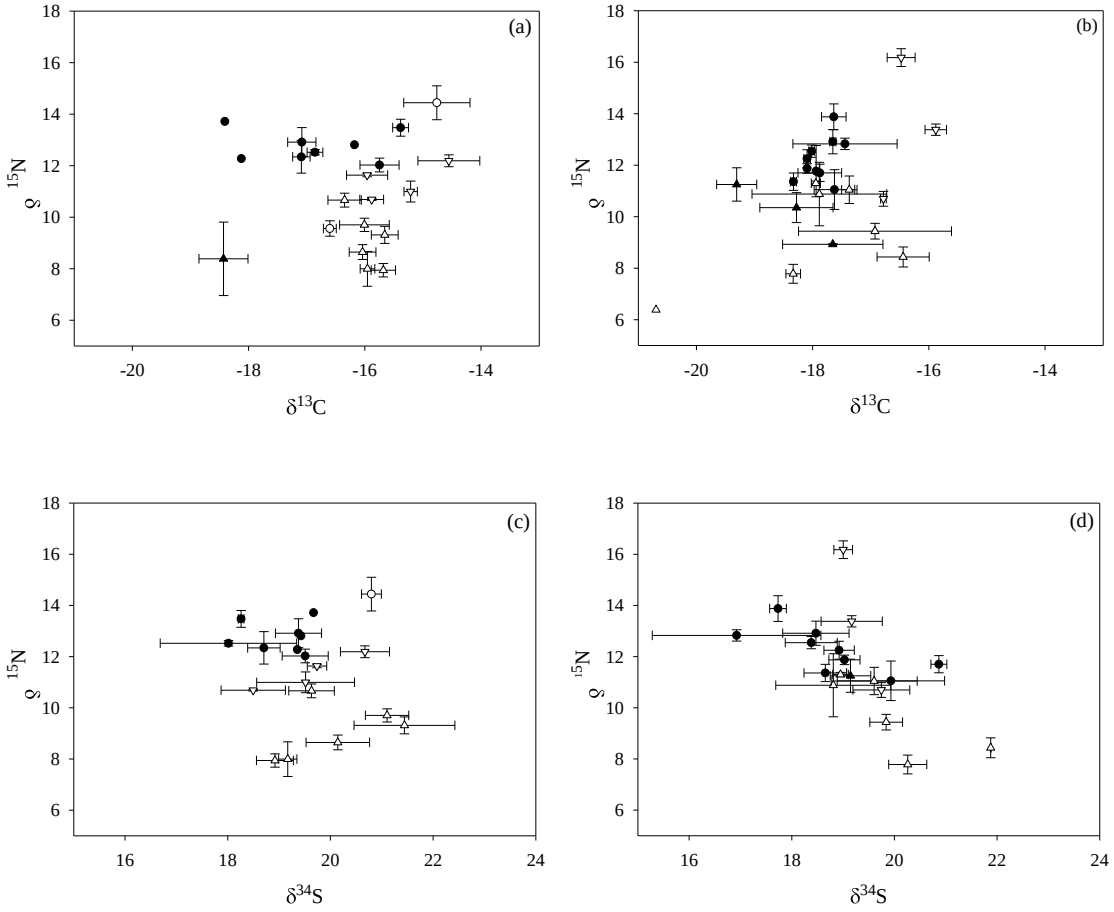

Fig. 2.3: Stable isotope values of predators (▲), predator/ scavengers (●), surface deposit feeders (△), subsurface deposit feeders (▽) and suspension feeders (○) from the southern station (a & c) and northern station (b & d) of the Mid-Atlantic Ridge collected in 2007. Open symbols represent fauna utilising the phytodetritus food chain and solid symbols represent fauna utilising the predator/ scavenger food chain. Values are means ± 1 standard deviation.

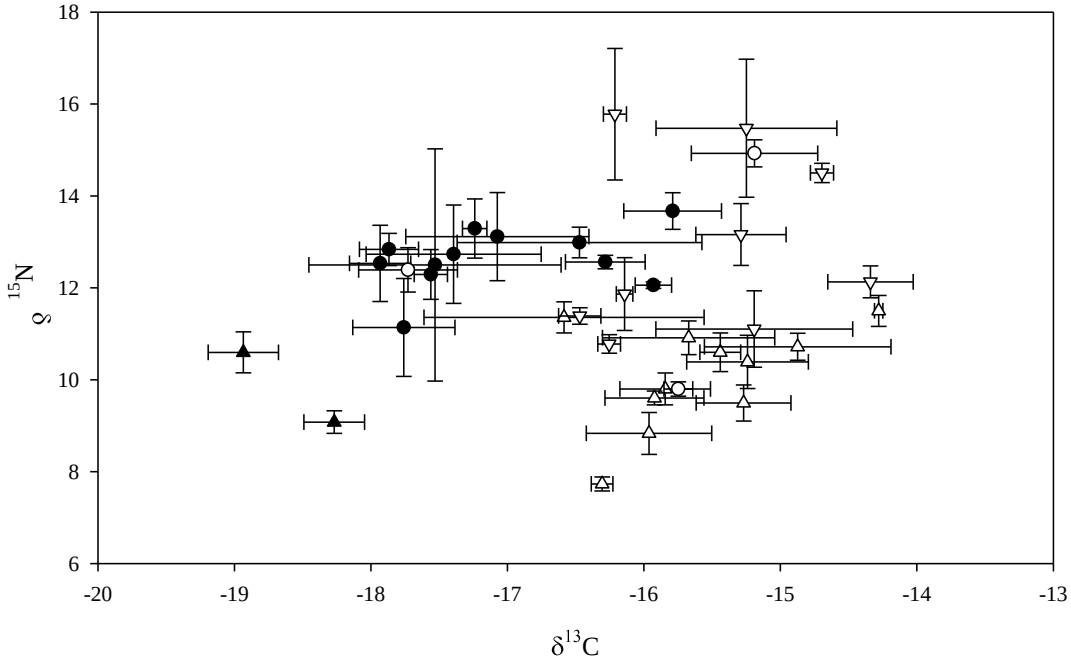

Fig. 2.4: $\delta^{15}N$ and $\delta^{13}C$ of predators (▲), predator/ scavengers (●), surface deposit feeders (△), subsurface deposit feeders (▽) and suspension feeders (○) collected in 2009 from the southern station of the Mid-Atlantic Ridge. Open symbols represent fauna utilising the detrital food chain and solid symbols represent fauna utilising the predator/ scavenger food chain. Values are means ± 1 standard deviation.

Table 2.3: Mean $\delta^{13}C$, $\delta^{15}N$ and $\delta^{34}S$ values (‰) of benthic fish and invertebrates collected in 2007 from the Mid-Atlantic Ridge at the southern station. Trophic guild abbreviations refer to surface deposit feeder (SDF), subsurface deposit feeder (SSDF), suspension feeder (SF), predator (P) and predator/ scavenger (PS). Standard deviations are in parentheses.

Taxonomic group	Species	Trophic guild	N	$\delta^{13}C$	$\delta^{15}N$	$\delta^{34}S$
Cnidaria						
Anthozoa	*Anthomastus agaricus*	SF	3	-16.60 (0.11)	9.56 (0.30)	19.35 (0.1)
Echinodermata						
Asteroidea	*Hyphalaster inermis*	SSDF	3	-15.96 (0.02)	10.69 (0.03)	19.59 (0.19)
Asteroidea	*Porcellanaster cerulus*	SSDF	3	-15.88 (0.21)	11.63 (0.07)	19.74 (0.19)
Asteroidea	*Plutonaster* sp.	SSDF	3	-14.55 (0.53)	12.19 (0.23)	20.67 (0.48)
Asteroidea	*Hymenaster membranaceus*	SDF	3	-16.01 (0.43)	9.70 (0.26)	21.10 (0.42)
Asteroidea	*Freyella* sp.	SF/ P	3	-14.76 (0.57)	14.02 (0.08)	20.80 (0.19)
Holothuroidea	*Staurocucumis abyssorum*	SDF	3	-16.59 (1.77)	7.92 (0.28)	18.92 (0.36)
Holothuroidea	*Benthothuria funebris*	SDF	3	-15.21 (0.12)	10.99 (0.40)	19.52 (0.95)
Holothuroidea	*Benthodytes gosarsi*	SDF	3	-15.66 (0.23)	9.31 (0.33)	21.44 (0.98)
Holothuroidea	*Peniagone* sp. A	SDF	3	-15.95 (0.13)	7.99 (0.68)	19.17 (0.18)
Holothuroidea	*Peniagone longipapillata*	SDF	3	-16.04 (0.23)	8.65 (0.29)	20.14(0.62)
Crustacea						
Decapoda	*Acanthephyra* sp.	P	3	-18.43 (0.42)	8.38 (1.43)	18.25 (0.70)
Decapoda	*Glyphocrangon sculpta*	SDF/ PS	3	-15.75 (0.06)	12.02 (0.27)	19.51 (0.45)
Decapoda	*Munidopsis rostrata*	SDF/ PS	3	-16.35 (0.29)	10.66 (0.27)	19.63 (0.44)
Decapoda	*Stereomastis nana*	PS	1	-18.41	13.72	19.43
Decapoda	*Willemoesia forceps*	PS	1	-16.18	12.81	19.67
Vertebrata						
Osteichthyes	*Antimora rostrata*	PS	1	-18.12	12.28	19.36
Osteichthyes	*Coryphaenoides armatus*	PS	3	-16.86 (0.14)	12.52 (0.11)	18.01 (1.33)
Osteichthyes	*Coryphaenoides brevibarbis*	PS	3	-17.09 (0.15)	12.34 (0.63)	18.70 (0.32)
Osteichthyes	*Halosauropsis macrochir*	PS	3	-17.08 (0.04)	12.90 (0.56)	19.38 (0.45)
Osteichthyes	*Polyacanthonotus challengeri*	PS	2	-15.38 (0.14)	13.47 (0.33)	18.26 (0.06)

Table 2.4: Mean $\delta^{13}C$, $\delta^{15}N$ and $\delta^{34}S$ values (‰) of benthic fish and invertebrates collected in 2007 at northern Mid-Atlantic Ridge station. Trophic guild abbreviations refer to surface deposit feeder (SDF), subsurface deposit feeder (SSDF), suspension feeder (SF), predator (P) and predator/ scavenger (PS). Standard deviations are in parentheses.

Taxonomic group	Species	Trophic guild	N	$\delta^{13}C$ (s.d.)	$\delta^{15}N$ (s.d.)	$\delta^{34}S$ (s.d.)
Foraminifera	*Rhizammina* sp.	SDF	1	-20.70	6.38	17.77
Echinodermata						
Asteroidea	*Bathybiaster vexillifer*	SSDF/ PS	6	-15.88 (0.18)	13.18 (0.22)	19.17 (0.60)
Asteroidea	*Hymenaster* cf *coccinatus*	SDF	3	-16.01 (0.43)	9.42 (0.26)	19.84 (0.32)
Echinoidea	*Urechinus naresianus*	SDF	3	-16.69 (0.53)	9.03 (1.07)	21.92 (0.08)
Holothuroidea	*Benthothuria funebris*	SDF	3	-16.78 (0.05)	10.70 (0.29)	19.74 (0.55)
Holothuroidea	*Paelopatides grisea*	SDF	3	-17.37 (0.13)	11.05 (0.53)	19.60 (0.84)
Holothuroidea	*Peniagone azorica*	SDF	5	-18.36 (0.23)	7.68 (0.30)	20.26 (0.37)
Ophuiroidea	Ophuiroid sp.	SDF	2	-17.94 (0.08)	11.35 (0.02)	18.95 (0.15)
Crustacea						
Amphipoda	*Eurythenes gryllus*	PS	3	-17.72 (0.49)	10.15 (1.02)	19.93 (1.05)
Decapoda	*Acanthephyra* sp.	P	2	-18.82 (0.67)	8.93 (0.02)	18.33 (0.14)
Decapoda	*Glyphocrangon sculpta*	SDF/ PS	2	-17.44 (0.90)	12.83 (0.22)	16.92 (1.64)
Decapoda	*Munidopsis bermudezi*	SDF/ PS	2	-17.88 (1.16)	10.88 (1.23)	18.81 (1.12)
Decapoda	*Stereomastis nana*	PS	3	-17.88 (0.38)	11.81 (0.24)	20.87 (0.15)
Lophogastrida	*Gnathophausia zoea*	P	3	-18.28 (0.63)	10.35 (0.58)	18.52 (0.17)
Sipuncula						
Sipunculidea	*Sipunculus norvegicus*	SSDF	3	-16.48 (0.24)	16.18 (0.34)	19.00 (0.18)
Vertebrata						
Osteichthyes	*Antimora rostrata*	PS	3	-18.10 (0.03)	11.87 (0.19)	19.03 (0.30)
Osteichthyes	*Bathylagus euryops*	P	3	-19.31 (0.34)	11.25 (0.65)	19.14 (0.40)
Osteichthyes	*Bathysaurus ferox*	PS	3	-17.64 (0.21)	13.88 (0.50)	17.73 (0.16)
Osteichthyes	*Coryphaenoides armatus*	PS	3	-18.02 (0.05)	12.55 (0.54)	18.83 (0.50)
Osteichthyes	*Coryphaenoides brevibarbis*	PS	3	-18.33 (0.05)	11.36 (0.34)	18.65 (0.41)
Osteichthyes	*Coryphaenoides mediterraneus*	PS	2	-18.10 (0.06)	12.25 (0.35)	18.92 (0.30)
Osteichthyes	*Halosauropsis macrochir*	PS	3	-17.56 (0.05)	12.91 (0.47)	18.47 (0.65)
Osteichthyes	*Histiobranchius bathybius*	PS	2	-17.94 (0.01)	11.77 (1.00)	17.98 (0.41)

Table 2.5: Mean $\delta^{13}C$ and $\delta^{15}N$ values (‰) of benthic fish and invertebrates collected in 2009 from the Mid-Atlantic Ridge at the southern station. Trophic guild abbreviations refer to surface deposit feeder (SDF), subsurface deposit feeder (SSDF), suspension feeder (SF), predator (P) and predator/ scavenger (PS). Standard deviations are in parentheses.

Taxonomic group	Species	Trophic guild	N	$\delta^{13}C$	$\delta^{15}N$
Annelida					
Polychaeta	Aphrodita sp.	SDF	3	-15.24 (0.45)	10.39 (0.58)
Polychaeta	Polynoidae sp.	SDF	2	-15.84 (0.04)	9.80 (0.35)
Cnidaria					
Anthozoa	*Anthomastus agaricus*	SF	3	-15.75 (0.11)	9.80 (0.16)
Echinodermata					
Asteroidea	*Hyphalaster inermis*	SSDF	3	-16.14 (0.06)	11.86 (0.79)
Asteroidea	*Porcellanaster cerulus*	SSDF	3	-15.19 (0.72)	11.10 (0.86)
Asteroidea	*Plutonaster bifrons*	SSDF	3	-14.87 (0.68)	10.72 (0.29)
Asteroidea	*Plutonaster* sp.	SSDF	3	-14.34 (0.31)	12.13 (0.35)
Asteroidea	*Hymenaster membranaceus*	SDF	3	-15.52 (0.14)	9.20 (0.22)
Asteroidea	*Freyella* sp.	SF/ P	3	-15.19 (0.46)	14.93 (0.29)
Holothuroidea	*Staurocucumis abyssorum*	SDF	3	-16.31 (0.08)	7.73 (0.15)
Holothuroidea	*Benthothuria funebris*	SDF	3	-15.89 (0.16)	10.78 (0.20)
Holothuroidea	*Benthodytes gosarsi*	SDF	3	-15.92 (0.36)	9.60 (0.15)
Holothuroidea	*Ellipinion delagei*	SDF	3	-15.27 (0.35)	9.49 (0.39)
Holothuroidea	*Gephirothuria alcocki*	SSDF	3	-15.25 (0.66)	15.47 (1.50)
Holothuroidea	*Paelopatides grisea*	SDF	3	-16.58 (1.03)	11.36 (0.34)
Holothuroidea	*Peniagone islandica*	SDF	3	-15.96 (0.46)	8.83 (0.46)
Holothuroidea	*Pseudostichopus aff peripatus*	SSDF	3	-16.47 (0.16)	11.39 (0.18)
Holothuroidea	*Psychropotes depressa*	SDF	3	-15.29 (0.33)	13.16 (0.67)
Holothuroidea	*Molpadia musculus*	SSDF	3	-16.21 (0.08)	15.78 (1.43)
Crustacea					
Decapoda	*Acanthephyra* sp.	P	3	-18.27 (0.22)	9.08 (0.25)
Decapoda	*Glyphocrangon sculpta*	SDF/ PS	3	-15.93 (0.13)	12.06 (0.07)
Decapoda	*Munidopsis rostrata*	SDF/ PS	3	-15.67 (0.63)	10.91 (0.37)
Decapoda	*Stereomastis nana*	PS	2	-17.87 (0.22)	9.29 (0.23)

Lophogastrida	*Gnathophausia zoea*	P	3	-18.93 (0.26)	10.60 (0.44)
Pendunculata	Stalked barnacle	SF/ P	3	-17.73 (0.36)	12.39 (0.48)
Mollusca					
Gastropoda	Gastropoda sp. 1	SDF	3	-14.28 (0.03)	11.50 (0.34)
Gastropoda	Gastropoda sp. 2	SDF	3	-15.44 (0.15)	10.60 (0.42)
Sipuncula					
Sipunculidea	*Sipunculus norvegicus*	SSDF	3	-14.70 (0.08)	14.50 (0.21)
Vertebrata					
Osteichthyes	*Antimora rostrata*	PS	3	-17.93 (0.22)	12.53 (0.83)
Osteichthyes	*Bathysaurus ferox*	PS	3	-17.53 (0.92)	13.13 (2.42)
Osteichthyes	*Coryphaenoides armatus*	PS	4	-16.47 (0.90)	12.99 (0.33)
Osteichthyes	*Coryphaenoides brevibarbis*	PS	3	-17.56 (0.12)	12.29 (0.54)
Osteichthyes	*Coryphaenoides carapinus*	PS	3	-16.28 (0.29)	12.57 (0.12)
Osteichthyes	*Coryphaenoides leptolepis*	PS	3	-17.24 (0.09)	13.29 (0.64)
Osteichthyes	*Halosauropsis macrochir*	PS	3	-17.07 (0.67)	13.11 (0.96)
Osteichthyes	*Histiobranchius bathybius*	PS	6	-17.76 (0.37)	11.47 (1.07)
Osteichthyes	*Polyacanthonotus challengeri*	PS	3	-15.79 (0.36)	13.67 (0.40)
Osteichthyes	*Spectrunculus grandis*	PS	3	-17.39 (0.64)	12.73 (1.07)

2.3.3. Mid-Atlantic Ridge trophic guilds

Trophic guilds differed in $\delta^{13}C$ within all stations (ANOVA, south 2007, $F_{4, 16} = 4.17$, $p < 0.05$; ANOVA, south 2009, $F_{4, 33} = 13.11$, $p < 0.001$; ANOVA, north 2007, $F_{3, 19} = 9.37$, $p < 0.001$). At the southern station in 2007 the only difference in trophic guilds was between predators (P) and subsurface deposit (SSDF), although there was only one species represented within P. In 2009, at the southern station P and predator/ scavengers (PS) had lower $\delta^{13}C$ than the surface deposit (SDF), SSDF and suspension feeders (SF) (Tukey HSD, $p < 0.05$), while there were no differences between the trophic guilds SDF, SSDF and SF. In contrast to the southern stations, the northern trophic guilds P, PS and SDF were not different from each other but had lower $\delta^{15}N$ values compared to SSDF (Tukey HSD, $p < 0.05$). $\delta^{34}S$ did not differ among trophic guilds within either station (ANOVA south 2007, $p = 0.12$; north 2007, $p = 0.43$).

$\delta^{15}N$ values of the trophic guilds differed within all stations (Welch's ANOVA, south 2007, $F_{4, 3.7} = 15.44$, $p < 0.05$; ANOVA, south 2009, $F_{4, 33} = 8.85$, $p < 0.001$; ANOVA, north 2007, $F_{3, 18} = 6.40$, $p < 0.01$). The P trophic guild had lower $\delta^{15}N$ values than PS at the southern station in 2007 (Bonferroni adjusted t-test, $p < 0.005$), but was similar to PS in $\delta^{15}N$ at the southern station in 2009 and northern station in 2007 (Tukey HSD, $p = 0.07$). P also had lower $\delta^{15}N$ values than SSDF at the southern station in 2007 (Bonferroni adjusted t-test, $p < 0.005$) and 2009 (Tukey HSD, $p < 0.05$) as well as at the northern station (Tukey HSD, $p < 0.05$). SDF had lower $\delta^{15}N$ values than PS and SSDF at southern station in 2007 (Bonferroni adjusted t-test, $p < 0.005$) and 2009 (Tukey HSD, $p < 0.05$) and the northern station (Tukey HSD, $p < 0.05$). Taken together with $\delta^{13}C$, the $\delta^{15}N$ *post-hoc* analysis suggested increasing isotopic values from P to PS and from SDF to SSDF, which illustrates a hierarchical pattern in trophic structure for predatory and

deposit feeding organisms that was broadly similar between stations and years (Figure 2.3 a & b, Figure 2.4).

2.3.4. Linearity of trophic assemblages

There were no significant relationships at the southern station between $\delta^{15}N$ and $\delta^{13}C$ across all species within each trophic assemblage (2007, $p = 0.39$; 2009, $p = 0.98$) or between $\delta^{15}N$ and $\delta^{34}S$ (2007, $p = 0.71$). However, at the northern station, relationships between $\delta^{15}N$ and $\delta^{13}C$ ($r = 0.43$, $p < 0.01$) and $\delta^{15}N$ and $\delta^{34}S$ ($r = -0.35$, $p < 0.01$) were significant. The assemblages were split into two groups according to whether they were dependent on phytodetritus (SDF, SSDF & SF), or were mobile predators and scavengers (P and PS) (Figure 3 & 4). For the phytodetritus-dependent group, there were significant relationships between $\delta^{15}N$ and $\delta^{13}C$ (2007, $r_s = 0.46$, $p < 0.01$; 2009, $r_s = 0.24$, $p < 0.05$) but not between $\delta^{15}N$ and $\delta^{34}S$ (2007, $p = 0.14$) at the southern station. While at the northern station there were relationships between $\delta^{15}N$ and $\delta^{13}C$ ($r_s = 0.68$, $p < 0.001$) and between $\delta^{15}N$ and $\delta^{34}S$ ($r_s = -0.44$, $p < 0.01$). For the predator/ scavenger group, at the southern station there were relationships between $\delta^{15}N$ and $\delta^{13}C$ in 2009 ($r_s = 0.64$, $p < 0.001$) but not in 2007 ($p = 0.15$). There was also no significant relationship between $\delta^{15}N$ and $\delta^{34}S$ at the southern station in 2007 ($p = 0.45$). At the northern station the predator/ scavenger group showed relationships between $\delta^{15}N$ and $\delta^{13}C$ ($r_s = 0.40$, $p < 0.05$) and between $\delta^{15}N$ and $\delta^{34}S$ ($r_s = -0.43$, $p < 0.01$).

2.4. Discussion

Carbon and sulphur stable isotopic data indicated that the benthic assemblages at the two MAR stations appear to be supported by photosynthetic primary production. This accords with the range of $\delta^{13}C$ values from other deep-sea systems where there is no energy input from chemosynthetic primary production including abyssal benthic communities of the North Atlantic (-23 to -13‰; Iken et al. 2001) and North Pacific (-

21 to -17‰; Drazen et al. 2008b), continental slopes of the Mediterranean (-22 to -16‰; Polunin et al. 2001) and the high Arctic (-23 to -16‰; Bergmann et al. 2009). However, $\delta^{13}C$ values of hydrothermal vent fauna can cover the same $\delta^{13}C$ range as non-vent fauna making interpretation of carbon isotopic values ambiguous (Erickson et al. 2009). This is relevant on MOR where vent organic matter entrained in hydrothermal plumes can be found settling 2 km away from the venting source (Roth & Dymond 1989), bathypelagic biomass associated with the hydrothermal plume can be enhanced (Burd et al. 1992) and consumers at inactive chemosynthetic sites can potentially utilise organic sulphur derived from sulphide of hydrothermal vent origin that has persisted within the sediment (Erickson et al. 2009). Macro-consumers feeding on photosynthetically derived organic sulphur have $\delta^{34}S$ values ranging from 16 to 19‰ (Fry 1988), while organisms consuming organic sulphur compounds of hydrothermal vent origin have $\delta^{34}S$ values between -9 and 10‰ (Erickson et al. 2009, Fabri et al. 2011). The MAR consumers fall within the former range confirming these stations were dependent on photosynthetic primary production and not influenced by unknown chemosynthetic sources that were transported to or persisted within the sediment.

The pathways by which organic matter reaches the seafloor, for example as phytodetritus or vertically interconnecting food chains, has implications for how it is utilised and may be reflected in the stable isotope values of the MAR benthic macro-consumers. Mediterranean deep-water bentho-pelagic (Polunin et al. 2001) and North Pacific abyssal (Drazen et al. 2008b) trophic assemblages have strong, positive relationships between the $\delta^{15}N$ and $\delta^{13}C$ values suggesting a linear food chain dependent on a single photosynthetic energy source (Polunin et al. 2001, Drazen et al. 2008b). The trophic assemblage at the northern section of the MAR had a positive relationship between $\delta^{15}N$ and $\delta^{13}C$ which suggested a single trophic pathway. However, a weak association between $\delta^{15}N$ and $\delta^{13}C$ values occurred at the southern station in 2007 and

2009 and was more akin to that found at the Porcupine Abyssal Plain (PAP) (Iken et al. 2001), which suggested the southern MAR station had a complex food web with more than one trophic pathway. Separation of the MAR benthic assemblages into phytodetritus and predator/ scavenger food chains revealed strong positive relationships between $\delta^{15}N$ and $\delta^{13}C$ at all stations, except the predator/ scavenger food chain at the southern station in 2007. Large scavenging and predatory fishes including *Histiobranchus bathybius, Bathysaurus ferox* and *Spectunculus grandis* were absent in the trawls but this is likely an issue of sample collection as they do occur on the MAR between the Azores and the CGFZ (Bergstad et al. 2008). In 2009 *H. bathybius, B. ferox* and *S. grandis* along with *Coryphaenoides carapinus* and *Coryphaenoides leptolepis* had $\delta^{13}C$ and $\delta^{15}N$ values intermediate to those measured for *Acanthephyra* sp. and *Coryphaenoides armatus*. Therefore, their absence in 2007 potentially resulted in the lack of a significant relationship between $\delta^{15}N$ and $\delta^{13}C$.

The MAR predator/ scavenger food chain comprised fishes and crustaceans, which in general were depleted in ^{13}C relative to the benthic deposit feeders. The $\delta^{13}C$ values of pelagic organisms in the top 1000 m over the MAR range from approximately -25 to -17‰ (Petursdottir et al. 2008, Letessier et al. 2012), indicating that MAR bentho-pelagic predators and scavengers may feed on these organisms. Bathypelagic fish biomass is also higher in the vicinity of the MAR and may provide an important food source for bentho-pelagic predators and scavengers (Sutton et al. 2008). However, there were differences in the stable isotope values among the various fishes within the predator/ scavenger food chain, which may indicate differences in trophic niches as well as connectivity to the phytodetritus food chain. For example, the larger scavengers *Antimora rostrata* and *Coryphaenoides armatus* have similar foraging strategies (Collins et al. 2005) but there were differences in stable isotope values between these species on the MAR. *A. rostrata* and *C. armatus* from the Porcupine Seabight differ in

certain fatty acids which are thought to reflect a greater proportion of benthic organisms in the diet of *C. armatus* (Stowasser et al. 2009). If there is a similar scenario on the MAR then it might explain the greater $\delta^{13}C$ and $\delta^{15}N$ values, which were found in *C. armatus*.

Diets and behaviour of small benthic deep-sea fish, e.g. *Polyacanthonotus challengeri* and *Halosauropsis macrochir*, and those not attracted to carrion, e.g. *Bathysaurus ferox* and *Coryphaenoides carapinus*, are less well understood. *H. macrochir*, *B. ferox* and *Coryphaenoides brevibarbis* had similar $\delta^{13}C$ values to the larger scavengers but *P. challengeri* and *C. carapinus* were enriched in ^{13}C relative to these fishes. The $\delta^{13}C$ and $\delta^{15}N$ values of *P. challengeri* and *C. carapinus* suggested that these consumers are higher in the phytodetritus food chain, indicating that some fish species may gain a certain proportion of their nutrition from benthic invertebrates. Stomach content analysis within the genus *Polyacanthonotus* indicates that it forages on epibenthic invertebrates and polychaetes (Carrasson & Matallanas 2002). In contrast, *C. brevibarbis* and *H. macrochir* stomachs contain copepods, decapods, fish, large amounts of unidentified material and only traces of epibenthic invertebrates, indicating a diet dominated by bathypelagic organisms (Mauchline & Gordon 1984a, Gordon & Duncan 1987).

Crustaceans were the other main taxonomic group that comprised the predator/ scavenger food chain. *Acanthephyra* sp. and *Gnathophausia zoea* had $\delta^{13}C$ values related to a pelagic energy source and were consistently depleted in ^{15}N relative to deep-sea fishes. *Acanthephyra* sp. and *G. zoea* may have been caught during the ascent or descent of the OTSB because they are occur at meso-pelagic depths above the southern and northern stations (Letessier et al. 2012). However, the genus *Acanthephyra* covers a wide depth range (420 to 4000 m: Sarda et al. 2005) and species are seen in baited

lander experiments at bathyal and abyssal sites (Jones et al. 2003). While *G. zoea* occurs at depths greater than 2500 m on the MAR (Aas 2006) and is also found within the stomachs of bentho-pelagic fishes below its peak abundance on continental margins (Mauchline & Gordon 1991). *Acanthephyra* sp. and *G. zoea* may form part of the predator/ scavenger food chain by impinging on the slopes or sinking as carrion. The benthic crustaceans *Glyphocrangon sculpta* and *Munidopsis rostrata* had higher $\delta^{13}C$ values compared to other species of crustaceans at the southern station and to *G. sculpta* and *Munidopsis bermudezi* at the northern station. Bathyal galatheids, including *Munidopsis* spp., have a mixed diet of detritus and infauna (Cartes et al. 2007) and are observed at marine carrion falls (Kemp et al. 2006). *G. sculpta* and *M. rostrata* trophic role may vary between the two stations depending on the availability of detrital, infaunal and carrion food sources.

Deposit feeders are dependent on phytodetritus once it arrives on the seafloor and therefore may utilise photosynthetic primary production at a different stage of degradation and mineralisation to that of deep-sea mobile predators and scavengers. There was a large difference in $\delta^{13}C$ values between MAR deposit feeders and sediments. At the southern station, primary deposit feeders were enriched in ^{13}C relative to the mean surface sediment by 4.55‰ (2007) and 4.18‰ (2009) and at the northern station by 3.59‰, which is greater than the mean trophic shift of between 0.5 and 1‰ that is used in many trophic studies. The difference between sediment and SDF $\delta^{13}C$ values on the MAR is greater than other deep-sea areas as differences between sediment and SDF range from approximately 1.50‰ on the North Pacific abyssal plain (Drazen et al. 2008b) to 2.00‰ on the Pakistan Margin (Jeffreys et al. 2009). At the northern station in 2007 the foraminifera *Rhizammina* sp. had an isotopic value between the surface sediment layer and the SDF with the lowest $\delta^{13}C$ values, *Peniagone azorica*, which suggested processing of the sediment before it was consumed by the deposit

feeding megafauna. Bacterial remineralisation of the organic matter pool will have an effect on the material that is ingested by the deposit feeders as bacterial production can have $\delta^{13}C$ values 2.3‰ greater than the carbon source (Coffin et al. 1990). This may partially explain large differences between sediment and deposit feeders (Lovvorn et al. 2005) as an increase in the amount of organic matter arriving at the sediment surface can result in a 30% increase in bacterially derived organic matter (Mayer 1993).

The difference in $\delta^{15}N$ values of the sediment and deposit feeders was complex on the MAR. Surface deposit feeders had $\delta^{15}N$ values that were between 0.58‰ and 2.35‰ greater than mean surface sediments, at the southern and northern stations in 2007, respectively. In 2009 surface deposit feeders $\delta^{15}N$ values were 11.07‰ lower than the mean surface sediment. At the southern station, $\delta^{15}N$ values of some sediments did not fall within the 0 to 14‰ range of recently accumulated marine sediments (Hoefs 2004) with values as low as -0.74‰ and as high as 23.14‰. There is a possibility that the negative $\delta^{15}N$ values may be an artefact of low total nitrogen (TN) concentration within the sediment samples. However, the mass of TN within the sediment samples was above the level of detection of the analytical system, which indicates the negative $\delta^{15}N$ values at the southern station in 2007 are likely to be accurate. Negative values for marine sediments are rare and these values are linked to pelagic processes rather than those occurring after deposition (Rau et al. 1987). However, the $\delta^{15}N$ values of the sediment trap moored 100 m above the sea floor ranged from 0.98 to 2.93‰, indicating that enrichment of ^{15}N occurred below this point. Understanding the different nitrogen pools within the sediment and the degree of organic matter reworking by heterotrophic micro-organisms or bacteria was outside the scope of this study and different inorganic species, e.g. NH_4^+ and NO_3^-, and organic nitrogen fractions were not isolated from the sediment. The MAR sediment $\delta^{15}N$ values represented the TN pool, which may not be

representative of organic nitrogen within the sediment (Horsfall & Wolff 1997) and that assimilated by deposit feeders.

The $\delta^{13}C$ and $\delta^{15}N$ values of SDF and SSDF represent a trophic spectrum, where consumers utilise different biochemical fractions within the sediment. Phytoplankton polyunsaturated fatty acids (PUFAs), for example, in the holothurians *Staurocucumis abyssorum* and *Peniagone vitrea* and the asteroid *Hymenaster membranaceus* indicate a heavy dependence on "fresh" phytodetritus (Howell et al. 2003, Drazen et al. 2008a). Low $\delta^{13}C$ and $\delta^{15}N$ values in *S. abyssorum*, *Peniagone* spp. and *H. membranaceus* in relation to other organisms within this study and others (Iken et al. 2001, Drazen et al. 2008b) also suggest they feed at the surface sediment layer. The $\delta^{13}C$ and $\delta^{15}N$ values of SSDF including asteroid *Hyphalaster inermis*, holothurian *Molpadia musculus* and sipunculan *Sipunculus norvegicus*, which ingest sediment deep below the surface, were amongst the highest for the MAR. Similarly, *Molpadia blakei* and a sipunculan have some of the highest $\delta^{15}N$ values of organisms collected at the PAP (Iken et al. 2001). $\delta^{13}C$ and $\delta^{15}N$ values of marine sediments generally increase with depth, indicating a more refractory food source, as well as concurrent a decrease in %TN (Cowie et al. 2009). Isotopic discrimination can depend on nitrogen availability with trophic discriminations of up to ~6‰ occurring when food is nitrogen deficient (Adams & Sterner 2000). Therefore, the high $\delta^{15}N$ values of SSDF may reflect metabolic processes of feeding on sediment with lower nitrogen concentration as well as ingesting a food source more enriched in ^{15}N relative to that consumed by SDF. Furthermore, bacterial 18:1 fatty acid isomers have been detected in high concentrations in *H. inermis* (Howell et al. 2003) and *M. blakei* (Ginger et al. 2000), indicating ingestion of large amounts of bacterial biomass. Trophic discrimination between food source and bacteria can be as large as 22‰ depending on the nitrogen source (Macko & Estep 1984), which may further explain such high $\delta^{15}N$ values in SSDF. Without a better understanding of

trophic discrimination in deep-sea deposit feeders ingesting sediment with different nitrogen content and the potential role of bacterial nutrition, trophic position of deposit feeders may not be ascertained.

There were a number of cases where there were large differences in $\delta^{15}N$ values but very similar $\delta^{13}C$ values within the MAR deposit feeders. For example, *Molpadia musculus* had $\delta^{15}N$ that were approximately 7‰ greater than *Staurocucumis abyssorum* and *Peniagone islandica* at the southern station in 2009, while *Sipunculus norvegicus* was ^{15}N enriched by 5.48‰ compared to *Benthothuria funebris* at the northern station in 2007. However, $\delta^{13}C$ values only differed by 0.3‰ between these species, which indicated they assimilated similarly labile carbon sources but the higher $\delta^{15}N$ values suggested that *M. musculus* and *S. norvegicus* assimilated a more refractory nitrogen source. Examining the $\delta^{13}C$ and $\delta^{15}N$ values of amino acids within a deposit feeder's tissue may provide insight into the degree of reworking of the organic matter within the sediment before it is ingested. Total hydrolysable amino acids are potentially an important source of organic carbon and nitrogen to deep-sea deposit feeders (Horsfall & Wolff 1997). In the case of nitrogen, the trophic discrimination of specific amino acids can be negligible (e.g. phenylalanine) providing basal resource information while others can be greater than 9‰ (e.g. glutamic acid) (McClelland & Montoya 2002, Styring et al. 2010). Thus the relative difference between $\delta^{15}N$ values of amino acids that undergo negligible and large trophic discrimination are hypothesised to provide information on relative trophic position (Chikaraishi et al. 2009) but in deposit feeders this may indicate the degree of previous heterotrophic reworking of amino acids within the sediment before assimilation. Compound-specific amino acid stable isotope analysis (CSAA-SIA) may therefore provide higher resolution information on the organic nitrogen compounds assimilated by deposit feeders and if further trophic pathways exist within the benthic deposit feeding assemblage.

2.5. Conclusions

The trophic assemblage of the MAR is broadly similar to other deep-sea habitats and is ultimately dependent on photosynthetic primary production being transported to the seafloor. The stable isotopic data presented here, indicated there may be two trophic pathways with mobile predator and scavenging fishes and crustaceans linked to aggregations of meso- and bathypelagic biomass associated with the MAR (Sutton et al. 2008) and benthic invertebrates dependent on the downward flux of phytodetritus. In fact these two trophic pathways would be interconnected at various stages of organic matter recycling within the water column and benthos as organic matter is ingested, processed and excreted becoming available to different trophic guilds. Therefore, the predator/ scavenger and phytodetrital food chains suggested within this paper potentially represent the dominant but not exclusive trophic pathways for highly mobile bentho-pelagic fishes and crustaceans and benthic invertebrates, respectively.

The MAR also highlights some interesting deposit-sediment trophic interactions. The greater spread of sediment $\delta^{15}N$ values at the southern station compared to the north but similar spread in faunal values suggests that bulk sediment or TN $\delta^{15}N$ values may not be reflective or too crude a measure to understand the nitrogen sources utilised by the deposit feeding fauna. This would help to explain why other studies have observed varying isotopic differences between deposit feeders and surface sediments (Lovvorn et al. 2005, Drazen et al. 2008b, Mincks et al. 2008, Jeffreys et al. 2009). Further exploration at a finer biochemical level using CSAA-SIA to examine differences in $\delta^{15}N$ values of deposit feeders may shed more light on nitrogen cycling within the sediment and whether benthic deposit feeders are utilising similar nitrogen sources.

Chapter 3: Spatial and temporal variability in size-based trophodynamics of deep-sea fishes from the Mid-Atlantic Ridge elucidated by stable isotopes.

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3.1. Introduction

Body size is fundamentally important in ecology as it influences metabolic processes (Brown et al. 2004), foraging ability (Collins et al. 2005) and predator-prey interactions (Cohen et al. 2003, Reid et al. 2007). Marine organisms can grow through five orders of magnitude during life (Jennings et al. 2008) leading to trophic size structuring of marine communities where body size can explain a greater proportion of variance in trophic position than species (Jennings et al. 2001b). A large proportion of trophic size structuring is driven by intra-population increases in trophic position with body size (Jennings et al. 2002b). Intra-population variation in trophic position may be common (Galvan et al. 2010) but size-based patterns are not independent of the broader food web dynamics. Spatial (Jennings et al. 2002a, Sherwood et al. 2007, Greenwood et al. 2010) and temporal (Jennings et al. 2007, Nakazawa et al. 2010) variation in size-based trends in trophic position are evident, suggesting feeding plasticity in fish (i.e. the variation in feeding behaviour in response to available prey items) may be common and highlights the importance of wider food web influences.

The quantity and quality of food arriving at the seafloor are important influences on deep-sea communities (Ruhl & Smith 2004, Wolff et al. 2011) and its variability can affect a variety of different consumers (Bailey et al. 2006, Billett et al. 2010). Deep-sea fish distribution and activity tend to reflect the overlaying surface primary productivity regimes (Priede et al. 1991, Henriques et al. 2002) with many species inhabiting the same depth range in different parts of the ocean (Priede et al. 2010). Biological rather

than physical factors may determine patterns of deep-sea fish distribution and this may be mediated through low food availability and subsequent high competition (Collins et al. 1999, Stowasser et al. 2009). Functional role is an important determinant of body size and trophic ecology in deep-sea fish (Collins et al. 2005, Yeh & Drazen 2009). Deep-sea scavengers increase in size with depth: the larger body size allows for greater swimming speed and endurance whilst searching for sporadic high energy pelagic carrion whilst maintaining greater energy reserves (Collins et al. 2005). In contrast, smaller more abundant prey items are evenly distributed for predators, therefore predators do not need to search widely for their prey item (Ruhl & Smith 2004, Collins et al. 2005).

Little is known about intra-population size-based trophodynamics in deep-sea fishes. Declining food availability with increasing depth (Lampitt et al. 1986) and decreasing latitude (Thurston et al. 1998) increases competition among bentho-pelagic deep-sea fish suggesting ecological factors may affect intra- and inter-population trophodynamics (Collins et al. 1999). Many species of deep-sea fish are thought to switch diet from benthic invertebrates to pelagic organisms during ontogeny (Sedberry & Musick 1978, Mauchline & Gordon 1984a, Martin & Christiansen 1997), but small sample sizes, regurgitated stomachs and large amounts of unidentifiable prey items undermine the ability to detect ontogenetic diet shifts and feeding plasticity (Mauchline & Gordon 1984b, Stowasser et al. 2009). Basic information is still lacking on size-based trends in trophic position and whether trophic connectivity between benthic and pelagic food chains varies with life history stage. A complementary approach is to use stable isotopes, which successfully elucidates food web dynamics (Iken et al. 2001, Polunin et al. 2001) and intra-population variation in trophic position (Drazen et al. 2008b, Jennings et al. 2008, Stowasser et al. 2009).

Stable isotopes of carbon ($^{13}C/^{12}C$ expressed as $\delta^{13}C$) and nitrogen ($^{15}N/^{14}N$ expressed as $\delta^{15}N$) provide an integrated view of the assimilated diet over time (Hesslein et al. 1991). Consumers are enriched in the heavier isotope in relation to their diet through trophic discrimination processes. In fish, carbon trophic discrimination is approximately 1.5‰ (Sweeting et al. 2007b) and is used to distinguish different primary production sources, i.e. benthic verses pelagic (Sherwood et al. 2007), but it can also indicate shifts in habitat, i.e. nursery to adult feeding grounds (Nakamura et al. 2008). Nitrogen trophic discrimination ($\Delta^{15}N$) is approximately 3.2‰ (Sweeting et al. 2007a), used to estimate relative trophic position from a known isotopic baseline and investigate how trophic position varies through life as a function of size (Jennings et al. 2008).

Here, stable isotopes of carbon and nitrogen were used to investigate intra-population trophodynamics in four abundant demersal deep-sea fish caught at a depth of approximately 2500 m (Bergstad et al. 2008) on the Mid-Atlantic Ridge (MAR). *Antimora rostrata* and *Coryphaenoides armatus* are opportunistic scavenging fishes, inhabiting overlapping depth zones (Collins et al. 1999, King et al. 2006). *Coryphaenoides brevibarbis* and *Halosauropsis macrochir* are predators with wide bathymetric distributions (Collins et al. 2005, Priede et al. 2010). The aims of the study are to (1) examine whether intra-population variation in stable isotopes of carbon and nitrogen are related to size in deep-sea fish, (2) identify whether relationships between size and stable isotopes vary temporally, and (3) whether these trends vary spatially within a species.

3.2. Methods

3.2.1. Sample collection and study area

Fish were collected from onboard the R.R.S. *James Cook* in the summers of 2007 (13 July to 18 August) and 2009 (1 August to 9 September) at three different stations on the MAR. Stations were located at 2400-2750 m depth and are oceanographically separated by the Arctic Sub-polar Front and physically by the Charlie-Gibbs Fracture Zone (CGFZ), between 48° and 54°N (Figure 3.1). Samples were collected at two stations north of the CGFZ approximately 140 km apart on the east and west axes of the ridge and south of the CGFZ on the eastern axis approximately 690 km from the northeast station (Table 3.1). Sampling was undertaken using a single warp semi-balloon otter trawl (OTSB) with a wing spread of 8.6 m and a headrope height of 1.5 m. The net body consisted of a 44 mm mesh, a codend mesh of 37 mm with an internal codend liner of 13 mm mesh. Further details can be found in (Merrett & Marshall 1981). Catches were sorted immediately once on board. Fish were identified, weighed and length was measured to the nearest mm. Standard length (SL) was taken for the morid *Antimora rostrata* and pre-anal fin length (PAFL) for the macrourids, *Coryphaenoides armatus* and *C. brevibarbis*, and the notocanthiid *Halosauropsis macrochir* because tails are often broken. White muscle tissue was dissected from the dorso-lateral region for stable isotope analysis and frozen at -80^{o}C in glass vials.

Tissue samples were freeze dried and ground to a homogeneous powder using a mortar and pestle. Approximately 0.7 mg of powder was weighed into a tin capsule for combustion. Dual stable carbon and nitrogen isotope ratios were measured by continuous-flow isotope ratio mass spectrometry using a Costech Elemental Analyser interfaced with either a Thermo Finnigan Delta Plus XP mass spectrometer or a Thermo

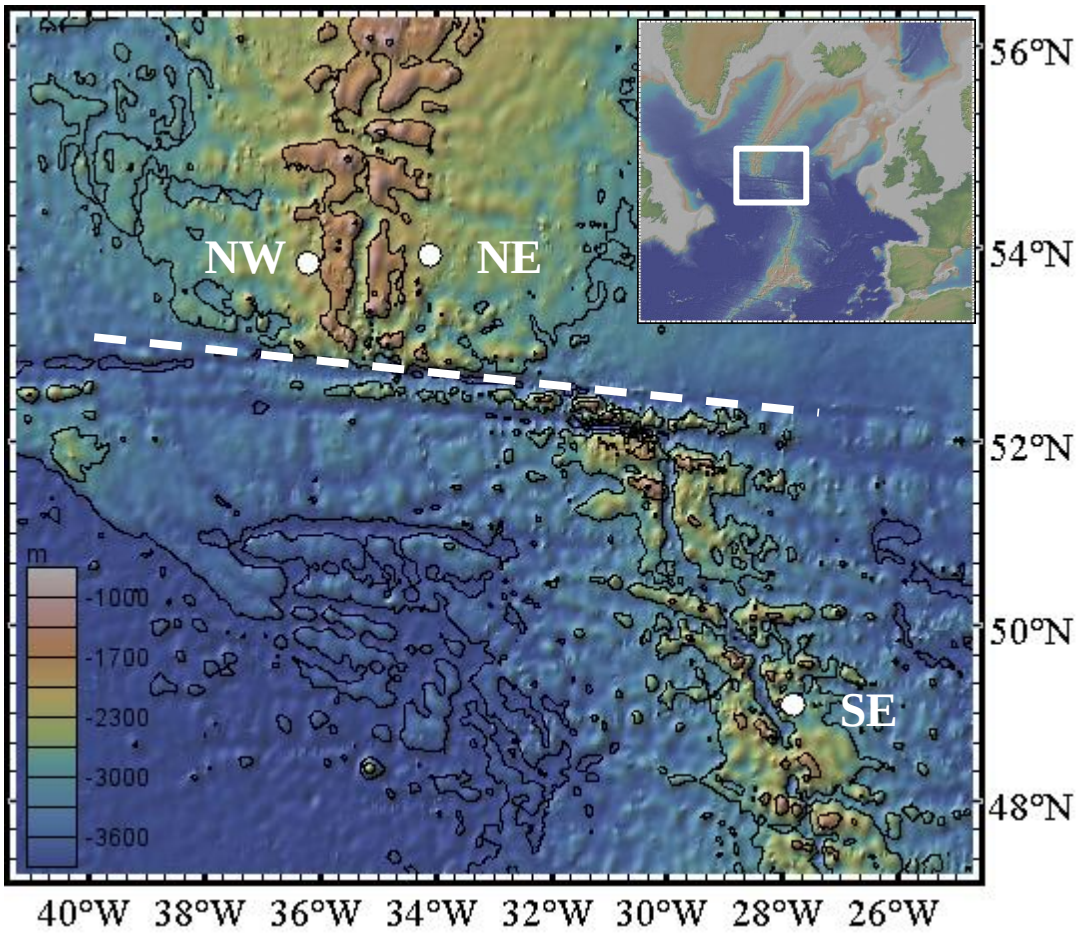

Fig. 3.1: Bathymetric map of the Mid-Atlantic Ridge with white circles indicating the northeast (NE), northwest (NW) and southeast (SE) stations and a dashed line indicating the position of the Charlie-Gibbs Fracture Zone. Insert represents the position of the study area in relation to the rest of the North Atlantic Ocean.

Finnigan Delta V Plus mass spectrometer (Natural Environment Research Council, Life Sciences Mass Spectrometry Facility, SUERC, East Kilbride). Two laboratory standards were analysed every ten samples in each analytical sequence for linearity and drift corrections. These alternated between paired alanine standards, of differing $\delta^{13}C$ and $\delta^{15}N$, and an internal laboratory gelatin standard. Laboratory standards are traceable to international standards v-PDB (Pee Dee Belemnite) and AIR (atmospheric nitrogen). An external standard of freeze dried and ground white fish muscle (*Antimora rostrata*) was also analysed ($\delta^{13}C$, n = 22, -18.88‰ ±1 s.d. 0.07; n = 22, $\delta^{15}N$ 13.29‰ ±1 s.d. 0.12).

Table 3.1: Semi-balloon otter trawl stations, positions and depths for samples collected on the Mid-Atlantic Ridge in 2007 and 2009.

Station	Deployment date	Depth (m)	Start position		End position	
			Latitude (N)	Longitude (W)	Latitude (N)	Longitude (W)
Southeast	22/07/2007	2718	48°54.59'N	27°50.00'W	49°15.85'N	27°50.00'W
Northwest	05/08/2007	2605	53°51.10'N	36°11.36'W	54°11.14'N	36°05.66'W
Northeast	10/08/2007	2405	54°06.33'N	33°58.27'W	53°47.47'N	34°02.89'W
Northeast	11/08/2007	2410	54°05.68'N	33°58.54'W	53°46.94'N	34°03.02'W
Northeast	12/08/2007	2404	54°05.68'N	33°58.54'W	53°47.71'N	34°02.83'W
Southeast	10/08/2009	2700	48°58.73'N	27°51.01'W	49°11.14'N	27°49.17'W
Southeast	10/08/2009	2700	48°58.05'N	27°51.06'W	49°14.36'N	27°49.29'W
Southeast	18/08/2009	2700	48°57.34'N	27°49.83'W	49°13.55'N	27°50.92'W
Northwest	28/09/2009	2602	54°19.62'N	36°00.87'W	54°04.96'N	36°07.98'W
Northwest	28/09/2009	2600	54°19.50'N	36°01.11'W	54°06.55'N	36°07.14'W
Northwest	29/08/2009	2602	54°19.23'N	36°01.17'W	54°03.65'N	36°08.42'W

3.2.2. Data analysis

Lipids are depleted in ^{13}C by between 6 and 8‰ relative to proteins (DeNiro & Epstein 1977). Intra-population variation in lipid content may impact the interpretation of bulk $\delta^{13}C$ values through influence on population variance structure and trends with body size. $\delta^{13}C$ data were lipid-normalised ($\delta^{13}C_{norm}$) to remove lipid bias using a modified arithmetic correction (Sweeting et al. 2006). This replaced the C:N for pure protein in the correction equation, with the lowest observed C:N in each species within each year and location (Equation 3).

$$\delta^{13}C_{norm} = ((\delta^{13}C_{sample} \times C{:}N_{sample}) + (7\times\ (C{:}N_{sample} - C{:}N_{lowest})))/\ C{:}N_{sample} \quad \text{(Eq. 3)}$$

The approach did not normalise among species to the same lipid content but aimed to reduce intra-population lipid bias. This is sufficient as length-based trends in $\delta^{13}C_{norm}$ were based on within species changes in slope rather than intercept (Greenwood et al. 2010).

Linear regression was used to investigate length-based trends in $\delta^{13}C_{norm}$ and $\delta^{15}N$ at each station and year for each species. Length was $\log_{10}$ transformed for the $\delta^{13}C_{norm}$ analysis to meet the assumptions of linear regression analysis while length remained unchanged for the $\delta^{15}N$ analysis. The shift in intra-population trophic position (ΔTP_c and ΔTP_n) was calculated using the relationship between length and $\delta^{13}C_n$ and $\delta^{15}N$ for each species and within each station assuming a $\Delta^{13}C$ of 1.5‰ and $\Delta^{15}N$ of 3.2‰ (Sweeting et al. 2007a, Sweeting et al. 2007b). Temporal relationships, within stations, were assessed by analysis of covariance (ANCOVA) with length as the covariate and year as the categorical variable. If the results were non-significant, data were pooled to produce a station regression. This was used to assess within species spatial patterns with length as a covariate and station as the categorical variable.

3.3. Results

Antimora rostrata were sampled over a similar size range at each station and year (Table 3.2). Relationships between $\delta^{13}C_{norm}$ or $\delta^{15}N$ and length were found at each station and year ($p < 0.01$) (Figure 3.2a & b; Table 3.3), with $\delta^{15}N$ changes the equivalent of between 0.34 and 0.58 ΔTP_n (Table 3.3). Temporal comparisons at the northwest station showed slopes (ANCOVA: $\delta^{13}C_{norm}$ $F_{1, 32} = 0.82$, $p = 0.37$; $\delta^{15}N$ $F_{1, 32} = 0.82$, $p = 0.37$) and elevation (ANCOVA: $\delta^{13}C_{norm}$ $F_{1, 32} = 0.24$, $p = 0.10$; $\delta^{15}N$ $F_{1, 32} = 0.47$, $p = 0.47$) were similar. Spatial differences in slopes across the three stations were marginally non-significant (ANCOVA: $\delta^{13}C_{norm}$ $F_{2,76} = 0.67$, $p = 0.51$; $\delta^{15}N$ $F_{2,76} = 2.74$, $p = 0.07$) but elevation differed (ANCOVA: $\delta^{13}C_{norm}$ $F_{2,76} = 17.42$, $p < 0.01$; $\delta^{15}N$ $F_{2,76} = 3.68$, $p < 0.05$).

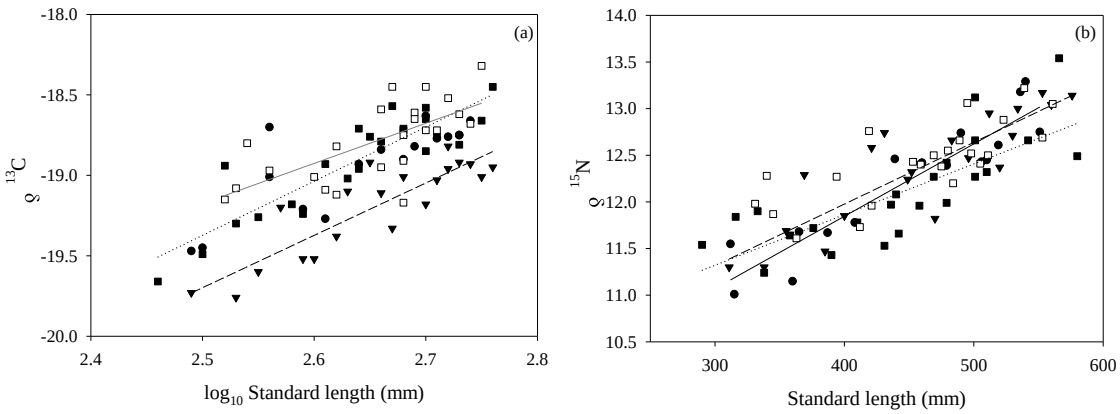

Fig 3.2: Plots of (a) $\delta^{13}C$ against $\log_{10}$ standard length (mm) and (b) $\delta^{15}N$ against standard length for *Antimora rostrata* with fitted regression lines for 2007 data at the northeast (■ & black dotted line) and northwest (● & black solid line) stations, and for 2009 data at the northwest (▼& black dashed line) and southeast (□ & grey solid line) stations.

The size range of *Coryphaenoides armatus* sampled was similar at each station (Table 3.2). Relationships between $\delta^{13}C_{norm}$ and length were found at all stations ($p < 0.01$) with the slopes negative at the southern and positive at the northern stations (Figure 3.3a & Table 3.3). This resulted in spatial differences among slopes (ANCOVA: $\delta^{13}C_{norm}$

$F_{2,57} = 41.67$, $p < 0.01$). No relationships between $\delta^{15}N$ and length were observed ($p > 0.05$) (Figure 3.3b) and spatial differences in slope were also not observed (ANCOVA: $\delta^{15}N$ $F_{2,57} = 1.75$, $p = 0.19$) nor were there differences in elevation (ANCOVA: $\delta^{15}N$ $F_{2,57} = 2.17$, $p = 0.12$).

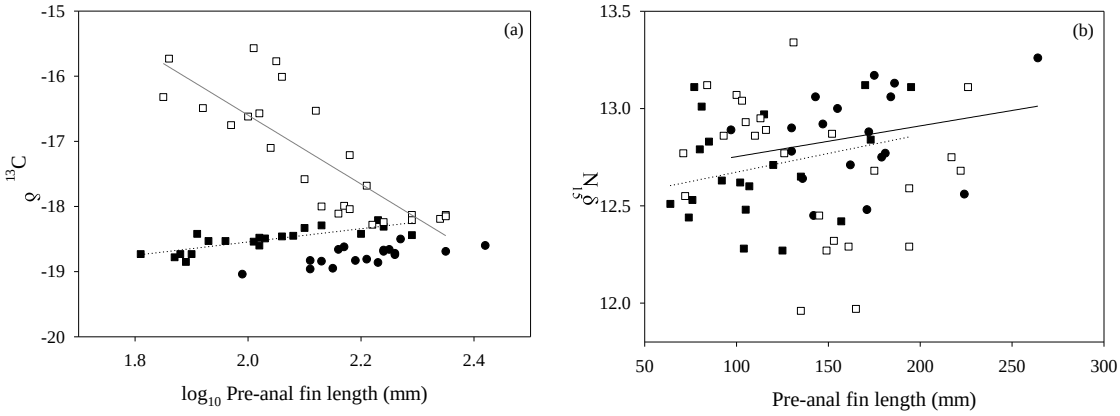

Fig 3.3: Plots of (a) $\delta^{13}C$ against $\log_{10}$ pre-anal fin length (mm) and (b) $\delta^{15}N$ against pre-anal fin length for *Coryphaenoides armatus* with fitted regression lines for 2007 data at the northeast (■ & black dotted line) station and for 2009 data at the northwest (▼& black dashed line) and southeast (□ & grey solid line) stations.

Differences in the size range sampled were observed in *Coryphaenoides brevibarbis*, with a much narrower size range sampled at the southern station in 2009 than the other stations (Table 3.2). Relationships between $\delta^{13}C_{norm}$ and length were apparent at the northwest station in both years ($p < 0.01$) but were positive in 2007 and negative in 2009 whereas no relationship was found at the southern or northeast stations (Figure 3.4a & Table 3.3). Spatial differences in slope were observed (ANCOVA: $\delta^{13}C_{norm}$ $F_{3,65} = 5.73$, $p < 0.01$). The relationships between $\delta^{15}N$ and length were significant in 2009 at the southern and northwest ($p < 0.01$) stations but not in 2007 at the northeast and northwest stations ($p > 0.05$) (Table 3.3). Therefore slopes differed among stations and years (ANCOVA: $\delta^{15}N$ $F_{3,65} = 4.78$, $p < 0.01$) (Figure 3.4b & Table 3.3). Shifts in ΔTP_n were between 0.09 and 0.34 (Table 3.3).

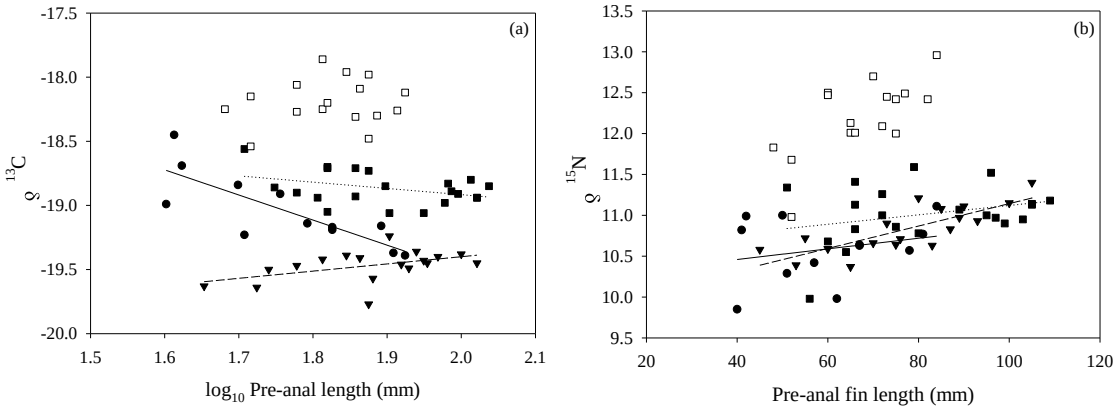

Fig 3.4: Plots of (a) $\delta^{13}C$ against $\log_{10}$ pre-anal fin length (mm) and (b) $\delta^{15}N$ against pre-anal fin length for *Coryphaenoides brevibarbis* with fitted regression lines for 2007 data at the northeast (■ & black dotted line) and northwest (● & black solid line) stations, and for 2009 data at the northwest (▼& black dashed line) and southeast (□ & grey solid line) stations.

There were differences in the size range of *Halosauropsis macrochir* sampled between the two stations with larger individuals collected at the northeast compared to the southern station (Table 3.2). Relationships between $\delta^{13}C_{norm}$ and $\delta^{15}N$ with length were positive ($p < 0.01$) in both years at the southern station while they were absent at the northeast station (Figure 3.5a & b; Table 3.3). Slope (ANCOVA: $\delta^{13}C_{norm}$ $F_{1, 27} = 0.80$, $p = 0.78$; $\delta^{15}N$ $F_{1, 27} = 1.25$, $p = 0.27$) and elevation (ANCOVA: $\delta^{13}C_{norm}$ $F_{1, 27} = 0.72$, $p = 0.40$; $\delta^{15}N$ $F_{1, 27} = 0.10$, $p = 0.76$) were similar at the southern station in 2007 and 2009. But spatial differences in slope (ANCOVA: $\delta^{15}N$ $F_{1,37} = 9.35$, $p < 0.01$) were observed between the southern and northern stations, which resulted in ΔTP_n varying between 0.15 and 0.70.

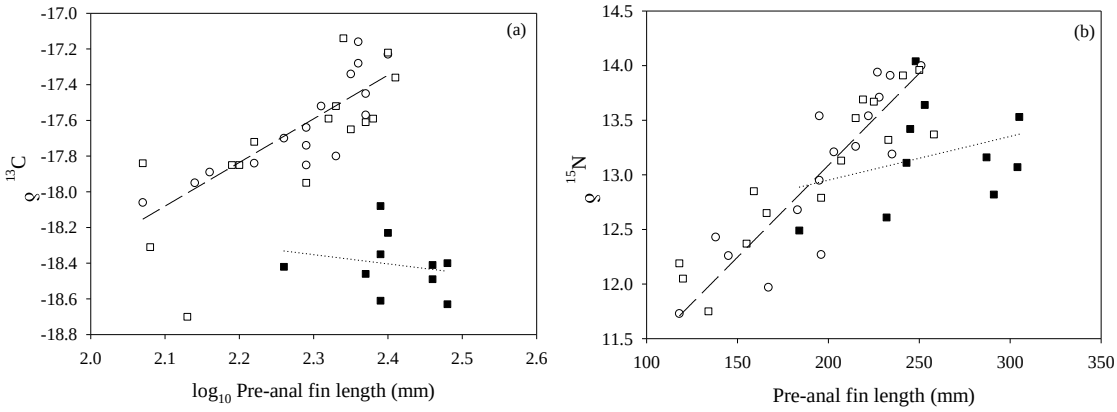

Fig 3.5: Plots of (a) $\delta^{13}C$ against $\log_{10}$ pre-anal fin length (mm) and (b) $\delta^{15}N$ against pre-anal fin length for *Halosauropsis macrochir* with fitted regression lines for 2007 data at the northeast (■ & black dotted line) and southeast (○ & long dashed line) stations, and for 2009 data at the southeast (□ & grey solid line) station.

Table 3.2: Size range, proportional maximum length range (ΔL_{max}), lipid-normalised $\delta^{13}C$ ($\delta^{13}C_{norm}$) range, $\delta^{15}N$ range, C:N (w/w) range for the four species of deep-sea fish collected on the Mid-Atlantic Ridge for size-based feeding analyses.

Species	Year	Station	Size range (mm)	ΔL_{max} (%)	$\delta^{13}C_{norm}$ range	$\delta^{15}N$ range	C:N (w/w) range
Antimora rostrata	2007	NE	290 to 580	48.33	-19.66 to -18.45	11.24 to 13.54	3.12 to 3.25
		NW	312 to 551	39.83	-19.47 to -18.63	11.01 to 13.29	3.14 to 3.23
	2009	NW	311 to 576	44.17	-19.76 to -18.82	11.30 to 13.17	3.11 to 3.18
		SE	331 to 561	38.33	-19.17 to -18.32	11.61 to 13.22	3.12 to 3.19
Coryphaenoides armatus	2007	NE	64 to 195	37.11	-18.85 to -18.21	12.27 to 13.12	3.12 to 3.23
	2009	NW	97 to 264	47.31	-19.04 to -18.50	12.45 to 13.26	3.14 to 3.26
		SE	71 to 226	43.91	-18.28 to -15.57	11.96 to 13.34	3.14 to 3.24
Coryphaenoides brevibarbis	2007	NE	51 to 109	39.73	-19.06 to -18.56	9.98 to 11.59	3.11 to 3.22
		NW	40 to 84	30.14	-19.48 to -18.64	9.85 to 11.11	3.15 to 3.24
	2009	NW	45 to 105	41.10	-19.77 to -19.24	10.37 to 11.40	3.16 to 3.22
		SE	48 to 84	24.66	-18.54 to -17.86	10.98 to 12.96	3.15 to 3.24
Halosauropsis macrochir	2007	NE	184 to 305	38.41	-18.63 to -17.86	12.49 to 14.04	3.22 to 3.58
		SE	118 to 251	42.22	-18.06 to -18.73	11.73 to 14.00	3.16 to 3.54
	2009	SE	118 to 258	44.30	-18.73 to -17.14	11.75 to 13.96	3.14 to 3.26

Table 3.3: Year, station, regression parameters for relationship between length and lipid-normalised $\delta^{13}C$ ($\delta^{13}C_{norm}$) or $\delta^{15}N$ and shift in trophic position (ΔTP_C or ΔTP_N) calculated from the regression equations for the four species of deep-sea fish collected on the Mid-Atlantic Ridge.

Species	Year	Station	Length (log_{10}) v $\delta^{13}C_{norm}$						Length v $\delta^{15}N$					
			a	b	r^2	F	p	ΔTP_C	a	b	r^2	F	p	ΔTP_N
Antimora rostrata	2007	NW	-26.00	2.67	0.63	$27.37_{1,14}$	<0.01	0.44	8.74	0.0078	0.85	$88.26_{1, 14}$	<0.01	0.58
		NE	-27.78	3.36	0.78	$79.17_{1, 21}$	<0.01	0.67	9.69	0.0054	0.60	$33.44_{1, 21}$	<0.01	0.49
	2009	SE	-25.41	2.87	0.53	$18.47_{1,21}$	<0.01	0.44	10.28	0.0047	0.56	$28.90_{1, 21}$	<0.01	0.34
		NW	-27.83	3.25	0.75	$59.29_{1,18}$	<0.01	0.58	9.33	0.0066	0.73	$53.01_{1, 18}$	<0.01	0.55
	All	NW	-26.69	2.87	0.44	$40.49_{1,34}$	<0.01	0.51	9.05	0.0071	0.79	$134.60_{1,34}$	<0.01	0.59
Coryphaenoides armatus	2007	NE	-20.58	1.02	0.63	$32.93_{1,18}$	<0.01	0.33	12.48	0.0019	0.02	$1.39_{1, 18}$	0.26	0.08
	2009	SE	-6.02	-5.29	0.68	$49.25_{1,23}$	<0.01	-1.77	12.99	-0.0021	0.03	$1.82_{1, 23}$	0.19	-0.10
		NW	-21.07	1.04	0.49	$17.08_{1,16}$	<0.01	0.30	12.59	0.0016	0.01	$1.10_{1, 16}$	0.31	0.08
Coryphaenoides brevibarbis	2007	NW	-15.59	-1.96	0.62	$19.17_{1,10}$	<0.01	-0.42	10.20	0.0064	-0.03	$0.71_{1,10}$	0.42	0.09
		NE	-17.94	-0.49	0.10	$3.10_{1,19}$	0.09	0.19	10.55	0.0058	0.04	$1.82_{1, 19}$	0.19	0.10
	2009	SE	-18.92	0.40	-0.04	$0.37_{1,14}$	0.56	0.06	10.19	0.0298	0.43	$12.54_{1, 14}$	<0.01	0.34
		NW	-20.95	0.76	0.36	$10.73_{1,16}$	<0.01	0.19	9.88	0.0125	0.60	$24.41_{1, 17}$	<0.01	0.23
Halosauropsis macrochir	2007	SE	-23.22	2.45	0.69	$34.03_{1,14}$	<0.01	0.54	9.72	0.0168	0.76	$47.52_{1,14}$	<0.01	0.70
		NE	-17.16	-0.52	-0.08	$0.37_{1,8}$	0.56	-0.07	12.15	0.0040	-0.01	$0.89_{1,8}$	0.37	0.15
	2009	SE	-23.76	2.66	0.56	$18.88_{1,13}$	<0.01	0.60	10.38	0.0136	0.84	$72.3_{1, 13}$	<0.01	0.60
	All	SE	-23.58	2.59	0.62	$49.21_{1,29}$	<0.01	0.58	10.11	0.0149	0.79	$114.20_{1,29}$	<0.01	0.65

3.4. Discussion

Overall MAR deep-sea fish exhibited size-based relationships between length and $\delta^{13}C_{norm}$ and/ or $\delta^{15}N$. The strength, direction and significance of these trends varied temporally and spatially in some species suggesting a degree of feeding plasticity that has not been previously observed in size-based analyses of deep-sea fish using stable isotopes.

$\delta^{13}C_{norm}$ and $\delta^{15}N$ size-based relationships were observed for *Antimora rostrata* from the MAR (Figure 3.2). The $\delta^{15}N$ of *A. rostrata* from the Porcupine Seabight (PSB) also increases with size and in conjunction with increases in the monounsaturated fatty acids (MUFAs) 18:1(n-9) and 20:1(n-9) and decreases in polyunsaturated fatty acids 20:5(n-3) and 22:6(n-3), implies a greater contribution of benthic organisms in the diet of larger fish (Stowasser et al. 2009). On the MAR, benthic organisms are enriched ^{13}C relative to pelagic fauna but do not necessary have greater $\delta^{15}N$ values (Chapter 2), which may explain size-based trends in $\delta^{13}C_{norm}$ but may not fully account for $\delta^{15}N$ trends. PSB *A. rostrata* also increase in 22:1(n-11) MUFA with size (Stowasser et al. 2009). MUFAs are present in high concentrations in copepods and their predators (e.g. fish and squid) (Petursdottir et al. 2008, Stowasser et al. 2009) potentially confounding the interpretation of increasing benthic influence with size. Instead, a shift from low to high trophic position pelagic carrion consumed in the diet could explain the results observed for *A. rostrata*. The concurrent size-based trends in $\delta^{13}C_{norm}$ and $\delta^{15}N$ observed in *A. rostrata* indicated that size-based trends in stable isotopes may be linked. The resultant station ΔTP_c and ΔTP_n for *A. rostrata* are very similar indicating increases in trophic position within a food web and are likely due to trophic discrimination processes rather than switches in production source. Pelagic fish and cephalopods dominate the diet of adult *A. rostrata* along with large amounts of unidentifiable material (Mauchline &

Gordon 1984b) reflecting their dependence on scavenging rather than predation. There are a number of shallow water fish species that consume greater proportions of fish and cephalopods with increasing size that also result in increases in $\delta^{15}N$ (Renones et al. 2002, Muto & Soares 2011). A shift from low to high trophic position pelagic carrion consumed in the diet could explain the results observed for *A. rostrata.* However, it is likely that in a food limited environment an organism will still consume some benthic prey opportunistically (Collins et al. 2005, Drazen et al. 2008b).

The relationship between $\delta^{13}C_{norm}$ and size of MAR *Coryphaenoides armatus* varied spatially (Figure 3.3), which suggested foraging behaviour and diet differed in smaller individuals between the north and south stations. Neither studies in the North Pacific nor the PSB found size-based trends in $\delta^{13}C$ (Drazen et al. 2008b, Stowasser et al. 2009). *C. armatus* diet varies spatially, containing mainly pelagic organisms in the Rockall Trough and western North Atlantic slope (Sedberry & Musick 1978, Mauchline & Gordon 1984a) but shifting from epi-benthic invertebrates to greater proportions of fish and cephalopod flesh in the Iceland Basin and on the North Pacific slope and abyssal plain (Pearcy & Ambler 1974, Martin & Christiansen 1997, Drazen et al. 2008b). Ocean and regional scale differences in diet appear to be reflected in the $\delta^{13}C_{norm}$ size-based trends found at the MAR stations, indicating a degree of feeding plasticity not observed in *A. rostrata*. $\delta^{13}C$ values of MAR benthic crustaceans and polychaetes are approximately -16‰ compared to -20 to -18‰ for bathypelagic fishes and crustaceans (Chapter 2; Letessier et al. 2012) indicating individuals at the southern station switched from benthic to meso- and bathypelagic prey as they grew. At the northern station, *Coryphaenoides armatus* $\delta^{13}C_{norm}$ trends were similar to *Antimora rostrata,* which suggested a higher dependence on pelagic prey. Differences in pelagic carrion between the northern and southern stations is unknown but if availability is lower at the southern,

then *C. armatus* may not be able to compete with the faster *A. rostrata* (Collins et al. 1999), resulting in *C. armatus* remaining on a benthic diet for longer at this depth.

MAR *Coryphaenoides armatus* did not show any relationships between $\delta^{15}N$ and size. Size-based shifts in $\delta^{15}N$ are present in *C. armatus* from the PSB (Stowasser et al. 2009) but analysis of samples from the North Pacific abyssal plain (Drazen et al. 2008b) are in agreement with this study. There are two confounding factors that may explain the differences between the PSB size-based trends and those of the North Pacific abyss and the MAR. Firstly, liver tissue was analysed in the PSB *C. armatus* (Stowasser et al. 2009) whereas muscle was sampled from the North Pacific (Drazen et al. 2008b) and MAR *C. armatus*. Liver and muscle tissue have different biochemical components (Stowasser et al. 2009), different isotopic turnover times (del Rio et al. 2009) and therefore provide different dietary information. Secondly, *C. armatus* was collected throughout its depth range in the PSB (Stowasser et al. 2009) while it was collected at discrete depths on the North Pacific abyssal plain (Drazen et al. 2008b) and on the MAR. As *C. armatus* becomes larger it undertakes an ontogenetic migration into deeper water (Collins et al. 1999, King et al. 2006). *C. armatus* size and $\delta^{15}N$ values increase with depth in the PSB (Stowasser et al. 2009) making it difficult to distinguish whether trends are a result of depth related shifts in $\delta^{15}N$ baseline values, reflected in some deep-sea taxa (Bergmann et al. 2009), or size-based isotope trends driven by increasing prey trophic position. *C. armatus* collected from the North Pacific abyssal plain and the MAR may be at equilibrium with the basal nitrogen source. The lack of size-based trends in $\delta^{15}N$ may be a result of no change in diet during the life history stage they spent at the depth sampled on the MAR or a shift to prey of similar $\delta^{15}N$ values. However, size-based $\delta^{13}C_{norm}$ trends were present on the MAR but even a large shift with size did not manifest in a size-based $\delta^{15}N$ shift.

Coryphaenoides brevibarbis exhibited spatial and temporal variability in the strength and direction of size-based trends (Figure 3.4). There was no consistent pattern in relationships between either $\delta^{13}C_{norm}$ or $\delta^{15}N$ with length among stations or between years, which may reflect a high degree of feeding plasticity in *C. brevibarbis* or an under sampled length range. The sample size needed to detect a size-based shift in $\delta^{15}N$ for a given slope increases exponentially below 40% of a species proportional length range (ΔL_{max}) (Galvan et al. 2010) but in 2007 less than 40% ΔL_{max} was sampled at the northern stations where trends were not significant. However, only 25% ΔL_{max} was sampled at the southern station yet an increase in $\delta^{15}N$ was detected suggesting other factors rather than sample size bias determined the different spatial and temporal trends.

In shallow water systems, similar temporal patterns in the significance and magnitude of intra-population shifts in trophic position are seen in some species (Jennings et al. 2007) indicating size-based trends are not consistent through time. Stable isotope values of macrourids of a similar size to *C. brevibarbis* (e.g. *Nezumia aequalis* and *Hymenocephalus italicus*) vary temporally in association with the proportions of the prey items they consume (Fanelli & Cartes 2010). Geographical variation in diet is observed in *C. brevibarbis* (N. Cousins pers. comms.). Therefore, it is likely that wider food availability and temporal or spatial shifts in isotopic values of prey may influence size-based trends in *C. brevibarbis*.

Little is known about the behaviour and diet of *C. brevibarbis* but limited stomach content data reveal a diet of amphipods, copepods and polychaetes with a greater proportion of cephalopods and decapods at larger sizes (Mauchline & Gordon 1984a, Martin & Christiansen 1997). However, on the MAR there are no detectable ontogenetic changes in diet but approximately 70% of the diet by mass is unidentifiable digested material (N. Cousins pers. comms.). The poor diet resolution is therefore

masking distinct spatial and temporal trends in size-based feeding observed in the stable isotope analysis. A switch from benthic to pelagic fauna observed in dietary studies (Mauchline & Gordon 1984a) is in agreement with the decreasing $\delta^{13}C$ values with size at the northern stations in 2007. The importance of benthic prey in smaller size classes may vary temporally and spatially as indicated by the differences in slope of the $\delta^{13}C$ size-based trends. Given the variability in the direction and magnitude of size-based stable isotope trends, *C. brevibarbis* is potentially an opportunistic forager consuming what is abundant at any given time.

Halosauropsis macrochir exhibited size-based trends in $\delta^{13}C_{norm}$ and $\delta^{15}N$ at the southern station but not at the northeast (Figure 3.5). Behavioural, dietary and morphological observations suggest this species is a benthic feeder (Sedberry & Musick 1978, Gordon & Duncan 1987, Crabtree et al. 1991). On the MAR, it consumes a mix of epifauna, infauna, cephalopods and fish (Bergstad et al. 2012) but infauna are not as prominent as on continental margins (Sedberry & Musick 1978). The increase in $\delta^{15}N$ with size at the southern station, may relate to the greater proportion of fish found within the diet of MAR fish at larger sizes (Bergstad et al. 2012). ΔTP_N increased by 0.70 at the southern station, which was greater than *A. rostrata* and twice the level of the other predator *C. brevibarbis.* However, spatial differences in size-based $\delta^{13}C_{norm}$ and $\delta^{15}N$ trends within *H. macrochir* are difficult to compare as they covered different life history stages. The greatest shift in stable isotope values with size can occur over smaller size classes which are driven by changes in habitat utilisation, ontogenetic shifts in diet and increasing gape size linked with higher isotopic turnover (Wells et al. 2008). The slope of the size-based trends may become less steep in larger sized individuals as the diet becomes constant or contains a consistent mix of multiple prey items. Furthermore, isotopic values of the prey may take longer to become integrated into the consumer's tissue as a result of less somatic growth. The absence of size-based stable

isotope trends at the northeast station is potentially a result of sampling only larger individuals where diet changes are not as pronounced (Bergstad et al. 2012).

3.5. Conclusion

In the present study size-based trends were observed indicating size is an important factor in understanding intra-population variation in stable isotopes values of deep-sea fish. Some species exhibited spatial and temporal patterns which may indicate varying degrees of feeding plasticity. The consistent size-based trends in *Antimora rostrata* for $\delta^{13}C_{norm}$ and $\delta^{15}N$ are in contrast to spatial differences in trends observed in the macrourids *Coryphaenoides armatus* and *Coryphaenoides brevibarbis*. Both these macrourids extend into deeper water than *A. rostrata*. If the size-based trends in stable isotope values are indicative of feeding plasticity then it may further explain their success where food availability is lower as they have the capacity to adapt to available prey. Furthermore, different species increased in trophic position by varying amounts but there did not appear to be any consistent pattern between predators or scavengers. The negative $\delta^{13}C_{norm}$ trends observed at the northern stations in 2007 in *C. brevibarbis* and in *C. armatus* at the southern station suggested a switch from benthic to pelagic prey items, which had lower $\delta^{13}C$ values. Concurrent size-based $\delta^{15}N$ trends were absent but this may be related to different basal stable nitrogen isotope values between pelagic and benthic prey rather than trends driven by increasing trophic position as suggested for *A. rostrata*. Differences in bulk $\delta^{15}N$ values between sources may be small but greater differences in $\delta^{15}N$ values between specific amino acids (Chikaraishi et al. 2009) may provide higher resolution to understand whether the lack of $\delta^{15}N$ trends are confounded by shifts in basal nitrogen sources (Dale et al. 2011). This study suggests functional role may play an important part in body size but there are other factors that

determine intra-population trophodynamics. Such factors may be related to the wider food web and flexibility of a species foraging behaviour.

Chapter 4: Spatial differences in hydrothermal vent trophic assemblages with contrasting vent fluid chemistry on the East Scotia Ridge (Southern Ocean)

Chapter 4: Spatial differences in hydrothermal vent trophic assemblages with contrasting vent fluid chemistry on the East Scotia Ridge (Southern Ocean)

4.1. Introduction

Hydrothermal vents are deep-sea chemical reducing habitats occurring on mid-ocean ridges and back-arc spreading centres. They contain high biomass but low biological diversity that is distinct from the surrounding deep sea in terms of fauna and environmental variables (German et al. 2011). Hydrothermal vent habitats are dominated by organisms evolved to tolerate extreme abiotic factors, and thrive along steep temperature and chemical gradients that are inaccessible to many deep-sea fauna. Microbial chemosynthesis replaces photosynthetic primary production at hydrothermal vents resulting in them being one of the few autochthonous ecosystems within an otherwise allochthonous deep sea (Rau & Hedges 1979, Cavanaugh et al. 1981). Reduced chemical compounds (e.g. hydrogen sulphide, methane) required for chemoautolithotrophy are supplied to microorganisms where high temperature venting or lower temperature diffuse flow mixes with colder oxygenated sea water (Karl 1995). Microorganisms utilise the chemical energy released during oxidation reactions to fuel the biosynthesis of organic carbon compounds, thus providing the base of the food web (Karl 1995). Hydrothermal vent fauna utilise microbial primary production through endo- and episymbiotic relationships (Rau & Hedges 1979, Rieley et al. 1999), consume free-living microorganisms from surfaces or the water column (Van Dover et al. 1988, Van Dover & Fry 1994), and indirectly through predation and scavenging (Colaco et al. 2002, Bergquist et al. 2007). The varity of feeding adaptations aid in the partitioning of resources among species and provide a framework in which elements are cycled.

Stable carbon isotopes have been used extensively to describe the energy sources sustaining hydrothermal vent fauna (Van Dover & Fry 1989, Colaco et al. 2002, Bergquist et al. 2007). Small trophic discrimination (0 to 1.5‰) between the food source and consumer (Peterson & Fry 1987) allows the $\delta^{13}C$ values of the vent fauna to be used to identify different carbon fixation pathways and sources (Robinson & Cavanaugh 1995, Naraoka et al. 2008, Hugler & Sievert 2011). The Calvin-Benson-Bassham (CBB) and reductive tricarboxylic acid (rTCA) cycles are the two dominant carbon fixation pathways at hydrothermal vents that fix carbon for the macro-consumer food web (Campbell & Cary 2004). The CBB cycle involves the enzyme ribulose-1,5-biphosphate carboxylase/ oxygenase (RuBisCO), which exists in two forms denoted I and II that differ in their specificity for CO_2 (Nakagawa & Takai 2008). Isotopic discrimination between substrate and organic products during reactions involving form I RuBisCO leads to $\delta^{13}C$ values in vent fauna between -35 and -27‰ (Robinson et al. 2003, Nakagawa & Takai 2008). Higher $\delta^{13}C$ values, less than -16‰ in vent fauna, were originally attributed to form II RuBisCO (Robinson & Cavanaugh 1995) but there is strong evidence that these values result from the reductive tricarboxylic acid (rTCA) cycle (Markert et al. 2007, Nakagawa & Takai 2008, Hugler et al. 2011). Methanotrophy is a further carbon fixation process at hydrothermal vents (Tunnicliffe et al. 2003, De Busserolles et al. 2009). The stable carbon isotopic composition of CH_4 can vary depending on whether it is of thermogenic, biogenic or magmatic origin and is thus reflected in the tissue of the organism hosting the endosymbionts (Naraoka et al. 2008, De Busserolles et al. 2009).

Stable isotopes of sulphur and nitrogen provide further information in describing trophic interactions at hydrothermal vents (Colaco et al. 2002, Van Dover 2002, De Busserolles et al. 2009, Erickson et al. 2009). Stable sulphur isotope values also identify energy sources energy sources because of the small trophic discrimination (-1 to 2‰) between

food source and consumer (Michener & Kaufman 2007). The large difference in stable isotope values of inorganic sulphur substrates between seawater sulphate (~20‰) and sulphides at hydrothermal vents (< 10‰) result in organic matter of photosynthetic (~16 to 19‰) and chemosynthetic (-9 to 10‰) origin having distinctive $\delta^{34}S$ values (Fry 1988, Erickson et al. 2009). Therefore, $\delta^{34}S$ provides further resolution in identifying food sources where $\delta^{13}C$ values of vent fauna are similar to non-vent fauna (Erickson et al. 2009). The greater trophic discrimination (2 to 5‰) in $\delta^{15}N$ between consumer and food source provides information on the trophic position of an organism relative to a primary consumer (Peterson & Fry 1987, Post 2002b).

The relative contribution of different carbon sources, food chain length and complexity of hydrothermal vent food webs varies globally depending on the species present and the host substrate (Fisher et al. 1994, Colaco et al. 2002, Van Dover 2002, Limen & Juniper 2006). Recently, hydrothermal vent communities have been discovered on the East Scotia Ridge (ESR); a back-arc spreading centre in the Atlantic sector of the Southern Ocean (German et al. 2000, Rogers et al. 2012). Two vent sites lacking the characteristic alvinocarid shrimps, bathymodiolid mussels and siboglinid worms found at Atlantic, Indian and Pacific hydrothermal vents occur on the ridge segments E2 and E9 (Rogers et al. 2012). Instead the vents are dominated by anomuran crabs (*Kiwa* n. sp.), stalked barnacles (*Vulcanolepas* n. sp.) and large peltospiroid gastropods, indicating a new biogeographic province (Rogers et al. 2012). It is therefore hypothesised that the ESR vent sites may contain novel trophic interactions. Furthermore, vent fluid chemistries at back-arc spreading centres can differ from those on mid-ocean ridges (German & Von Damm 2003). There are differences in the end-member chemistry of the hydrothermal vent fluid between the E2 and E9 vent sites as well as between northern and southern sections of E9 (Rogers et al. 2012). Vent fluid chemistry is thought to influence $\delta^{13}C$, $\delta^{15}N$ and $\delta^{34}S$ values of vent fauna (Trask & Van

Dover 1999, De Busserolles et al. 2009). Therefore, differences in chemical properties of the end-member fluids may result in differences in the ESR trophic assemblages among these sites.

The overarching objective of the present research was to investigate the spatial patterns in the trophic assemblages of three ESR vent sites. Specifically, the following aims were to: (1) compare the spread of $\delta^{13}C$, $\delta^{15}N$ and $\delta^{34}S$ values among sites to examine potential differences in trophic structure; (2) compare $\delta^{13}C$ and $\delta^{34}S$ values of vent fauna and benthic non-vent fauna at the northern E9 site to understand differences in photosynthetic and chemosynthetic energy sources; (3) examine differences in $\delta^{13}C$, $\delta^{15}N$ and $\delta^{34}S$ values of vent fauna that were sampled at two or more stations.

4.2. Methods

4.2.1. Study sites and sample collection

The study focused on hydrothermal vent sites located on the E2 and E9 segments of the ESR (Figure 4.1). The vent site at E2 was situated at 56° 05.35'S and 30° 19.20'W at a depth of 2600 m. Seafloor topography was complex with a series of terraced features and lobed pillow basalts filling a major north-south steep-sided fissure. The main venting occurred at an intersection between this fissure and a west-east running fault or scarp. Chimney structures emitting temperatures up to 352.6^{o}C were found along the scarp edge and diffuse flow areas (3.5^{o}C to 19.5^{o}C) were associated with the base of chimneys and scattered throughout the area (Rogers et al. 2012). E9 was located approximately 440 km south at 60° 02.50' S and 29° 58.80' W at a depth of 2400 m. E9 was relatively flat with sheet lava, a series of lava drain back features and collapsed pillow basalts. There were a series of north-south fissures with venting mainly occurring on the most western fissure. High temperature fluids up to 382.8^{o}C were emitted through a series of chimneys while diffuse flow (5^{o}C to 19.9^{o}C) located around their

base and at discrete areas throughout the site (Rogers et al. 2012). Differences in the end-member fluid chemistry emitted from chimneys were found between E2 and E9 as well as the northern and southern sections of E9 (Table 4.1). E9 is therefore subdivided into E9 north (E9N) and south (E9S) sites. Background water temperatures were 0.0°C at E2 and between -0.1°C and -1.3°C at E9 (Rogers et al. 2012).

Table 4.1: Chemical composition of the vent fluid end-members at E2, E9N and E9S hydrothermal vent sites taken from Rogers et al. (2012).

Site	Maximum temperature (°C)	pH	Chlorinity (nM)	H_2S (mM)
E2	353	2.9	531	7.0
E9N	383	3.4	98	9.5
E9S	351	3.2	179	13.6

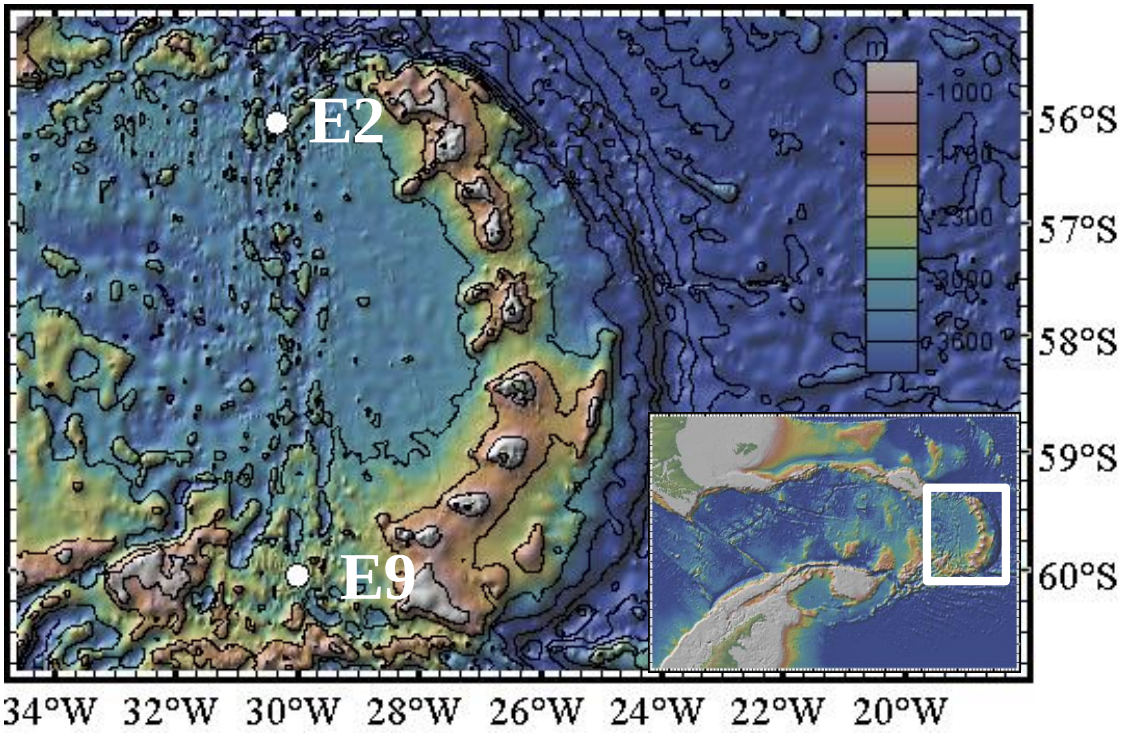

Fig. 4.1: Bathymetric map illustrating the positions of the E2 and E9 vent sites at the northern and southern ends of the East Scotia Ridge (ESR), located in the Atlantic sector of the Southern Ocean. The insert indicates the study area position in relation to South America and the Antarctic Peninsula.

Samples were collected onboard the R.R.S *James Cook* during the 2010 austral summer (7 January to 21 February) using the remotely operated vehicle (ROV) *Isis*. Vent fauna

were collected by suction sampler or scoop with species separated into a series of acrylic chambers or perspex boxes to avoid predation or contamination. Further samples were collected using large collapsible and small metal baited traps deployed from ROV *Isis*. Non-vent fauna were collected from metres to tens of metres away from active venting where there were no obvious signs of hydrothermal influence, i.e. no bacterial mat, and where ambient temperature was consistent with normal Antarctic bottom waters (~0°C). Non-vent samples were collected on separate dives from those for vent fauna to avoid contamination. Potential food sources were collected by scraping material and epibionts from rocks and from the underside of the decapod *Kiwa* n. sp. Particulate suspended material was collected from the acrylic chambers, which was sampled incidentally during faunal collection. Samples were sorted on board to the lowest possible taxonomic resolution.

4.2.2. Sample processing

Samples were frozen at -80°C whole or after dissection, depending on their size, for stable isotope analysis. Muscle was removed from the chelipeds of *Kiwa* n. sp., foot dissected from Peltospiroidea n. sp., tube feet removed from the asteroids Stichasteridae n. sp. and *Freyella* cf *fragilissima* and tentacles were removed from the anemones. Legs were removed from the pycnogonids *Colossendies* cf *concedis* and *C.* cf *elephantis*, while *Sericosura* spp was sampled whole. The gastropods Provannid sp. 1 and 2, *Lepetodrilus* n. sp. 1, and small Peltospiroidea n. sp. (< 7 mm shell length), and the stalked barnacle *Vulcanolepas* n. sp. were removed from their shells and sampled whole. Tissue samples were later freeze dried and ground to a homogenous powder using a pestle and mortar. Aliquots of fauna, particulate suspended material and materials scrapped from rocks were tested for carbonates prior to analysis with 0.1 N HCl. If the sample effervesced this indicated carbonates were present and it was subsequently

acidified by further addition of acid until the effervescing ceased. Samples were redried at 50^{o}C for 48 hours. If the sample did not effervesced no acidification was carried out. Aliquots for carbon stable isotope analysis were not lipid extracted before analysis because large differences in δ^{13}C values of trophic end-members exist between carbon sources at hydrothermal vents. Therefore, any confounding lipid effects due to metabolic processes would not affect the interpretation of the ultimate carbon sources of the vent fauna.

Approximately 0.7 mg of powder was weighed into separate tin capsules for carbon and nitrogen SIA. For sulphur SIA, 2 mg of sample and 4 mg of the catalyst vanadium pentoxide were weighed into each tin capsule. Dual stable carbon and nitrogen isotope ratios were measured by continuous-flow isotope ratio mass spectrometry using a Costech Elemental Analyser interfaced with Thermo Finnigan Delta Plus XP (Natural Environment Research Council, Life Sciences Mass Spectrometry Facility, SUERC, East Kilbride, United Kingdom). Two laboratory standards were analysed every ten samples in each analytical sequence. These alternated between paired alanine standards, of differing δ^{13}C and δ^{15}N, and an internal laboratory gelatin standard. Sulphur SIA was conducted by Iso-Analytical (Crewe, United Kingdom) using a SERCON Elemental Analyser coupled to a Europa Scientific 20-20 mass spectrometer. Laboratory standards of barium sulphate (two sets of differing δ^{34}S) and silver sulphide were used for calibration and drift correction. An internal standard of whale baleen was used for quality control (n = 28, 16.34‰ ± s.d. 0.21). Stable isotope ratios were expressed in delta (δ) notation as parts per thousand/ permil, (‰). All internal standards are traceable to the following international standards v-PDB (Pee Dee Belemnite), AIR (atmospheric nitrogen) and NBS-127 (barium sulphate), IAEA-S-1 (silver sulphide) and IAEA-SO-5 (barium sulphate). An external standard of freeze dried and ground white fish muscle

(*Antimora rostrata*) was also analysed ($\delta^{13}C$, n = 24, -18.94‰ ± s.d. 0.09; $\delta^{15}N$, n = 24, 13.11‰ ± s.d. 0.38; $\delta^{34}S$, n = 30, 18.20‰, ± s.d. 0.59).

4.2.3. Data analysis

Data were assessed for normality using a Shapiro-Wilk test. A Fligner-Killen homogeneity of variance test was used to analyse the spread of the stable isotope values among the sites, based on species means. Spatial differences in stable isotope values of each species were analysed with one-way ANOVAs followed by *post-hoc* analysis using Tukey's honest significant differences (HSD) when variance was homogenous among sites. Welch's ANOVA followed by two sample t-tests was used when there was heterogeneity of variance among sites. A Bonferroni correction ($p = 0.05/n$) was undertaken in order to take into account the multiple pairwise comparison. A Wilcoxon test was used when data were non-normally distributed.

4.3. Results

4.3.1. Spatial differences in sites

The spread of mean $\delta^{13}C$ values of the vent fauna differed amongst the three sites (Fligner-Killeen test, $\chi^2 = 6.46$, $p < 0.05$). E2 had the narrowest range of $\delta^{13}C$ values (-29.89 to -19.01‰), whereas at E9N and E9S $\delta^{13}C$ ranged from -31.39 to -9.92‰ and -30.03 to -10.51‰, respectively (Figure 4.2). The pattern of vent fauna $\delta^{13}C$ values was consistent across the three sites in that Peltospiroidea n. sp. had the lowest values while *Kiwa* n. sp. had the highest (Figure 4.2, Table 4.2). Vent fauna including *Lepetodrilus* sp. 1, *Vulcanolepas* n. sp., *Pacmanactis* sp., and *Colossendeis* spp had intermediate $\delta^{13}C$ values (Figure 4.2, Table 4.2). The spread of mean $\delta^{34}S$ values of the vent fauna did not differ among the sites (Fligner-Killeen test, $p = 0.16$) (Figure 4.3). E9N had the narrowest range of $\delta^{34}S$ values (Table 4.2) *Kiwa* n. sp. had the lowest $\delta^{34}S$ at E2 and

E9S while *Lepetodrilus* n. sp. 1 had the lowest $\delta^{34}S$ values at E9N (Figure 4.3, Table 4.2). The highest vent fauna $\delta^{34}S$ values were in *Pacmanactis* sp. (E2), *Vulcanolepas* n. sp. (E9N) and *Sericosura* spp (E9S) (Figure 4.3, Table 4.2). The spread of mean $\delta^{15}N$ values did not differ significantly among the three sites ($p = 0.84$) (Figure 4.2, Table 4.2). The provannid gastropods at E2 and E9S had the lowest $\delta^{15}N$ values while Peltospiroidea n. sp. had the lowest values at E9N (Figure 4.2, Table 4.2). Stichasteridae n. sp. consistently had the highest $\delta^{15}N$ values relative to the other vent fauna at each site (Figure 4.2, Table 4.2).

4.3.2. Differences between vent and benthic non-vent fauna at E9N

$\delta^{13}C$ and $\delta^{15}N$ values of vent fauna overlapped with non-vent benthic fauna at E9N (Figure 4.2, Table 4.2 & 4.3), which meant there was no significant difference ($\delta^{13}C$, $p = 0.40$; $\delta^{15}N$, $p = 0.60$). However, $\delta^{34}S$ values of non-vent benthic fauna did not overlap with vent fauna at E9N indicating a significant difference (t-test, $t = -9.37$, $p < 0.01$) (Figure 4.3, Table 4.2 & 4.3).

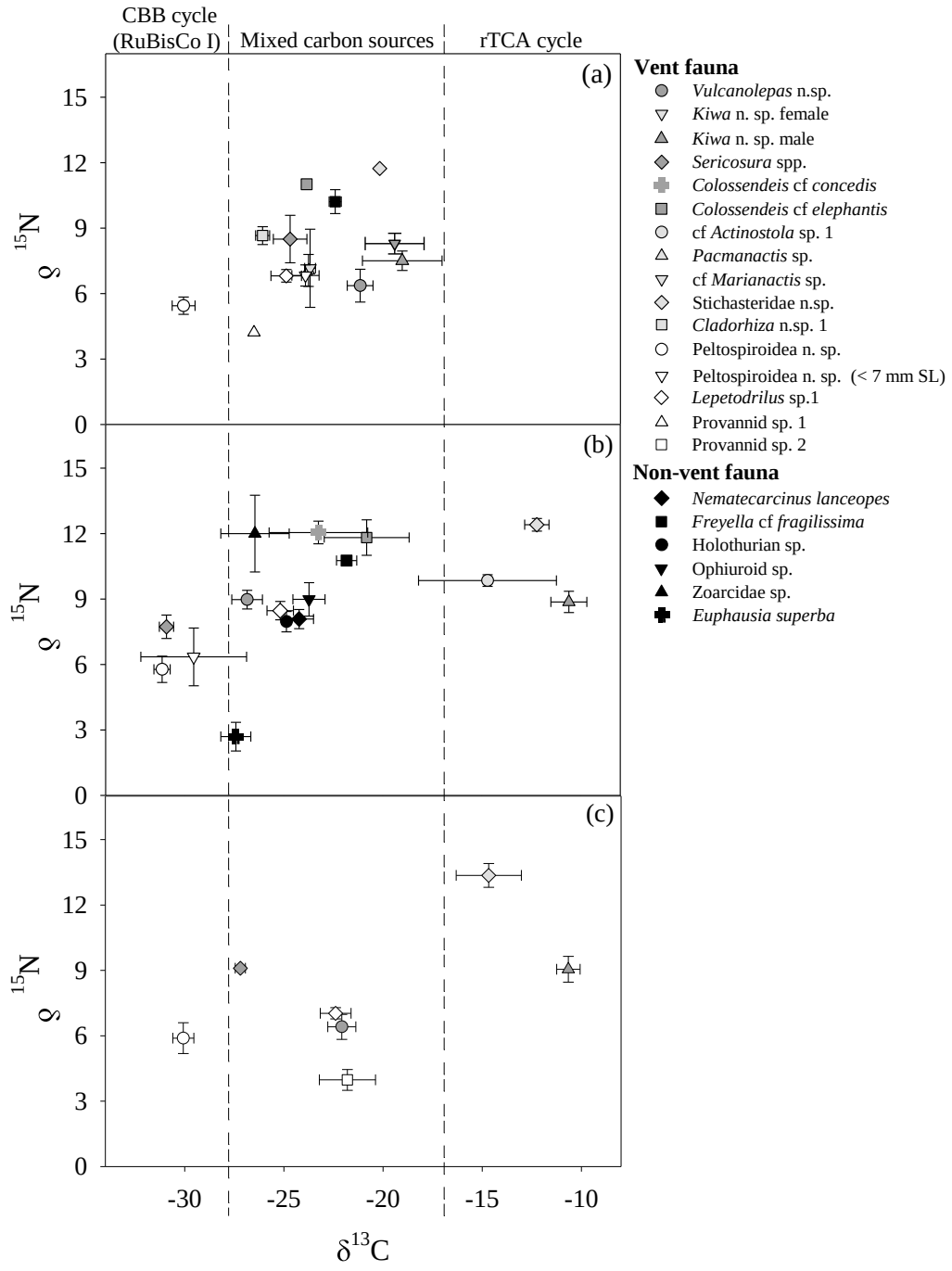

Fig. 4.2: $\delta^{13}C$ and $\delta^{15}N$ values of hydrothermal vent and off-vent fauna collected from the (a) E2, (b) E9N and (c) E9S ridge segments of the East Scotia Ridge, Southern Ocean. Dashed vertical lines represent potential ranges of $\delta^{13}C$ values indicative of carbon sources representing the Calvin-Benson-Bassham (CBB) cycle utilising form I RuBisCO, the reductive tricarboxylic acid (rTCA) cycle and a mixed carbon source. Values are means ± 1 standard deviation.

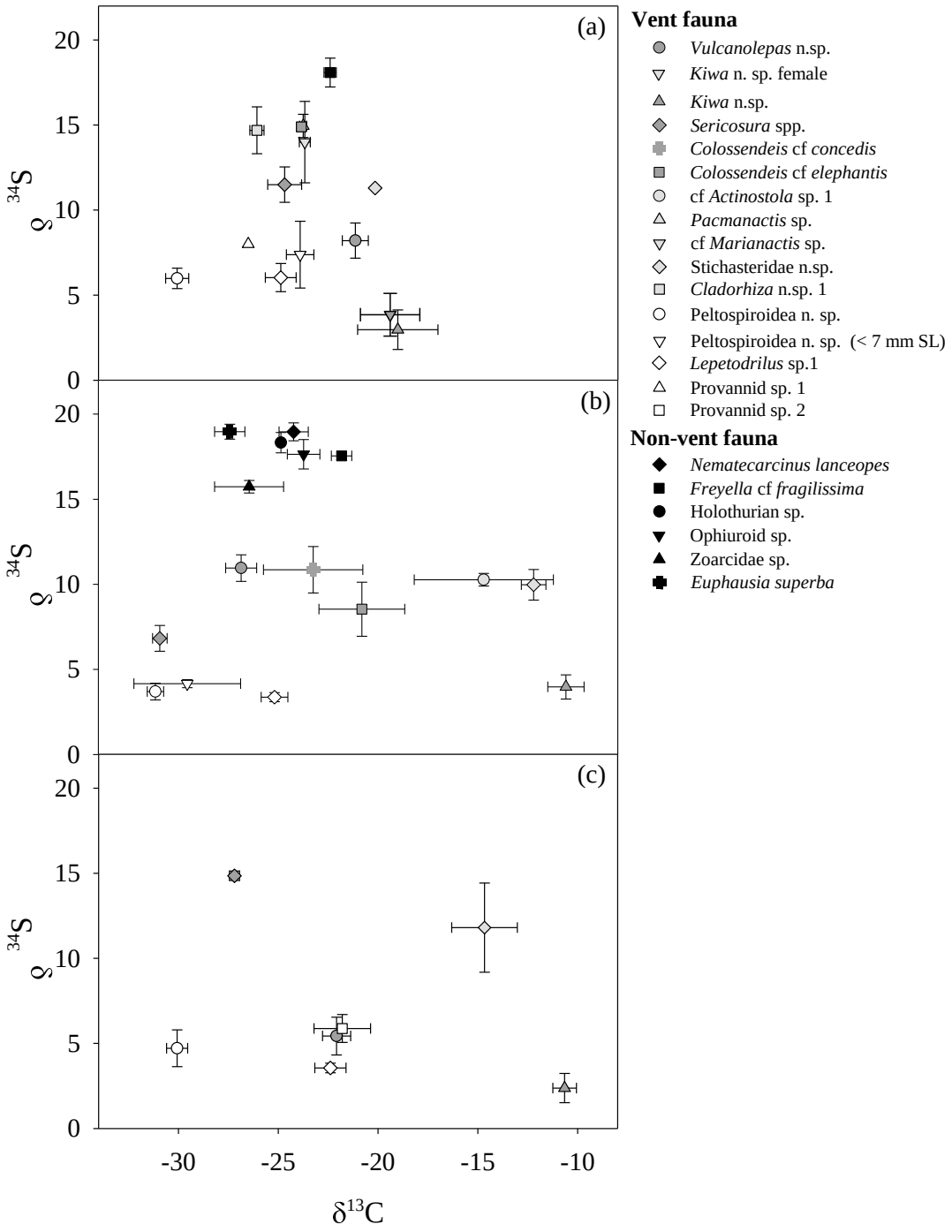

Fig. 4.3: $\delta^{13}C$ and $\delta^{34}S$ values of hydrothermal vent and off-vent fauna collected from the (a) E2, (b) E9N and (c) E9S ridge segments of the East Scotia Ridge, Southern Ocean. Values are means ± 1 standard deviation.

Table 4.2: Mean $\delta^{13}C$, $\delta^{15}N$ and $\delta^{34}S$ values (‰) values of hydrothermal vent fauna collected from the E2 and E9 ridge segments of the East Scotia Ridge, Southern Ocean. Standard deviations are in parentheses and - indicates no data.

Taxonomic group	E2				E9N				E9S			
	N	$\delta^{13}C$	$\delta^{15}N$	$\delta^{34}S$	N	$\delta^{13}C$	$\delta^{15}N$	$\delta^{34}S$	N	$\delta^{13}C$	$\delta^{15}N$	$\delta^{34}S$
Cirripedia												
Vulcanolepas n. sp.	22	-21.13 (0.64)	6.34 (0.74)	8.21 (1.03)	23	-26.85 (0.76)	8.95 (0.43)	10.95 (0.78)	23	-22.08 (0.70)	6.41 (0.57)	5.43 (1.11)
Decapoda												
Kiwa n. sp. female	20	-19.40 (1.49)	8.29 (0.47)	3.85 (1.26)	0	-	-	-	0	-	-	-
Kiwa n. sp. male	18	-19.02 (2.00)	7.50 (0.46)	2.97 (1.16)	22	-10.59 (0.91)	8.87 (0.49)	3.97 (0.71)	30	-10.68 (0.60)	9.06 (0.58)	2.38 (0.86)
Pycnogonida												
Sericosura spp	6	-24.68 (0.85)	8.50 (1.29)	11.88 (0.44)	9	-30.92 (0.53)	7.73 (0.54)	6.82 (0.76)	2	-27.19 (0.26)	9.10 (0.10)	14.86 (0.28)
Colossendeis cf *concedis*	0	-	-	-	6	-23.25 (2.50)	12.05 (0.52)	10.86 (1.37)	0	-	-	-
Colossendeis cf *elephantis*	1	-23.84	11.01	14.90	3	-20.81 (2.15)	11.81 (0.81)	8.54 (1.59)	0	-	-	-
Anthozoa												
cf *Actinostola* sp. 1	0	-	-	-	4	-14.71 (3.48)	9.85 (0.27)	10.27 (0.37)	0	-	-	-
Pacmanactis	5	-23.75 (0.18)	7.06 (0.73)	14.94 (0.68)	0	-	-	-	0	-	-	-
cf *Marianactis*	5	-23.67 (0.28)	7.16 (1.79)	14.00 (2.39)	0	-	-	-	0	-	-	-
Asteroidea												
Stichasteridae n. sp.	1	-20.15	12.29	11.30	5	-12.21 (0.62)	12.40 (0.37)	9.97 (0.90)	5	-14.67 (1.64)	13.36 (0.55)	11.81 (2.62)
Gastropoda												
Peltospiroidea n. sp.	19	-30.06 (0.58)	5.44 (0.40)	5.99 (0.60)	22	-31.15 (0.40)	5.77 (0.59)	3.70 (0.49)	15	-30.07 (0.53)	5.89 (0.71)	4.72 (1.08)
Peltospiroidea n. sp (< 7mm)	4	-23.90 (0.69)	6.83 (0.48)	7.38 (1.96)	5	-29.55 (2.67)	6.35 (1.32)	4.16 (0.24)	0	-	-	-
Provannid sp. 1	1	-26.51	4.22	8.00	0	-	-	-	0	-	-	-
Provannid sp. 2	0	-	-	-	0	-	-	-	4	-21.79 (1.41)	3.97 (0.48)	5.88 (0.82)
Lepetodrilus sp.1	5	-24.88 (0.77)	6.81 (0.29)	6.35 (0.48)	4	-25.18 (0.67)	8.46 (0.42)	3.36 (0.33)	4	-22.39 (0.78)	7.03 (0.26)	3.56 (0.28)
Cladorhizidae												
Cladorhiza n. sp. 1	5	-26.07 (0.35)	8.66 (0.41)	14.69 (1.37)	0	-	-	-	0	-	-	-
Bacteria												
Particulate suspended material	3	-23.19 (5.41)	-0.11 (4.88)	9.96 (1.11)	0	-	-	-	0	-	-	-
Rock scrapings	0	-	-	-	1	-23.15	2.35	0.77	1	-31.12	1.92	-
Kiwa n. sp epi-symbiont	5	-18.86 (5.27)	3.29 (1.46)	7.53 (0.33)	0	-	-	-	5	-9.87 (0.34)	5.22 (0.81)	6.64 (0.24)

Table 4.3: Mean $\delta^{13}C$, $\delta^{15}N$ and $\delta^{34}S$ values (‰) values of non-vent deep-sea fauna collected from the E2 and E9 ridge segments of the East Scotia Ridge, Southern Ocean. Standard deviations are in parentheses.

Taxonomic group	Species	Site	N	$\delta^{13}C$	$\delta^{15}N$	$\delta^{34}S$
Arthropoda						
Decapoda	*Nematecarcinus lanceopes*	E9	5	-24.22 (0.73)	8.08 (0.44)	18.96 (0.52)
	Euphausia superba	E9	3	-27.42 (0.76)	2.69 (0.66)	18.97 (0.13)
Echinodermata						
Asteroidea	*Freyella* cf *fragilissima*	E2	3	-22.40 (0.31)	10.21 (0.55)	18.09 (0.85)
	Freyella cf *fragilissima*	E9	2	-21.82 (0.52)	10.76 (0.19)	17.54 (0.22)
Holothuroidea	Holothurian sp.	E9	3	-24.86 (0.08)	7.96 (0.47)	18.32 (0.60)
Ophiuroidea	Ophiuroid sp.	E9	3	-23.73 (0.81)	8.98 (0.77)	17.64 (0.86)
Vertebrata						
Osteichthys	Zoarcidae sp.	E9	4	-26.45 (1.73)	12.00 (1.76)	15.73 (0.37)

4.3.3. Spatial differences in species

Six species of vent fauna were examined for differences in stable isotope values among the three sites (Table 4.4 & 4.5). *Vulcanolepas* n. sp. exhibited spatial differences in $\delta^{13}C$, $\delta^{15}N$ and $\delta^{34}S$ but there was no consistent pattern in the differences among sites (Table 4.4). Male and female *Kiwa* n. sp. at E2 did not differ in $\delta^{13}C$ but males were lower in $\delta^{15}N$ and $\delta^{34}S$ than females (Table 4.5). Therefore females were not part of the spatial analysis. Male *Kiwa* n. sp. showed spatial differences in each stable isotope (Table 4.4). The spread of $\delta^{13}C$ values differed (Fligner-Killen test $\chi^2 = 10.91$, $p < 0.01$) with a greater range at E2 than E9N and E9S (Table 4.2). E2 *Kiwa* n. sp. were depleted in ^{13}C and ^{15}N compared to E9N and E9S, the last two being similar to each other (Table 4.4). The epibionts attached to the ventral surface of male *Kiwa* n. sp., exhibited a similar pattern to *Kiwa* n. sp. with a greater spread of $\delta^{13}C$ values at E2 than E9S (F-test: $F = 244.46$, $p < 0.01$). $\delta^{13}C$ and $\delta^{15}N$ values were also higher at E2 than E9S but the reverse was true for $\delta^{34}S$ (Table 4.2 & 4.5). *Sericosura* spp $\delta^{13}C$ and $\delta^{34}S$ values varied amongst sites but $\delta^{15}N$ values marginally did not (Table 4.4). $\delta^{13}C$ and $\delta^{34}S$ values were lowest at E9N but highest at E2 for $\delta^{13}C$ and E9S for $\delta^{34}S$ (Table 4.2). Peltospiroidea n.

sp. showed spatial differences in $\delta^{13}C$ and $\delta^{34}S$ but not in $\delta^{15}N$ (Table 4.4). $\delta^{34}S$ values differed among all sites but E9N $\delta^{13}C$ values were lower than those at E9S and E2 (Table 4.4). Pair-wise comparisons of stable isotope values of Stichasteridae n. sp. revealed differences between all sites for $\delta^{13}C$ and $\delta^{15}N$ but differences in $\delta^{34}S$ were only found between E2 and E9N (Table 4.5).

Table 4.4: Results of ANOVA and *post-hoc* Tukey host significant differences test for the differences in stable isotope values of vent fauna among the three sites on the East Scotia Ridge.

Species	$\delta^{13}C$				$\delta^{15}N$				$\delta^{34}S$			
	F	DF	P	*Post-hoc*	F	DF	P	*Post-hoc*	F	DF	P	*Post-hoc*
Vulcanolepas n. sp.	428.85	2, 64	< 0.01	E9N < E9S < E2	141.34	2, 64	< 0.01	E2 = E9S < E9N	168.89	2, 62	< 0.01	E9S < E2 = E9S
Kiwa n. sp. male	162.91	2, 32.61	< 0.01	E2 < E9S = E9N*	54.33	2, 68	< 0.01	E2 < E9N = E9S	19.52	2, 66	< 0.01	E2 = E9S < E9N
Sericosura sp.	215.00	2, 15	< 0.01	E9N < E9S < E2	3.39	2, 15	0.06	NA	100.61	2, 15	< 0.01	E9N < E2 < E9S
Peltospiroidea n. sp.	31.04	2, 53	< 0.01	E9N < E9S = E2	2.92	2, 53	0.06	NA	49.27	2, 53	< 0.01	E9N < E9S < E2
Lepetodrilus sp. 1	17.41	2, 10	< 0.01	E2 = E9N < E9S	32.10	2, 10	< 0.01	E2 = E9S < E9N	31.99	2, 10	< 0.01	E9N = E9S < E2

*Welch's ANOVA with *post hoc* analysis by t:test with Bonferroni correction (p = 0.05/3 = 0.017)

Table 4.5: Results of the pair-wise comparisons using t-tests for between sites differences in stable isotope values of vent fauna at the East Scotia Ridge.

Species	Comparison	$\delta^{13}C$		$\delta^{15}N$		$\delta^{34}S$	
		t	p	t	p	t	p
Kiwa n. sp.	E2 female v male	-0.50	0.62	5.13	< 0.01	2.23	< 0.05
Kiwa n. sp. episymbionts	E2 v E9S*	-3.81	< 0.05	na	$< 0.05^{\Psi}$	na	$< 0.05^{\Psi}$
Stichasteridae n. sp.	E2 v E9N	28.92	< 0.01	5.14	< 0.01	-3.29	< 0.05
	E2 v E9S	6.67	< 0.01	5.94	< 0.01	0.385	0.73
	E9N v E9S*	3.13	< 0.05	-3.49	< 0.05	-1.33	0.26
Colossendeis cf *elphantis*	E2 v E9N	2.45	0.13	1.71	0.23	-6.93	< 0.05

*Welch's t:test
$^{\Psi}$Wilcoxon test

4.4. Discussion

4.4.1. Carbon utilisation and production sources

The stable carbon isotopic values of the East Scotia Ridge (ESR) vent fauna were consistent with a chemosynthetic food source (Fisher et al. 1994, Colaco et al. 2002, Van Dover 2002). There appeared to be at least two isotopically distinct carbon sources of vent origin, which was reflected in the extreme $\delta^{13}C$ values. Peltospiroidea n. sp. exhibited the lowest $\delta^{13}C$ values (~ -30‰), which were consistent with carbon fixation via the CBB cycle using form I RuBisCO (Robinson & Cavanaugh 1995, Robinson et al. 2003). Similar values are present in other molluscs containing sulphur-oxidising endosymbiotic bacteria including bathymodiolid mussels, provannid gastropods and vesicomyid clams, which range between -35 to -27‰ (Fisher et al. 1994, Colaco et al. 2002, Suzuki et al. 2006). *Kiwa* n. sp. and their epibionts consistently had the highest $\delta^{13}C$ values at each site. $\delta^{13}C$ values greater than -16‰ are consistent with those fixed via the rTCA cycle (Markert et al. 2007, Hugler et al. 2011) but such values were not observed at E2, which suggested the relative contribution of carbon fixed via the rTCA and CBB cycles assimilated by *Kiwa* n. sp. may differ between E2 and E9. Similar values to *Kiwa* n. sp. are apparent in vent fauna inhabiting chimney surfaces or living close to high temperature venting including *Rimicaris exoculata*, *Riftia pachyptila* and some paralvinellid species (Fisher et al. 1994, Colaco et al. 2002, Levesque et al. 2006).

The intermediate $\delta^{13}C$ values (-27 to -16‰) observed in organisms such as *Vulcanolepas* n. sp., *Lepetodrilus* n. sp. 1, *Sericosura* spp and *Pacmanactis* sp., may be the result of a mixed microbial diet utilising both CBB and rTCA carbon sources, an uncharacterised chemoautotrophic carbon source (Van Dover & Fry 1994) or assimilation of photosynthetic primary production (Erickson et al. 2009). *Lepetodrilus* n. sp. 1 and *Vulcanolepas* n. sp. $\delta^{34}S$ values were less than 10‰ and indicative of a

chemosynthetic food source (Fabri et al. 2011), which meant their $\delta^{13}C$ values were likely to be a result of mixing of the CBB and rTCA carbon sources or another uncharacterised carbon source. All non-vent fauna collected within the vicinity of the ESR hydrothermal vents had $\delta^{34}S$ values greater than 16‰, which suggested vent fauna with $\delta^{34}S$ values between 10 and 16‰ may assimilate some photosynthetically derived material from their diet. The anemones, *Colossendis* cf *elephantis* and the sponge *Cladorhiza* n. sp. 1 at E2 and *Sericosura* spp at E9S all had $\delta^{34}S$ values of approximately14‰, which suggested the incorporation of photosynthetic organic matter may be important.

4.4.2. Trophic position and nitrogen cycling

Poor understanding of nitrogen cycling (German & Von Damm 2003) has implications for identifying basal organisms for assessing trophic position. The broad range of $\delta^{13}C$ values and narrow range of $\delta^{15}N$ values at the ESR vent sites were not consistent with estimated $\Delta^{13}C$ (0.5‰) and $\Delta^{15}N$ (3.4‰) trophic discrimination factors (Post 2002b) and suggested that the majority of the organisms were not linked together by predator-prey interactions. At other basalt-hosted hydrothermal vents, there is a similar pattern of increasing $\delta^{13}C$ with $\delta^{15}N$ (Colaco et al. 2002, Van Dover 2002, Levesque et al. 2006). However, the slope of this relationship varies among sites because of the large range of $\delta^{15}N$ values at the Mid-Atlantic Ridge (~ -12 to 8‰; Colaco et al. 2002) and the Central Indian Ridge (~ -5 to 13‰; Van Dover 2002) compared to a much narrower range and only positive $\delta^{15}N$ values at the ESR and eastern Pacific vents (Fisher et al. 1994, Bergquist et al. 2007). A greater spread of $\delta^{15}N$ values does not necessarily mean more trophic positions at the Mid-Atlantic Ridge and Central Indian Ridge vents but may be related to either differences in the inorganic nitrogen source or the source isotopic value used by endosymbiotic and free-living bacteria. Isotopic discrimination during nitrogen

metabolism of micro-organisms can be dependent on the inorganic source (Macko et al. 1987, Hoch et al. 1994, Hoch et al. 1996) and temperature (Yun et al. 2011). It is unknown what effect this has on free-living and endosymbiotic bacteria at hydrothermal vents, whether these microbes use similar inorganic nitrogen sources and how these factors are propagated through the food web. This is further confounded by the use of different tissues (e.g. whole animals, muscle, gills) within hydrothermal vent studies in order to construct food webs (Michener & Kaufman 2007). The spread of $\delta^{15}N$ values of vent fauna across the whole community, therefore, may not reflect the number of trophic positions but instead reflect mixing of isotopically different nitrogen sources used by chemoautolithotrophs.

Although it may not be applicable to estimate the number of trophic positions based on the range of $\delta^{15}N$ vent fauna values, it may be possible to identify specific predator-prey interactions that conform to the trophic discrimination factors 3.4‰ ($\delta^{15}N$) and 0.5‰ ($\delta^{13}C$) (Post 2002b). Stichasteridae n. sp. was observed feeding on *Kiwa* n. sp.(Rogers et al. 2012), had similar $\delta^{13}C$ values and was approximately 3.5‰ heavier in $\delta^{15}N$, which indicated a strong trophic link. *Colossendeis* cf *elephantis* and *Sericosura* spp were collected from among anemones at E2. *Colossendeis* spp and *Sericosura* spp are known to feed on anemones at whale falls (Braby et al. 2009) and vent sites on the Juan de Fuca Ridge (Bergquist et al. 2007). $\delta^{13}C$ and $\delta^{15}N$ values suggested anemones may be the main food source at E2. These potential predator-prey interactions suggest that ESR food chains may be highly specialised or habitat dependent covering only two trophic positions.

4.4.3. Spatial patterns in species stable isotopic composition

Large differences in $\delta^{13}C$ values for *Kiwa* n. sp., Stichasteridae n. sp. and *Sericosura* spp were found among sites and are potentially a result of differences in carbon fixation

at the base of the food web which is transferred to higher trophic positions. $\delta^{13}C$ values of *Kiwa* n. sp. and its epibionts differed by ~9‰ between E2 and E9S. The epibiont community also differed with epsilon-Proteobacteria dominating at E9 and gamma-Proteobacteria largely absent, compared to a mix of gamma- and epsilon-Proteobacteria at E2 (K. Zwirglmaier pers. comms.). All epsilon-Proteobacteria to date use the rTCA cycle to fix carbon while gamma-Proteobacteria tend to use the CBB cycle (Hugler & Sievert 2011). *Riftia pachyptila* has similar spatial differences in its $\delta^{13}C$ values among vent sites, but this is attributed to its endosymbionts shifting between rTCA and CBB cycles (Markert et al. 2007) rather than changes in the microbial community it consumes. The gastropod *Alvinoconcha* spp has $\delta^{13}C$ values up to 20‰ different between sites, which relates to whether epsilon- or gamma-Proteobacteria are the endosymbionts housed within the gills (Suzuki et al. 2005). It is unclear why *Kiwa* n. sp. epibiont diversity is different between E2 and E9 but spatial differences in dissolved sulphides and methane of the vent fluids can affect microbial communities (Trask & Van Dover 1999, Flores et al. 2011). The difference in carbon fixation appeared to be transferred through *Kiwa* n. sp. to Stichasteridae n. sp. Such a predator-prey interaction may also explain the large difference in $\delta^{13}C$ values between E2 and E9N in *Sericosura* spp, which had $\delta^{13}C$ values indicative of a mixed carbon food source at E2 and the CBB cycle using RuBisCO form I at E9N. However, it is uncertain in the case of *Sericosura* spp whether these are direct trophic links through predator-prey interactions with anemones and Peltospiroidea n. sp., which they were collected from amongst, or if it is the result of grazing on microbial communities and on the detritus associated with these organisms.

Smaller differences in stable isotope values were observed among sites in Peltospiroidea n. sp., *Lepetodrilus* n. sp. 1 and *Vulcanolepas* n. sp. Peltospiroidea n. sp. contains a single strain of gamma-Proteobacteria within its gills (K. Zwirglmaier pers. comms.),

which means spatial differences in $\delta^{13}C$ and $\delta^{34}S$ could not be the result of differences in the type of endosymbiont (Trask & Van Dover 1999). The differences were likely a result of site-specific variations in the source inorganic carbon and sulphur stable isotope values used by the endosymbionts during chemoautotrophy or temperature-related effects on isotopic discrimination. Small differences among sites for the grazer *Lepetodrilus* n. sp. 1 and suspension feeder *Vulcanolepas* n. sp. are harder to explain because of the various factors that are likely to influence their food source. $\delta^{13}C$ values indicated these organisms consume a mixed diet of free-living microbes and particulate material. However, differences in $\delta^{13}C$ values within sites may be related to the organism's distribution within the vent (Levesque et al. 2006), which will affect the composition of the microbial community (Flores et al. 2011), stable isotope values of the inorganic substrate used during chemoautotrophy (Levesque et al. 2005) and temperature effects on trophic discrimination. *Lepetodrilus* n. sp. 1 and *Vulcanolepas* n. sp. were collected from single points within each vent site and, therefore, it is not clear whether the difference in stable isotope values among sites is greater or less than that within sites.

4.5. Conclusion

This study provides a snap-shot of the hydrothermal vent trophic assemblages on the ESR. It is difficult to identify factors that caused the differences in the *Kiwa* n. sp. epibiont communities, which ultimately resulted in greater spread of $\delta^{13}C$ values at the E9 sites compared to E2. Higher concentrations of dissolved sulphides in vent fluids can lead to broader ranges in the $\delta^{13}C$ values, with the community having increasing numbers of organisms with $\delta^{13}C$ values greater than -16‰ (De Busserolles et al. 2009). On the ESR, E9 has higher hydrogen sulphide and lower chlorine concentrations than E2 meaning greater concentrations of available reduced compounds for microbial

primary production (Rogers et al. 2012). Higher concentrations of reduced compounds may be one of the drivers of the observed differences in the trophic structure at the ESR vents as well as other vent sites. However, hydrothermal vent communities also undergo changes in community composition with age (Shank et al. 1998) and fluctuating hydrothermal activity (Cuvelier et al. 2011), which will have an effect on trophic structure. At present it is unknown whether the communities at E2 and E9 sites represent different successional stages, are a product of varying chemistry or a mix of both.

The ESR trophic assemblages showed similarities in trophic structure to other vent sites as well as a series of novel trophic interactions. The wide range of $\delta^{13}C$ values (-30.56 to -10.59‰) and narrow range of $\delta^{15}N$ values (3.97 to 13.36‰) of ESR vent fauna was more akin to food webs of Pacific hydrothermal vents (Van Dover & Fry 1989, Fisher et al. 1994) but still fell within the range observed at other mid-ocean and back-arc basin vents (Colaco et al. 2002, Limen & Juniper 2006, Bergquist et al. 2007). $\delta^{13}C$ values of Peltospiroidea n. sp. and *Kiwa* n. sp. indicated they assimilated two distinct carbon sources of vent origin. The heavier $\delta^{13}C$ values in *Kiwa* n. sp. may represent a greater proportion of carbon fixed via the rTCA cycle at the E9 sites than E2 but the underlying mechanism of why this occurred is still unknown. More work is required to link vent fluid chemistry to pools of microbial primary production and then into the trophic structure at hydrothermal vents.

Chapter 5: Isotopic variability in macro-consumers of the East Scotia Ridge hydrothermal vents

Chapter 5: Isotopic variability in macro-consumers of the East Scotia Ridge hydrothermal vents

5.1. Introduction

Inter- and intra-population interactions are important factors determining ecosystem function and energy flow (Jennings et al. 2001b, Araujo et al. 2011). When population densities are high, inter-population competition results in abiotic (e.g. space, temperature) and biotic (e.g. prey availability) resources being partitioned among species into their realised ecological niches in order to reduce competition (Schoener 1974). The breadth of an ecological niche is often dictated by a collection of trophic interactions linking animals to their environment (Quevedo et al. 2009, Araujo et al. 2011). Such trophic interactions tend to distinguish trophic specialists and generalists; the latter consuming a large variety of prey and the former a narrower range (Bearhop et al. 2004). However, niche partitioning of resources among co-occurring individuals of the same species is also apparent (Bolnick et al. 2003, Matich et al. 2011), as a population of generalists in fact may consist of specialists selecting different resources from their shared environment (Bolnick et al. 2003, Inger et al. 2006, Araujo et al. 2011).

Intra-population niche variation can occur as a result of ontogeny and/or between sexes via differences morphology or in habitat utilisation (Bodin et al. 2007b, Araujo et al. 2011). Changes in resource use with body size results in strong structuring effects on communities (Jennings et al. 2001b) where prey can turn from competitor to predator in later life (Werner & Gilliam 1984, Boyle & von Boletzky 1996). Larger individuals often increase their foraging range (Hatase et al. 2002) and can defend more productive (Thurber et al. 2011) or larger territories (Grelon et al. 2006). Differences in habitat utilisation or morphology among sexes can occur as a result of brooding behaviour

(Ramirez-Llodra et al. 2000) or nutritional demands for reproduction (Forero et al. 2002). Consumers have the potential to link multiple nutritional pathways, which may affect energy flow through the system (Quevedo et al. 2009, Lorrain et al. 2011). Quantifying intra-population trophic niche variation using conventional trophic techniques such as stomach or scat content analysis is fraught with difficultly because large sample sizes are needed to achieve an accurate estimate of the contribution of various prey items in the diet, prey are not always identifiable, and the analysis only provides dietary information immediately prior to sampling (Staniland 2002, Bearhop et al. 2004, Inger et al. 2006).

Stable isotope analysis has become an important tool in studying intra-population niche variation (Bolnick et al. 2003, Bearhop et al. 2004). Stable carbon, nitrogen and sulphur isotope values of a consumer's tissue represent a time-integrated dietary signal of their prey, the proportions of the prey assimilated from the diet, sources of primary production and foraging location (Hesslein et al. 1993, Hatase et al. 2002, Bearhop et al. 2004, Newsome et al. 2007). Stable isotope values of a population represent information about diet (biotic) and habitat (abiotic) (Newsome et al. 2007). Isotopic variation amongst individuals can also provide information on individual specialisation within a population (Bolnick et al. 2003, Araujo et al. 2011). However, variability in stable isotope values can also be a function of size-based shifts in diet and habitat utilisation. Incorporating size, sex or location into an analysis, allows a greater understanding of potential drivers of among-individual variability in stable isotope values.

Isotopic data are often represented as bivariate plots of $\delta^{13}C$ and $\delta^{15}N$ along with estimates of variance. $\delta^{13}C$ is plotted along the *x*-axis indicating the energy sources while the *y*-axis contains $\delta^{15}N$ and represents the relative trophic position. Isotopic data are analysed to investigate hypotheses examining differences in energy sources or

trophic positions among feeding guilds, species or size by using univariate (e.g. t-tests, ANOVA) or bivariate (e.g. correlation or regression) statistics. Alternative statistical methods have now been developed that examine the dispersion of stable isotope values (Layman et al. 2007, Turner et al. 2010, Jackson et al. 2011) and in turn represent a species' isotopic niche (Newsome et al. 2007). Convex hull (Layman et al. 2007) and standard ellipse (Jackson et al. 2011) areas are methods that aim to define a species isotopic niche in *n*-dimensional space but are usually defined by $\delta^{13}C$ and $\delta^{15}N$ values in *xy*-space. The isotopic niche is then parameterised by a central location and area ($‰^2$) of the convex hull or standard ellipse. Convex hull and standard ellipse area simultaneously analyse $\delta^{13}C$ and $\delta^{15}N$ values allowing a comparison of the isotopic variability among or between a grouping variable. Univariate and bivariate statistics can then be employed to examine whether specific variables (e.g. sex or size) can account for the differences in stable isotope values (Hammerschlag-Peyer et al. 2011).

Hydrothermal vents can have a complex trophic structure, with isotopic variability among- and within-species covering a large range (Colaco et al. 2002, Bergquist et al. 2007, De Busserolles et al. 2009). Investigating a vent organism's food sources and niche is important for describing an individual's role within the broader food web and factors that affect it. The decapod *Kiwa* n. sp., peltospiroid gastropods and stalked barnacles *Vulcanolepas* n. sp. are three species associated with hydrothermal vents on the East Scotia Ridge (ESR), dominating chimney surfaces and areas of diffuse flow (Rogers et al. 2012). *Kiwa* n. sp. is hypothesised to graze on episymbionts and there are spatial differences in size and sex within the venting area (Rogers et al. 2012), which may result in differences in isotopic variability. Peltospiroidea n. sp. and *Vulcanolepas* n. sp. occur in areas of diffuse flow (Rogers et al. 2012). The peltospiroid gastropods contain a single strain of sulphur-oxidising bacteria in its gills (K. Zwirglmaier pers.

comms.) and *Vulcanolepas* n. sp. feed on free living bacteria in suspension or epibionts growing on its cirri.

The current investigation examined spatial differences in isotopic variability among sites (i.e. the spread of stable isotope values) rather than mean differences in stable isotope values. Stable isotopes were used to determine whether the isotopic variability differed among sites and to what extent this variability was a function of length and/ or sex. The objectives were to compare differences in isotopic variability (1) between sexes in *Kiwa* n. sp., (2) among sites for male *Kiwa* n. sp., Peltospiroidea n. sp. and *Vulcanolepas* n. sp., (3) examine length-based trends in stable isotope values for each species, (4) and spatial differences in length-based trends among sites.

5.2. Methods

5.2.1. Sample sites, collection and processing

Samples were collected from onboard the R.R.S *James Cook* during the 2010 austral summer (7 January to 21 February) using the remotely operated vehicle (ROV) *Isis*. Sites were divided into E2, E9N and E9S based on differences in the chemistry of the end-member fluids (Rogers et al. 2012). For a description of the study sites and sample collection see **Chapter 4 section 4.2.1**. Length measurements (mm) were taken for *Kiwa* n. sp., Peltospiroidea n. sp. and *Vulcanolepas* n. sp. using vernier callipers. *Kiwa* n. sp. was sorted into sexes before carapace length was measured. Shell length was measured in Peltospiroidea n. sp. along the central axis from the shell apex to the outer lip. The length of the capitulum was measured in *Vulcanolepas* n. sp. Further information on tissue dissection, storage, sample processing and stable isotope analysis, can be found in **Chapter 4 sections 4.2.1. and 4.2.2.**

5.2.2. Data analysis

The isotopic variability in *xy*-space was represented by calculating the standard ellipse area using $\delta^{13}C$ and $\delta^{15}N$ values using the SIAR package (Parnell & Jackson 2011) implemented in the R statistical package version 2.13.2 (R Core Development Team 2011). Data were first checked for normality by using a multivariate Shaprio-Wilk test. The standard.ellipse function within the SIAR package was used to calculate and plot a sample size corrected standard ellipse area (SEA_C). The function returns values for: a SEA_C; the lengths of the semi-major (*a*) and semi-minor (*b*) axes of the SEA_C; the angle in radians between *a* and the *x*-axis and whether the inclination of the SEA_C was above (positive) or below (negative) the *x*-plane; the eccentricity (*E*) of the standard ellipse between $0 < E < 1$, where $E = 0$ was a perfect circle and $E \rightarrow 1$ indicated the standard ellipse became more elongated (Parnell & Jackson 2011). θ was converted to degrees for reporting results. The area of overlap between two SEA_C was calculated using the overlap function (Parnell & Jackson 2011) and was used to compare sites and sexes. The convex hull area was also calculated and plotted to delineate the extreme values at each site. The siber.ellipse function was used to create a matrix of Bayesian standard ellipses (SEA_B) based on 10000 posterior iterations and calculated the probability that the proportion of posterior samples of SEA_B differed between sites (Parnell & Jackson 2011). The Bayesian method allowed a direct probabilistic interpretation of the differences in SEA_B by pair-wise comparisons. For mathematical details on the calculation of SEA_C and SEA_B see Jackson et al. (2011).

Comparison of the mean length among sites for each species collected for stable isotope analysis was undertaken. E2 female and male length was compared using a two sample t-test. Length was compared among sites for male *Kiwa* n. sp., Peltospiroidea n. sp. and *Vulcanolepas* n. sp. using an ANOVA with *post-hoc* analysis by Tukey honest

significant difference (HSD) test. Linear regression was undertaken to investigate the relationship between length and stable isotopes at each site in order to determine whether length was an important factor in describing the SEA_C. The difference in slope of length-isotope relationships among sites was undertaken by analysis of covariance (ANCOVA) with length as the covariate and site as the categorical variable. All regression and ANCOVA diagnostic plots were examined for normality and homogeneity of variance. If departures from homogeneity of variance were suspected in the ANCOVA analysis, then a Fligner-Killeen test was undertaken on the standardised residuals.

5.3. Results

Kiwa n. sp. overlapped in length ranges among sites (Table 5.1) but mean length was smaller in females compared to males at E2 ($t = -2.85$, $p < 0.01$) and mean length differed among sites for males ($F_{2, 67} = 17.42$, $p < 0.01$). *Post-hoc* analysis revealed mean length was greater for E2 males than at E9N and E9S (Tukey HSD, $p < 0.05$), while there were no differences in mean length between E9N and E9S ($p = 0.70$). The length range overlapped at each site for Peltospiroidea n. sp. (Table 5.1) but mean length differed among sites ($F_{2, 53} = 17.42$, $p < 0.01$). Peltospiroidea n. sp. mean length was greater at E2 compared to E9N and E9S ($p < 0.05$) but there was no difference in mean length between E9N and E9S ($p > 0.05$). There was no difference in mean length among sites for *Vulcanolepas* n. sp.

5.3.1 Isotopic variability between sexes in *Kiwa* n. sp.

$\delta^{13}C$ and $\delta^{15}N$ values for male and female *Kiwa* n. sp. are depicted in Figure 5.1a along with their respective convex hulls and SEA_C. Similar SEA_B were found between female and male *Kiwa* n. sp. at E2 (probability = 0.80) (Figure 5.1b) but there was only an 18% overlapped in SEA_C (Figure 5.1a). A qualitative assessment using E and θ was

examined to see whether shape or inclination differed between sites with similar SEA_C. *E* was similar between female and male *Kiwa* n. sp. but θ indicated the relationship between $\delta^{13}C$ and $\delta^{15}N$ was positive for females and negative for males (Figure 5.1a, Table 5.1).

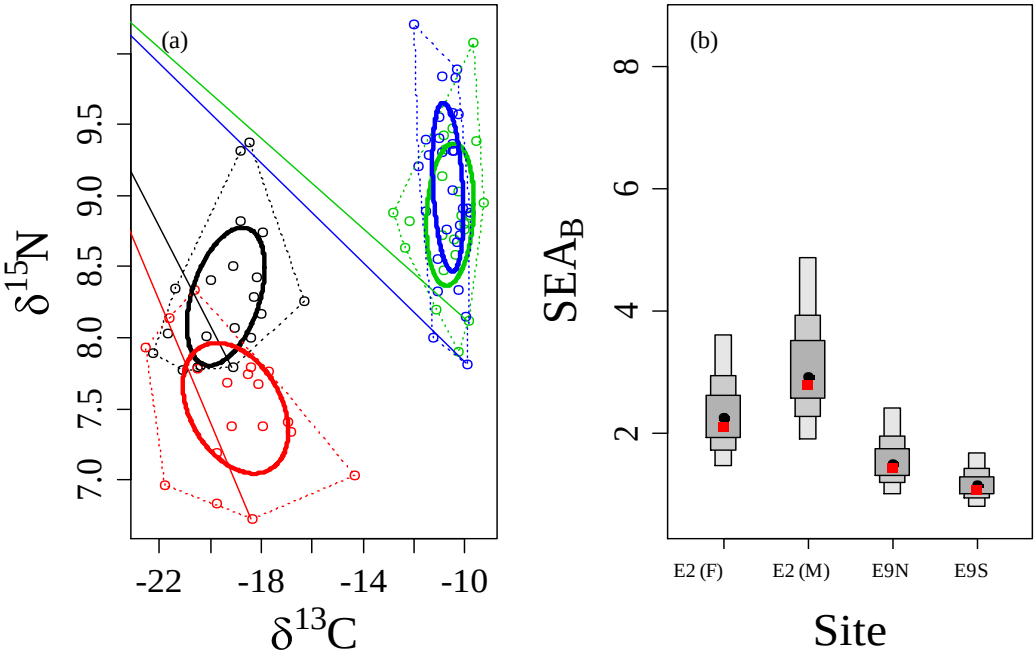

Fig. 5.1: (a) $\delta^{13}C$ and $\delta^{15}N$ values for female *Kiwa* n. sp. (black) and male *Kiwa* n. sp. from E2 (red), male *Kiwa* n. sp. from E9N (green) and male *Kiwa* n. sp. from E9S (blue) with their respective standard ellipse area (SEA_C) (solid line) and convex hull (dotted line). (b) The posterior Bayesian estimates of the standard ellipse area (SEA_B). The shaded boxes represent 50%, 75% and 95% credible intervals in decreasing order of size, with the mode indicated by a black circle and SEA_C by a red square.

5.3.2. Isotopic variability among sites

$\delta^{13}C$ and $\delta^{15}N$ values for male *Kiwa* n. sp. are depicted in Figure 5.1a along with their respective convex hulls and SEA_C. Significant differences between SEA_B were found by pairwise tests, which indicated male E2 *Kiwa* n. sp. ellipses were greater than males at E9N (probability = 0.99) and E9S (probability = 0.99) (Figure 5.1b). Neither E9N nor E9S SEA_C overlapped with E2 (Figure 5.1a). Similar SEA_B were found between male

Table 5.1: The standard ellipse area and parameters for *Kiwa* n. sp., Peltospiroidea n. sp. and *Vulcanolepas* n. sp. collected from the E2, E9N and E9S vent sites on the East Scotia Ridge, Southern Ocean.

Species	Site	n	Length range (mm)	Mean length (s.d.)	SEA_C	Theta (θ)	Eccentricity (E)	SEA_B (mode)	SEA_B 95 % Credible Intervals
Kiwa n. sp (female)	E2	20	26-56	48.73 (6.79)	2.11	8.31	0.98	2.26	1.44 to 3.61
Kiwa n. sp (male)	E2	18	35-73	57.29 (11.37)	2.80	-4.67	0.96	3.02	1.88 to 4.82
Kiwa n. sp (male)	E9N	22	20-52	39.88 (9.73)	1.45	6.75	0.85	1.57	1.00 to 2.37
Kiwa n. sp (male)	E9S	30	22-54	42.15 (9.44)	1.08	-43.04	0.68	1.17	0.81 to 1.70
Peltospiroidea n. sp.	E2	19	25-40	33.36 (4.24)	0.69	24.91	0.84	0.95	0.62 to 1.54
Peltospiroidea n. sp.	E9N	22	14-36	24.08 (6.44)	0.77	78.40	0.75	1.03	0.67 to 1.55
Peltospiroidea n. sp.	E9S	15	19-42	26.73 (7.90)	1.00	57.58	0.89	1.34	0.82 to 2.30
Vulcanolepas n. sp.	E2	22	10-23	17.01 (3.35)	1.55	72.80	0.55	1.63	1.05 to 2.49
Vulcanolepas n. sp.	E9N	23	10-25	16.88 (3.89)	1.07	-1.23	0.82	1.21	0.82 to 1.87
Vulcanolepas n. sp.	E9S	23	12-26	17.50 (7.90)	1.34	-5.08	0.58	1.45	0.94 to 2.22

Kiwa n. sp. at E9S and E9N (probability = 0.85) and these SEA_C overlapped by 82%. *E* was greater in male *Kiwa* n. sp. at E9N than E9S. θ indicated a positive relationship at E9N between $\delta^{13}C$ and $\delta^{15}N$ and a negative relationship at E9S.

The convex hulls and SEA_C based on $\delta^{13}C$ and $\delta^{15}N$ values for Peltospiroidea n. sp. are plotted in Figure 5.2a. All ellipses were similar between sites (Figure 5.2b): E2 and E9N (probability = 0.56), E2 and E9S (probability = 0.85) and E9N and E9S (probability = 0.83). Only E2 and E9S SEA_C overlapped by 37% (Figure 5.2a). A qualitative assessment of SEA_C using θ indicated that the relationships between $\delta^{13}C$ and $\delta^{15}N$ were all positive but the inclination of the SEA_C varied among the sites (Table 5.1).

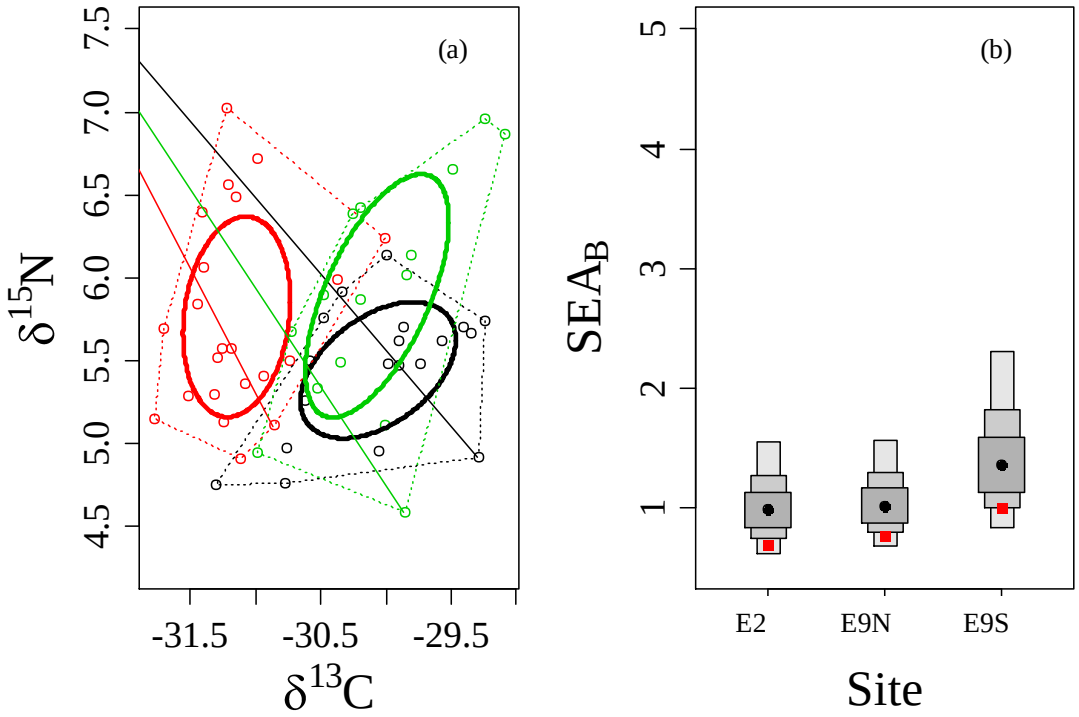

Fig. 5.2: (a) $\delta^{13}C$ and $\delta^{15}N$ values for Peltospiroidea n. sp. collected at E2 (black), E9N (red) and E9S (green) with their respective standard ellipse area (SEA_C) (solid line) and convex hull (dotted line). (b) The posterior Bayesian estimates of the standard ellipse area (SEA_B). The shaded boxes represent 50%, 75% and 95% credible intervals in decreasing order of size, with the mode indicated by a black circle and SEA_C by a red square.

$\delta^{13}C$ and $\delta^{15}N$ values of *Vulcanolepas* n. sp. are plotted in Figure 5.3a along with their convex hulls and SEA_C. All pair-wise tests of SEA_B between sites were similar (Figure 5.3b): E9N and E2 (probability = 0.56), E9S and E2 (probability = 0.67) and E9S and E9N (probability = 0.62). Similarly to Peltospiroidea n. sp., only E2 and E9S SEA_C overlapped by 25% (Figure 5.3a). Both *E* and θ varied among the sites. θ at E2 was positive but negative at E9N and E9S while *E* was similar at E2 and E9S but was greater at E9N (Table 5.1).

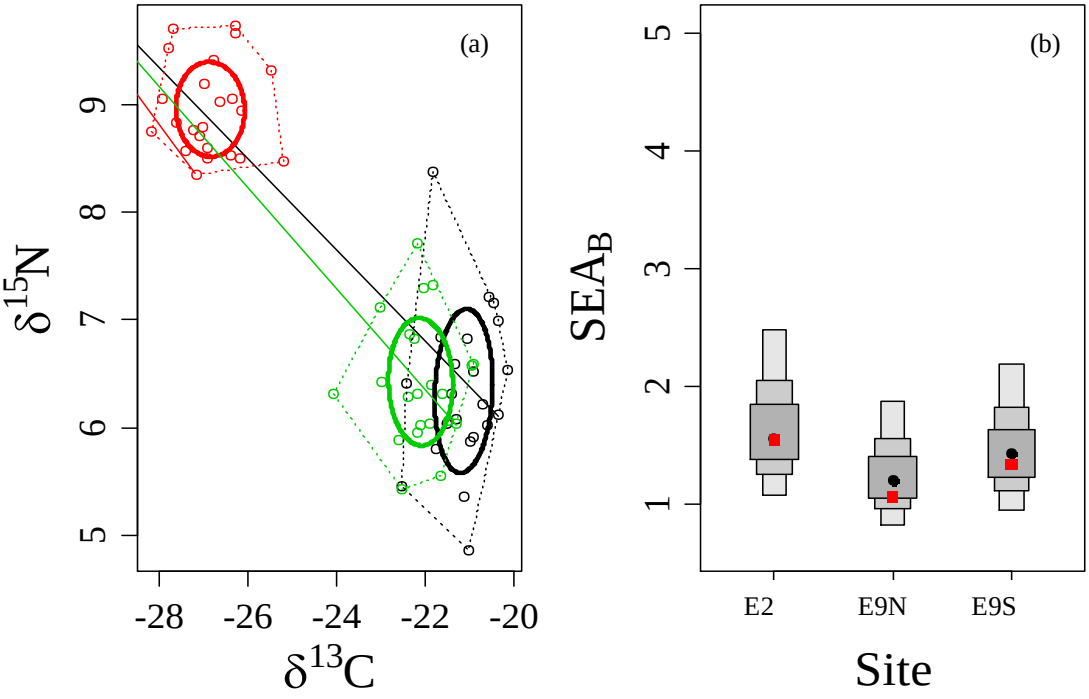

Fig. 5.3: (a) $\delta^{13}C$ and $\delta^{15}N$ values for *Vulcanolepas* n. sp. collected at E2 (black), E9N (red) and E9S (green) with their respective standard ellipse area (SEA_C) (solid line) and convex hull (dotted line). (b) The posterior Bayesian estimates of the standard ellipse area (SEA_B). The shaded boxes represent 50%, 75% and 95% credible intervals in decreasing order of size, with the mode indicated by a black circle and SEA_C by a red square.

5.3.3. $\delta^{13}C$, $\delta^{15}N$ and $\delta^{34}S$ length-based trends

$\delta^{13}C$ values of male *Kiwa* n. sp. increased with length at each site (Figure 5.4a) but female values $\delta^{13}C$ values were independent of length (Figure 5.4a, Table 5.2). *Kiwa* n. sp. $\delta^{15}N$ values were negatively related to length at E2 and E9N but length-based relationships were absent at E9S and in E2 females (Figure 5.4b, Table 5.3). Length-based trends in $\delta^{34}S$ were only present in female *Kiwa* n. sp. at E2 (Figure 5.4c, Table 5.4).

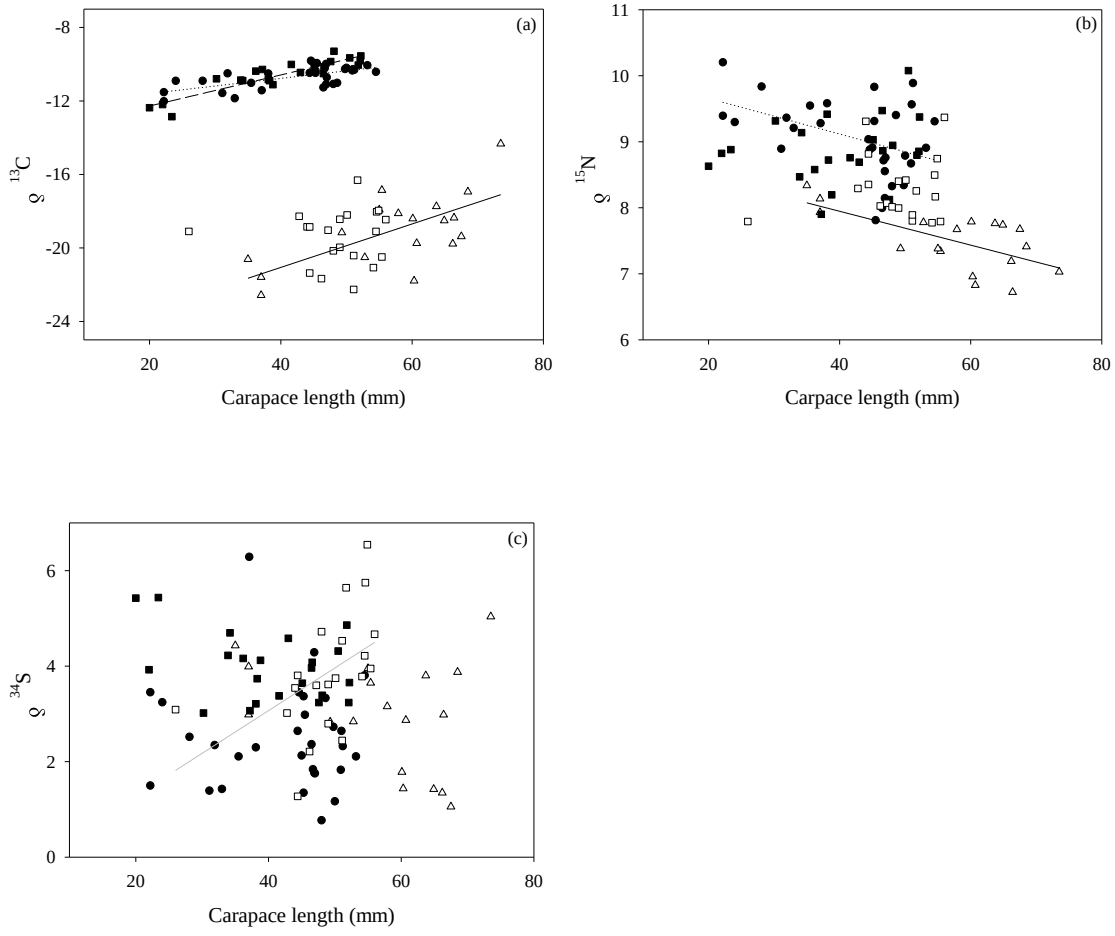

Fig 5.4: Plots of (a) $\delta^{13}C$, (b) $\delta^{15}N$ and (c) $\delta^{34}S$ against carapace length (mm) for *Kiwa* n. sp. with significant fitted regression lines for samples collected from E2 (male Δ & solid black line; female □ & grey solid line), E9 north (male ■ & dashed line) and E9 south (male ● & dotted line) venting sites.

Table 5.2: Regression parameters for the relationship between length and $\delta^{13}C$ for *Kiwa* n. sp., Peltospiroidea n. sp. and *Vulcanolepas* n. sp. collected from the E2, E9N and E9S vent sites on the East Scotia Ridge.

Species	Site	a	b	r^2	F	p
Kiwa n. sp (female)	E2	-21.52	0.0422	-0.04	$0.24_{1, 18}$	0.68
Kiwa n. sp (male)	E2	-25.78	0.1181	0.41	$12.93_{1, 16}$	< 0.01
Kiwa n. sp (male)	E9N	-13.97	0.0846	0.81	$90.43_{1, 20}$	< 0.01
Kiwa n. sp (male)	E9S	-12.42	0.0413	0.41	$20.88_{1, 28}$	< 0.01
Peltospiroidea n. sp.	E2	-32.98	0.0874	0.38	$11.84_{1, 17}$	< 0.01
Peltospiroidea n. sp.	E9N	-30.29	-0.0357	0.29	$9.72_{1, 20}$	< 0.01
Peltospiroidea n. sp.	E9S	-31.56	0.0557	0.66	$28.50_{1, 13}$	< 0.01
Vulcanolepas n. sp.	E2	-22.24	0.0651	0.07	$2.65_{1, 20}$	0.12
Vulcanolepas n. sp.	E9N	-28.28	0.0847	0.15	$4.90_{1, 21}$	< 0.05
Vulcanolepas n. sp.	E9S	-22.93	0.0469	0.00	$1.04_{1, 20}$	0.32

Table 5.3: Regression parameters for the relationship between length and $\delta^{15}N$ for *Kiwa* n. sp., Peltospiroidea n. sp. and *Vulcanolepas* n. sp. collected from the E2, E9N and E9S vent sites on the East Scotia Ridge.

Species	Site	a	b	r^2	F	p
Kiwa n. sp (female)	E2	7.84	0.0093	-0.04	$0.33_{1, 18}$	0.58
Kiwa n. sp (male)	E2	8.97	-0.0256	0.39	$11.95_{1, 16}$	< 0.01
Kiwa n. sp (male)	E9N	8.41	0.0114	0.00	$1.09_{1, 20}$	0.31
Kiwa n. sp (male)	E9S	10.19	-0.0269	0.16	$6.54_{1, 28}$	< 0.05
Peltospiroidea n. sp.	E2	3.94	0.0452	0.19	$5.10_{1, 17}$	< 0.05
Peltospiroidea n. sp.	E9N	5.91	-0.0059	-0.05	$0.08_{1, 20}$	0.78
Peltospiroidea n. sp.	E9S	4.75	0.0427	0.17	$3.83_{1, 13}$	0.07
Vulcanolepas n. sp.	E2	3.46	0.1693	0.56	$28.09_{1, 20}$	< 0.01
Vulcanolepas n. sp.	E9N	8.63	0.0191	-0.02	$0.65_{1, 21}$	0.43
Vulcanolepas n. sp.	E9S	4.67	0.0994	0.30	$10.14_{1, 20}$	< 0.01

Table 5.4: Regression parameters for the relationship between length and $\delta^{34}S$ for *Kiwa* n. sp., Peltospiroidea n. sp. and *Vulcanolepas* n. sp. collected from the E2, E9N and E9S vent sites on the East Scotia Ridge.

Species	Site	a	b	r^2	F	p
Kiwa n. sp (female)	E2	-0.50	0.0893	0.19	$5.45_{1, 18}$	< 0.05
Kiwa n. sp (male)	E2	4.53	-0.0273	0.01	$1.23_{1, 16}$	0.28
Kiwa n. sp (male)	E9N	5.07	-0.0275	0.10	$9.34_{1, 20}$	0.08
Kiwa n. sp (male)	E9S	2.26	0.0028	-0.04	$0.03_{1, 27}$	0.87
Peltospiroidea n. sp.	E2	5.90	0.0027	-0.06	$0.01_{1, 17}$	0.94
Peltospiroidea n. sp.	E9N	3.92	-0.0092	-0.04	$0.27_{1, 19}$	0.61
Peltospiroidea n. sp.	E9S	4.86	-0.0052	-0.08	$0.02_{1, 13}$	0.89
Vulcanolepas n. sp.	E2	8.90	-0.0404	-0.03	$0.35_{1, 19}$	0.56
Vulcanolepas n. sp.	E9N	11.23	-0.0165	-0.04	$0.11_{1, 20}$	0.74
Vulcanolepas n. sp.	E9S	6.02	-0.0320	-0.04	$0.18_{1, 20}$	0.67

Significant positive (E2 & E9S) and negative (E9N) trends in $\delta^{13}C$ values with length were observed in Peltospiroidea n. sp. (Figure 5.6a, Table 5.2). Peltospiroidea n. sp. $\delta^{15}N$ values increased with length at E2, the relationship was marginally non-significant at E9S and no relationship was observed at E9N (Figure 5.6b, Table 5.3). $\delta^{34}S$ values were independent of length in Peltospiroidaea n. sp. at all sites (Figures 5.5c, Table 5.4).

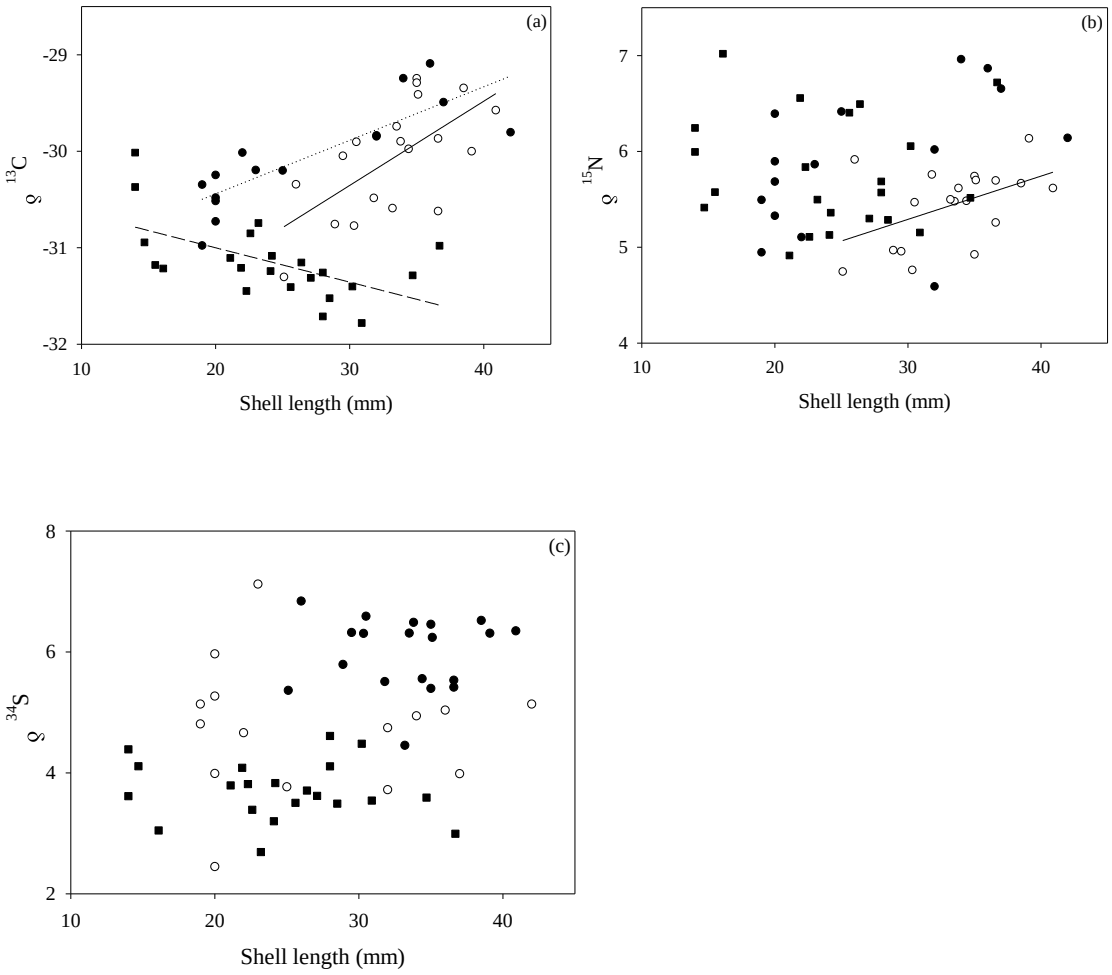

Fig 5.5: Plots of (a) $\delta^{13}C$, (b) $\delta^{15}N$ and (c) $\delta^{34}S$ against shell length (mm) for Peltospiroidea n. sp. (gastropod) with significant fitted regression lines for samples collected from E2 (○ & solid black line), E9 north (■ & dashed line) and E9 south (● & dotted line) venting sites.

Vulcanolepas n. sp. $\delta^{13}C$ values increased with length at E9N but not at E2 or E9S (Figure 5.6a, Table 5.2). *Vulcanolepas* n. sp. $\delta^{15}N$ values increased with length at E2 and E9S but no trend was detected at E9N (Figure 5.6b, Table 5.3). *Vulcanolepas* n. sp. did not show any length-based relationships with $\delta^{34}S$ (Figures 5.6c, Table 4).

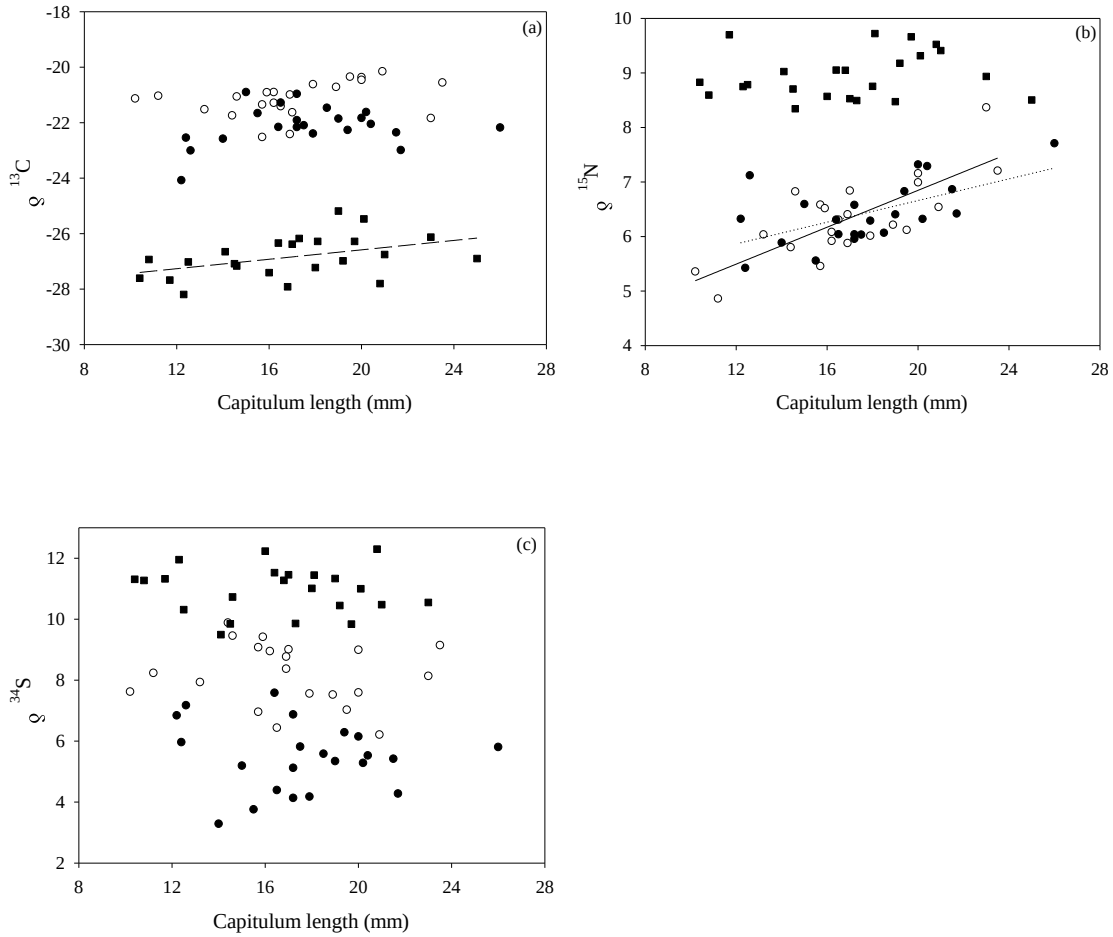

Fig 5.6: Plots of (a) $\delta^{13}C$, (b) $\delta^{15}N$ and (c) $\delta^{34}S$ against shell height (mm) for *Vulcanolepas* n. sp. (stalked barnacle) with significant fitted regression lines for samples collected from E2 (○ & solid black line), E9 north (■ & dashed line) and E9 south (● & dotted line) venting sites.

5.3.4. Spatial and sex differences in $\delta^{13}C$ and $\delta^{15}N$ length-based trends

Differences between male and female *Kiwa* n. sp. slopes' at E2 were not detected (ANCOVA $\delta^{13}C$: $F_{1, 33} = 3.24$, $p = 0.08$). Comparisons of the $\delta^{13}C$-length relationships among sites for male *Kiwa* n. sp., indicated slopes differed among sites (ANCOVA $\delta^{13}C$: $F_{2, 64} = 4.84$, $p = 0.01$). However, this must be treated with caution as standardised residuals were greater at E2 ($\chi^2 = 27.30$, $p < 0.01$), which violated the assumption of homogeneity of variance about the regression line (Underwood 1997). The slope of the $\delta^{15}N$-length relationship varied between E2 males and females (ANCOVA $\delta^{15}N$: $F_{1, 33} =$

4.26, $p < 0.05$). *Kiwa* n. sp. males exhibited differences in slopes among sites (ANCOVA $\delta^{15}N$: $F_{2, 64} = 4.35$, $p < 0.01$).

There were spatial differences in slopes of the regression between $\delta^{13}C$ and length for Peltospiroidaea n. sp. (ANCOVA $\delta^{13}C$: $F_{2, 50} = 18.65$, $p < 0.01$). However, there was no difference among sites for the $\delta^{15}N$-length relationships (ANCOVA $\delta^{15}N$: $F_{2, 50} = 2.06$, $p = 0.14$).

For *Vulcanolepas* n. sp. there were no differences observed among sites for the $\delta^{13}C$-length relationship (ANCOVA $\delta^{13}C$: $F_{2, 62} = 0.22$, $p = 0.80$) but slopes observed for the $\delta^{15}N$-length relationships varied spatially (ANCOVA $\delta^{15}N$: $F_{2, 62} = 7.22$, $p < 0.01$).

Spatial and sex differences in the trends were not undertaken because of the absence of significant $\delta^{34}S$-length regression trends in all but female *Kiwa* n. sp. at E2.

5.4. Discussion

The ESR hydrothermal vents at E2 and E9 are newly discovered communities (Rogers et al. 2012) and will need further exploration to fully understand population dynamics and structure. Larger individuals were collected from E2 for male *Kiwa* n. sp. and Peltospiroidea n. sp. than from E9 for stable isotope analysis but it is not clear how representative the sample collection was of the size distribution of these species at each site. Subsequent video analysis revealed smaller size classes in *Kiwa* n. sp. at E2 (Rogers et al. 2012) but unlike E9 these were not collected at the time of sampling. A similar sample collection bias may be present in Peltospiroidea n. sp. The SEA_C, *E* and θ therefore do not represent the population isotopic variability but only the variability of the samples. The focus here will therefore mainly be on understanding how length-based trends in stable isotopes relate to SEA_C, *E* and θ and what may be driving these trends.

5.4.1. Isotopic variability between sexes in *Kiwa* n. sp.

Mean $\delta^{15}N$ was greater in female *Kiwa* n. sp. than males (Chapter 4, Table 4.4). This was supported by a small overlap in SEA_C but the variability in $\delta^{13}C$ and $\delta^{15}N$ defined by both SEA_C and *E* was similar between male and female *Kiwa* n. sp for the length ranges sampled. However, differences in the angle and direction of θ suggested indicated that there were differences in how $\delta^{13}C$ and $\delta^{15}N$ values relate to each other. The negative values of θ in E2 male *Kiwa* n. sp. may be explained by an increase in $\delta^{13}C$ and a decrease in $\delta^{15}N$ with length, whereas the positive values of θ in females may have resulted from both $\delta^{13}C$ and $\delta^{15}N$ increasing with length, even though trends were not significant. Such length-based relationships may confound the amount of overlap in *xy*-space because there were differences in lengths sampled, as males were significantly larger than females. It may be that if smaller size classes were sampled for both sexes, there would be greater overlap in SEA_C. Therefore, the differences in direction of θ may also represent a divergence in trophic ecology with size between the sexes. The differences in isotopic variability may in part be explained by differences in the spatial distribution of the sexes at E2. Males were found closest to active venting on chimneys and in areas of high diffuse flow while females only occurred in diffuse flow areas adjacent to the chimneys (Rogers et al. 2012). The $\delta^{13}C$ of *Kiwa* n. sp. tissue and its epibiont communities was similar (K. Zwirglmaier pers. comms.) for male and female *Kiwa* n. sp., which indicated they were assimilating carbon sources with a similar isotopic value. However, differences in $\delta^{15}N$ indicated that the nitrogen source assimilated by larger males differs to larger females.

5.4.2. Isotopic variability among male *Kiwa* n. sp.

There were differences in SEA_C between E2 and E9 but not between E9N and E9S, which may be related to the greater range of $\delta^{13}C$ values found in E2 *Kiwa* n. sp. The

high *E* value for E2 *Kiwa* n. sp. SEA_C indicated it was highly elongated, with θ suggeting an inclination similar to the *x*-plane. Therefore, $\delta^{13}C$ values had a strong influence on SEA_C and individuals at E2 may assimilate a broader range of carbon sources. When the slope of the length-based $\delta^{13}C$ trends were compared among sites, the spread of standardised residuals around the regression line was also greater at E2 than at E9, which added further support to the larger SEA_C in *Kiwa* n. sp. at E2 compared to E9 being the result of greater variability in $\delta^{13}C$ values. The greater SEA_C in E2 male *Kiwa* n. sp. may be related to the epibiont community on the ventral surface; these are a mix of gamma-, epsilon and alpha-Proteobacteria while at E9 epsilon-Proteobacteria dominate (K. Zwirglmaier pers. comm.). Gamma- and epsilon-Proteobacteria fix carbon via different metabolic pathways resulting in isotopically distinct food sources (Hugler & Sievert 2011), which would fit with the expectation that a broader carbon source was available within the epibiont community at E2 and is therfore reflected by the greater SEA_C.

The length-based trends in $\delta^{13}C$ and $\delta^{15}N$ can explain part of the isotopic variability in male *Kiwa* n. sp. SEA_C as well as *E* and θ. For example, the relationship between $\delta^{13}C$ and $\delta^{15}N$ as described by *E* and negative θ for E2 and E9N male *Kiwa* n. sp. reflected the $\delta^{13}C$ increase and $\delta^{15}N$ decrease with length. Length-based trends in $\delta^{13}C$ and $\delta^{15}N$ are also found in other caridean vent shrimps (Polz et al. 1998, Van Dover 2002, Stevens et al. 2008). For example, *Rimicaris exoculata* increases in $\delta^{13}C$ by 6 to 7‰ with increasing length as it changes from a photosynthetic diet during larval and juvenile stages to a chemosynthetic diet as an adult when $\delta^{13}C$ becomes relatively constant (Polz et al. 1998, Vereshchaka et al. 2000, Van Dover 2002). The $\delta^{13}C$-length trends in *R. exoculata* are in contrast to the gradual increase in $\delta^{13}C$ with size observed in *Kiwa* n. sp. males. Even though *Kiwa* n. sp. $\delta^{13}C$-length relationships varied with site,

the trends still suggest they were not driven by a dilution of photosynthetic primary production over the size range sampled.

Instead, length-based trends in $\delta^{13}C$ and $\delta^{15}N$ may be related to the spatial distribution of male *Kiwa* n. sp. within the vent. There was a distinct gradient in the sizes of male *Kiwa* n. sp. at the vent sites: the largest individuals were only found in association with chimneys, medium sized in the diffuse flow at the base of the chimneys and juveniles only at the vent periphery (L. Marsh pers. comms.). *Kiwa* n. sp. may migrate to hotter, more productive areas closer to the vent openings as they increase in size, in order to find optimal environmental conditions for their epibionts (Van Dover 2000). Furthermore, the bacterial diversity of the *Kiwa* n. sp. epibiont community decreases with increasing size, becoming increasingly dominated by epsilon-Proteobacteria (K. Zwirglmaier pers. comms.), which utilise the reductive tricarboxylic acid (rTCA) carbon fixation pathway (Hugler & Sievert 2011). The increase in $\delta^{13}C$ with size may represent a greater proportion of assimilated carbon fixed by the rTCA pathway but it is unknown how this may affect $\delta^{15}N$ values. The shift in $\delta^{15}N$ may have occurred as a result of different inorganic nitrogen sources as these are not uniform within vents (Johnson et al. 1988). Nitrogen trophic discrimination ($\Delta^{15}N$) between prey and consumer can also have a negative relationship with increasing temperature (Power et al. 2003). Therefore, if the inorganic nitrogen source remains the same then the negative relationship between size and $\delta^{15}N$ values in *Kiwa* n. sp. may have been caused by a decrease in $\Delta^{15}N$ between inorganic source and its epibionts, a decrease in $\Delta^{15}N$ between its epibionts and *Kiwa* n. sp. or a combination of both.

5.4.3. Isotopic variability in Peltospiroidea n. sp.

The SEA_C and *E* of Peltospiroidea n. sp. were similar among sites in line with the expectation of a constant diet supplied by a single strain of endosymbionts (K.

Zwirglmaier pers. comm.). E2 and E9S SEA_C overlapped even though the mean shell length was greater in E2 samples but there was no overlap in SEA_C between E9N and either E2 or E9S. The isotopic values of the organic material transferred from symbiont to host at E2 and E9S were therefore similar. The overlap in SEA_C between E2 and E9S accords with the statistical tests on differences among means, which indicates no difference in mean $\delta^{13}C$ between E2 and E9S but lower $\delta^{13}C$ values at E9N (Chapter 4, Table 4.4). However, θ indicated there were differences in the relationship between $\delta^{13}C$ and $\delta^{15}N$ at each site, which may relate to size-related differences in trophic ecology. The increase in θ from E2 to E9S to E9N matched the length-based $\delta^{13}C$ and $\delta^{15}N$ trends: increase in $\delta^{13}C$ and $\delta^{15}N$ with length at E2, increase in $\delta^{13}C$ and marginally non-significant increase in $\delta^{15}N$ with length at E9S and a decrease in $\delta^{13}C$ with length and no significant relationship between $\delta^{15}N$ and length at E9N.

Length-based trends in $\delta^{13}C$ and $\delta^{15}N$ of endosymbiont hosting invertebrates are present at vent sites on the Mid-Atlantic Ridge and East Pacific Rise (Fisher et al. 1990, Trask & Van Dover 1999, De Busserolles et al. 2009). The mussel *Bathymodiolus azoricus* from the Mid-Atlantic Ridge increases in $\delta^{13}C$ and $\delta^{15}N$ values with length, as a result of differences in the proportion of methane- and sulphur-oxidising bacteria within its gills (De Busserolles et al. 2009). Peltospiroidea n. sp. contains a single strain of sulphur-oxidising bacteria (K. Zwirglmaier pers. comm.), therefore other factors than changes in host-symbiont diversity must relate to the observed length-based trends. The length-based trends may be a result of physiological factors that influence trophic discrimination by the endosymbiont. For example, increasing $\delta^{13}C$ in *Riftia pachyptila* is potentially a result of CO_2 limitation of the endosymbionts (Fisher et al. 1990) or increasing diffusion distance for CO_2 travelling from the environment through the host tissue to the endosymbionts (Trask & Van Dover 1999, Scott 2003). Furthermore, substrate limitation may be affected by endosymbiont density but the relationship

between body size, endosymbiont density and $\delta^{13}C$ and $\delta^{15}N$ values of the host tissue have not been explored. Increases in microbial cell density can affect isotopic discrimination because microbes cause an area of substrate depletion around the cell (Kampara et al. 2009). If endosymbiont density changes with size then this may affect stable isotope values of the host tissue. Other explanations include the accumulation of the heavier isotope (^{13}C and ^{15}N) in the tissue over time (Trask & Van Dover 1999), which are attributable to an increase in the volume of structural tissue (Emmery et al. 2011). However, these explanations all suggest an increase in $\delta^{13}C$ with length whereas at E9N $\delta^{13}C$ decreased with length. The latter has not been observed in other endosymbiont hosting organisms at hydrothermal vents. Further work is therefore required to understand the drivers of isotopic variability in the endosymbiont hosting organisms.

5.4.4. Isotopic variability in *Vulcanolepas* n. sp.

SEA_C was similar among sites but there was no overlap between E9N and either E2 or E9S. There was also no difference in the lengths sampled at each site indicating SEA_C was not biased by the length range sampled. Differences in mean $\delta^{15}N$ were in agreement with the position of E9N SEA_C in *xy*-space because $\delta^{15}N$ was greater at E9N than E2 and E9S (Table 4.4), but the overlap in SEA_C between E2 and E9S was not reflected in the statistical difference observed in mean $\delta^{13}C$ (Chapter 4, Table 4.4). In the case of *Vulcanolepas* n. sp., E and θ did not match the length-based trends in $\delta^{13}C$ and $\delta^{15}N$. There was a large difference in θ between E2 and E9S even though E and the length-based stable isotope trends were similar.

The spatial differences in the length-based trends indicated there are differences in the trophic ecology but it was difficult to determine what was driving them. Bacteria and mineral particles are found in peritrophic membranes but animal remains are absent

suggesting that stalked barnacles collect small particles from the water column by extending and retracting their cirri (Tunnicliffe & Southward 2004). However, stalked barnacles harbour filamentous bacteria on their cirri, which are thought to provide their food source rather than suspension feeding (Southward & Newman 1998, Suzuki et al. 2009). The filamentous bacteria form dense mats on the cirri making setose feeding by filtering particles difficult (Southward & Newman 1998). This indicates that differences in length-based stable isotope trends may be linked to processes within the consortia of bacteria on the cirri or trophic discrimination between bacteria and stalked barnacle. In the case of $\delta^{15}N$, insufficient protein within the diet (Adams & Sterner 2000) can result in lower growth rates, higher nitrogen turnover rates within the consumer's tissue and increasing $\Delta^{15}N$ (del Rio et al. 2009). Spatial differences in growth rates as a result of prevailing environmental conditions are proposed in stalked barnacles at volcanic seamounts (Tunnicliffe & Southward 2004). If the *Vulcanolepas* n. sp. diet became deficient in protein with increasing length at E2 or E9S, then $\Delta^{15}N$ may increase in large individuals resulting in the length-based trends in $\delta^{15}N$.

5.5. Conclusions

The use of standard ellipse area and its parameters in conjunction with length-based analyses, complemented the analysis of variability in stable isotope values. *E* and θ helped to differentiate the shape and inclination of the SEA_C among sites when there was no difference in the standard ellipse area for *Kiwa* n. sp. and Peltospiroidea n. sp. The interaction of *E* and θ may offer important ecological information, which SEA_C does not provide. As *E* becomes more elongated ($E \rightarrow 1$) and depending on θ, the relationship between $\delta^{13}C$ and $\delta^{15}N$ can be viewed as a correlation. In the case of *Kiwa* n. sp. and Peltospiroidea n. sp. there appeared to be a link between *E* and θ, which was reflected in the length-based $\delta^{13}C$ and $\delta^{15}N$ trends. Therefore, *E* and θ have the potential

to identify if pairs of stable isotope values are related, which may lead to further hypothesis testing. At present, E and θ only provided a qualitative assessment of differences among SEA_C as there is no Bayesian approach to assessing the probability that E or θ differ among sites. Incorporating these into the statistical analysis would expand the options for testing differences among standard ellipses. A better understanding of how E and θ interact when E becomes more circular ($E \rightarrow 0$) is also required. It may be that as E becomes more circular, θ is more difficult to interpret because there are more possibilities of θ. Further work is needed to understand and refine the use of E and θ in ecological studies.

Chapter 6: Thesis overview, limitations and wider implications

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This thesis examines aspects of the trophodynamics of two contrasting benthic deep-sea habitats: non-vent areas of Mid-Atlantic Ridge (MAR), North Atlantic Ocean, and hydrothermal vents on the East Scotia Ridge (ESR), Southern Ocean. Data presented within this thesis provides the first investigations of trophodynamics of benthic non-vent habitats of a mid-ocean ridge and hydrothermal vents in the Antarctic. The aim of this chapter is to firstly provide an overview of the main findings of the core research themes, which were elucidating energy sources (Chapters 2 & 4), examining spatial differences in trophodynamics among assemblages (Chapter 2 & 4) and within species (Chapters 2, 3, 4 & 5), and investigating the role of body size in describing isotopic variability of selected macro-consumers (Chapters 3 & 5). Secondly, it will identify some of the limitations of the study. Finally, it will aim to place the work of this thesis into the wider context.

6.1. Thesis overview

6.1.1. Elucidating energy sources: photosynthesis v chemosynthesis

The use stable sulphur isotopes in addition to carbon and nitrogen provided much greater resolution in trying to identify energy sources at the MAR and ESR sites. The isotopic evidence was that photosynthetic primary production in the surface waters sustained the benthos through bentho-pelagic coupling on the MAR (Chapter 2). The $\delta^{34}S$ values of the deep-sea fauna confirmed that there was no chemosynthetic primary production being assimilated by the animals at these MAR stations (Chapter 2) and corroborates the view that the influence of chemosynthetic primary production along the MAR on non-vent benthic organisms may be small (Bergstad & Godo 2003). On the ESR, $\delta^{34}S$ values of vent fauna helped to identify potential organisms that were

assimilating photosynthetic primary production (Chapter 4). Some species of hydrothermal vent fauna have $\delta^{13}C$ values that overlap with those of non-vent deep-sea organisms (Figure 6.1), making the interpretation of energy sources ambiguous (Erickson et al. 2009). Where $\delta^{13}C$ values are ambiguous, $\delta^{15}N$ is often used to infer whether the assimilated diet is of vent origin because $\delta^{15}N$ can be lower in vent fauna than organisms and particulate organic matter (POM) from the surrounding deep-sea (Van Dover & Fry 1989, Bergquist et al. 2007). However, $\delta^{15}N$ values of vent fauna from the ESR overlapped with non-vent fauna hampering interpretation of energy sources at the gross level. The use of $\delta^{34}S$ revealed some ESR species with similar $\delta^{13}C$ and $\delta^{15}N$ values to non-vent fauna were potentially assimilating photosynthetic primary production from their diet (e.g. *Cladorhiza* n. sp. 1) while others were not (e.g. *Lepetodrilus* n. sp. 1). Given the importance of inorganic sulphur compounds in bacterial metabolism at hydrothermal vents, it is surprising that this is one of few studies that have undertaken stable sulphur isotope analysis at the community level. It is therefore recommended that future investigations should analyse stable carbon, nitrogen and sulphur isotopes whenever a mix of chemosynthetic and photosynthetic primary production is suspected in order to provide the resolution required to the identify these energy sources.

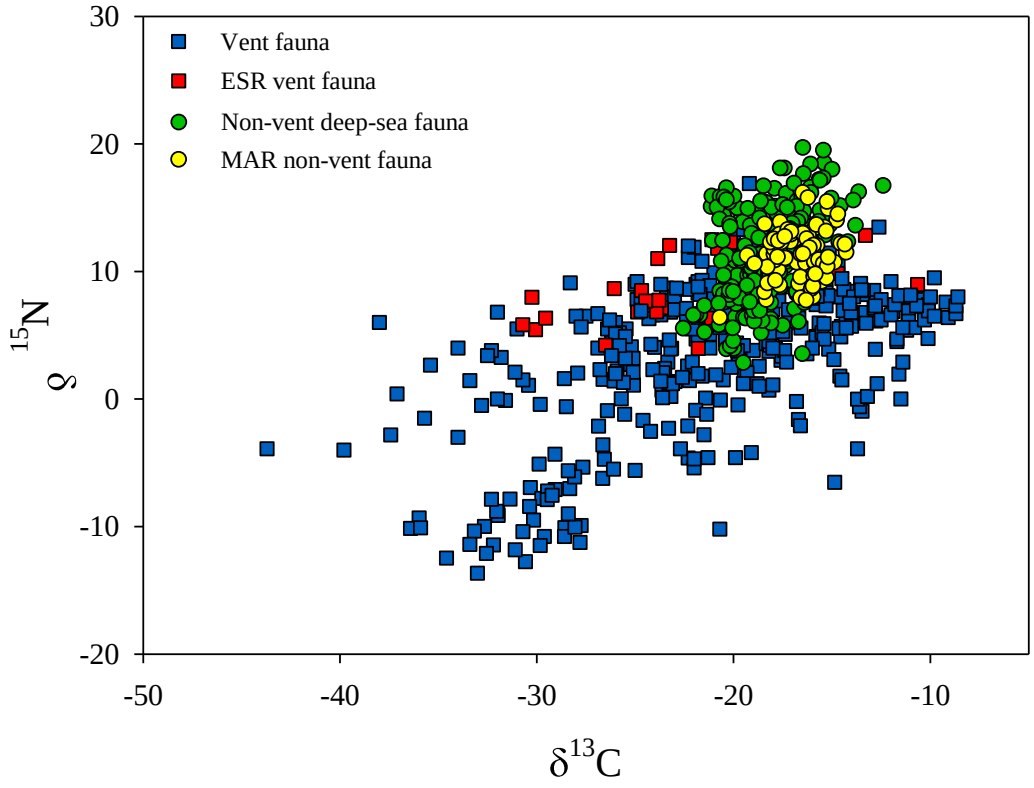

Fig. 6.1: $\delta^{13}C$ and $\delta^{15}N$ values of vent (Van Dover & Fry 1989, Fisher et al. 1994, Van Dover & Fry 1994, Trask & Van Dover 1999, Colaco et al. 2002, Van Dover 2002, Levesque et al. 2003, Levesque et al. 2006, Limen & Juniper 2006, Bergquist et al. 2007, Erickson et al. 2009) and non-vent fauna (Iken et al. 2001, Polunin et al. 2001, Drazen et al. 2008b, Madurell et al. 2008, Jeffreys et al. 2009) extracted from the published literature, in addition to the non-vent Mid-Atlantic Ridge (MAR) and vent East Scotia Ridge (ESR) fauna from this study.

6.1.2. Spatial differences in trophodynamics

Spatial differences in $\delta^{13}C$ values of the MAR benthic and bentho-pelagic fauna were apparent as deep-sea fauna had higher $\delta^{13}C$ values at the southern relative to the northern station (Chapter 2). Latitudinal shifts in POM $\delta^{13}C$ values occur as a result of the interaction between temperature, CO_2 solubility and different metabolic pathways of phytoplankton (Rau et al. 1989, Goericke et al. 1994). The result is POM $\delta^{13}C$ increase at lower latitudes and can differ across frontal systems and climatic zones (Rau et al. 1989, Rau et al. 1997). The station south of the Arctic Sub-Polar Front and the Charlie-

Gibbs Fracture Zone (CGFZ) has higher surface water temperatures than the northern station and is dominated by nano- and picophytoplankton instead of diatoms (Tilstone et al. 2009, Martinez-Vicenta et al. 2012). $\delta^{13}C$ values of surface POM are higher at the southern station compared to the north, which are also reflected in the mesopelagic zooplankton (Letessier et al. 2012). Spatial differences in the $\delta^{13}C$ values of POM and mesopelagic zooplankton are therefore transferred to the benthos via the downward flux of phytodetritus or through predator-prey interactions in the water column.

The MAR benthic trophic assemblages appeared to function in a similar way to those in abyssal and continental slope habitats. Surface deposit feeders and predator/ scavengers often had lower $\delta^{15}N$ values than subsurface deposit feeders. If these organisms were part of a single food chain then subsurface deposit feeders, including *Sipunculus norvegicus* and *Molpadia musculus*, would be considered the top predator, whixh is highly unlikely for a deposit feeder. However, at the southern station in 2009 predators/ scavengers were ^{13}C depleted relative to benthic deposit feeders. This suggests there were potentially two dominant trophic pathways associated with the MAR benthic assemblage; a mobile predator/ scavenger food chain linked to bathypelagic prey and pelagic carrion, and a deposit feeding food chain supported by the downward flux of phytodetritus (Chapter 2). Therefore, the predator/ scavenger food chain may represent the endpoint of a series of meso- and bathypelagic predator-prey interactions.

The two trophic pathways will be interconnected at various points as carbon is recycled and remineralised in the water column and within the benthos. For example, meso- and bathypelagic invertebrates will consume phytodetritus and in turn be predated on by bathypelagic fishes and crustaceans, which will in turn be consumed by bentho-pelagic fishes that will defecate above the seafloor producing organic matter available to deposit feeders (Figure 6.2). Alternatively phytodetritus will descend directly to the seafloor

without being repackaged by pelagic fauna, which may not render it immediately accessible to benthic and bentho-pelagic fishes. Bacteria and benthic invertebrates ingest, process and redistribute phytodetritus by incorporating it into biomass (Smith et al. 2002, Witte et al. 2003, Drazen et al. 2008a). This may form an important dietary component of benthic fishes (e.g. *Polyacanthonotus challengeri* (Chapter 2) or be consumed at certain life history stages (e.g. *Coryphaenoides armatus* Chapter 3) (Figure 6.2).

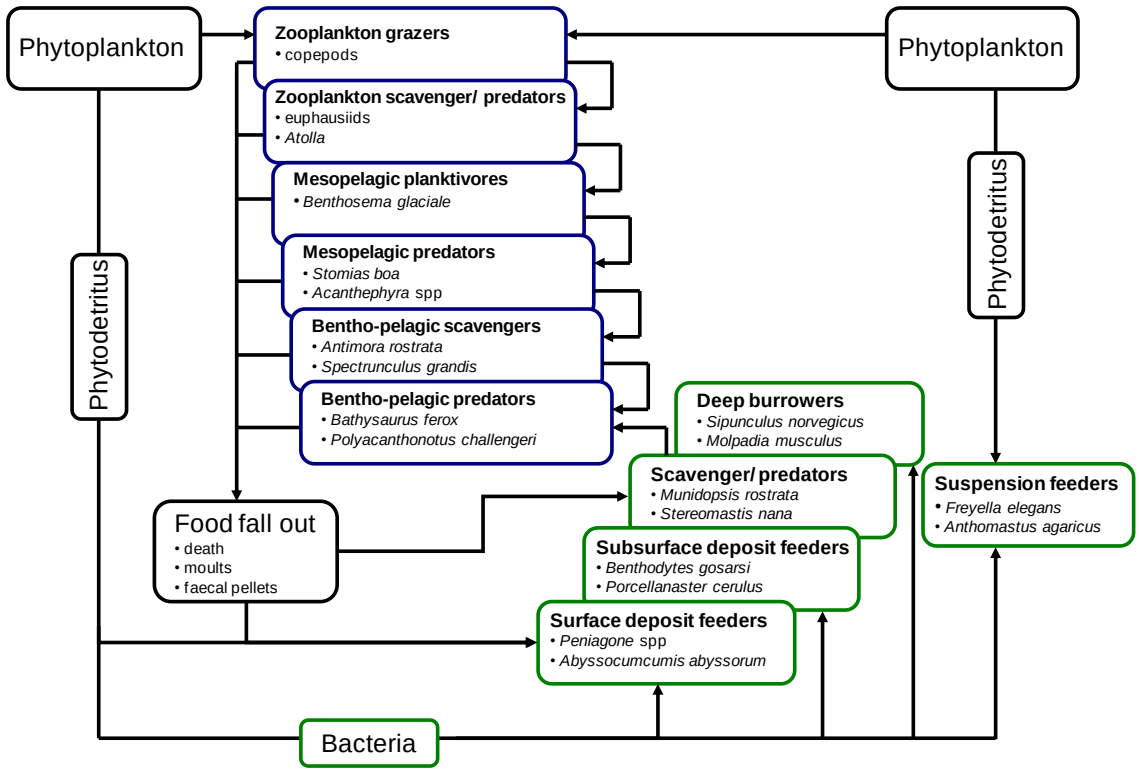

Fig 6.2: Conceptual diagram of the trophic pathways that exist within the deep sea. The black boxes indicate potential food souces while the black arrows indicate the direction of energy flow and potential trophic interactions. The blue boxes depict the transfer of organic matter via predator-prey interactions through the water column, culminating in interactions with the benthos. The green boxes represent the transfer of organic matter through benthic invertebrate, which at first is not available to benthic predators.

There appeared to be differences in the way the north and south MAR benthic trophic assemblages were structured (Chapter 2), which may be related to carbon export from the euphotic zone. The mobile predator/ scavenger food chain overlapped with the

deposit feeding food chain at the northern station but did not at the southern station (Chapter 2). The causes of such differences in trophic structure were not clear as the estimates of the amount of carbon produced in the surface waters over the stations is similar (G. H. Tilstone pers. comm.) as is the quantity of organic matter arriving at the seafloor (R. Abell pers. comm.). However, there are latitudinal changes in the phytoplankton, macrozooplankton and pelagic fish communities (Sutton et al. 2008, Letessier et al. 2012, Martinez-Vicenta et al. 2012). The differences in phytoplankton and planktonic grazing communities are likely to impact the rate at which labile carbon is exported to the benthos as phytodetritus (Thibault et al. 1999, Buesseler et al. 2007, Phillips et al. 2009). Latitudinal changes in abundance, biomass, community composition and behaviour (i.e. vertical migrations) of zooplankton and larger nekton (e.g. fishes) in relation to prevailing productivity regimes may play an important role in the way organic matter is transfered to the seafloor. The interaction between the phytodetrital flux and organic matter transfer through the meso- and bathypelagic food webs and whether this affects benthic food web structure needs further exploration.

Spatial differences in the stable isotope values of vent fauna were observed on the ESR (Chapter 4). The isotopic value of a consumer at a hydrothermal vent is a product of the following factors:

i. the inorganic source (e.g. CO_2, CH_4, NO_3^-) and its isotopic value used by the chemoautotroph,
ii. the isotopic discrimination process occurring during metabolic reactions involving inorganic substrates to create organic compounds (e.g. CBB or rTCA cycles) within the chemoautotroph,
iii. trophic interactions between chemoautotrophs and consumer (e.g. endosymbiont-host, predator-prey) that will incorporate i and ii,

iv. and the physiological processes associated with trophic discrimination by the consumer of the nutritional resource (e.g. effects of temperature or food quality).

Depending on the magnitude, if one or more of these factors varies spatially (Chapter 4) or at a different life history stage (Chapter 5), then it is likely to result in differences in isotopic values among sites, species or individuals. For example, there were small (~1‰) spatial differences in $\delta^{13}C$ for the Peltospiroidea n. sp. between E9N and the other two sites. Peltospiroidea n. sp. hosts a single strain of sulphur-oxidising bacterium, which precludes differences in the species of endosymbionts among sites causing differences in stable isotope values. Therefore, the differences in $\delta^{13}C$ values are likely a result of either differences in the inorganic source isotopic value or the physiology of Peltospiroidea n. sp. Conversely, large differences in $\delta^{13}C$ values of *Kiwa* n. sp. observed between E2 and E9 are unlikely to be solely driven by differences in the isotopic value of the inorganic carbon source, but are more likely to reflect the different carbon fixation pathways of the epibiont communities attached to the setae of *Kiwa* n. sp.

Overall, the absence of $\delta^{13}C$ values greater than -16‰ at E2 suggests that the contribution of carbon fixed via the rTCA cycle to the macro-consumer food web may be less than at E9. Oxygen sensitivity of enzymes involved in the rTCA cycle may restrict this carbon fixation pathway to hotter areas, where temperatures are greater than $20^{o}C$ and there are more reducing chemical compounds (Hugler & Sievert 2011). Temperature and pH of the end-member fluids is similar at the three venting sites but the chemistry differs (Rogers et al. 2012) suggesting reducing compounds within the vent fluids may play an important role in governing the microbial composition at E2 and E9 at the base of the food web. The rTCA cycle is still potentially the dominant carbon fixation pathway at E2 as it may provide a significant contribution to carbon fixation on and within chimney structures and the sub-seafloor but is not readily

available to the macro-consumer food web. Quantifying the relative contribution of the CCB and rTCA cycles within various ecosystem compartments (e.g. macro-consumer food web, sub-seafloor, buoyant plume) and placing this within the context of the global ocean carbon cycle remains an ongoing challenge.

6.1.3. Isotopic variability and body size

Size-based trends in $\delta^{13}C$ and $\delta^{15}N$ were observed in MAR deep-sea fishes (Chapter 3) and ESR hydrothermal vent invertebrates (Chapter 5). The mechanism behind shifts in isotopic composition with size may be different at the two settings. In fishes, isotopic size-based trends are often the result of gape-dependent feeding, i.e. as an individual grows it can consume larger prey at higher trophic positions. However, prey handling constraints may not be important in the trophic interactions of hydrothermal vent organisms because the majority obtain their nutrition from microbial sources. The key issue is that isotopic variability can be linked to size, which may reveal important insights into diet, migration and physiology of an individual at different life history stages.

The MAR deep-sea fish analysis adds to a body of literature addressing the role of size in explaining intra-population variability in the trophodynamics of fishes (Chapter 3). At present this has not been adequately addressed within the deep-sea and the two studies using stable isotopes produced conflicting results (Drazen et al. 2008b, Stowasser et al. 2009). Spatial and temporal variability in the slope and direction of size-based trends in $\delta^{13}C$ and $\delta^{15}N$ for *Coryphaenoides armatus* and *Coryphaenoides brevibarbis* revealed aspects of feeding plasticity that cannot be investigated by baited lander experiments or stomach content analysis alone. In contrast, *Antimora rostrata* had similar $\delta^{13}C$ and $\delta^{15}N$ size-based trends among sites and years, which may represent a more consistent feeding behaviour or diet.

The advantage of greater feeding plasticity is that it may allow individuals to adapt to spatial and temporal fluctuations in prey availability. Deep-sea fish abundance and distribution may be controlled by bottom-up processes (Bailey et al. 2006). The allochthonous food supply to the deep sea means bottom-up controls on fish abundance may originate via climatic affects on euphotic communities, which are linked to the benthos via bentho-pelagic coupling (Bailey et al. 2006, Drazen et al. 2008b, Bailey et al. 2009, Billett et al. 2010). The majority of deep-sea fishes consume a larger proportion of pelagic than benthic prey either through scavenging or bentho-pelagic predation (Pearcy & Ambler 1974, Mauchline & Gordon 1984a, Haedrich & Merrett 1992), but the contributions of benthic or pelagic prey can vary with life history stage as eluded by stable isotopes (Chapter 3). Therefore, if diet changes with size, then deep-sea fishes may be more susceptible to fluctuating prey availability at different life history stages.

Isotopic variability in hydrothermal vent invertebrates on the East Scotia Ridge was also explicable by body size at some but not at all sites (Chapter 5). The processes that drive differences in mean stable isotope values (outlined in **6.2.2. East Scotia Ridge**) are also pertinent to isotopic variability among individuals within a species. Size-based $\delta^{13}C$ trends observed in Peltospiroidea n. sp. were the first to be reported for a gastropod at a hydrothermal vent but are known in other endosymbiont hosting organisms (Trask & Van Dover 1999, De Busserolles et al. 2009). They were probably the result of associated physiological factors between host and endosymbiont or temporal changes in the isotopic value of the carbon source. $\delta^{15}N$ size-based trends varied spatially in the stalked barnacle *Vulcanolepas* n. sp. A greater number of factors could contribute to $\delta^{15}N$-length trends in *Vulcanolepas* n. sp. than the peltospirid gastropod because the stalked barnacle contains a consortium of epibionts rather than endosymbionts. Any one or a combination of the factors outlined in the section **6.2.2. East Scotia Ridge** could

cause an increase in $\delta^{15}N$ with size. A parsimonious explanation may be the interaction of growth, protein turnover and food quality (Gaill et al. 1997, Tunnicliffe & Southward 2004, Trueman et al. 2005, Stowasser et al. 2006, del Rio et al. 2009).

The stable isotope trends in size and variability in *Kiwa* n. sp. may be explained by a combination of the epibiont community and spatial segregation of sizes within the vent system. Juvenile *Kiwa* n. sp. are found at the periphery of the hydrothermal vent and migrate to hotter areas at larger sizes where there will be greater concentrations of reducing chemical compounds for their epibionts. There is also a change in composition of the epibiont community as the portion of epsilon-Proteobacteria increases with host size. The increase in $\delta^{13}C$ with size fits with the expectation that the rTCA carbon fixation pathway is more prominent in hotter areas of the vent (Hugler & Sievert 2011). Spatial differences in stable isotope values relating to migration are also observed in crustaceans in other marine systems (Fry et al. 1999, Guest & Connolly 2006, Bodin et al. 2007b). These migrations are on much larger scales than those observed within hydrothermal vents but they also tend to reflect differences in primary production sources and isotopic baselines (Le Loc'h & Hily 2005, Bodin et al. 2007b).

Isotopic variability in Peltospiroidea n. sp., *Vulcanolepas* n. sp. and *Kiwa* n. sp. were also investigated using a new statistical technique (Chapter 5). A number of studies have developed new approaches to analysing isotopic data over the past five years (Layman et al. 2007, Schmidt et al. 2007, Turner et al. 2010, Jackson et al. 2011) ever since the theory that isotopic variability may provide extra information on trophic ecology (Bearhop et al. 2004, Sweeting et al. 2005). Standard ellipse areas were calculated in this study, which simultaneously analyse $\delta^{13}C$ and $\delta^{15}N$ data, and were compared using Bayesian inference (Jackson et al. 2011). Standard ellipse area has the potential to reveal patterns in isotopic data that may not be clear when focusing on a

single isotope. For example in Chapter 5, the greater variability around the $\delta^{13}C$-size regression lines in E2 male *Kiwa* n. sp. were reflected in a larger standard ellipse areas at E2 than E9. Whereas the size-based trends in Peltospiroidea n. sp. were mirrored by the parameters E and θ indicating the relationship between $\delta^{13}C$ and $\delta^{15}N$ may be linked.

6.2. Limitations of the study

6.2.1. Sample collection

Collecting deep-sea organisms is logistically challenging. Samples from the MAR were collected using a variety of equipment including trawls, traps and coring equipment whereas the ESR samples were collected with the use of a remotely operated vehicle (ROV) and therefore sampling equipment that could deployed from it. The research programmes sampling the MAR and ESR were the first concerted effort to characterise these deep-sea communities. Although the sampling approaches differed between the MAR and ESR, they share issues of gear selectivity, which leads to the question to what extent have we sampled the community?

On the MAR, trawling was logistically challenging owing to the nature of the bottom topography and the direction of the prevailing weather conditions in relation to the direction of trawling. This resulted in instances of damaged and lost gear, which in turn affected sample sizes and replication. The trawling speed can also have an effect on the catch composition with faster trawling speeds catching higher numbers of mobile fishes but will underestimate the abundance or biomass of invertebrates. Furthermore, sponges, deep-sea corals and other suspension feeders were not collected because of their position on hard substrates which were inaccessible to trawling. Unfortunately there is no way around such sampling issues. However, subsequent analysis of images from baited lander experiments and ROV video footage suggests that trawl samples

provided a good indication of the megafaunal component of the MAR soft-bottom community that were present in the area with the exception of large skates and fragile, gelatinous enteropneusts and dense aggregations of the small holothurian *Kolga* sp. (N. J. Cousins & C. H. S. Alt).

Sampling on the ESR can largely be described as a success given the potential weather conditions that could limit ROV operations at high latitudes. Sampling via ROV can be limiting as a result of manoeuvring the ROV into the correct position without damaging the habitat or dislodging the animal, because of the limited space on the ROV to store samples and the type of animal that can be collected by the ROV. This meant that large specimens of the anemone cf *Actinostola* n. sp. were collected at only one site at E9 and vagrant species occasionally observed at the periphery of the vents including macrourids or octopus were not sampled. Many species collected from the ESR vents sites were also new to science (Rogers et al. 2012). Therefore, a balance was required between assessing the species composition of the community for taxonomic and biogeographic purposes and undertaking destructive tissue sampling for stable isotope and genetic analyses to elucidate food sources and gene flow. The result was for some species there were low sample sizes or in the case of the polychaetes no samples were collected for stable isotope analysis. Furthermore, subsequent analysis back in the laboratory revealed that the provannid gastropod and the pycnogonid *Sericosura* sp. that were originally thought to be one were in fact multiple species.

6.2.2. Potential food sources

Within this study a number of potential food sources were sampled in order to try and constrain dietary items for deposit feeding MAR and grazing and sestonivore ESR macro-consumers. The only food source that revealed a potential link was the epibiont community on the setae of *Kiwa* n. sp. The trophic link between *Kiwa* n. sp. and its

epibionts, however, will need further investigation, either through analysis of lipid biomarkers or by identifying epibionts in the stomach, before it can be described with greater certainty as an episymbiotic relationship. Sediment samples collected from the MAR and rock scrapings and suspended particulate matter from the ESR were not adequate to constrain the isotopic values of fauna. This is probably a combination of not collecting a wide enough range of samples, the complex biochemical nature of particulate matter and selective feeding or digestion of organic matter by the organisms collected. For example on the MAR, there is evidence that burrowing holothurians and sipunculans can reach as deep as 150 mm into sediment (M. A. Shields pers. comms.), whereas sediment samples for stable isotope analysis were only collected from the top 10 mm. A better understanding of how $\delta^{15}N$ values of sediment changed with depth might have provided greater insight into differences among deposit feeding fauna. In conjunction with other organic nitrogen compounds (e.g. protein and carbohydrates), this might have given greater insight into whether food quality had an effect on $\delta^{15}N$ values of deposit feeders. Similarly at the ESR, there was insufficient sampling of potential food sources. There is a diverse array of habitats found within a venting area and the composition and nutritional quality of particulate matter can vary accordingly (Levesque et al. 2005, Limen et al. 2007). A more concerted sampling effort collecting potential food sources from various micro-habitats in association with venting sites will be required in order to constrain food sources for organisms that are not in a symbiotic relationship.

6.2.3. Investigative technique

The PhD has exclusively used stable isotope analysis to examine trophic interactions. Focusing on one technique to elucidate trophodynamics has limited the breadth and scope of the overall investigation. Including the analysis of lipids within the PhD may

have provided a greater insight into the trophic role of many of the organisms collected from the MAR and ESR. For example, lipid analysis may have aided in the identification of bacterial fatty acids in the nutrition of deposit feeders on the MAR, refined the relative importance of different sources of production (i.e. chemosynthetic v photosynthetic) at E2 hydrothermal vents on the ESR and may have aided in the interpretation of size-based trends in $\delta^{13}C$ values. There are also other techniques that may have helped to strengthen the findings of this PhD but were out with the scope of the PhD; mainly the use of proteomics and functional gene analyses. These techniques are used to identify potential metabolic pathways, including carbon fixation, within endo- and episymbiotic bacteria at hydrothermal vents (Markert et al. 2007, Hugler et al. 2011, Gardebrecht et al. 2012). Using a proteomic approach to identify the carbon fixation pathway utilised by the endo- and episymbiotic bacteria would help underpin the $\delta^{13}C$ values of a number of vent organisms (Markert et al. 2007).

6.3. Wider implications

Investigating spatial patterns in trophodynamics of species and communities potentially has important implications for understanding current large-scale geographic patterns of abundance, biomass and diversity. The processes that have led to the current distribution of marine organisms are a result of a complex mix of evolutionary and ecological processes (Wiens & Donoghue 2004). Evolutionary or historical processes tend to examine tectonic or climatic events that lead to the isolation or connection of geographically separated communities and its effect on speciation and adaptive radiation (Rex & Etter 2010, Wiens 2011). Therefore, evolutionary processes are important in determining species distributions at basin and global scales as well understanding how they reached their current locations (Monge-Najera 2008, Rex & Etter 2010). However, an important driver of evolution is the need for species to diverge

ecologically and partition resources in order to avoid competition (Rex & Etter 2010). Resource partitioning can take the form of utilising slightly different forms of a similar resource (Wigham et al. 2003, Hudson et al. 2004) or using the same resource at a different time or place (Laws & Sinha 1993). Coexistence of species can be maintained by species specialising in their own use of resources, with nutritional resources likely to be one of the most important. Therefore, ecological processes including dispersion potential, competition, trophic interactions and symbiotic relationships, may to varying extents, define the current range and distribution of many species in association with climatic or environmental variables (Chase & Myers 2011, Wiens 2011).

The way ecological processes interact and how this defines species distributions are often overlooked in studies examining large-scale patterns of species diversity or richness as well as biogeography. Often species diversity or richness is correlated with an environmental variable or latitude indicating a correlative rather than causative pattern in species distributions (Valentine 2009, Rex & Etter 2010, Wiens 2011). The underlying ecological processes driving turnover of species along large-scale gradients are often not explored. For example, only 45% of species diversity at hydrothermal vents along the East Pacific Rise, based on presence-absence data, can be explained by latitude or venting state, indicating that other biotic (e.g. predation and competition) and abiotic (e.g. habitat heterogeneity) factors may be important in defining hydrothermal vent communities (Matabos et al. 2011). Ecological processes, therefore, may exert strong influences on species assemblages at transition zones between biogeographical provinces or climatic zones limiting the ecological opportunity of some species to colonise suitable or adapt to new habitat and disperse further (Wiens & Donoghue 2004, Matabos et al. 2011).

A key question is, therefore, as species turnover along environmental gradients do the new species occupy a similar ecological and functional role and to what extent do the resultant spatial or temporal changes in community structure affect ecosystem function? This is a pertinent question that crosses a number of scientific disciplines as ecosystem function aims to understand the intra- and inter-specific interactions of organisms and their role in elemental cycling; part of which can be studied by examining trophic interactions. For example, comparing the ESR trophic assemblage with other basalt hosted hydrothermal vents from different biogeographical provinces suggests there may be regional differences in trophic structure, as indicated by differences in the relationship between $\delta^{13}C$ and $\delta^{15}N$ (Figure 6.3). If trophic structure is linked to ecosystem function then the differences in the relationship between $\delta^{13}C$ and $\delta^{15}N$ may indicate that elemental cycling and trophodynamics are different at MAR and Indian Ocean vents compared to the Pacific and ESR vents. Investigating spatial patterns in trophic interactions among species and especially in areas that span changes in climatic conditions or biogeographical transition zones may help determine the relative importance of ecological interactions (e.g. predation and competition) and environmental variables (e.g. primary productivity, vent fluid chemistry) in driving differences in trophic structure and maintaining species composition.

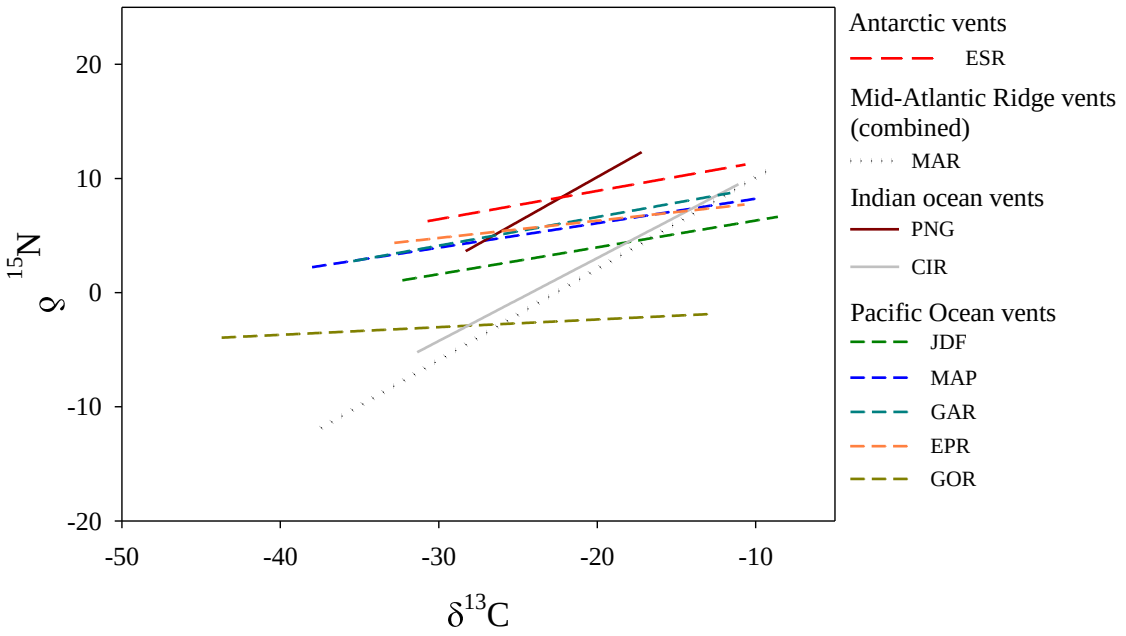

Fig. 6.3: The fitted trends lines of the relationship between $\delta^{13}C$ and $\delta^{15}N$ values of vent fauna extracted from the published literature for the Mid-Atlantic Ridge (MAR), Papua New Guinea (PNG), Central Indian Ridge (CIR), Juan de Fuca Ridge (JDF), Mariana Arc (MAP), Galapagos Rift (GAR), East Pacific Rise (EPR) and Gorda Ridge (GOR) (Van Dover & Fry 1989, Fisher et al. 1994, Van Dover & Fry 1994, Trask & Van Dover 1999, Colaco et al. 2002, Van Dover 2002, Levesque et al. 2003, Levesque et al. 2006, Limen & Juniper 2006, Bergquist et al. 2007, Erickson et al. 2009), in addition to the vent East Scotia Ridge (ESR) vent fauna from this study.

6.4. Concluding remarks

Within this thesis stable isotope analysis was used to elucidate trophic pathways and understand whether sources of production supporting organisms differed at certain life history stages. As the climate changes through natural fluctuations (e.g. North Atlantic Oscillations) or anthropogenic warming, species ranges and productivity regimes are expected to alter accordingly (Dulvy et al. 2008, Alheit et al. 2012, Hinder et al. 2012). This will have an effect on shallow marine systems to the deep sea but how this will manifest into changes in ecological processes and trophodynamics is currently hard to predict. Spatial comparisons of trophic assemblages and trophic ecology of different

species at various life history stages are an important starting point in understanding how community change may affect ecosystem function. Such studies provide a framework in which energy flow can be traced, provide valuable information on resource utilisation at different life history stages, indicate the degree of feeding plasticity by examining species at various points in their geographical range and provide a baseline from which change can be monitored.

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Appendix I: Research grants written during PhD

1. Deep sea macro-consumer food web structure of the Mid-Atlantic Ridge under varying primary productivity regimes: Grant-in-kind for sample analysis awarded by NERC Life Sciences Mass Spectrometry Steering Committee EK127-10/08
2. Deep sea macro-consumer food web structure of the Mid-Atlantic Ridge under varying primary productivity regimes- EXTENSION: Grant-in-kind for sample analysis awarded by NERC Life Sciences Mass Spectrometry Steering Committee EK150-12/09
3. Spatial differences in size structure and niche space of a seamount and its adjacent continental slope fish community: a stable isotope approach: $1200 awarded by Census of Marine Life on Seamounts (CenSeam)
4. Spatial differences in size structure and niche space of a seamount and its adjacent continental slope fish community: a stable isotope approach- EXTENSION: $10 000 awarded by CenSeam
5. Chemosynthetically-driven Ecosystems South of the Polar Front: Grant-in-kind for sample analysis awarded by NERC Life Sciences Mass Spectrometry Steering Committee LSMFBRISO43_04/10_R_09/10

Appendix II: Publications as well as oral and poster presentations during PhD

i. Publications

Billett DSM, Bett BJ, Reid WDK, Boorman B, Priede IG (2010) Long-term change in the abyssal NE Atlantic: The 'Amperima Event' revisited. Deep-Sea Res Part II 57:1406-1417

Galvan DE, Sweeting CJ, Reid WDK (2010) Power of stable isotope techniques to detect size-based feeding in marine fishes. Mar Ecol Prog Ser 407:271-U230

Letessier TB, Pond DW, McGill RAR, Reid WDK, Brierley AS (2012) Trophic interaction of invertebrate micronekton on either side of the Charlie Gibbs Fracture Zone / Subpolar Front of the Mid-Atlantic Ridge. J Mar Syst 94:174-184

Rogers AD, Tyler PA, Connelly DP, Copley JT, James R, Larter RD, Linse K, Mills RA, Naveira Garabato A, Pancost RD, Pearce DA, Polunin NVC, German CR, Shank T, Boersch-Supan PH, Alker BJ, Aquilina A, Bennett SA, Clarke A, Dinley RJJ, Graham AGC, Green DRH, Hawkes JA, Hepburn L, Hilario A, Huvenne VAI, Marsh L, Ramirez-Llodra E, Reid WDK, Roterman CN, Sweeting CJ, Thatje S, Zwirglmaier K (2012) The discovery of new deep-sea hydrothermal vent communities in the Southern Ocean and implications for biogeography. PLoS Biol 10:e1001234

Reid WDK, Wigham BD, McGill RAR, Polunin NVC (2012) Elucidating trophic pathways in benthic deep-sea assemblages of the Mid-Atlantic Ridge north and south of the Charlie-Gibbs Fracture Zone. Mar Ecol Prog Ser 463:89-103

ii. Oral presentations

Reid WDK, Wigham BD, Polunin NVC, Sweeting CJ & McGill RAR (2010) Spatial variability in size-related shifts in deep-sea fish trophodynamics elucidated by stable isotope analysis. Deep-sea Biology Symposium, Reykavik, Iceland.

Reid WDK, Wigham BD, Neat F & Sweeting CJ (2012) Intra-population variation in stable isotope values explained by body size in Rockall Trough deep-sea fish. World Fisheries Congress, Edinburgh, UK.

iii. Poster Presentations

Reid WDK, Wigham BD, Polunin NVC, Sweeting CJ & McGill RAR (2009) Size related dietry shifts in deep-sea fish. MAR-ECO workshop, Kristiasand, Norway.

Reid WDK, Wigham BD, Polunin NVC, Sweeting CJ & McGill RAR (2009) Benthic trophodynamics of the Mid-Atlantic Ridge: a stable isotope approach. MAR-ECO workshop, Kristiasand, Norway.

Zhang J, Reid WDK, Wigham BD & Burgess G (2009) Diversity of psychrophilic bacteria in Mid-Atlantic Ridge sediments and their production of fatty acids. MAR-ECO workshop, Kristiasand, Norway.

Reid WDK, Wigham BD, Polunin NVC, Sweeting CJ & McGill RAR (2010) Benthic trophodynamics of the Mid-Atlantic Ridge: a stable isotope approach. Deep-sea Biology Symposium, Reykavik, Iceland.

Sweeting CJ, Reid WDK & Galvan D (2012) Exploring the ubiquity and form of size-based feeding in marine fishes. World Fisheries Congress, Edinburgh, UK.