Stable Isotope Data as Reef Food-Web Descriptors in a Dynamic Tropical Environment

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Abstract

Despite the increasing use of stable isotope data as diet and trophic position descriptors in food-web ecology, their generic value relies on basic assumptions of constant trophic step enrichment, steady state conditions and accuracy of isotopic analyses for the estimation of trophic level (TL). This thesis explores the implications of these assumptions for understanding reef community trophodynamics in Oman, where upwelling events produce seasonal patterns among potential food sources.

Nitrogen isotopes ($\delta^{15}N$) revealed 3-4 TL's (6.16 to 17.8‰) and the wide range of carbon isotope ($\delta^{13}C$) values (-21.92 to -6.43‰) indicated that there were both benthic and pelagic sources of production. Primary producer and primary consumer $\delta^{15}N$ fluctuated seasonally by 2.24‰ (9.02-11.26‰) however, this variability was not consistently observed at higher TLs, and therefore the $\delta^{15}N$ of high-TL consumers may not accurately reflect their trophic position. Long-lived marine bivalves had no temporal isotopic variability, allowing the trophic position of higher consumers to be estimated using their $\delta^{15}N$ as a baseline. Baseline organisms also allowed spatial comparison of part of the trophic structure of two reef communities; Barr al Hickman had $\delta^{15}N$ values 2.7‰ enriched compared to a similar community at Bandar Kayran 360km to the north. Across the Western Indian Ocean, macroalgae $\delta^{15}N$ values correlated well with differences in underlying nutrient regimes of surrounding waters. Trophic-step fractionation in herbivorous fish was 4.69-5.25‰, higher than the generally accepted value of 3.4‰ and was explained by a dynamic model incorporating feeding rate, diet quality and excretion rate, which are inherently different between herbivorous and carnivorous fish. $\delta^{15}N$ was strongly correlated with body size in some fish species but across the entire community body size was a poor descriptor of trophic position. Use of diet-specific trophic-step fractionation values and sulphur isotopes ($\delta^{34}S$) greatly improved the resolution of food-web models.

Acknowledgements

When I undertook this PhD project three and half years ago I knew it would only be possible with the help and patience of a number of other people. I'd like to thank them all for their time and support but will also give a special mention to a few.

First and foremost I would like to thank my supervisors, Nicholas Polunin and John Pinnegar. At times it has been a steep learning curve for me, but with their guidance, advice and keen interest in my subject they have set me well on the way to a career in marine science. I'd also like to thank my examiners, Aaron Fisk and Richard Bevan for making the final task of the viva-voce an interesting and enjoyable discussion. This project was funded by a NERC CASE studentship (NER/S/A/2003/11885) through Newcastle University and the Centre for Environment Fisheries and Aquaculture Sciences (CEFAS), without their support this thesis may never have been written.

In total I made six trips to Muscat, Oman spending a total of six and a half months there over a period of a year and a half. At first it seemed a daunting thought to be working in such a challenging environment but my fieldwork in Oman was made feasible and thoroughly enjoyable by the friends I met there.

My main link with Oman was good friend Saoud Al-Habsi, who was able to initiate many of the contacts I made there. In particular, Jenny McIlwain provided invaluable support and great friendship and always had time for me when things weren't going quite right. Jenny, her husband, Andy Halford and their family made me feel very welcome and spending time with them was always a pleasure.

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This thesis is dedicated to my Mum, who has shown amazing strength and perseverance, and to Joe, who will be sadly missed but fondly remembered.

1 Using stable isotope analysis as a food web description tool in an unusual tropical upwelling setting

1.1 Reef food webs

Coral reefs ecosystems have sufficient productivity and structural habitat to support the greatest diversity of vertebrate and invertebrate fauna in the oceans. Yet, the apparent paradox exists that these highly productive ecosystems occur in the relatively oligotrophic conditions of the tropical oceans (Hatcher 1997). Nitrogen and phosphorus are both known to be important nutrients for coral growth, yet corals have adapted a tolerance to thrive in low nutrient conditions. Nutrient sources from the open ocean are taken up into the reef system largely by planktivores including fish. Water currents advect nutrients to and from the reef and organisms sequester a small proportion of this flux in both organic and inorganic forms, to produce living tissue. Reef fish feed on two plankton sources, diurnally on oceanic plankton brought to the reef by currents and nocturnally on more numerous and often larger reef-associated zooplankters (Hobson 1991). Both sources of plankton are utilised by reef organisms and enter the reef ecosystem through direct predation of plankton consumers or through consumption of their faeces (Robertson 1982). Overall there is only a small net flux of input and outputs to the ecosystem (Hatcher 1997). The productivity and biomass of reef ecosystems remain high as reef organisms are very efficient at energy capture and recycling, as a result of a high level of specialisation, symbiosis and complex food webs (Hallock 1988, Paulay 1997). Nutrients are recycled through microbial processes and remineralisation; as a result coral reefs are recognised to have few demands on the surrounding waters (Azam et al. 1983, Crossland et al. 1991).

Corals exist in a huge variety of forms, from large massives to intricate branching structures which grow at different rates controlled by primary production. Corals assimilate carbon in its inorganic form (CO_2) during photosynthesis and convert it into its organic form for reef building. Reef structure is created by the process of coral accretion whereby a calcium carbonate skeleton is created. True framework reefs thrive where specific environmental conditions for growth, including temperature, salinity, light, aragonite saturation (carbonate) and essential

nutrients such as phosphorus are optimal (Kleypas et al. 1999). Where these conditions are non-optimal true reef formation and structure can be absent but 'coral communities' or 'marginal' reefs (see Perry and Larcombe 2003 for definitions) can still persist (Benzoni et al. 2003, Kleypas et al. 1999). Many of these coral communities can be found at boundaries of light and temperature tolerances, often in high latitude sites outside the tropics, or where colder upwelled water brings excess nutrients creating a 'pseudo-latitude' effect (Glynn 2004, Perry and Larcombe 2003, Sheppard and Salm 1988).

There is a great diversity of organisms associated with reefs and their coexistence is often described in relation to the availability of resources, commonly food (Sale 1977). A number of factors favour coral reefs as areas of high diversity, the waters are moderately warm and abiotic factors remain relatively constant, in the absence of seasonal change (Paulay 1997). Species diversity is often so great that there is significant overlap within functional groups and much redundancy within coral reef ecosystems. In these conditions a reduction in diversity may not necessarily have an adverse effect on ecosystem function, as long as crucial guilds remain. The occurrence of a species in a reef ecosystem will depend upon both evolutionary history and ecological maintenance (Ricklefs and Schulter 1993). Ultimately, coexistence of species will depend on a number of factors and processes such as environment favourability, niche diversity, niche breadth, evolution of historical stability, origination, extinction, productivity, habitat heterogeneity and inter-specific interactions (Brown 1988).

1.2 The coastal waters of Oman, a unique environment.

The Sultanate of Oman lies between 16 and 27°N (Figure 1.1) in the North West Indian Ocean, forming the north east corner of the Arabian Peninsula. To the South, Oman borders Yemen where the land is separated from the African continent by the Gulf of Aden, linking the Arabian Sea to the Red Sea. The majority of the land mass of the country is desert with a hot, dry climate. However, the region surrounding Salalah in the south is renowned for cloud and rain, brought by the monsoon winds for about four months of the year, resulting in well-developed forests on the coastal slopes and a climate unlike the rest of Arabia (Sheppard et al. 1992).

The dominant oceanographic features to influence the coast of Oman are the upwelling events brought about by the seasonal reversal of the dominant winds. In the summer (June–September) the SW monsoon ('*Khareef*') prevails, resulting in intense upwelling along much of the Arabian Sea coastline bringing cold nutrient-rich, deep water to replace nutrient depleted surface waters (Currie et al. 1973). The monsoon winds usually begin in May and continue to dominate from a south westerly direction, almost parallel to the Arabian Sea coastline, until August/September (Currie et al. 1973, Weller et al. 1998). The winds of speeds of about $3ms^{-1}$ produce a strong sea current, the Oman Coastal Current, which flows in a northerly direction along the Arabian Sea coast (Savidge et al. 1990). Eckman forces result in surface water moving hundreds of kilometres offshore and being replaced by deep, cold nutrient rich water from below. When the Oman coastal current reaches the point of Ras Al Hadd the current flow moves offshore to form the Ras Al Hadd Jet. This oceanographic feature exports the upwelled water offshore, creating a seasonal boundary between the Gulf of Oman and Arabian Sea coasts. The intensity of the upwelling is greatest on the Arabian Sea coast where the continental shelf is narrow (Sheppard et al. 1992). In the Gulf of Oman the intensity is much reduced; shorter less predictable upwelling events occur along with seasonal coastal currents and eddies (Kindle and Arnone 2001). These eddies are cyclonic and are associated (perhaps generated) by the Ras al Hadd jet (Bohm et al. 1999, Kindle and Arnone 2001). In the winter months the winds reverse and the NE monsoon ('*Shamal*' winds) dominates, resulting in smaller upwelling events within the Gulf of Oman. Both periods of the monsoon system elevate the biological productivity of the region. During the inter-monsoon periods the conditions return towards a more oligotrophic state, typical of regions at this latitude. In the north of Oman, the Musandam peninsula lies outwith the influence of the upwelling events and temperatures and productivity here are more stable year round (Glynn 1993). The SW monsoon upwelling has been the focus of many oceanographic expeditions to the Arabian Sea (including the John Murray expedition 1933–1934, International Indian Ocean Expedition (IIOE) and Joint Global Ocean Flux Study (JGOFS)) which, along with the advent of satellite imagery and remote sensing technology (e.g Sea-viewing Wide Field of View Sensor (SEAWIFS), Moderate Resolution Imaging Spectro-radiometer (MODIS) and Coastal Zone Color Sensor (SZCS)),

have led to a better understanding of this complex environment (see overview by Wiggert et al. 2005).

The coastal sea surface temperatures prior to the SW monsoon reach an annual high of around 32°C in June (Fig. 1.2). During the upwelling months (July-October) the temperatures can drop to as low as 16°C on the SE Arabian coast, the waters here are eutrophic and dominated by macroalgal growth and phytoplankton blooms for 5-6 months of the year (Barratt 1984 cited in Sheppard and Salm 1988, Rezai et al. 2004). The Dhofar coastline is particularly productive, and has a prolific growth of kelp (*Ecklonia*) and macroalgae during these months (Sheppard and Salm 1988). The temperatures do not drop as severely along the Gulf of Oman coast as this region is more sheltered from the full force of the SW monsoon winds. The lowest temperatures are recorded along this coast when the monsoon winds reverse (Jan-Feb) and the NE monsoon causes small localised upwelling events. Productivity, measured by chlorophyll-*a* concentrations, peaks in the summer months during the SE monsoon upwellings (Fig.1.3). In the Gulf of Oman there is a second chlorophyll-*a* peak in January and February as the NE monsoon reverses.

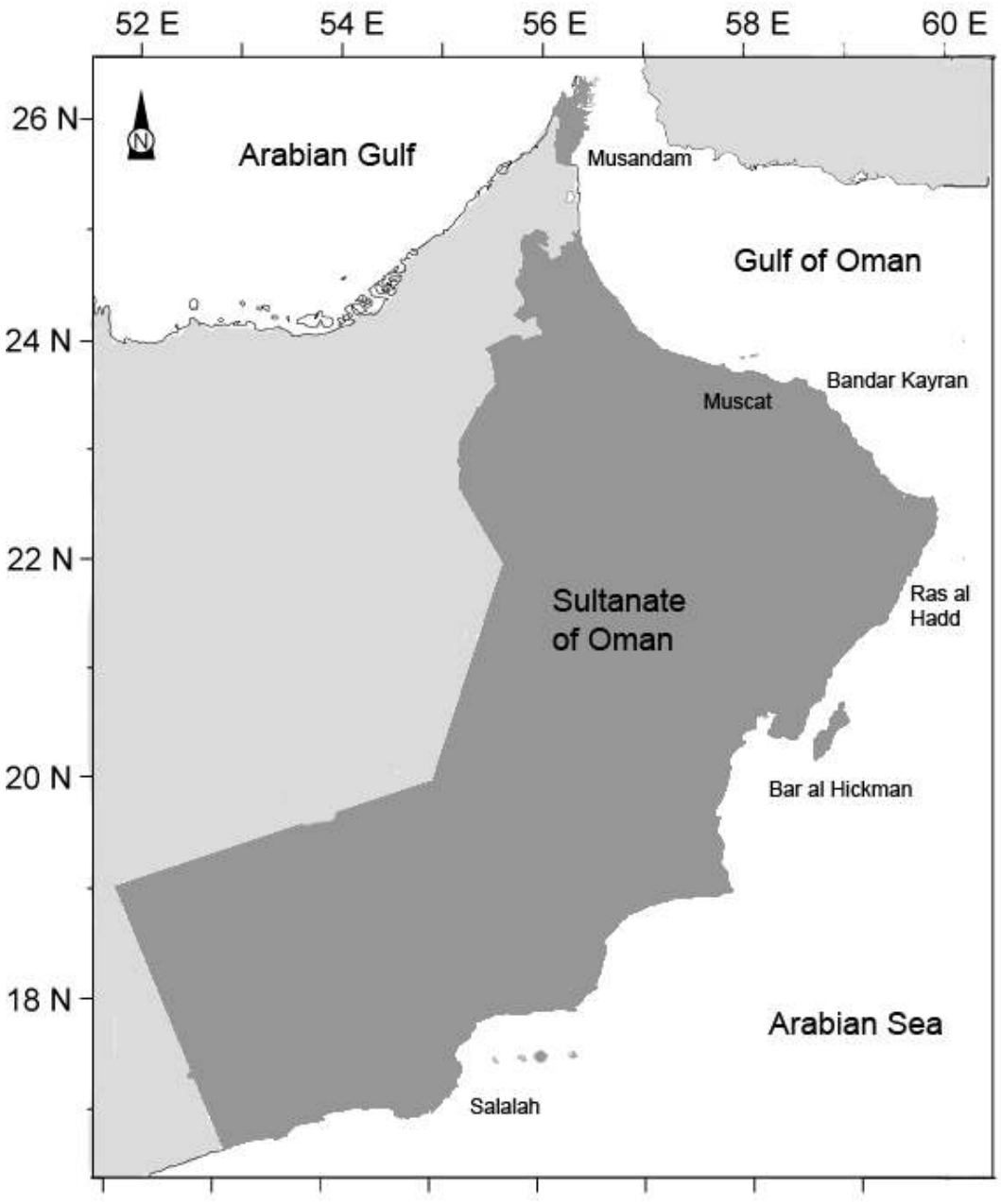


Fig. 1.1: The Sultanate of Oman with study sites Bar al Hickman and Bhandar Kayran.

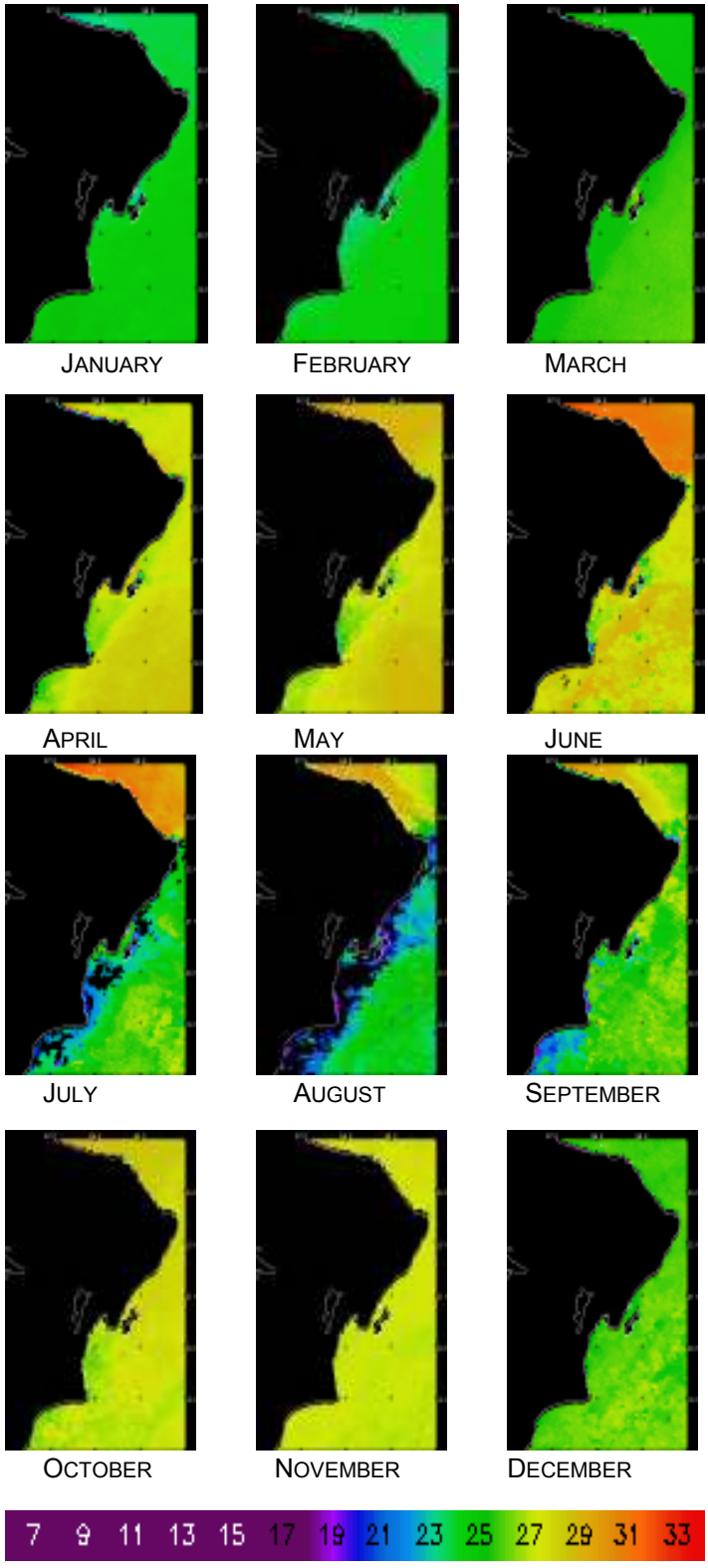


Fig. 1.2: Monthly mean sea-surface temperature images of the Oman peninsula. Images from left to right, top to bottom are Jan–Dec 2004. NOAA AVHRR imagery courtesy of Plymouth Marine Laboratory Remote Sensing Group.

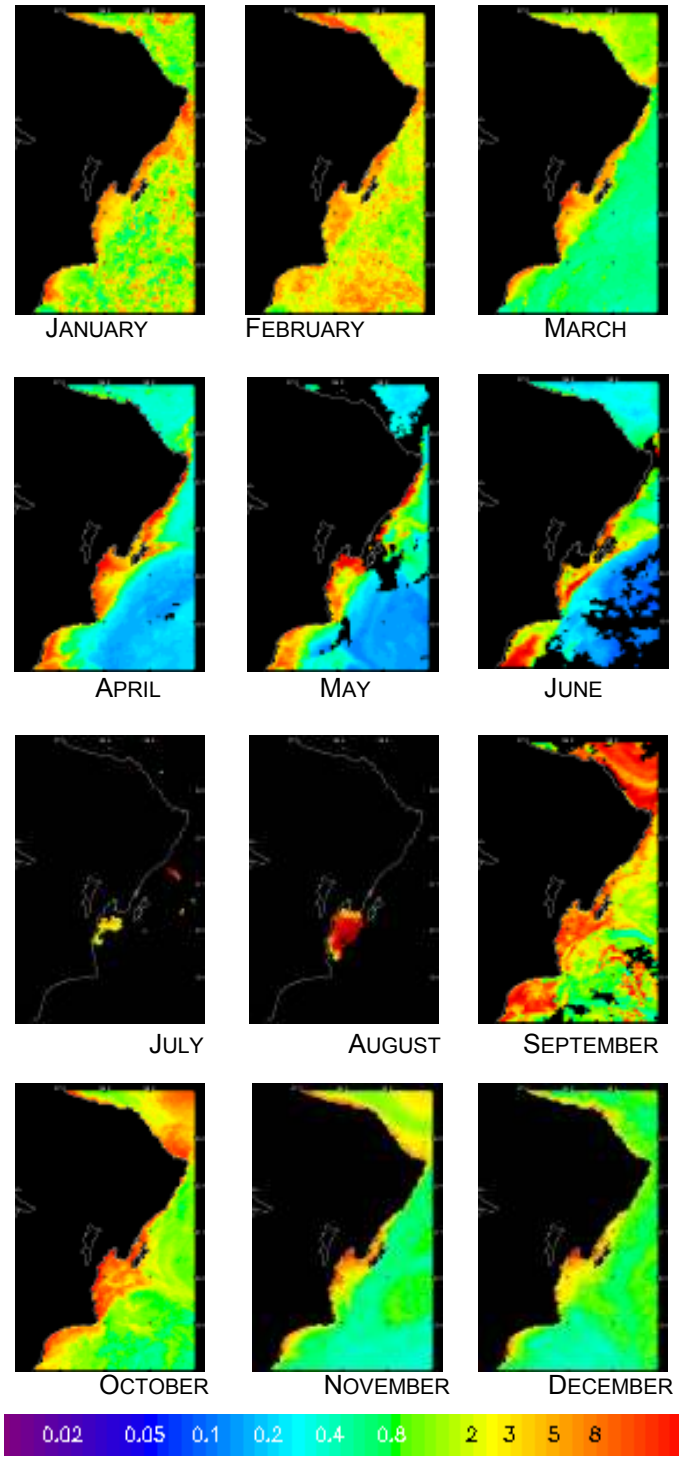


Fig. 1.3: Mean monthly chlorophyll-*a* images of the Oman peninsula, Jan-Dec 2004. Black patches in May- September images are where cloud-cover obscured readings. NASA MODIS satellite imagery courtesy of Plymouth Marine Laboratory Remote Sensing Group.

1.3 Coral communities of Oman.

Charles Darwin reported in the 1880's that there was no significant coral growth on the Omani coastline (1889) and until relatively recently our knowledge of these coral communities has been poor. Prior to the 1970's only a few coral species reported (Sheppard and Salm 1988). Subsequently there have been 107 species of coral recorded in the Gulf of Oman and there are likely to be about 20 more on the Arabian Sea coast, of these about 10‰ are new species currently being described (Rezai et al. 2004). There are four distinct coastal regions where reef ecosystems occur in Oman.

Musandam, in the north of the country, is the peninsula that forms the entrance to the Persian Gulf (Fig. 1.1). This region of Oman is separated from the rest of the country by the United Arab Emirates. The landscape consists of fjord like inlets ('*Khwars*') which support coral growth on steep slopes to depths of ~30m (Sheppard and Salm 1988). Summer temperatures are high and sustained throughout the summer months as the area is not affected by upwelling. The reefs here are well developed.

In the Gulf of Oman, the Muscat 'Capital Area' has bays with fringing reefs notably at Bandar Jissah, Bandar Kayran and there is also significant coral growth at offshore Damaniyat islands which lie ~20km from the coast. These islands are recognised as having some of the best developed reefs in the region and are protected from fishing.

On the Arabian Sea coast, reefs are found in the upwelling zone at Bar al Hickman and Masirah Island. These include large monospecific reefs of the coral *Montipora* (Sheppard et al. 2000).

Reefs are rare in the south of Oman, however substantial coral growth is present at the Hallaniyat island chain (Kuria Muria islands) 40km offshore from Salalah.

The framework and non framework coral communities are described in details by Benzoni et al (2003). The reefs of Oman are rarely accreting structures,

the majority of the coral communities are 'non-framework' and grow on hard substrate with between 30-40% coral cover at depths of 4-12m., few species occur below this depth (Rezai et al. 2004). *Porites* is the largest reef-building coral but most of the reefs in Oman remain only marginally developed. In the 'Capital Area' *Pocillopora* and *Acropora* are the dominant coral forms, and these genera often form monospecific reefs, or 'coral carpets' (Benzoni et al. 2003). South of Bar al Hickman *Montipora* forms monospecific reefs that cover large areas (Rezai et al. 2004).

Oman's corals are particularly resilient, tolerating a seasonal temperature range of 23-31°C, which due to the presence of a strong thermocline in the summer months can often be experienced on a daily basis (Fig. 1.4) (Coles 1997, Quinn and Johnson 1996). Bandar Kayran on the Muscat coastline has however been subject to frequent bleaching events (1996, 1998, 2000, and 2004) but usually with a high proportion of recovery (Rezai et al. 2004). The seasonal influx of cold water is likely to constrain the development of reefs in the region but is also suggested to protect the corals from the high temperature stress seen in the neighbouring Persian Gulf (Glynn 1993).

Optimal temperatures for coral growth are in the range 25-29°C; hence upwelling will affect the development of reefs. The low latitude reefs in Oman exhibit a pseudo-latitude effect with characteristics such as reduced coral cover and diversity and high macroalgae growth, typical of high latitude reefs (Sheppard and Salm 1988). The reef building potential may be low in Omani waters because of high levels of bioerosion fuelled by high primary productivity (Rezai et al. 2004). The high incidence of turf and macroalgae growth can sustain a large population of the grazing sea urchin *Diadema*. *Diadema* can be extremely destructive to reefs and will erode calcite in search of food and shelter. There have also been several outbreaks of the crown of thorns starfish, *Acanthaster planci,* which have locally decreased coral cover (Glynn 1993). Additionally, there is considerable competition between macroalgae and corals for space and increased nutrient levels have had a negative impact on the growth of corals promoting macroalgal growth (Sheppard and Salm 1988).

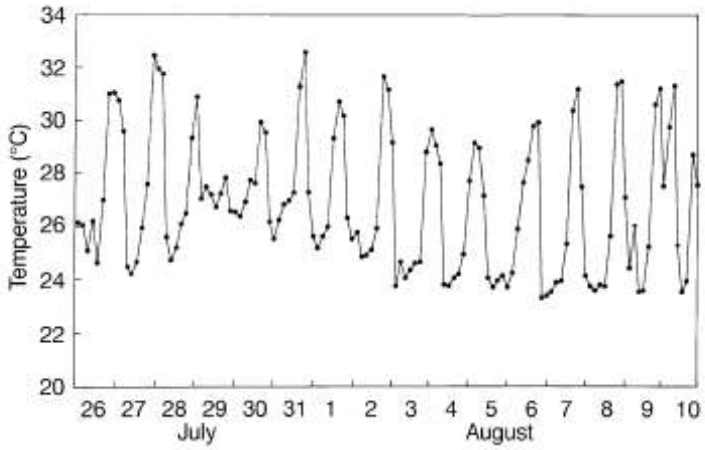


Fig. 1.4: Daily temperature fluctuations during July and August at Fahal Island recorded at a depth of 10m. Taken from (Coles 1997).

The reef fish fauna of Oman consists of species with distributions in the Indian Ocean, Pacific Ocean, Persian Gulf, Red Sea and Gulf of Aden, as well as a high proportion of endemics (Randall 1995, Randall and Hoover 1995). The fish diversity in Oman is slightly reduced in comparison to the neighbouring Red Sea (~900 species compared to over 1000) and substantially less species rich than the biodiversity hotspots (over 3000 fish species) of Indonesia and the Philippines (Sale 1980). Most reef fish families are represented in Omani waters' however in some cases this is only by one or two species (Randall 1995, Sheppard et al. 1992). Fish biomass is generally high in Oman, reflecting the productivity of the surrounding waters (Sheppard and Salm 1988).

The published literature on the ecology of Oman's reefs is limited (Coles 1997, Glynn 1993, Quinn and Johnson 1996), and information on the feeding ecology of many of the fish species of the reef is extremely scarce. Much of the marine research has been focussed on some of the other processes influencing the coastline, in particular the pattern of seasonal upwelling on the SE coast caused by the 'Khareef' (Brock and McClain 1992, Currie et al. 1973, Savidge et al. 1990, Schils and Coppejans 2003). There are very few coral communities in the world that experience such dynamic environmental fluctuations on a seasonal basis. Hence in Oman the opportunity exists to study the food web that these unique coral

communities support. Little is known about how (or if) the upwelling events influences food availability, diet breadth and associated feeding interactions between species.

1.4 Methods for studying food webs

Traditional methods

Food webs are trophodynamic diagrams depicting the feeding relationships of organisms within an ecosystem (Pimm et al. 1991). Food webs are often the best models available from describing ecosystem structure and function, providing information about energy flow and ultimate sources of production. At their simplest food webs models will describe the diet of a single species. Sophisticated multi-species food web models, such as ECOPATH (Pauly et al. 2000), can also incorporate biomass data and allow whole ecosystems to be assessed in detail. Such models can be used to predict the effects of perturbations, such as fishing. The structural organisation within food webs is referred to in terms of trophic levels (TL) where by each consumer is a trophic level higher than its food. Most food webs have between 4 and 5 TLs.

TL estimations have traditionally been assigned on the basis of feeding observations, scat (Jobling 1987) or gut contents analysis (Deb 1997, Hyslop 1980). Direct feeding observations in the wild may be possible for some species that are relatively site attached or have sessile food sources (herbivores), yet for fish species that are roving predators or where feeding grounds are not known, difficult to reach (deep-pelagic feeders) or feed nocturnally this technique is impossible. Scat analysis tends to be the preferred non-destructive sampling method for marine mammals (Burns et al. 1998, McFadden et al. 2006) yet faeces analysis is scarcely practical when studying fish. Many fish only feed intermittently or may regurgitate their prey upon capture making gut analysis very difficult (Bowman 1986).

Stomach contents data can either be qualitative (by simply listing dietary components) or quantitative whereby volumetric or mass data can be taken to calculate a continuous measure of a population's TL (Hyslop 1980). Trophic level in this way can be calculated using Equation 1.1.

$$TL_{\alpha} = \sum (V_i \times T_i) + 1 \qquad (1.1)$$

Where $TL\alpha$ is the mean TL of the consumer alpha, Vi = volumetric contribution of the *ith* food item to diet, Ti is the TL of the *ith* food item, +1 is applied where the primary producer TL is designated as one. Estimation of TL based on Equation 1.1 relies on the assumption that the trophic position of all prey is known and any error here may be compounded in estimating the consumers TL (Vander Zanden et al. 1997).

Stomach contents analysis is typically a sacrificial method of diet analysis and a large sample size and temporal re-sampling are necessary to obtain quality results (Hyslop 1980). Partial digestion of food items can make them indistinguishable; for example algal components which are high in carbonates deteriorate rapidly in the gut. Also, diet items that are small in size (detritus), digested quickly (e.g algal fragments or gelatinous plankton) or ground up during the ingestion process, notably by the pharyngeal mill in labrids (Choat et al. 2004) can make them indistinguishable; hence they tend to be under-represented in analysis. The taxonomic precision of the stomach contents analysis may also impact the estimated trophic level; thus where a worm is labelled a polychaete rather than a predatory polychaete of TL 3 the fish consumer trophic level may be underestimated. Obtaining accurate data from gut contents analysis is especially fraught when the consumer is very small e.g. some fish species and invertebrates (Polunin and Pinnegar 2002). Gut contents analysis is also limited by the fact that food present in the gut may not be absorbed, giving a biased picture of the consumer's trophic status (Deb 1997, Stoner and Zimmerman 1988). The time frame that stomach contents reflects is very short, often only the most recent prey items are recorded, hence giving little idea of longer tem feeding patterns throughout the year. This is markedly so in species which have a seasonal component to their diet or are opportunistic feeders (Deb 1997). These problems can lead to consumers being assigned incorrectly within food webs or ecosystem models, thus distorting food web structure (Kwak and Zedler 1997).

1.5 Stable isotopes as food web descriptors

Stable isotope analysis (SIA) has become an increasingly popular tool to study food webs. The technique involves sampling a small section of tissue, from which information on long-term diet preferences and information about trophic level can be inferred. There are a number of advantages of using this biochemical technique over traditional methods such as gut contents analysis in that sampling can be non-destructive (although in the marine environment usually not) and the dietary information obtained reflects a much longer time period (a year or longer in some fish species) (Hesslein et al. 1993).

Isotopes are forms of an element that differ in relative mass due to number of neutrons contained within the atomic nucleus. Those that do not decay with time are termed 'stable' isotopes. Naturally occurring stable isotopes which lend themselves to biological and ecological research are those of hydrogen, carbon, nitrogen, oxygen and sulphur. Stable isotopes of most elements occur as one highly abundant isotope and one or two much less common isotopes (Table 1.1). The elements carbon, nitrogen and sulphur have two or more isotopes. The minor isotopes contain one or more neutrons extra resulting in them being one or more atomic mass units heavier. Nitrogen occurs in two forms, the abundant ^{14}N and the less abundant heavier ^{15}N. Carbon is most abundant as ^{12}C but has a heavier isotope ^{13}C. Sulphur has four stable isotopes (^{32}S, ^{33}S, ^{34}S and ^{36}S), ^{32}S being most abundant and $^{34}S/^{32}S$ ratio being commonly used in marine and estuarine studies.

The recognised technique for measuring the small difference in concentration of the rarer isotopes is Isotope Ratio Mass Spectrometry (IRMS), originally conducted by Neir (1939) with later developments by Preston (1992). IRMS methodology and preparation techniques are described more fully in chapter 2 of this thesis. The ratio of heavy (e.g ^{15}N or ^{13}C) to light (^{14}N or ^{12}C) isotopes occurring within a sample is recorded and compared to a standard reference material to give the 'isotope signature' or ratio of the sample. Delta notation (δ) is used to express the isotope signature of a sample and is calculated by:

$$\delta X = \left(\frac{R_{sample}}{R_{reference}} - 1 \right) \times 1000 \qquad (1.2)$$

where X is ^{15}N or ^{13}C, R_{sample} is the corresponding ratio $^{15}N:^{14}N$ or $^{13}C:^{12}C$ in the sample and $R_{reference}$ is the ratio of the international reference standard (see chapter 2). δ has units of per mil (‰) and allows measurements of isotopic ratios to remain within a manageable magnitude.

Table 1.1: Relative abundance of stable isotopes of H, N, C, O and S and examples of their uses in ecology.

Element	Isotope	Natural Abundance	Example of Uses
Hydrogen	^{1}H ^{2}H	99.985 0.015	Food webs, sewage tracer, medical tracer, mechanisms of primary production or water uptake in plants, reconstruction of climate change patterns.
Nitrogen	^{14}N ^{15}N	99.63 0.37	Food webs, trophic level, migration, paleodiet, medicinal tracer, mechanisms of primary production, compound specific SIA.
Carbon	^{12}C ^{13}C	98.89 1.11	Food webs studies, trophic level, primary nitrogen source and nitrogen fixation, migration, paleodiet, medical tracer, pollutant tracer, compound specific SIA
Oxygen	^{16}O ^{17}O ^{18}O	99.757 0.0037 0.205	Temperature indicator, rainfall, salinity, otoliths, fish stock tracer, reconstruction of climate change patterns.
Sulphur	^{32}S ^{33}S ^{34}S ^{36}S	94.93 0.76 4.29 0.02	Food webs, sediments, anthropogenic SO2 emissions, deep sea vents and sulphur bacteria

In absolute terms the changes in abundance of ^{13}C or ^{15}N are very small. A shift of 1‰ for carbon is 3 times as great an isotopic change than the equivalent 1‰ shift in nitrogen due to their relative natural abundances (Table 1.1).

A sample that has more of a heavy isotope (e.g. ^{13}C or ^{15}N) than other samples is termed ‘heavy’ or ‘enriched’ in the isotope. A sample with less of the ‘heavy’ isotope compared to other samples is termed ‘light’ or ‘depleted’.

1.6 Trophic level calculation

Assimilation results in the slight accumulation of heavier isotopes in preference to the lighter isotopes which are lost in excretory products; in other words the ‘heavier’ isotopes tend to bioaccumulate up the food chain. Molecules of ^{13}C and ^{15}N form slightly stronger bonds and hence react slightly more slowly than those containing ^{12}C and ^{14}N. The fractionation of $^{15}N/^{14}N$ between tissue and diet has a relatively constant rate of ~3.4‰ (Post 2002). This allows consumers to be classed into trophic levels (TL) depending on their $\delta^{15}N$ with reference to a basal organism of known trophic level (Equation 1.3)

$$TL_{consumer} = \frac{\left(\delta^{15}N_{consumer} - \delta^{15}N_{baseline}\right)}{\Delta_n} + TL_{baseline} \quad (1.3)$$

Where $\delta^{15}N_{consumer}$ is the isotope signature of the consumer $\delta^{15}N_{baseline}$ is the isotope signature of the baseline and $TL_{baseline}$ is the trophic level of the baseline. Δ_n is the per TL fractionation.

While it has become recognised that the use of ^{15}N to define an organism's TL eliminates bias seen with the use of gut contents analysis data, stable isotope analysis can not differentiate between two prey items with the same isotopic signature. Dietary analysis of stomach or gut contents is necessary to resolve the diet to detailed taxonomic level (Pinnegar and Polunin 2000). These two methods of TL estimation can be used to complement each other (Fisk et al. 2002, Grey et al. 2002, Monteiro et al. 1991, Vander Zanden et al. 1997). The power of stable isotope data for diet analysis will be improved by introducing further elements, such as carbon and sulphur as there may be differences in prey types in at least one of these elements.

1.7 Fractionation

The exact mechanisms of dietary isotope fractionation, which lead to enrichment (= trophic step enrichment), remain poorly understood. However, several papers have highlighted and modelled various processes that are known to contribute to the overall fractionation between consumer and diet (Balter et al. 2006, Olive et al. 2003, Ponsard and Averbuch 1999). A consensus view is that fractionation occurs during assimilation, metabolism and excretion of N (or C) (Ponsard and Averbuch 1999, Schoeller 1999). Two types of fractionation occur, 'equilibrium isotope fractionation' related to reversible reactions and 'kinetic isotope fractionation' where reactions are irreversible (Owens 1987). Kinetic isotope fractionation is involved in the biological nitrogen and carbon cycles, where a substrate reacts to form a product at an instantaneous rate and this form of fractionation is the more important of the two in food web studies (Owens 1987). As this reaction proceeds the unreacted substrate becomes more enriched (heavier), simultaneously the product is depleted i.e. becomes lighter (Figure 1.5). In a closed system this reaction would go to completion and the product would gradually

become less 'depleted', eventually having the same isotopic value as the original substrate (Owens 1987, Schoeller 1999).

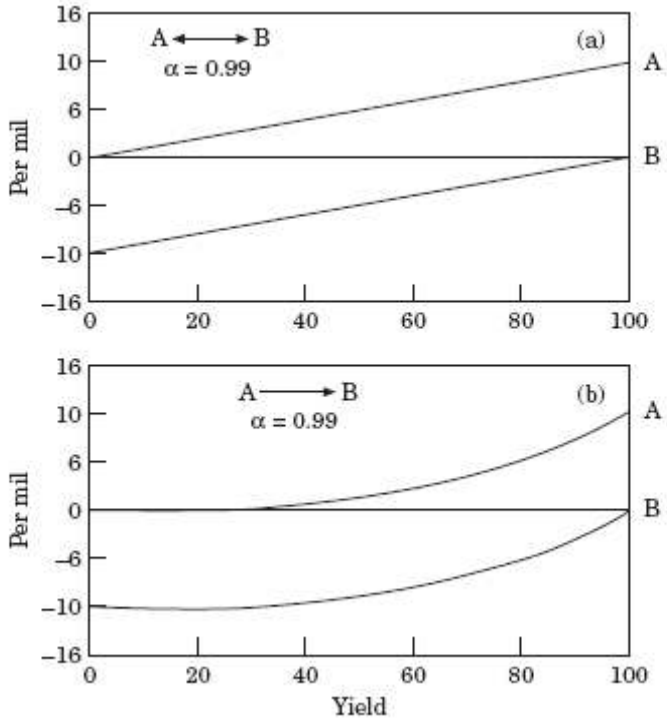


Fig. 1.5. Fractionation reactions (a) equilibrium (reversible) fractionation (b) kinetic (non reversible) fractionation as substrate (A) reacts to form product (B). Figure taken from Schoeller (1999).

A single kinetic fractionation can be described by

$$\Delta n = \frac{\delta X(A) - \delta X(B)}{1 + \delta X(A) \times 10^{-3}} \qquad (1.4)$$

Where, Δn is the fractionation for the reaction from substrate (A) to product (B) and δX is the corresponding isotope ratio. Δn will be positive in sign when the lighter isotope reacts more quickly.

Nitrogen in the body is mostly in the form of amino acids or proteins and within an organism many fractionation events will take place during the processes of their assimilation and excretion. Trophic fractionation is the cumulative depletion or enrichment of all of these processes compared to the diet.

1.8 Variance in stable isotope fractionation

The mean trophic fractionation value of 3.4‰ (+/- 1.1 ‰), was first proposed by Minagawa and Wada (1984) and has since been shown to be a robust assumption when applied across food-webs (Post 2002). This value is commonly used to estimate TL (Cabana and Rasmussen 1994, 1996, Vander Zanden et al. 1997), however, further studies have shown that there is considerable variation about this mean and its validity in TL calculations has been questioned (Gannes et al. 1997, McCutchan et al. 2003, Vander Zanden and Rasmussen 2001, Vanderklift and Ponsard 2003). The use of inaccurate trophic fractionation values will lead to misinterpretation of isotope data, therefore, establishing sources of systematic variance in fractionation may increase the power of the tool (McCutchan et al. 2003, Olive et al. 2003). Identifying departures from the assumed constant trophic fractionation has been attempted and possible important factors extend to a range of physiological and behavioural processes including taxonomy, body size, age, diet quality (in terms of C:N ratios), excretory mechanism, temperature and feeding rate (McCutchan et al. 2003, Sweeting et al. 2007, Vander Zanden and Rasmussen 2001, Vanderklift and Ponsard 2003). Many of these factors are interrelated e.g. lower trophic level species tend to also be smaller in size, feed on high carbon foods, of which they absorb less and process material quickly, hence it is intuitive for ecologists to look for biological correlates in fractionation variation between species (Jennings et al. 2002c).

Research of variance in fractionation at the molecular level has focussed on cellular processes during assimilation, metabolism and excretion. Nitrogen fixation has a low fractionation; hence organic nitrogen derived from atmospheric di-nitrogen will have similar values to N_2 in the air (Balter et al. 2006, Macko et al. 1986). Conversely, denitrification reactions have a large isotopic fractionation as the N-O bond associated with this process is particularly strong. Hence, $\delta^{15}N$ values in marine systems where denitrification is predominant are particularly high. Kinetic fractionation values for various speciation changes within nitrogen pathways in the body have been calculated, including those associated with protein break down and amino acid catabolism (Dewiche 1970). The transamination of NH_2 from glumatic acid to aspartic acid in an animals' cells proceeds 1.0083 times faster

with $^{14}NH_2$ than $^{15}NH_2$ (Macko et al. 1986); this a possible explanation for tissue enrichment during starvation or nutritional stress as seen in experiments (Fantle et al. 1999, Fuller et al. 2005, Gannes et al. 1997, Hobson et al. 1993, Hobson and Clark 1992, Oelbermann and Scheu 2002). Individual measurements of $\delta^{15}N$ in amino acids are rare and highly variable, hence it is likely that the amino acid make up and different combinations of proteins in different tissues will lead to variable fractionation values for different tissues (Gaebler et al. 1966, Macko et al. 1987, Moeri et al. 2003). It is generally thought that 'essential' amino acids incorporated directly from the diet are fractionated only to a small extent. On the contrary, non-essential amino acids, constructed within the body, have a higher fractionation values associated with more complex pathways and increased number of reactions during synthesis (Fantle et al. 1999).

The removal of nitrogen from the body as an end product of protein deamination typically involves a ^{15}N-depleted form, either ammonia, uric acid or urea which are soluble to form urine. Kinetic fractionation during preferential excretion of ^{15}N-depleted urea resulting in a relative retention of ^{15}N in the body may partly explain consumer enrichment compared to diet (Balter et al. 2006). This hypothesis is supported by the evidence that urine is ^{15}N-depleted relative to diet (Sponheimer et al. 2003). Differences in the biological form of excretion have explained some variation in fractionation in several meta-analyses (Owens 1987, Vanderklift and Ponsard 2003). Minagawa and Wada (1984) first proposed excretory mechanisms as a potential source of variance in fractionation but found no systematic differences. Vanderklift and Ponsard (2003) however, found significant fractionation differences according to form of excretion, e.g. urea, ammonia, versus uric acid. Ammonia is toxic to the body and will either be excreted immediately after it is produced (as in aquatic animals) or undergo further reaction to form a non-toxic product such as urea. This further reaction will result in more fractionations resulting in an even more ^{15}N-depleted product. In general organisms from marine environments have lower values of enrichment than those in freshwater or terrestrial environments (Vanderklift and Ponsard 2003). This effect may be due to marine examples having a high number of crustaceans and ammonotelics, which have a smaller fractionation due to excretion of ammonia (Vanderklift and Ponsard 2003).

Additional biological effects which may alter fractionation at a cellular level include temperature, dietary C: N content and metabolic rate (Silfer et al. 1992). Temperature affects biochemical and physiological processes in organisms and can effect fractionation factors, both directly and indirectly by controlling reaction rates (Mariotti et al. 1982). Kinetic and equilibrium isotope fractionation can vary in magnitude with temperature; as an example, denitrification of nitrate to N_2 is temperature dependant and fractionations can vary by 5‰ over 10°C (Mariotti et al. 1981). For simple molecules, bond strength measurements can be used to predict temperature-dependant differences (Mook et al. 1974). Temperature effects have been observed in *Drosophila* (Power et al. 2003) and in seabass, *Dicentrarchus labrax* (Barnes 2006) where $^{15}N/^{14}N$ fractionation is greater at lower temperatures. Seasonal variability in isotopic signature has been widely observed (Lorrain et al. 2002, Peterson and Fry 1987) and may be explained by temperature fluctuations.

Following on from the knowledge that cellular processes can result in enrichment of body tissues in animals that are undergoing nutritional stress or starvation, differences have been observed in animals consuming foods of different C: N ratios (Oelbermann and Scheu 2002). The empirical results are mixed (e.g. Scheu and Folger 2004). By analysing published data Vanderklift and Ponsard (2003) found a significant negative relationship between food quality and fractionation, while McCutchan et al. (2003) found no relationship in their meta-analysis. Individual studies that set out to measure the effect of C: N ratios of diet in single organisms, found fractionation to decrease with increasing food quality (Adams and Sterner 2000, Pearson et al. 2003). This would suggest that organisms of different trophic groups differ in fractionation values and could explain why herbivores do not conform to the conventional fractionation factor of 3.4‰ (Jennings et al. 1997, Pinnegar and Polunin 2000, Post 2002) (this theory is developed in full in Chapter 5). Quality and quantity are likely to be interlinked factors as animals ingesting a poorer quality diet (e.g. herbivores) will have to consume a greater quantity (higher ration of food) in order to meet their nutritional needs. Fish fed different quantities of the same food have been shown to vary in fractionation, however this may have been an artefact of differing metabolic rates (Gaye-Siessegger et al. 2004).

Metabolic and tissue turnover rates are correlated factors that decrease with increased size of an organism (Clarke and Johnston 1999, Cohen et al. 1993, Woodward et al. 2005) and since larger individuals are typically older than smaller individuals it was thought that age may have an effect on fractionation (Minagawa and Wada 1984). However, theory suggests that age should not confound fractionation and adult and growing animals should have the same enrichment in $\delta^{15}N$ (Hobson and Clark 1992, Ponsard and Averbuch 1999). Hence enrichment correlated with increase in age or size is due to a change in diet (Hobson and Welch 1995). Contrary to this, Overman and Parrish (2001) found older individuals with the same diet to be of higher trophic level, suggesting that ^{15}N accumulates with age or that food processing changes in some way.

In many studies, notably multi-species food webs, consumer enrichments do not follow the expected pattern of 3.4‰ and this discrepancy remains unexplained (Jennings et al. 1997, Needoba et al. 2003, Pinnegar et al. 2001, Pinnegar and Polunin 2000, Yokoyama et al. 2005). Parasitic organisms are more complicated than heterotrophic organisms as they tend not to follow the usual fractionation patterns observed between consumer and diet (in this case parasite and host). Nutrient selectivity of parasites and the precise trophic pathways are poorly understood (Boag et al. 1998, Deudero et al. 2002, Pinnegar et al. 2001) but it is thought that since fluid feeders do not need to break down proteins, fractionation will be less (McCutchan et al. 2003, Pinnegar et al. 2001, Spence and Rosenheim 2005).

1.9 Dual use of C and N as food web descriptors

Nitrogen and carbon are the most commonly utilised isotopes in food web studies. Nitrogen plays a fundamental role in food web dynamics, being an important and sometimes limiting component of many dietary items (Owens 1987) and essential for protein synthesis. Carbon is important as a structural component and is essential for many cellular processes as well as an energy source. The way in which ^{13}C and ^{15}N pools are enriched in an animal compared to its food source allow interpretations of food web interactions to be made (Owens 1987, Rau et al. 1983). Trophic fractionation of nitrogen isotopes (~3.4‰) (Post 2002) is typically higher than for carbon isotopes (~1‰) (DeNiro and Epstein 1978) hence nitrogen is

more commonly used as a descriptor of trophic level (Equation 1.2) (Vander Zanden and Rasmussen 1999). The more conservative nature of carbon isotopes allows ultimate diet sources to be traced. These properties enable the two isotopes to be used in a complementary manner for different purposes; in conjunction they can provide useful information about consumer diet.

Natural variation in stable isotope composition occurs between systems such that organisms from marine systems are generally more enriched in ^{15}N than in freshwater systems which tend to be more enriched than those in terrestrial systems (Owens 1987). In terrestrial systems ^{13}C can be used to differentiate between animals feeding on plants which use different photosynthetic pathways e.g. C3 vs. C4 (Herrera et al. 2003, Peterson and Fry 1987). Plants using the C_3 photosynthetic pathway typically exhibit a depleted $\delta^{13}C$ value of around -27‰ whereas the C_4 or crassulacean acid (CAM) plants exhibit a δ value of around -12‰. In aquatic plants $\delta^{13}C$ varies from -8‰ to -30‰ depending upon the method of carbon uptake (bicarbonate vs carbon dioxide) and photosynthetic pathway used (Maberly et al. 1992). Similarly ^{13}C has been used to differentiate between two major sources of energy production in lakes as the littoral carbon sources are enriched in ^{13}C compared to pelagic carbon sources. $\delta^{13}C$ can be used to differentiate between benthic and pelagic food source in marine food webs (Jennings et al. 1997).

1.10 Sulphur Isotopes

The inclusion of sulphur as a stable isotope food web tracer has until recently been very limited. The preparation of samples for sulphur analyses was very laborious and required large sample volumes however recently an increased level of automation has made sulphur isotopes less costly and less time consuming (Harrigan et al. 1989, Yun et al. 2005). Sulphur in the marine environment is typically about 20‰ greater than the international standard, Canyon Diablo Troilite (Peterson and Fry 1987). Fixation of sulphur by plankton has a small isotope fractionation effect and plankton has values typically of 17-19‰ (Peterson and Fry 1987). Terrestrial sulphur sources are depleted compared to oceanic sulphate; plants have low ^{34}S values (2-6‰) which make them distinct from marine plants and depend on the bedrock type and value of rainwater sulphates (2-16‰). Sulphur reduction results in porewater sulphates being significantly enriched in $\delta^{34}S$ with

typical values of 30-60‰ (Fry et al. 1982). In contrast, seawater sulphides (bacterially reduced sulphates) are extremely depleted with a negative range of signatures (-30 to-10‰). Oceanic fish have typical $\delta^{34}S$ values of 17-19‰, i.e. similar to oceanic plankton. Since most authors assume no or negligible fractionation of sulphur it is mostly used as a source tracer in food webs studies (Hesslein et al. 1993, Kwak and Zedler 1997, Mekhtiyeva et al. 1976, Peterson and Howarth 1987). However, some studies have found fractionation to be highly variable and suggest that further study is required to determine factors that influence fractionation (McCutchan et al. 2003). Where carbon and nitrogen data result in tied sources, sulphur is able to separate them as sulphur signatures for producers tend to be further apart (Connolly et al. 2004). Since $\delta^{13}C$ and $\delta^{34}S$ used in conjunction have a high ratio of 'among' to 'within' producer variation they are ideal for distinguishing between sources, yet, as both elements have limited fractionation their use without nitrogen would give little indication of trophic level (Connolly et al. 2004, Kwak and Zedler 1997). The majority of food web studies that have used sulphur isotopes have been focussed on salt marsh, seagrass or estuarine environments that have several distinct $\delta^{34}S$ sources (Post 2002).

1.11 Dietary mixing models

A common application of stable isotopes is to pinpoint specific source contributions to a consumer's diet, particularly those feeding on multiple items. When the isotopic signature of the consumer is known, and an appropriate fractionation factor can be applied, the signature of the diet mixture can be obtained. Linear mixing models estimate the required proportions of all potential sources to result in a particular isotope mixture (Phillips 2001). These models are useful, with reference to food webs, to determine potential proportions of different dietary items that could result in the observed diet.

Linear models

Mixing models are linear equations designed to give estimations of source contributions while satisfying isotopic mass balance A linear model can be solved for n elements to partition $n+1$ sources. To solve for two sources (A and B)

contributing to a mixture (M) using a single isotope (δ) the following equation is used:

$$\delta_M = f_A \delta_A + f_B \delta_B \quad (1.5)$$

$$1 = f_A + f_B \quad (1.6)$$

where δ_M , δ_A and δ_B represent the mean isotope signature of the mixture and source A and B respectively and f_A and f_B are the proportions of A and B in M. Hence a dual isotope linear model (e.g. using $\delta^{13}C$ and $\delta^{15}N$) can only partition up to three food sources (Kwak and Zedler 1997) here the equations used would be expanded to:

$$\delta_M = f_A \delta_A + f_B \delta_B + f_C \delta_C \quad (1.7)$$

$$\lambda_M = f_A \lambda_A + f_B \lambda_B + f_C \lambda_C \quad (1.8)$$

$$1 = f_A + f_B + f_C \quad (1.9)$$

The mixture and sources must first be aligned by applying a fractionation factor to the mixture that allows the mixture to lie within the 'mixing triangle' created by the sources around the mixture.

This mathematical approach is limited to deriving diet proportions for consumers with a small number of diet items.

1.12 Synopsis

This study set out to contribute to the current knowledge of near-shore reef and coral community ecosystems of Oman using stable isotopes as food web tracers. The unusual and dynamic environment of the region brought about by seasonal upwelling events provided a number of interesting additional problems to address, requiring current assumptions in isotope ecology to be challenged.

How can isotopes be used to describe trophic structure of food webs in a dynamic environment?

How does the seasonal upwelling influence the isotopic signatures of the main sources of production to the reefs? Does upwelling influence the isotope

signatures of a reef community? Are there spatial differences in community structure or feeding relationships between the Gulf of Oman Coast and Arabian Sea Coast?

Can variations in trophic-step fractionation be explained by differences physiological processes?

Difference in feeding rates and quality of food are linked to variations in $\Delta\delta^{15}N$. Do specific feeding guilds show empirical differences in fractionation depending on their food quality? Can modelling physiological parameters explain differences in fractionation?

Can mean and variance in isotopic signatures tell us more about trophic niches?

How useful are variances about the men of population isotopic data in describing trophic niches? Can variability in isotopic data tell us more differences in feeding habits with size and age?

How useful are multi-source stable isotope mixing models at describing food web linkages across ecosystems?

Can models that do not give unique solutions still be useful to determine feeding links? How do fractionation assumptions affect model performance?

The overall structure of this thesis, with these questions in mind, developed from the original ideas about variability in trophic fractionation to encompass the temporal aspect that the study location provided. Chapter 2 provides a review of the current preparation and methodological techniques of stable isotopes in ecological studies and outlines the methods used in this thesis. It was necessary to use two mass spectrometers for the isotope analysis required for this thesis as with over 1000 samples to analyse there were considerable time and cost constraints. Despite in theory measurements from these instruments being comparable it was necessary to calibrate data from the two machines and this posed an interesting and important methodological aside to the main ecological focus of this thesis. In chapter 3 the influence of temporal variability brought by the upwelling in Oman on the use of

stable isotopes analyses of food-webs is explored. The spatial analysis in chapter 4 follows on from the previous chapter and compares the isotopic signatures of similar species in two food-webs as well as developing the idea of using a long-lived species as a baseline from which to calculate trophic level. Trophic fractionation is examined in detail in herbivorous fish in chapter 5 and a model was created to explain the differences in fractionation between trophic guilds. Until now there has been no description of the trophic structure of the reef communities in Oman, chapter 6 is largely descriptive and uses isotopic data to examine feeding patterns, trophic niches and dietary overlap, within the community. The effect of size and $\delta^{15}N$ and feeding variability in the Bandar Kayran food web are also investigated. Chapter 7 draws on the results of the previous chapters and re-analyses the isotope data in various mixing models to determine more accurately the proportions of the diet and the reliance of some organisms on particular diet sources. This chapter incorporates additional sulphur isotope data, to see if accuracy of the models improves with the use of three rather than two tracers. This chapter compares and critiques the various food web models created. To conclude chapter 8 synthesises the new findings of this thesis and suggests directions for future studies.

2 Methodological theory

2.1 Introduction

Increased automation of mass spectrometers and further technological advances have increased the speed and decreased the cost of stable isotope analysis (SIA). Techniques such as continuous-flow isotope ratio mass spectrometry (CF-IRMS) have allowed two isotopes (typically C and N) to be routinely analysed simultaneously from a single sample (Preston 1992). The incorporation of sulphur analysis is becoming routine in leading ecological laboratories, with current research focussed on the configuration of mass spectrometers to handle the determination of 4 isotopes (C, N, S and H) from a single sample (Fry 2006). While such developments will be welcomed by ecologists, multiple isotope determination has caused some concern related to the applicability of sample preparation techniques that were suitable for single isotope analysis but may have confounding affects on the determination of a second/subsequent isotope(s). A range of recent papers have discussed stable isotope sample processing methodology (Carabel et al. 2006, Feuchtmayr and Grey 2003, Kaehler and Pakhomov 2001, Sweeting et al. 2004, 2006), analytical error (Jardine and Cunjak 2005), and variation and experimental design (Lancaster and Waldron 2001), with a view to increasing standardisation of preparation techniques to allow more comparability between studies and results. Technical descriptions of analytical and referencing techniques can be found in detail in Preston (1992) and Werner and Brand (2001). Here the procedures for stable isotope analysis and sample preparation are reviewed and the methods used in this thesis are outlined. One issue that has been scarcely addressed is variation arising from differences between mass spectrometer measurements. As sample analysis for this thesis was carried out at two facilities and using two different machines, it was necessary to cross-check the results obtained from each to ensure data comparability.

2.2 Analytical measurement

Carbon and nitrogen isotope ratios are now routinely analysed together with little loss of linearity or accuracy. Unfortunately this has also led to a poorer understanding of the analytical process and the error and precision therein (Jardine

and Cunjak 2005). To obtain stable isotope ratios, homogenised samples are accurately weighed into tin capsules and combusted to release elements in their gaseous form. The pure gas of an element is entered into the mass spectrometer at one end of the flight tube (which is a vacuum). The gas is ionised by bombardment with electrons, creating a fine beam of positively charged ions which then enters a magnetic field. The magnetic field deflects the ions into circular paths depending on their mass. The ions of separate masses are then collected at the other end of the flight tube in Faraday cups. Amplifiers convert the ionic impacts on these cups into a voltage, which is converted to a frequency (Preston 1992). Absolute measurements are not necessary as it is the ratio of the two isotopes that is the critical parameter (Ehleringer and Rundel 1989). This raw ratio is compared to a standard material (V-PBD) for carbon and atmospheric di-nitrogen (N_2) for nitrogen and converted to δ units (units per mil or ‰) (Equation 1.2).

2.3 Calibration and Standards

The automation of analysis has led to an increase in the number of samples analysed which, in turn, has necessitated the need for tightening up the strategy for referencing among isotope ratio mass spectrometry (IRMS) laboratories (Werner and Brand 2001). In theory, all mass spectrometers should report the same values when analysing the same biological material (within error range), as all IRMS facilities should be calibrated to International Atomic Energy Agency (IAEA) standards. At the advent of stable isotope analysis, standards were chosen for their consistency of isotopic value after repeated measurement. Di-nitrogen is used as a reference for nitrogen because it is the most stable and abundant form of nitrogen in the natural environment and can not be significantly altered by known processes (Mariotti 1984). The original reference material for carbon was a cephalopod fossil called *Belemnitella americana*, from the Pee Dee formation in South Carolina (hence PDB) (Craig 1957, cited in Werner and Brand 2001). This source has since been exhausted and the modern day standard is Vienna-PDB which was created by the IAEA in Vienna as a replacement. Primary reference materials are generally of limited supply, so IRMS laboratories are expected to maintain day-to-day calibration by use of secondary or internal standards which exhibit known value with respect to the accepted precision of the international standards. A secondary

standard should be of a very homogeneous material, ideally of a single chemical compound that reflects a similar chemical identity and isotopic ratio to the samples being analysed (Werner and Brand 2001). Most laboratories have a range of secondary standards to analyse, in conjunction with different sample materials; examples include leucine, flour, bovine and cod muscle. It is common for mass spectrometers to 'drift with time' during an automated sample run, whereby measurements will change slightly during the course of the analysis period. In order to account for drift during an analysis run (typically of ~90 samples), samples and standards are alternately introduced (about two standards per 6-9 samples), then compared to obtain an accurate difference measurement (Fry and Sherr 1989). The use of internal standards in this way should make comparisons of results feasible across laboratories and studies.

2.4 Precision

Replication of isotopic results is imperative for the values to be of generic use to ecologists. This is especially so when relatively small changes in isotopic value are being recorded. Precision of a machine is usually within 0.2‰ (Preston 1992). Experimental precision should be reported by the lab based on the repeatability of lab standards but can also be checked by the ecologist by repeating (or blind repeating) their own samples. Authors have been encouraged to submit blind replicates of a portion of their samples to keep a check on repeatability achieved by the IRMS laboratory (Jardine and Cunjak 2005), this is best done using an extremely homogenous material of known isotopic signature.

2.5 Dealing with high C, low N content of samples

The amount of gas of an individual element produced from a sample during combustion depends on the C and N content, and the mass of the particular sample. The volume of gas produced affects the frequency peaks recorded, which in turn determines the precision of the analysis. To analyse C and N simultaneously the peaks must be of similar amplitude. Most fish and invertebrate tissues have a low C: N ratio (~3-6) and frequency peaks of broadly similar amplitude are obtained. However, samples of a high C: N ratio (e.g. algae, coral and detritus) require a greater mass of sample in order to obtain a higher N peak and hence a more precise

measurement for this element. The C peak can be diluted by a higher pressure of the carrier gas helium or the sample can be analysed twice, once for C and repeated with a greater mass for N (Preston 1992).

2.6 Tissue types

Whole organism analysis will reflect the contribution of the diet to all tissue compartments of the consumer combined. In most studies only organisms small in size are homogenised whole, for most other organisms one tissue is selected for analysis. A wide range of tissues have been used as recorders of stable isotope abundance in animals: baleen in whales (Schell et al. 1989), tooth enamel (Cerling and Harris 1999), fish otoliths (Dufour et al. 1998), bird feathers (Pearson et al. 2003) and blood, heart, liver, muscle and bone collagen from a range of animals. The isotopic content of can be highly variable among different tissues of one organism (Pearson et al. 2003, Pinnegar and Polunin 1999). These differences are attributed to their different biochemical composition, different metabolic processes of fractionation and 'dietary routing' (Gannes et al. 1997). After assimilation, internal enzymatic steps can alter isotope ratios (i.e. whenever chemical bonds are broken or formed). Tissues can be apportioned varying amounts of these isotopes. It is intuitive to use tissues that have relatively constant isotope value, and constitute a large mass of the animal. In fish, dorsal white muscle tissue is suggested as the most appropriate to use for stable isotope analysis because it contains small amounts of lipid and inorganic carbonates (Pinnegar and Polunin 1999) and also constitutes the greatest mass of any body tissue, up to 90% of body mass. Tissues such as blood, feathers or fin clippings can be sampled in a non-destructive manner and allow the same individual animals to be tracked through space and time (Hobson 1999). Tissues such as collagen, chitin and shell are ideally suited to studies of a paleoecological nature as they are typically well preserved with time in the fossil record (Schoeninger and DeNiro 1984). Intraspecific tissue variation has also been noted in plants. Fronds and leaves are known to vary in $\delta^{15}N$ in macroalgae (Fredriksen 2003) and by 1.63‰ along the length of seagrass fronds (Yamamuro et al. 2004). Similar variation is present between the leaves and wood of trees (Leavitt and Long 1986).

2.7 Tissue turnover rates

The isotopic composition of tissues is the result of two processes, metabolic breakdown (or turnover) of tissues and new tissue growth. Turnover rates are taxon and tissue-specific, and may also change during the course of an organism's life. A new isotope signature can be seen in tissues following a change in diet. In a non-growing animal this is governed by the 'metabolic' turnover rate (Hobson and Clark 1992, Tieszen et al. 1983). In a growing animal, somatic growth also has to be considered. Fry and Arnold (1982) demonstrated this with fast-growing juvenile and slow-growing adult brown shrimps. Maturation and growth may play a role as a growing fish will have a higher tissue turnover rate than an adult fully-grown fish (Hesslein et al. 1993, Ponsard and Averbuch 1999).

Isotope turnover rates are most commonly measured in a laboratory setting by conducting diet switch experiments, where the rate of incorporation of a new isotopically-distinct diet is measured. Diet switch experiments have been conducted on a range of animals but are rarely conducted under the same conditions and with similar foods, confounding the ability to make systematic comparisons (McIntyre and Flecker 2006). An alternative to the diet switch experiment that relies on diets having natural isotopic differences is the 'dilution experiment' where the consumer is fed an artificially enriched diet (^{15}N labelled or ^{13}C labelled) and the rate of loss of the label through time is measured to quantify tissue turnover rate. An enriched label can be added to the usual diet of the study organism hence not affecting food quality (in terms of C: N), a factor that is known to affect N turnover rates. The dilution method has the added advantage that the animal does not need to be in equilibrium with its diet at the start of the experiment (McIntyre and Flecker 2006), and can be applied to field settings (Merriam et al. 2002, Mulholland et al. 2000). However, caution should be applied when comparing methods as uptake (calculated from a standard diet switch) and elimination rates (calculated by dilution) may not be equal within the same organism (MacNeil et al. 2006).

Generally the more metabolically active a tissue is, the faster the turnover rate. An isotopic signature of a new diet can be seen in the blood within a few days but the muscle tissue of a fully grown fish may take a few months for the isotopic signature to reach equilibrium after a switch in diet (Hesslein et al. 1993).

Metabolically slow tissues such as bone collagen have a slow turnover rate and isotope signatures reflect feeding habits over a long period (months–years). Hair and feathers remain biochemically unchanged as they are formed, providing the opportunity to study a recent time series of isotope signatures. Cross sections of tissues such as teeth, horn and otoliths can provide a similar, but longer term time series as they are laid down by accretion. Two or more tissues can be studied in conjunction to determine short or long term change in diet (MacNeil et al. 2005, Tieszen et al. 1983). It should be noted that fractionation values for different tissue compartments within the body differ; muscle is depleted in $\delta^{15}N$ relative to liver and collagen (for more details see chapter 1) and bone collagen tends to be more enriched in ^{13}C than muscle tissue (Sholto-Douglas et al. 1991). Whole-animal isotopic signatures will be skewed towards the tissue compartment of the greatest mass.

2.8 Tissue preservation

Prior to stable isotope analysis, samples must be stored in a way in which their isotopic ratios will not be altered. Fixatives such as ethanol and formaldehyde have been shown to react with the biochemical properties of various tissues and are not suitable preservatives (Kaehler and Pakhomov 2001, Sweeting et al. 2004). Freezing does not appreciably alter the isotopic ratio of tissues with time and is the recommended method of tissue preservation (Bosley and Wainright 1999, Kaehler and Pakhomov 2001). For the treatment of plankton, fixatives and freezing have been shown to alter isotope ratios by up to 1.5‰ (Feuchtmayr and Grey 2003); it is suggested that where possible fresh material should be utilised. In preparation for isotope analysis all samples must be dehydrated. This can be achieved either by oven drying (~60 °C) to a constant mass or by freeze drying. Freeze drying is thought to be the more accepted method as all water is eliminated in the process whereas this cannot be guaranteed with oven drying. However, Carabel et al. (2006) found no difference in isotopic ratios with the different water extraction methods, so either is probably acceptable. Homogeneity of samples can be achieved through grinding the dried sample with a pestle and mortar or for larger samples a ball mill may be used. Samples rich in lipids will be hard to homogenise, the use of liquid nitrogen as a coolant during homogenisation will aid the process.

2.9 Lipids

^{13}C can vary in tissues due to the presence of lipids and inorganic carbon. To account for this variability it is common to remove lipids and carbonates. A high lipid fraction in a tissue may adversely affect the results of stable isotope analysis as lipid synthesis discriminates against ^{13}C in favour of the lighter isotope ^{12}C (DeNiro and Epstein 1977, Tieszen et al. 1983). Lipid reserves are typically ^{13}C depleted by 2-8‰ compared to other biochemical components (Focken and Becker 1998). Liquid extraction methods are common for small samples, using a solvent base such as methanol/chloroform to remove the bulk of the lipid (Bligh and Dryer 1959, Folch et al. 1957). Lipid contains very little or no nitrogen and no discrimination between ^{14}N and ^{15}N is apparent. However the process of lipid extraction can alter $\delta^{15}N$, so separate analysis is needed if both isotope values are required. Alternative methods of lipid removal include arithmetic correction which is used to predict lipid-free $\delta^{13}C$ (McConnaughey and McRoy 1979, Sweeting et al. 2006).

2.10 Inorganic carbonates

Inorganic carbon tends to be more enriched in ^{13}C than other fractions (DeNiro and Epstein 1978). Decalcification of samples with high inorganic carbon content, or of small body size where it is difficult to dissect inorganic structures, is achieved by acidifying the sample (using 1M HCl) and re-drying before analysis (DeNiro and Epstein 1978). However acidification increases the variability in results (reducing statistical power) and also significantly alters the $\delta^{15}N$ value in the tissue so samples must be analysed for ^{13}C and ^{15}N separately (with and without decalcifying) (Bunn et al. 1995, Pinnegar and Polunin 1999).

2.11 Methods applied in this thesis

2.11.1 Sample Preparation

Fish were typically sampled in triplicate and individuals were selected that were >70 % of L_{max} (Randall 1995) to minimise size effects on $\delta^{15}N$. Similarly, invertebrates were collected according to size class. For fish, dorsal white muscle tissue was selected for analysis, and a 2-5g sample was dissected just below the dorsal fin.

All dried samples were ground to a homogeneous powder with a pestle and mortar. Only invertebrate species with high carbonate content (i.e. extensive shell) were treated with acid to remove excess carbonates. This was done by adding 1M HCl drop wise to a sub-sample of the dried material until effervescence ceased (~3 h); then the sub-sample was rinsed with filtered water and re-dried in a 60°C oven to a constant mass. Initially plankton samples were acid-treated but no effervescence was observed and thus all remaining plankton samples were treated without acid.

All samples for this study were analysed on one of two machines, an Automated Nitrogen Carbon Analysis (ANCA) 20-20 Isotope ratio mass spectrometer (Scottish Crops Research Institute (SCRI), Dundee, Scotland) and a Thermo-Finnegan mass spectrometer (Scottish Universities Earth Research Centre (SUERC), East Kilbride, Scotland).

Small aliquots of fish and invertebrate materials (~1 mg for SCRI and ~0.7mg for SUERC) were weighed into tin capsules for simultaneous carbon and nitrogen analysis. Acidified invertebrates were analysed twice, once for $\delta^{13}C$ (acidified) and once for $\delta^{15}N$ (non acidified). All algae, detritus and coral samples were weighed into two aliquots, ~1mg and ~ 2mg to determine $\delta^{13}C$ and $\delta^{15}N$ separately, because the much smaller quantities of N in these samples were beyond the sensitivity range of the machines.

In order to account for any disparities in $\delta^{15}N$ and $\delta^{13}C$ between the two mass spectrometers three laboratory standards, LE1, L4 and cod muscle (*Gadus morhua*) were analysed on both machines. LE1 and L4 are internal laboratory standards routinely analysed at the SCRI facility and cod muscle has been the internal standard for the mass spectrometer facility at Newcastle University for over ten years. All have shown very consistent data (Gillian Thompson and C. Scrimegour pers. comm.). And the dried material is known not to change in isotopic signature with time (Sweeting et al. 2004). In addition to the laboratory standards, two field samples, ≥200µm zooplankton and white muscle of the rabbitfish *Siganus canaliculatus* were also analysed on both machines.

2.12 Machine calibration

Machine precision was 0.2 and 0.3‰ for C and N respectively at SUERC and 0.2‰ for both C and N at SCRI (based on internal lab standards). The two machines reported different $\delta^{15}N$ values in a consistent manner across a wide range of values (0- 20‰). The samples analysed at SUERC were found to be significantly enriched by 1.149‰ in ^{15}N compared to samples analysed at SCRI values (Table 2.1).

Table 2.1: Mean $\delta^{13}C$ and $\delta^{15}N$ ($\pm$ standard deviation) of 5 materials analysed on two separate mass spectrometers, SUERC and SCRI.

	n	$\delta^{13}C_{SUERC}$	$\delta^{15}N_{SUERC}$	$\delta^{13}C_{SCRI}$	$\delta^{15}N_{SCRI}$
LE1	8	-28.92 $\pm$ 0.21	18.93 $\pm$ 0.30	-28.50 $\pm$ 0.18	19.96 $\pm$ 0.20
L4	10	-30.06 $\pm$ 0.12	-0.38 $\pm$ 0.34	-29.60 $\pm$ 0.11	0.99 $\pm$ 0.18
Cod (*Gadus morhua*)	12	-16.67 $\pm$ 0.26	14.93 $\pm$ 0.35	-16.40 $\pm$ 0.19	16.04 $\pm$ 0.46
Siganus canaliculatus	3	-13.84 $\pm$ 0.31	12.00 $\pm$ 0.76	-13.44 $\pm$ 0.47	13.75 $\pm$ 0.61
Zooplankton	26	-19.59 $\pm$ 1.71	9.90 $\pm$ 0.56	-19.24 $\pm$ 1.82	10.62 $\pm$ 0.73

Regression analysis revealed that the slope of the $\delta^{15}N_{SUERC}$: $\delta^{15}N_{SCRI}$ line did not differ from 1:1 (Figure 2.1a). Calibration of the discrepancy in $\delta^{15}N$ between machines was achieved using the correction factor in Equation 2.2 ($r^2 = 0.99$)

$$\delta^{15}N_{SCRI} = 0.9861\,\delta^{15}N_{SUERC} + 1.14 \qquad (2.1)$$

Where $\delta^{15}N_{SCRI}$ is the isotopic signature measured at SCRI and $\delta^{15}N_{SUERC}$ is the isotope signature obtained at SUERC. There were no significant differences in $\delta^{13}C$ between machines (Figure 2.1b) therefore these results were not adjusted in any way.

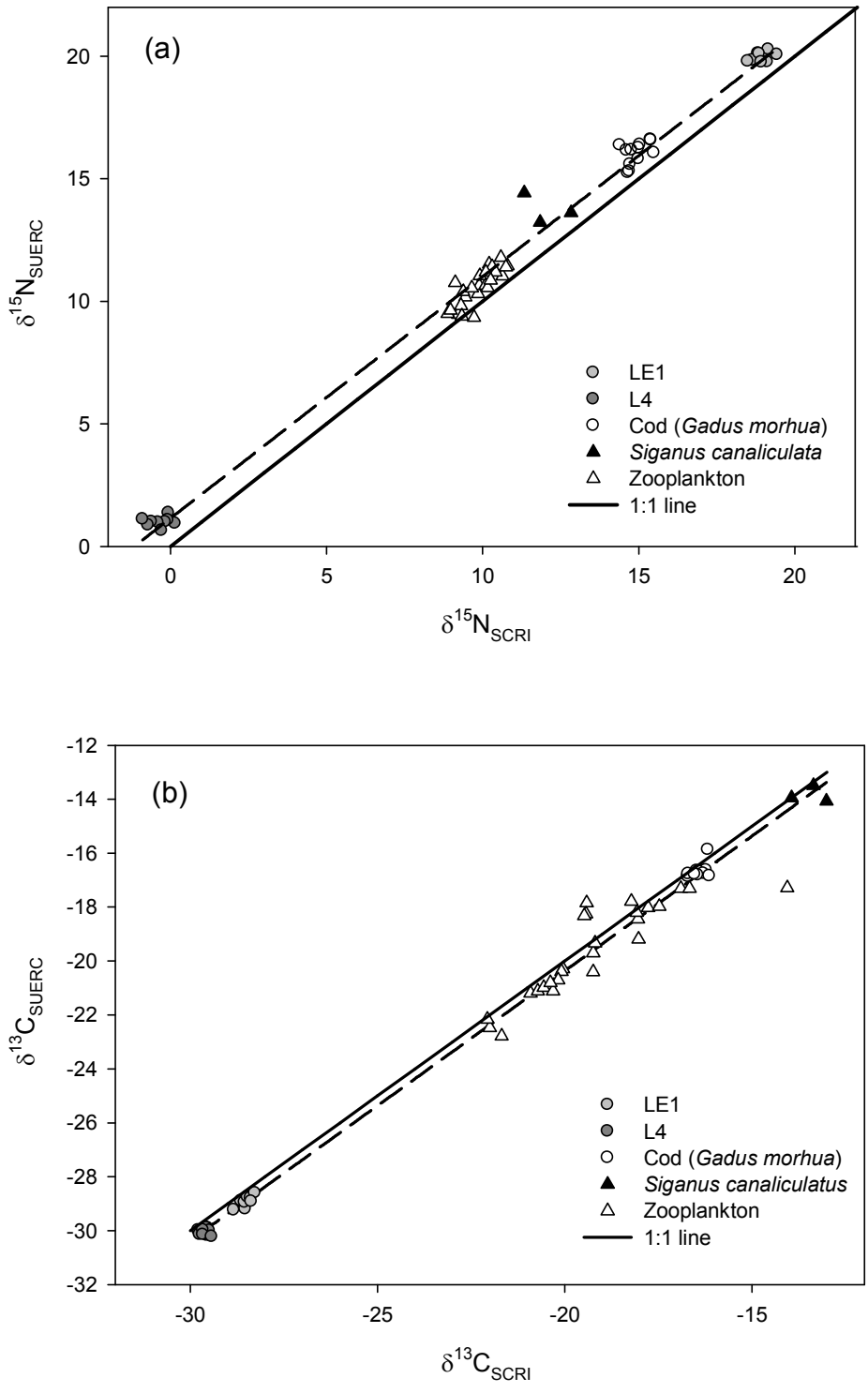


Fig. 2.1: Comparison of (a) $\delta^{15}N$ and (b) $\delta^{13}C$ values of the same materials analysed on two different mass spectrometers, SCRI and SUERC. The solid line represents a 1:1 ratio, the broken line is the regression line $y = 0.98x + 1.14$ of the observed data.

2.13 Discussion

The use of multiple IRMS machines in a single study is not common, but it is not clear why there was such a large discrepancy in the $\delta^{15}N$ between the mass spectrometers, as both are calibrated to international standards and maintain high levels of accuracy with these. Increasingly, ecologists use stable isotope analysis as an additional tool in food-web analysis and may not be aware of such effects; there is the potential especially for erroneous comparisons with previously published data. The rare cases that utilise multiple machines within a single study are scarcely supported by data to confirm that the two machines are reporting similar values for samples or standards (e.g. Davenport and Bax 2002). Multiple machine use also occurs where conducting analysis in the same geographic region as other authors (Deudero et al. 2004) or through time (Barnes 2006) or if comparing new data with previously published data (Takai et al. 2000). Genner et al. (1999) report repeating analysis by Bootsma et al (1996) and finding a difference in $\delta^{15}N$, although this difference is not attributed to analysis on different mass spectrometers. If the samples were stored correctly there should have been no reason for the $\delta^{15}N$ to change with time (Sweeting et al. 2004). Inter-machine variability is not always overlooked (Pinnegar 2000) and calibration by regression is a robust method to align data (provided that the difference is proven to be linear).

Clearly machine variability needs to be borne in mind by researchers. In this case the difference of 1.14‰ in $\delta^{15}N$ can be translated to about a third of a trophic level; if such differences are common there will be implications for studies using multiple machines, and for studies that draw on isotope data from a number of sources. In the case of meta-analysis studies (e.g. France 1997) where it is necessary to draw together results produced from many machines, the issue of inter-machine variability should at least be acknowledged even if it is not practical to quantify the associated error. Meta-analyses of non-absolute isotopic data such as fractionation (McCutchan et al. 2003, Post 2002, Robbins et al. 2005, Vander Zanden and Rasmussen 2001), calculated trophic levels (Pinnegar et al. 2003) and tissue turnover (Dalerum and Angerbjorn 2005, McIntyre and Flecker 2006) will not be affected by machine differences as differences are relative rather than absolute.

A recommendation by Jardine and Cunjack (2005) for authors to be more explicit in their reporting of isotope data, including precision and all treatments and additional sources of error and including whether or not multiple machines are used is supported by this study. Additionally authors are reminded always to ensure they include sufficient internal standards within analytical runs. These standards provide an indication of the precision of the machine but will also allow an independent check on variation across runs and between machines. If all analyses are planned for a single run, submission of standards is still recommended as it is common that a portion of samples need to be rerun due to machine error (samples not dropping, fully combusting or insufficient sample size.

3 Temporal variance in stable isotope ratios

3.1 Introduction

In conjunction, $\delta^{13}C$ and $\delta^{15}N$ isotopes can provide specific information about a consumer's diet. Consumers typically reflect similar $\delta^{13}C$ values to their diet, allowing ultimate sources or producers at the base of the food-web to be traced. There is a predictable trophic fractionation of approximately 3.4‰ in $\delta^{15}N$ values from diet to consumer for most organisms (Post 2002), allowing inferences about trophic level (TL) to be made. This biochemical method of TL estimation is more robust than traditional gut contents or scat analysis as isotopes provide a time-integrated measure of diet (Pinnegar and Polunin 2000). If there is a substantial change in the isotopic value of the diet over time or size then the usefulness of stable isotopes to determine TL will diminish. Significant temporal variation of the isotope value of a consumer's diet will occur where the consumer is switching prey types on a seasonal basis (Vizzini and Mazzola 2003) or where the dietary organism is constant but fluctuates in $\delta^{13}C$ and/or $\delta^{15}N$ values with time. Ultimately any temporal variability will be due to changes in the isotopic values of the autotrophs in the system caused by obtaining C and N from different source pools (Owens 1987).

$\delta^{13}C$ of marine plants (macroalgae and phytoplankton) is dependent on the $\delta^{13}C$ of the dissolved inorganic carbon of the surrounding waters and the plants' preference for use of CO_2 or HCO_3- for photosynthesis (Maberly et al. 1992). Algae that utilise CO_2 have a depleted ^{13}C whereas algae that utilise HCO_{3-} are typically more enriched. Most marine algae and phytoplankton range from -8‰ to -30‰ (Fry and Sherr 1989). Nitrate (NO^{3-}) and ammonium (NH^{4+}) are the two main pools of nitrogen for aquatic autotrophs (Dugdale and Goering 1967). Nitrate, enriched in ^{15}N (deep water nitrate has a relatively uniform signature of 4.8‰ + 0.2‰ (Sigman et al. 2000)) is the preferred nitrogen source for producers (Dugdale and Goering 1967). When available nitrate is exhausted, producers utilize recycled ammonia, resulting in a lower $\delta^{15}N$ signature (Michener and Schell 1994). Zooplankton $\delta^{15}N$ can vary markedly as part of a seasonal cycle in some temperate seas (Goering et al. 1990, Rolff 2000) and upwelling areas (O'Reilly et al. 2002). In addition, the phytoplankton upon which zooplankton feed can vary in species composition

shifting from diatom to dinoflagellate dominance and vice versa, on a seasonal basis (Dauby et al. 1990).

Although the mechanisms of temporal variability in basal materials is well understood, it is not known to what extent $\delta^{15}N$ variability is propagated or attenuated in marine food webs. The magnitude of the basal variability and the size, growth, trophic position and tissue turnover rate of the consumer are all likely to influence the way in which consumer's isotopic signatures are affected.

Given enough time, a new isotope signature will eventually be observed in a consumer's tissue after a change in the isotopic signature of the diet. In a non-growing organism this will be a relatively slow process relying solely on metabolic turnover (Hobson and Clark 1992, Tieszen et al. 1983). In a growing animal however, somatic growth may lead to an increased turnover rate (Perga et al. 2005). In fish, white muscle is the most commonly used tissue as the variability of isotope composition is low (Pinnegar and Polunin 1999). However, different tissues have different turnover rates and may be useful to describe diet changes over different time scales (Dalerum and Angerbjorn 2005). Active tissues such as blood and liver have high turnover rates (Logan et al. 2006, Sweeting et al. 2005); an isotope signature can be incorporated within days. Less metabolically active tissues such as muscle or bone collagen may only incorporate a change in isotope signature over a scale of months to years (Hesslein et al. 1993). Simultaneous analysis of multiple tissues may prove useful to help identify when an animal is likely to be in a steady state with its diet (MacNeil et al. 2005).

Turnover rates can be taxon-specific hence organisms or tissues with high turnover rates would be expected to exhibit some temporal variation in isotope signature while other organisms or tissues with slow turnover rates may attenuate variability in the basal signal. As trophic level increases, tissue turnover would be expected to decrease because growth rates are typically slower thereby resulting in a dampening and delay of any seasonal differences. Zooplankton are fast growing, have high turnover rates and short life histories and hence often have high seasonal variability of $\delta^{15}N$ signatures (Cabana and Rasmussen 1996, O'Reilly et al. 2002). Muscle tissue of a fully grown fish may take a few months for the new isotopic signature to be attained (Hesslein et al. 1993). Large variations in $\delta^{15}N$ of

invertebrates and small fish have been found with season (Vizzini and Mazzola 2003) but it is not known how larger fish respond to a diet that varies in isotope signature with time.

Two approaches have been pursued in order to correct for any basal variability when using stable isotopes to calculate TL. Firstly a primary consumer with a long life span can be used, such as gastropods or bivalve molluscs (Cabana and Rasmussen 1994, 1996, Jennings and Warr 2003, Vander Zanden et al. 1997). In the pelagic marine setting there are few such long-lived species suitable to constitute a baseline. The second method calculates the TL of higher consumers, using the mean isotopic signature of primary producers over a given time period as a baseline (Matthews and Mazumder 2005). In this case the magnitude and duration of the producer variability will affect TL estimates.

The Omani coastline, in the Arabian Sea, is subject to annual upwelling events between June and September, driven by the SW monsoon bringing large seasonal variations in temperature and nutrient availability (see chapter 1 and Quinn and Johnson 1996, Wiggert et al. 2005). It is hypothesised that the isotopic signatures of primary producers and consumers in the region will exhibit high seasonal variability and that any variability at the base of the food web will be attenuated with increasing trophic level. The study had the following objectives:

(i) determine the extent of seasonality in the $^{15}N/^{14}N$ isotope ratios of zooplankton and macroalgae.

(ii) assess the magnitude of seasonality in isotope signatures of a range of consumers using single and multiple tissues.

(iii) compare the temporal variability of basal materials with higher consumers to determine where attenuation of isotope signals occurs.

(iv) ascertain whether it is appropriate here to use an annual mean isotope signature of source materials as a baseline to estimate trophic position of fish.

3.2 Methods

3.2.1 Oceanographic Data

Satellite data were used to characterise the magnitude of the upwelling on the Omani coastline. Sea surface temperature and in-water chlorophyll-*a* concentrations from the Advanced Very High Resolution Radiometer (AVHRR) and Sea-viewing Wide Field-of-view Sensor (SeaWiFS) satellite were obtained from the Natural Environment Research Council (NERC) remote sensing data analysis service at Plymouth. The data were aggregated over 8 day windows during a two year time period from January 2004 to December 2005.

Chlorophyll-*a* data were averaged over a spatial grid of 23.78-23.49°N, 58.48-58.93°E. This area was chosen as smaller spatial resolutions close to Bandar Kayran were often obscured by cloud cover during the monsoonal months and did not return sufficient data for analysis.

3.2.2 Field Collections

All data were collected over a 14 month period (April 2004 – June 2005). Plankton tows were conducted from RV *Al-Jamiah* every two-four weeks. Triplicate zooplankton samples were collected using a 60cm diameter plankton net with a 200µm mesh towed at a constant speed (5km hr^{-1}) at about 5m depth within the bay of Bandar Kayran (23.49°N, 58.48°E, Fig 3.1). Planktonic material retained on the mesh was scraped into a vial and kept on ice. In the laboratory plankton samples were washed through two sizes of mesh filter, 200µm and 500µm, to attain two size classes of material; the samples were then kept frozen (-30°C). Samples of the pelagic fish *Sardinella gibbosa, Herklotsichthys quadrimaculatus, Sphyraena jello* and *Euthynnus affinis,* were purchased every three months from Muttrah fish market, Muscat. All fish were bought fresh and had been caught in the local area. Reef fish, invertebrates and algae were collected in triplicate every three months from a small reef in the Bay of Bandar Kayran (Site A; Fig 3.1). A range of species over a range of sizes and trophic groups were sampled to include 3 predators (*Cephalopholis hemistiktos*, *Scolopsis ghanam*, *Cheilodipterus macrodon*), 4 plankivores (*Chromis flavaxilla*, *Chromis xanthopterygia, Dascyllus trimaculatus, Dascyllus marginatus*), 3 herbivores (*Acanthurus sohal, Pomacentrus arabicus,*

Zebrasoma xanthurum) and 5 omnivores (*Cheilodipterus novemstriatus*, *Chaetodon nigropunctatus, Apogon cyanosoma, Abudefduf vaigiensis, Chaetodon melapterus, Parupeneus margaritatus*). Large fish were captured by spearing, smaller fish and invertebrates were collected by anaesthetising with a clove oil/alcohol mixture. All animals were killed immediately and placed on ice. Dominant macroalgae in the genera *Hypnea*, *Gracilaria* and *Sarconema* were hand picked from rocks and boulders then placed on ice. In the laboratory fork length (mm) and weight (g) were recorded for all fish; white muscle tissue was dissected from below the dorsal fin then frozen for $^{13}C/^{12}C$ and $^{15}N/^{14}N$ isotope analysis. Additional samples of liver tissue were taken for $^{13}C/^{12}C$ and $^{15}N/^{14}N$ isotope analysis from five randomly selected fish species (*C. melapterus, C. nigropunctatus, D. trimaculatus, P. margaritatus* and *S. ghanam*) during the last three sampling periods (November, February and May). Weight and length measurements were recorded for all invertebrates to ensure organisms were of a similar size during each sampling period. Where possible invertebrate muscle samples were removed and frozen for $\delta^{13}C$ and $\delta^{15}N$ isotope analysis; for smaller organisms (shrimps, amphipods and worms) the whole body was analysed. Algae samples were washed and any epiphytes and infauna were removed with forceps prior to freezing. All frozen materials were then prepared and analysed for C and N isotope ratios as described in Chapter 2.

3.2.3 Baselines for Trophic Level Estimation

To assess how effective the annual mean zooplankton signature was as a baseline it was compared with a seasonal mean of zooplankton baseline and a primary consumer *Barbatia decussata* baseline. The TLs of five consumers (*C. melapterus*, *C. nigropunctatus*, *D. trimaculatus*, *P. margaritatus* and *S. ghanam*) were calculated for each season (Equation 1.3) using each baseline. The annual mean $\delta^{15}N$ value for 200µm zooplankton was 10.62‰, seasonal mean $\delta^{15}N$ values of 200µm zooplankton consumer were, August= 9.62‰, November =11.22‰, February =9.02‰ and May =10.87‰ and the mean $\delta^{15}N$ of *Barbatia decussata* was 11.23‰.

3.3 Statistical analysis

The hypothesis that the isotopic composition of the fish varied as a function of species and season was tested using two-way ANOVA's with season (April, August, November, February and May, 5 levels) and species as fixed factors. In addition one way ANOVA's were used to test the null hypothesis of no significant differences in $\delta^{15}N$ and $\delta^{13}C$ of each species between seasons. Where this null hypothesis was rejected, a Fisher's *post hoc* comparison was used to compare the means in different seasons.

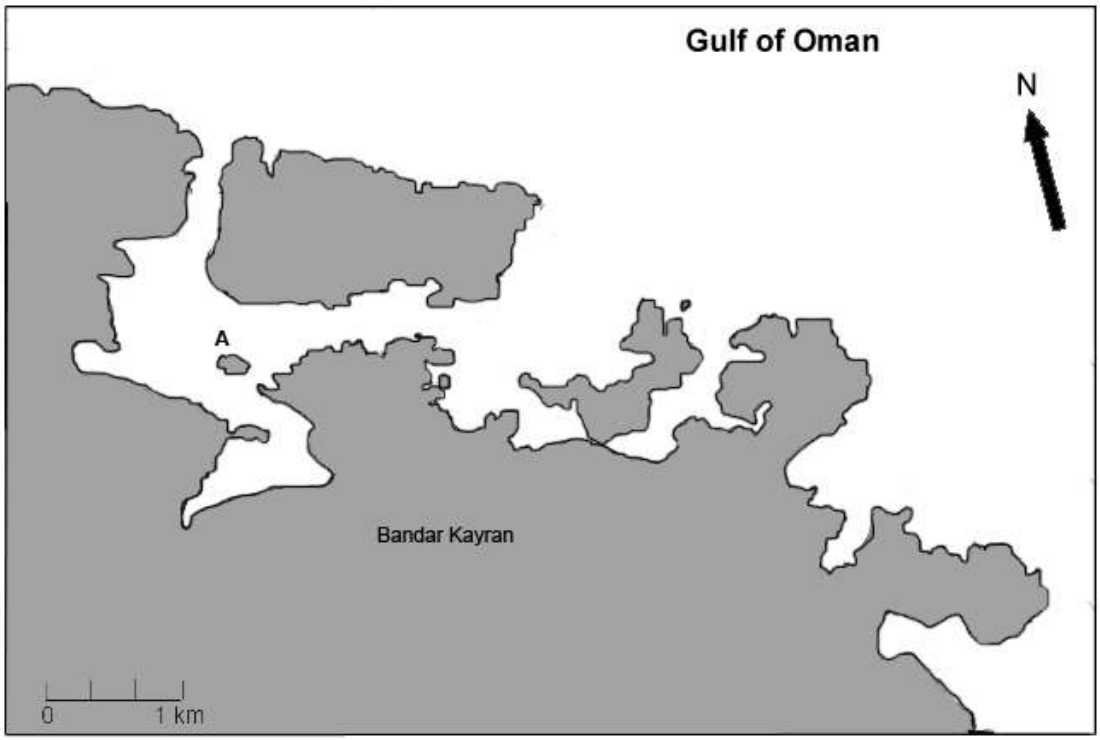


Fig. 3.1: Study site A at Bandar Kayran where collections were made. Plankton tows were conducted within the bay of Bandar Kayran adjacent to the reef area.

3.4 Results

3.4.1 Satellite data

The highest concentrations of chlorophyll-*a* were observed in September 2004 (<7.3mg chl-*a* m^{-3}) and Feb/March 2005 (<10.7mg chl-*a* m^{-3}) (Fig.3.2). Mean chl-*a* remained low (mean value <2mg chl-*a* m^{-3}) during other times in the sampling period.

Sea surface temperature (SST) exhibited an annual mean of 26.5°C and varied from a summer high of 31.8°C in July 2004 to a low of 22.8°C in February 2005 (Fig. 3.3). Key features of the seasonal cycle were the ten week period from 27/7/2004 to 8/11/2004 when the SST remained constant at ~28°C. This was followed by a steady decline in temperature to the February low of ~23°C.

3.4.2 Seasonality in $\delta^{13}C$ and $\delta^{15}N$ of pelagic plankton and fish

The 500µm size-fraction of zooplankton was enriched in $\delta^{15}N$ (annual mean =11.12‰) relative to the smaller 200µm size fraction (annual mean =10.59‰) (Figure 3.4a). Both size fractions showed similar seasonal variation. The maximum variation in $\delta^{15}N$ of the 200µm size class of zooplankton was 2.24‰, the most enriched values were observed in October (mean =11.26‰) and most depleted in February (mean =9.02‰). The $\delta^{13}C$ of zooplankton differed by a maximum of 5.2‰ throughout the year but the enrichment pattern was opposite to that of $\delta^{15}N$, being most depleted in November (mean = -21.92 ‰) and most enriched in March (mean = -16.79‰) (Figure 3.4b). Of the four pelagic fish species sampled, only one showed significant differences in $\delta^{15}N$, *Herklotsichthys quadrimaculatus* (ANOVA $F_{1,4}$=14.99, p=0.018). $\delta^{13}C$ varied significantly in three of the four species (Table 3.1).

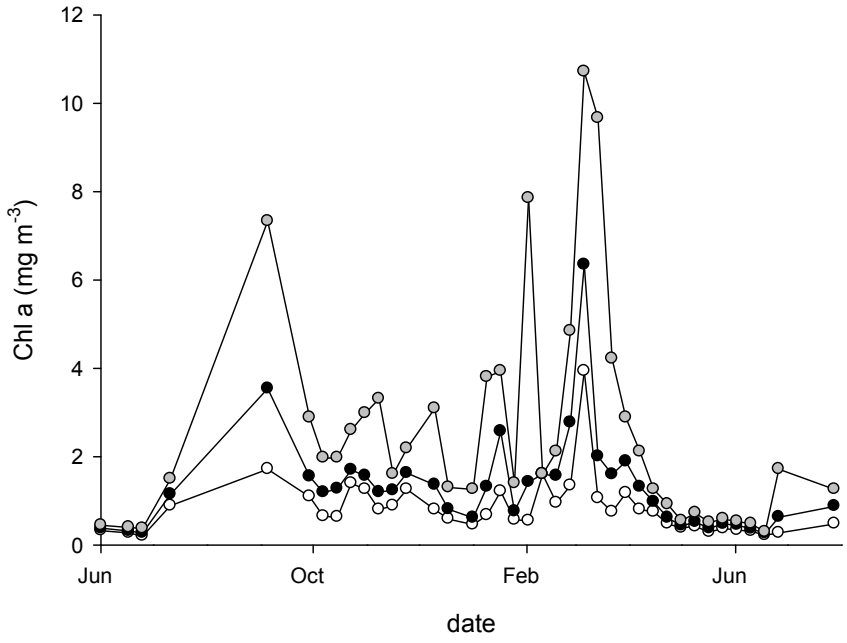


Fig. 3.2 Mean (black circles), minimum (white circles) and maximum (grey circles) chlorophyll-*a* concentrations (mg m^{-3}) at Bandar Kayran from June 2004 to July 2005. Data shown are 8-day aggregates of daily recordings. Data sourced from SeaWiFS satellite courtesy of RSDAS, Plymouth.

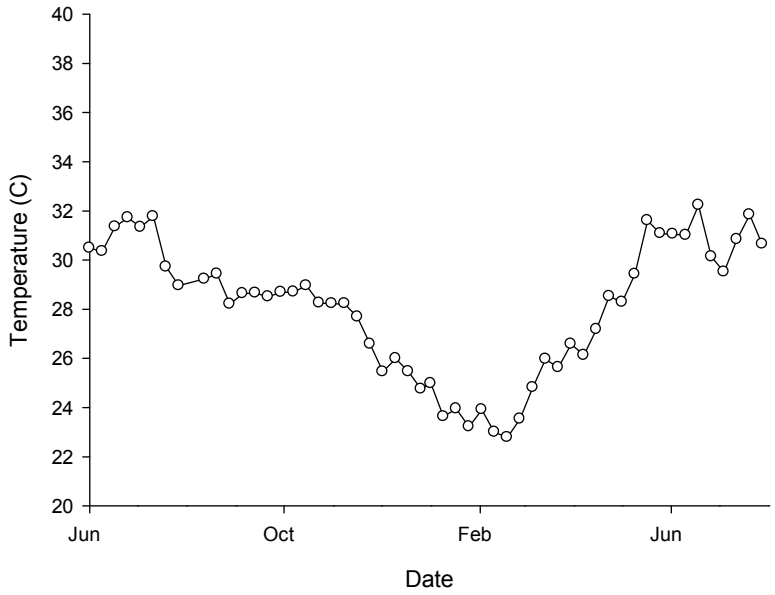


Fig.3.3: Mean weekly sea surface temperatures (°C) at Bandar Kayran from June 2004 to July 2005. Data shown are 8-day aggregates of daily recordings. Data sourced from AVHRR satellite courtesy of RSDAS, Plymouth.

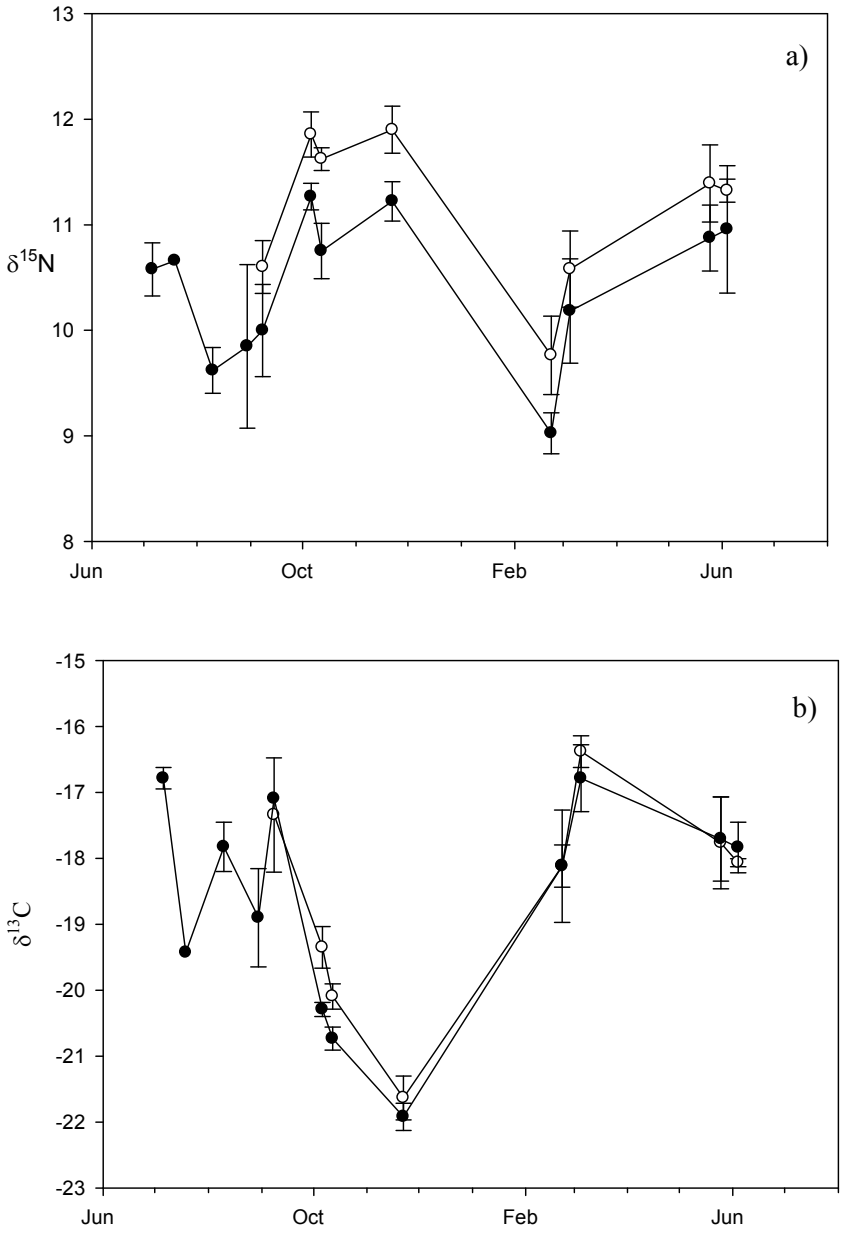


Fig. 3.4: Time series of (a) $\delta^{15}N$ and (b) $\delta^{13}C$ ($\pm$ SD) of zooplankton 200µm (black circles) and 500µm (white circles) collected at Bandar Kayran from June 2004 to June 2005.

Table 3.1: Seasonal variability in carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) isotope signatures (mean ± standard deviation) of fish species collected from Bandar Kayran quarterly from April 2004 to May 2005(n values are the total number of samples analysed).

		April		August		November		February		May	
		$\delta^{13}C$	$\delta^{15}N$	$\delta^{13}C$	$\delta^{15}N$	$\delta^{13}C$	$\delta^{15}N$	$\delta^{13}C$	$\delta^{15}N$	$\delta^{13}C$	$\delta^{15}N$
Fishes	n										
Abudefduf vaigiensis	15	-16.49 ± 0.41	14.60 ± 0.25	-15.42 ± 0.29	14.82 ± 0.52	-16.10 ± 0.32	14.92 ± 0.35	-16.39 ± 0.31	14.35 ± 0.45	-15.99 ± 0.44	14.78 ± 0.13
Acanthurus sohal	15	-12.41 ± 0.48	13.97 ± 0.11	-12.71 ± 0.22	12.91 ± 0.31	-12.54 ± 0.71	13.75 ± 0.71	-12.60 ± 0.81	13.02 ± 0.67	-12.34 ± 0.42	12.63 ± 0.65
Apogon cyanosoma	13			-14.64 ± 1.25	14.19 ± 0.04	-15.22 ± 0.24	14.89 ± 0.07	-16.46 ± 0.10	14.47 ± 0.51	-15.78 ± 0.37	14.58 ± 0.56
Cephalopholis hemistiktos	15	-14.36 ± 0.25	17.15 ± 0.10	-14.21 ± 0.46	16.86 ± 0.11	-14.54 ± 0.60	16.74 ± 0.08	-14.56 ± 1.75	16.30 ± 0.37	-15.38 ± 0.19	16.17 ± 0.34
Chaetodon melapterus	15	-11.53 ± 0.59	13.72 ± 0.25	-10.96 ± 0.41	13.89 ± 0.20	-10.85 ± 0.59	13.98 ± 0.24	-12.47 ± 1.38	13.75 ± 0.53	-10.39 ± 1.14	13.49 ± 0.89
Chaetodon nigropunctatus	14	-14.51 ± 0.12	15.89 ± 0.20	-13.58 ± 0.52	15.99 ± 0.32	-13.66 ± 0.25	16.29 ± 0.36	-14.06 ± 0.11	16.06 ± 0.22	-14.42 ± 0.82	16.22 ± 0.25
Cheilodipterus macrodon	15	-13.91 ± 0.23	16.99 ± 0.18	-13.41 ± 0.67	17.10 ± 0.41	-15.23 ± 0.84	16.48 ± 0.31	-14.43 ± 0.08	16.92 ± 0.36	-13.85 ± 0.11	16.47 ± 0.19
Cheilodipterus novemstriatus	15	-15.22 ± 0.10	14.15 ± 0.71	-14.33 ± 0.29	15.06 ± 0.17	-14.78 ± 1.89	13.99 ± 0.69	-13.64 ± 1.21	15.07 ± 0.48	-14.79 ± 0.16	13.80 ± 0.03
Chromis flavaxilla	13	-17.30 ± 0.17	14.44 ± 0.31	-17.17 ± 0.31	13.37 ± 0.45	-17.42 ± 0.11	14.50 ± 0.12	-18.80 ± 0.25	13.87 ± 0.06	-17.24 ± 0.13	14.40 ± 0.32
Chromis xanthopterygia	15	-16.80 ± 0.43	14.79 ± 0.07	-16.18 ± 0.16	14.56 ± 0.34	-16.75 ± 0.77	14.90 ± 0.81	-18.27 ± 1.31	13.80 ± 0.45	-16.85 ± 0.62	14.19 ± 0.42
Dascyllus marginatus	16	-16.36 ± 0.64	14.90 ± 0.28	-16.32 ± 0.27	15.30 ± 0.05	-17.27 ± 0.56	14.98 ± 0.42	-19.43 ± 0.19	15.26 ± 1.21	-16.50 ± 0.09	15.66 ± 0.21
Dascyllus trimaculatus	15	-15.79 ± 0.34	15.18 ± 0.08	-15.62 ± 0.23	15.47 ± 0.21	-16.77 ± 0.54	15.04 ± 0.22	-16.08 ± 0.20	15.40 ± 0.18	-15.85 ± 1.21	14.88 ± 0.63
Euthynnus affinis	12			-16.52 ± 1.75	16.54 ± 0.46	-16.89 ± 1.34	16.31 ± 0.09	-17.20 ± 0.90	15.62 ± 0.42	-15.47 ± 0.20	14.99 ± 1.31
Herklotsichthys quadrimaculatus	6			-17.33 ± 0.32	13.47 ± 0.40					-14.86 ± 0.48	14.55 ± 0.28
Istigobius decoratus	14	-14.06 ± 1.15	14.95 ± 0.27	-12.88 ± 0.69	14.92 ± 0.94	-13.94 ± 0.45	14.19 ± 0.74	-13.94 ± 0.35	14.34 ± 0.45	-14.22 ± 0.06	14.43 ± 0.56
Parupeneus margaritatus	15	-11.76 ± 0.34	15.93 ± 0.22	-13.58 ± 0.57	15.71 ± 0.06	-14.42 ± 0.18	15.85 ± 0.38	-14.91 ± 1.89	15.62 ± 0.79	-13.87 ± 0.46	15.90 ± 0.28
Pomacentrus arabicus	14	-15.16 ± 2.32	14.80 ± 0.47	-13.49 ± 1.37	14.68 ± 0.47	-13.29 ± 0.21	13.92 ± 0.87	-13.81 ±	13.57 ±	-13.60 ± 0.63	14.32 ± 0.64
Sardinella gibbosa	12			-16.12 ± 0.66	14.16 ± 0.25	-17.79 ± 0.38	14.50 ± 0.40	-17.38 ± 0.38	13.84 ± 0.77	-15.93 ± 0.44	14.39 ± 0.81
Scolopsis ghnam	15	-12.35 ± 0.46	17.13 ± 0.26	-12.66 ± 0.99	16.97 ± 0.17	-12.24 ± 0.66	17.02 ± 0.56	-11.71 ± 0.31	16.58 ± 0.17	-13.27 ± 0.48	16.52 ± 0.58
Sphyreana jello	11	-13.53	17.00	-15.31 ± 0.42	16.84 ± 0.87	-17.68 ± 1.13	16.03 ± 0.69	-18.54	15.75	-15.81 ± 1.34	15.89 ± 0.77
Zebrasoma xanthurum	15	-14.22 ± 0.42	13.68 ± 0.51	-14.82 ± 1.21	13.21 ± 0.54	-13.40 ± 0.38	13.32 ± 0.54	-13.35 ± 0.61	12.60 ± 0.65	-14.03 ± 0.97	13.87 ± 0.81

Table 3.2: Seasonal variability in carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) isotope signatures (mean ± standard deviation) of invertebrate samples collected from Bandar Kayran quarterly from April 2004 to May 2005 (n values are the total number of samples analysed).

		April		August		November		February		May	
Invertebrates	n	$\delta^{13}C$	$\delta^{15}N$	$\delta^{13}C$	$\delta^{15}N$	$\delta^{13}C$	$\delta^{15}N$	$\delta^{13}C$	$\delta^{15}N$	$\delta^{13}C$	$\delta^{15}N$
Alpheus spp	14	-14.97 ± 0.20	11.08 ± 0.3			-15.84 ± 0.16	11.34 ± 0.60	-16.49 ± 0.65	10.33 ± 1.58	-15.87 ± 0.61	12.74 ± 0.4
Barbatia decussata	15	-15.67 ± 0.17	11.52 ± 0.50	-15.05 ± 1.50	11.92 ± 2.51	-15.38 ± 1.99	12.11 ± 2.30	-16.12 ± 0.61	9.99 ± 1.28	-16.34 ± 0.35	10.59 ± 1.26
Bonellidae / Phylum Echiura	15	-13.30 ± 0.60	11.86 ± 1.41	-14.49 ± 1.10	12.77 ± 1.39	-15.56 ± 0.63	9.86 ± 0.21	-15.40 ± 2.73	10.06 ± 1.09	-15.32 ± 0.58	12.96 ± 0.38
Brittlestars *(Ophiurida)*	16	-7.91 ± 2.89	12.03 ± 1.50	-6.14 ± 0.44	12.28 ± 1.87	-7.75 ± 0.65	11.33 ± 0.65	-7.41 ± 0.77	14.44 ± 1.14	-5.69 ± 2.21	12.27 ± 1.86
Diadema setosum	13	-12.72 ± 0.48	9.08 ± 0.17	-13.54 ± 0.37	8.46 ± 0.32	-14.26 ± 1.46	8.39 ± 1.17	-12.91 ±	7.13 ±	-14.56 ± 0.65	8.69 ± 0.4
Goniopora spp	18	-13.17	10.34	-14.53 ± 0.60	10.67 ± 0.16	-13.34 ± 0.27	10.38 ± 0.28	-15.6 ± 1.2	10.70 ± 0.92	-14.37 ± 0.91	9.91 ± 0.6
Phymodius monticulosus	14	-11.65	11.06	-12.47 ± 1.40	10.63 ± 1.79	-13.98 ± 0.72	12.68 ± 1.74	-14.75 ± 0.01	12.94 ± 0.98	-11.63 ± 0.74	9.09 ± 1.52
Laevichlamys spp	15	-15.32 ± 0.03	11.86 ± 0.13	-14.97 ± 0.38	11.72 ± 0.17	-15.37 ± 0.52	11.20 ± 0.25	-16.28 ± 0.28	10.33 ± 1.06	-16.29 ± 0.82	10.78 ± 0.2
Pinctada margaritfera	12	-15.77 ± 0.20	9.44 ± 0.86	-15.63 ± 0.46	11.28 ± 0.11	-15.80	10.42	-15.86 ± 0.16	11.10 ± 0.42	-15.89 ± 0.27	10.93 ± 0.73
Polychaete	9			-14.75 ± 0.45	11.50 ± 0.48	-15.36 ± 0.23	14.16 ± 0.60			-16.13 ± 1.96	12.84 ± 1.4
Sponge	19	-16.53 ± 0.11	11.19 ± 0.23	-9.43 ± 0.43	6.61 ± 0.94	-10.24 ± 0.51	9.67 ± 0.81	-9.29 ± 0.93	7.90 ± 0.55	-17.85 ± 0.10	9.66 ± 0.3
Thais (Mancinella) alouina	15	-14.31 ± 0.86	14.29 ± 0.83	-14.12 ± 0.21	14.34 ± 0.37	-13.64 ± 0.35	14.68 ± 0.32	-13.92 ± 0.53	14.67 ± 0.05	-14.84 ± 0.33	14.41 ± 0.2
Trapezia cymodoce	15	-12.89 ± 1.48	11.67 ± 0.96	-15.07 ± 0.71	11.01 ± 0.8	-14.46 ± 0.97	9.84 ± 0.54	-15.36 ± 1.34	9.90 ± 0.69	-13.63 ± 1.25	10.30 ± 1.4
Urchin shrimp	12	-14.76 ± 0.28	13.08 ± 0.14	-14.65 ± 0.29	12.53 ± 0.91	-15.50 ± 0.44	14.40 ± 1.51	-15.50	13.08	-14.98 ± 0.29	13.21 ± 0.67
Xeniid gorgonian	14	-17.71 ± 4.62	9.42 ± 0.51	-17.12 ± 0.31	9.51 ± 0.82	-17.02 ± 0.65	11.70 ± 2.59	-17.17 ± 1.02	9.85 ± 0.78		

3.4.3 Seasonality in $\delta^{13}C$ and $\delta^{15}N$ of reef organisms

Macroalgae genera were most enriched in ^{15}N in November with a mean of 9.71‰ and most depleted in February with a mean of 7.02‰. $\delta^{13}C$ varied between genera and *Hypnea* was the only genus to show significant variation with season (Fig. 3.5).

The reef fish species ranged in size from the damselfish, *Dascyllus marginatus* (FL=30-45mm) to the grouper *Cephalopholis hemistiktos* (FL=290-395mm). Within each fish species there were no significant differences in the size of animals among sampling periods.

Overall the majority of $\delta^{15}N$ and $\delta^{13}C$ variation in fish and invertebrates was explained by species ($F=186.15$, $p<0.001$), although season ($F=4.17$, $p=0.003$) and the interaction of species and season were also significant ($F=2.94$, $p<0.001$). The mean $\delta^{15}N$ across all fish species was lower during February than at any other time of year (Table 3.3). At the individual species level, temporal variation in $\delta^{15}N$ with season was statistically significant in only two of the 17 reef fish species, namely *Chromis flavaxilla* and *Cephalopholis hemistiktos* (Table 3.4). Nine reef fish species showed significant differences in $\delta^{13}C$ throughout the year but there were no consistent patterns in $\delta^{13}C$ with any particular season (Table 3.1). Invertebrates had a greater range of $\delta^{15}N$ and $\delta^{13}C$ values than fish and varied from 8.53‰ (*Diadema setosum*) to 14.83‰ (*Thais aloui*) and in mean $\delta^{13}C$ from -6.98‰ (brittle stars) to -17.25‰ (Xeniid gorgonian) (Table 3.1). Five invertebrate species exhibited significant seasonal variation in $\delta^{15}N$ (*Bonella* sp*., Diadema* sp*., L. ruschenbergerii,* sponge and *Pocillopora* sp.) and five varied in $\delta^{13}C$ with season (*Goniopora* sp, *L. ruschenbergerii*, sponge, *Trapezia cymodoce*, urchin shrimp). *Post hoc* Fisher's tests suggested that mean $\delta^{15}N$ values were lower in February than during other sampling periods for these invertebrate species. $\delta^{13}C$ tended to be lower in November than during the summer months of May and August, although this result was less consistent among species.

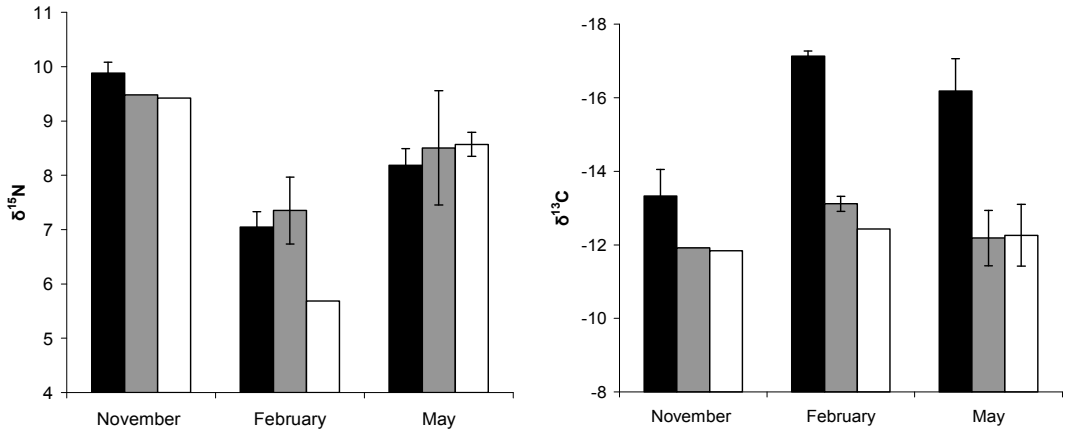


Fig. 3.5: Seasonal variations in mean (error bars represent ± 1SD) $\delta^{15}N$ and $\delta^{13}C$ for the macroalgae genera *Hypnea* (black, n=9), *Sarconema* (grey, n=9) and *Gracilaria* (white, n=5).

Table 3.3: Seasonal $\delta^{15}N$ and $\delta^{13}C$ values (mean ± standard error) of all fish species collected at Bandar Khyran between April 2004 and May 2005.

Season	$\delta^{13}C$			$\delta^{15}N$		
April	-14.52	±	0.28	15.13	±	0.17
August	-14.23	±	0.23	15.00	±	0.18
November	-14.54	±	0.26	14.95	±	0.17
February	-15.09	±	0.34	14.77	±	0.19
May	-14.57	±	0.25	14.87	±	0.17

Fish liver tissue had consistently lower $\delta^{15}N$ values but had higher and more variable in $\delta^{13}C$ relative to muscle tissue (Fig. 3.6). Neither $\delta^{15}N$ nor $\delta^{13}C$ of liver varied with season for the five sampled species.

3.4.4 Baselines for Trophic Level Estimation

Barbatia decussata and the annual mean zooplankton were more suitable as baselines than the seasonal mean zooplankton values. The seasonal mean zooplankton resulted in fish species varying in TL by up to 0.7 throughout the year, even though the consumers used here have been shown to be relatively constant in $\delta^{15}N$ with time (Table 3.5). *Barbatia decussata* gave slightly lower estimates of TL than the annual mean zooplankton value; however both estimates remained constant with season.

Table 3.4: ANOVA results for seasonal effects on $\delta^{15}N$ and $\delta^{13}C$ values of fish and invertebrates species at Bandar Kayran sampled between April 2004 and May 2005.

	$\delta^{15}N$			$\delta^{13}C$		
Species	F	df	p	F	df	p
Abudefduf vaigiensis	1.11	4,10	ns	4.06	4,10	<0.05
Acanthurus sohal	3.31	4,10	ns	0.2	4,10	ns
Apogon cyanosoma	1.76	3,8	ns	4.14	3,8	<0.05
Cephalopholis hemistiktos	7.31	4,10	< 0.05	0.82	4,10	ns
Chaetodon melapterus	0.71	4,10	ns	2.34	4,10	ns
Chaetodon nigropunctatus	0.85	4,9	ns	2.15	4,9	ns
Cheilodipterus macrodon	2.82	4,10	ns	5.95	4,10	<0.05
Cheilodipterus novemstriatus	3.36	4,10	ns	1.05	4,10	ns
Chromis flavaxilla	8.72	4,7	< 0.05	23.77	4,7	<0.001
Chromis xanthopterygia	2.68	4,10	ns	3.11	4,10	ns
Dascyllus marginatus	0.77	4,10	ns	30.81	4,10	<0.001
Dascyllus trimaculatus	1.73	4,10	ns	1.54	4,10	ns
Euthynnus affinis	2.83	3,8	ns	1.19	3,8	ns
Herklotsichthys quadrimaculatus	14.99	1,4	< 0.05	54.21	1,4	<0.001
Istigobius decoratus	0.52	4,9	ns	4.49	4,9	<0.05
Parupeneus margaritatus	0.29	4,10	ns	5.1	4,10	<0.05
Pomacentrus arabicus	1.26	4,8	ns	0.87	4,8	ns
Sardinella gibbosa	0.7	3,8	ns	10.96	3,8	<0.001
Scolopsis ghanam	1.47	4,10	ns	2.52	4,10	ns
Sphyraena jello	0.98	4,6	ns	5.11	4,6	<0.05
Zebrasoma xanthurum	1.9	4,10	ns	1.81	4,10	ns
Invertebrates						
Alpheus spp	4.15	2,6	ns	1.53	2,6	ns
Barbatia decussata	0.82	4,10	ns	0.62	4,10	ns
Bonellidae / Phylum Echiura	6.03	4,10	< 0.05	1.31	4,10	ns
Brittlestars *(Ophiurida)*	2	4,10	ns	1.44	4,10	ns
Diadema setosum	4.85	4,10	< 0.05	3.36	4,10	ns
Goniopora spp	1.15	4,10	ns	19.29	4,10	<0.001
Xeniid gorgonian	1.96	4,10	ns	4	4,10	ns
Laevichlamys ruschenbergerii	4.74	4,10	< 0.05	5.56	4,10	< 0.05
Phymodius monticulosus	3.41	2,6	ns	4.22	2,6	ns
Pinctada margaritfera	0.37	2,6	ns	0.62	2,6	ns
Pocillopora	30.52	1,7	0.001			
Polychaete	3.98	2,6	ns	0.64	2,6	ns
Sponge	24.15	4,10	<0.001	193.2	4,10	<0.001
Thais (Mancinella) alouina	1.72	4,10	ns	2.39	4,10	ns
Trapezia cymodoce	2.25	4,10	ns	5.67	4,10	< 0.05
Urchin shrimp	2.66	2,6	ns	5.35	2,6	< 0.05

Table 3.5: Seasonal trophic level estimates of 5 reef fish species at Bandar Kayran based on a seasonal zooplankton baseline, an annual mean zooplankton baseline and a primary consumer (*Barbatia decussata*) baseline. All samples were collected between April 2004 and May 2005.

Baseline	Baseline value	Season	*C. melapterus*	*C. nigropunctatus*	*D. trimaculatus*	*P. margaritatus*	*S. ghanam*
200 zooplankton	9.62	August	3.26	3.87	3.72	3.79	4.16
200 zooplankton	11.22	November	2.81	3.49	3.12	3.36	3.71
200 zooplankton	9.02	February	3.39	4.07	3.87	3.94	4.22
200 zooplankton	10.87	May	2.77	3.57	3.18	3.48	3.66
200 zooplankton annual mean	10.6	August	2.97	3.59	3.43	3.50	3.87
200 zooplankton annual mean	10.6	November	2.99	3.67	3.31	3.55	3.89
200 zooplankton annual mean	10.6	February	2.93	3.61	3.41	3.48	3.76
200 zooplankton annual mean	10.6	May	2.85	3.65	3.26	3.56	3.74
Barbatia decussata	11.23	August	2.78	3.40	3.25	3.32	3.69
Barbatia decussata	11.23	November	2.81	3.49	3.12	3.36	3.70
Barbatia decussata	11.23	February	2.74	3.42	3.23	3.29	3.57
Barbatia decussata	11.23	May	2.66	3.47	3.07	3.37	3.56

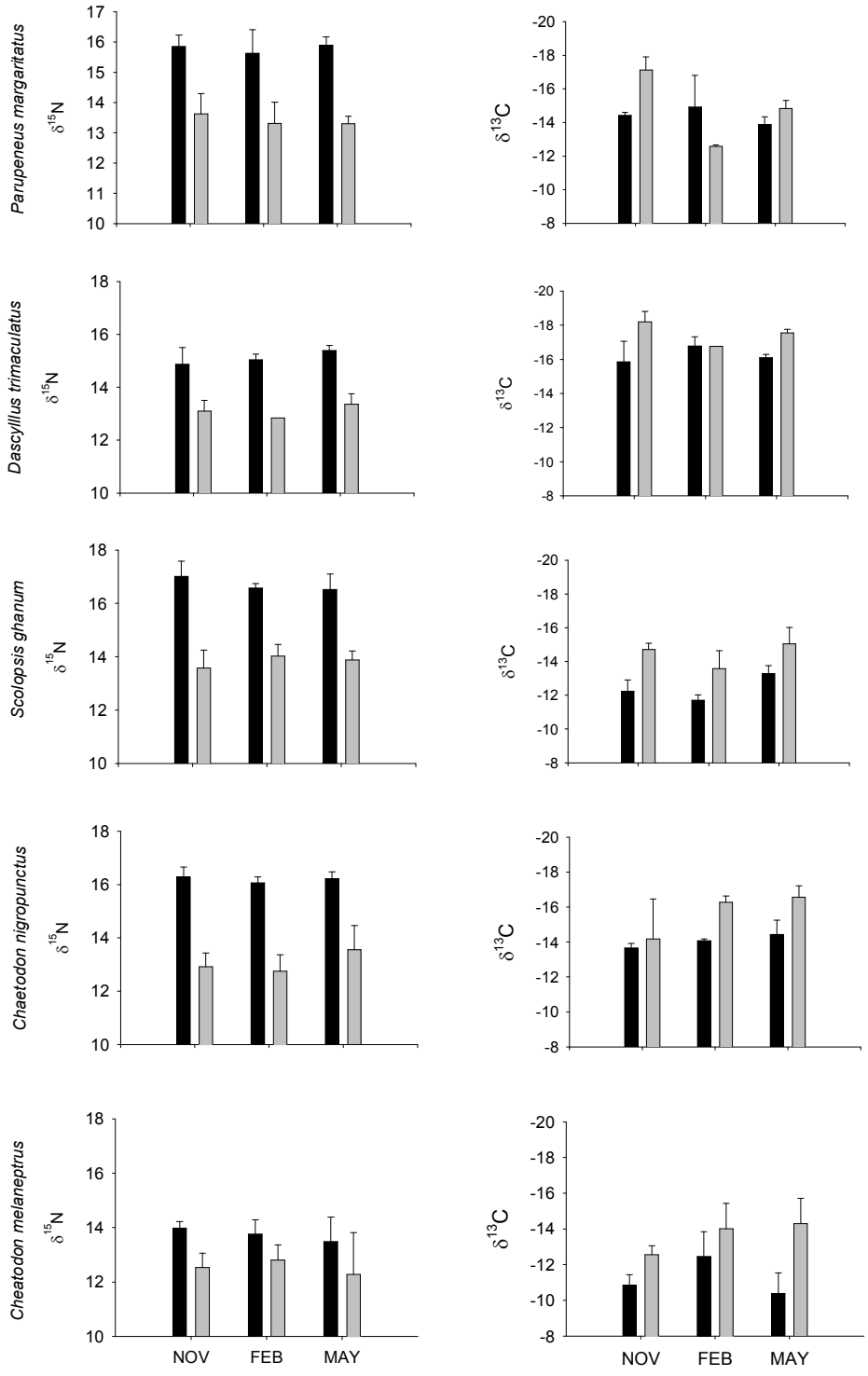


Fig. 3.6: Comparison of isotopic data (mean $\delta^{13}C$ and $\delta^{15}N \pm 1SD$) of muscle (black bars) and liver (grey bars) tissue for five reef fish species collected from Bandar Kayran between November 2004 and May 2005.

3.5 Discussion

The upwelling events in Oman result in marine primary producers drawing their N from different nitrogen pools throughout the year. By comparing zooplankton $\delta^{15}N$ and satellite data it can be concluded that the temporal variability of zooplankton and macroalgae $\delta^{15}N$ is strongly driven by the upwelling process, supporting the view of Michener and Schell (1994) that isotopic data can be used to characterise upwelling events. As the sea surface temperature dropped from ~31°C to 28°C during September, chlorophyll-*a* peaks at a maximum of 7.8mg chl-*a* m^{-3}, within the 5-20mg chl-*a* m^{-3} range previously published for this region by Savidge *et al*. (1990). Upwelling releases nutrients, including deep water nitrate, to the surface where they are readily utilised resulting in an increase in primary productivity. The influx of isotopically enriched nitrate is reflected in both zooplankton and macroalgae which both have $\delta^{15}N$ peaks during October, a slight lag behind the chl-*a* peak. The enrichment of zooplankton $\delta^{15}N$ is either due to a shift in the $\delta^{15}N$ of dietary phytoplankton reflecting a change in available nutrients or that the community composition of phytoplankton is shifting from isotopically depleted dinoflagellates to enriched diatoms (Casas et al. 1997, Dauby et al. 1990). However neither $\delta^{15}N$ nor species composition of phytoplankton were examined in this study. In either case the source shift in $\delta^{15}N$ is large enough to result in a 2.4‰ shift in the 200µm size class of zooplankton (mostly copepods) and a shift in the 500µm zooplankton of similar magnitude.

Macroalgae obtain nitrogen from the surrounding water column (McGlathery et al. 1996) and many species are able to uptake N in both ammonia and nitrate forms. Hence these producers will also reflect an increase in nitrate concentration in their isotopic values during upwelling. The seasonal shift of $\delta^{15}N$ in macroalgae was at least 2.7‰, very similar in magnitude to the shift in $\delta^{15}N$ of the zooplankton, again reflecting a major change in the underlying nutrient regime.

The large decrease in zooplankton $\delta^{13}C$ between September and December coincided with the period of elevated $\delta^{15}N$ levels in zooplankton. Phytoplankton obtain their carbon from dissolved inorganic carbon in the water column, or by sequestering bicarbonate. Upwelling will affect water carbonate chemistry such that the colder upwelled water will have a lower carbonate concentration (Peeters et al.

2002). Regions of high chlorophyll-*a* and low dissolved inorganic carbon are typical of upwelling areas, where the increased primary production leads to enriched ^{13}C in phytoplankton (Ostrom et al. 1997). Zooplankton sampled during the upwelling period in August and during March when chlorophyll-*a* values peaked, had higher $\delta^{13}C$ values than the rest of the year.

Overall it was expected that smaller primary consumers would exhibit more temporal isotopic variability in their tissues than their larger bodied high TL counterparts. The pelagic planktivorous food chain generally followed this pattern. Herring (*H. quadrimaculatus*) sampled in two periods (August and February) were significantly different, mirroring the shift in $\delta^{15}N$ of the zooplankton, however the sardine (*Sardinella* sp.) if anything, tended to decrease in $\delta^{15}N$ during this period. These two clupeid species have similar life histories and growth rates, the contrasting patterns in $\delta^{15}N$ may be a result of one species feeding in a slightly different location during part of the year or being more selective in choice of plankton prey. The larger pelagic species, *S. jello* and *E. affinis,* both top predators showed no significant change with season. These animals feed on pelagic prey, such as sardines and herrings, which are assumed to derive their N sources from purely planktivorous sources. These results suggest that attenuation of short-term isotopic variability in the planktonic food chain occurs at some point between these two trophic levels.

On the reef, the nitrogen stable isotope composition of fish species was notably enriched compared to similar species from coral reef locations elsewhere (Cocheret de la Moriniere et al. 2003, Nagelkerken et al. 2006, Schoeninger and DeNiro 1984). The high $\delta^{15}N$ values can be attributed to the elevated basal levels of $\delta^{15}N$ and are likely to be typical of consumers in upwelling areas. In general ^{15}N and ^{13}C values were enriched during summer (upwelling) months and depleted during winter (non-upwelling) months. Within the fish there were some systematic trophic responses to temporal variability whereby small zooplanktivorous species of fish tended to vary in $\delta^{15}N$ with season. However significant variability was not evident across all small-sized species but was observed in some large species, hence temporal variability of consumers is not simply a function of size.

Of the invertebrates that showed variation in $\delta^{15}N$ with season, two species are likely to have high dependence on benthic food sources (*Diadema* sp. and *Bonella* sp.) such as algae and detritus, while three are primarily planktivorous (*L. ruschenbergerii*, *Pocillopora* sp. and sponge). The lowest $\delta^{15}N$ values were observed in February for these five species, coinciding with lowest values of $\delta^{15}N$ recorded in the zooplankton and macroalgae. $\delta^{15}N$ of small fast-growing primary consumers such as corals varies spatially depending on the proximity of nutrients (Sammarco et al. 1999) and will tend to track temporal variations. Longer lived primary consumers such as bivalve molluscs can attenuate variability (Cabana and Rasmussen 1996). Over the time frame of this study tissue metabolism and/or the magnitude of temporal source variability was high enough for the scallop tissues to vary over time. The bivalve species also exhibited a lower $\delta^{13}C$ signature during February, suggesting a change in dietary source, perhaps indicative of a switch in preferred or available plankton species. A second, smaller bivalve species *Barbatia decussate* did not exhibit any temporal variability in either $\delta^{15}N$ or $\delta^{13}C$; clearly it cannot be assumed that all primary consumers will respond to seasonal changes in the same way.

Previous studies have noted that fish muscle $\delta^{15}N$ can vary temporally (Perga and Gerdeaux 2004, Vizzini and Mazzola 2003). Incorporation of dietary signatures into muscle tissue is dependant on the metabolic turnover rate and growth of the consumer; hence different fish species can be expected to respond to shifts in basal isotopic signature at different rates. Turnover rates are often determined in laboratory conditions using an artificially enriched diet therefore the magnitude of readjustment is much greater than would normally be observed for natural isotopic shifts. Wild animals are likely to feed on a greater mix of food items, which may mask any basal temporal variation, resulting in little or no apparent variability in the consumer's isotopic signature. An exception to this would be when all of the food sources are integrating temporal change at the same rate, which may be the case in a purely planktivorous food chain. The majority of tissue turnover has been attributed to growth of an organism (Harvey et al. 2002, Hesslein et al. 1993), although metabolic replacement of N has also been observed (Maruyama et al. 2001, McIntyre and Flecker 2006) with specific turnover rates being related to metabolic scaling and body size. Tissue turnover rates for fish are

estimated to be between three and six months for immature rapid-growing fish both in the laboratory (Hesslein et al. 1993, Sweeting 2004, Logan et al. 2006, McIntyre and Flecker 2006) and wild conditions (Maruyama et al. 2001). Wild adult populations are estimated to have muscle turnover rates of up to several years (Hesslein et al. 1993). The majority of the fish sampled in this study were within 70% of their L_{max} (Randall 1995) and it is unlikely that the tissue turnover rates would be less than three months and hence short term temporal variation in their diet is not expected to be apparent, particularly in regard to large herbivores and predators.

Liver tissue turnover in some fish can be as short as one month (Perga and Gerdeaux 2005), similar to findings in birds (Hobson and Clark 1992) and mammals (Tieszen et al. 1983), whereas Hesslein et al (1993) found fish liver turnover to be of the same rate as muscle tissue. Previous field studies have demonstrated that two tissues of differing turnover rates can be used to monitor trophic changes in an organism's diet (Dalerum and Angerbjorn 2005), whereby a change in the isotopic difference between the tissues over a given time scale reveals a short term change in diet (MacNeil et al. 2005). In this study the five fish species for which two tissues were sampled did not show seasonal changes in either liver or muscle tissue, nor was there any variance in the isotopic difference of the two tissues with season, indicating that these species were in equilibrium with the isotope ratios of their long-term diet even though they were characterised as feeding on different diets (e.g. *Dascyllus trimaculatus* feeds on plankton and small invertebrates and *Chaetodon melapterus* is a corallivore).

In addition to temporal changes due to upwelling, the Arabian Sea also has a seasonal temperature cycle with temperatures during November-February being 5-9°C colder than the summer temperatures. Metabolic rates are slower at colder temperatures (Clarke and Johnston 1999) and isotopic turnover rates increase with temperature in laboratory experiments (Bosley et al. 2002). Thus organisms can not be assumed to have the same metabolic rate all year round, and during colder periods fish may not turnover tissue at a sufficient rate to reflect recent diet changes (Perga and Gerdeaux 2005).

The annual mean of zooplankton $\delta^{15}N$ provided a good proxy for a baseline in the food web and proved a better estimate of TL than those estimated using seasonal averages of zooplankton $\delta^{15}N$ as a baseline. This analysis was only conducted for a few consumer species none of which showed any temporal variability in isotopic signature. It has been recommended that a long lived primary consumer will be a better baseline than zooplankton with fast turnover (Cabana and Rasmussen 1996). However when no such organisms are available zooplankton has been shown to be an effective baseline from which to characterise food webs (Pinnegar et al. 2003). Comparison of *Barbatia decussate* and the mean $\delta^{15}N$ of zooplankton only differed by 0.2‰, so here either would be acceptable.

3.6 Conclusions

$\delta^{15}N$ and $\delta^{13}C$ provide a good measure of the magnitude of temporal variability at the base of the food web. This was substantial especially in autotrophs and small primary consumers, which impacts the use of stable isotopes as a TL estimator for organisms at higher trophic levels. Some planktivorous consumers exhibited a seasonal shift in $\delta^{15}N$ values, although there was some dampening of the seasonal effect. In this study, fish of higher TL did not generally vary in $\delta^{15}N$ with season. There can be considerable variability in the strength and duration of upwelling events and the number of upwellings within a given year (Brock and McClain 1992, Coles 1997). While this study showed isotopic variation in fish species to be mainly present in planktivores, repeating the analysis over a more intense upwelling year might reveal temporal variability propagated further up the food web. The interpretation of temporal diet patterns using stable isotopes is reliant on further experimental studies to elucidate systematic differences in tissue isotopic turnover rates of consumers. At present it is not possible to predict how species will respond to seasonal variation without some knowledge of diet and life history.

4 Evidence of spatial variability in stable isotope signatures from coral reef organisms in the northwest Indian Ocean

4.1 Introduction

Within the marine environment the degree to which ^{15}N and ^{13}C isotope concentrations vary spatially depends on the location and on the local productivity regime (Owens 1987). Large scale marine studies have shown that there is an increase in phytoplankton $\delta^{13}C$ from polar to equatorial latitudes, which is especially pronounced in the southern hemisphere (Rau et al. 1982). In tropical and subtropical waters recycled ammonium, depleted in ^{15}N, is the main source of nitrogen for primary producers (Dugdale and Goering 1967). Secondary consumers are known to be relatively depleted in ^{15}N when ammonium is the main nitrogen source for producers (Mullin et al. 1984). A latitudinal pattern of $\delta^{15}N$ variation has been proposed whereby high latitude well-mixed and upwelling areas exhibit higher $\delta^{15}N$ values because nitrates are brought to the surface from the deep (Mizutani et al. 1991, Wada and Hattori 1991), whereas highly stratified tropical and equatorial waters tend to be depleted in ^{15}N because there is no replenishment of nutrients from deep waters and the only sources of nitrogen are recycled ammonium and nitrite.

Spatial differences in isotopic ratios in marine systems have been noted over a range of geographic scales. Within tropical latitudes coral tissue $\delta^{15}N$ varies by 6.6‰ globally, attributed to local conditions such as light intensity and sources of N rather than longitude or latitude *per se* (Heikoop et al. 2000). On the Great Barrier Reef near-shore corals exhibit higher $\delta^{15}N$ values where N is derived largely from terrigenous inputs, whilst lower $\delta^{15}N$ values were recorded further from the coast, where N is predominantly obtained from fixation by the symbiotic algae associated with scleractinian corals (Risk et al. 1994, Sammarco et al. 1999). A similar trend has also been observed in corals from the Caribbean (Mendes et al. 1996). Other isotopic gradients have been observed in coastal regions, especially within the vicinity of sewage treatment outflows, which are known to be depleted in ^{15}N (Gartner et al. 2002,

Gaston and Suthers 2004) and regions of cold water intrusions or land derived nutrification, typically enriched in ^{15}N (Sammarco et al. 1999).

If the environmental and oceanographic regime of an area has been well described then these factors can account for some of the isotopic variation (Vizzini et al. 2005). Jennings and Warr (2003) showed that, out of the environmental factors they measured, salinity and temperature were significant factors in explaining ^{15}N variability (4.18‰ to 11.0‰ $\delta^{15}N$) in scallops from the North Sea. In this way knowledge of spatial variation in stable isotope data can be applied to environmental management issues such as monitoring sewage outflows (Costanzo et al. 2005, Rogers 2003, Umezawa et al. 2002) and assessing autotroph inputs into estuarine (Melville and Connolly 2003) and seagrass/mangrove food chains.

A common application of stable isotope data involves the use of natural variations in ^{15}N and ^{13}C to describe migrations and feeding habits of animals over considerable geographical distances (Ainley et al. 2003, Hobson 1999, Takai et al. 2000). Differences in $\delta^{15}N$ of up to 3‰ have been found in reef fish of the same species and size at locations only a few kilometres apart (Jennings et al. 1997, Vizzini and Mazzola 2006, Vizzini et al. 2005). As fish species associated with reefs tend to be site attached, the food source of individuals usually comes from a very localized area. Any difference in $\delta^{15}N$ values in individuals of the same species at different sites is therefore attributable to differences in diet composition or dietary items being of different isotopic values (Thomas and Cahoon 1993). Comparison of trophic interactions using isotope data among sites that are subject to different nutrient regimes, and hence have different basal $\delta^{15}N$ signatures, can be difficult (Cabana and Rasmussen 1996). To compensate for any underlying differences in $\delta^{15}N$ at the base of the food web an organism common to both sites can be used to provide a comparable baseline (Matthews and Mazumder 2003, Post 2002). Primary producers which have high isotopic turnover rates, particularly phytoplankton, fluctuate greatly in $\delta^{15}N$ in space and time (see chapter 3) and are therefore not suitable baseline organisms (Cabana and Rasmussen 1996). Aquatic bivalves and gastropods are better candidates as they are slow growing and relatively long lived, which allows their body tissues to

assimilate any short term variability (Cabana and Rasmussen 1996, Post 2002, Vander Zanden and Rasmussen 1999). The $\delta^{15}N$ values of higher consumers can therefore be aligned by assessing any difference between sites compared to a common baseline organism.

The primary aim of this chapter is to determine the magnitude of variability in $\delta^{13}C$ and $\delta^{15}N$ of marine organisms across a range of spatial scales. The Northern Arabian Sea is subject to seasonal, periodic upwelling events driven by the onset of the SW monsoon (see chapters 1 and 3 and Michener and Schell 1994). These upwelling events are expected to result in a unique situation in Oman whereby coral reefs exist in highly productive seas and for part of the year dense macroalgae beds grow adjacent to the reefs. The North East coast of Oman is subject to less intense upwelling events and presents an opportunity to compare reef ecosystems with naturally different nutrient regimes.

Specifically this study set out to determine:

(i) how upwelling might influence $\delta^{15}N$ values of primary producers in Oman compared to other non-upwelling sites in the West Indian Ocean,

(ii) the impact of regional upwelling on $\delta^{15}N$ values and trophic level (TL) in coral-associated organisms, between two coral reef communities separated by a distance of 360 km and,

(iii) within an upwelling area, the variation in $\delta^{15}N$ and $\delta^{13}C$ in consumer species at three sites separated by 5 -10 kilometres.

4.2 Methods

4.2.1 Variability of $\delta^{15}N$ in autotrophs within the Indian Ocean

Macroalgal samples were collected from seven North-West Indian Ocean locations, Bandar Kayran, Barr al Hickman and the Hallaniyat islands in Oman, and

from one site each in Kenya, Mauritius, Chagos and Seychelles (Fig. 4.1) between November 2004 and February 2005. Six target species (*Gracilaria* sp., *Sarconema filiforme*, *Hypnea pannosa*, *Padina* sp., *Ulva lactuca* and *Turbinaria ornata*) were collected while snorkelling on shallow (3-6m) coral reefs. When the target algae were not present other locally abundant species were collected. Where possible whole plants were collected and included in the analysis as different parts of the plant can vary in both $\delta^{13}C$ and $\delta^{15}N$ (Fredriksen 2003). Samples were oven or air-dried depending on local facilities and returned to Newcastle where they were stored in a desiccator prior to isotope analysis.

4.2.2 Study Sites

The two main study sites in Oman were located at Bandar Kayran (BK) on the NE coast, near the capital Muscat and Bar al Hickman (BAH) on the South East coast of Oman (Fig. 1.1), both sites having significant coral growth (Sheppard and Salm 1988) yet quite different oceanographic regimes. Cold water upwelling events occur during summer months, and also to a lesser extent during the NE monsoon period in January and February. Temperatures in the winter are 22-24°C, while summer temperature fluctuates over the range 23-31°C, such fluctuations can also occur within a single day (Quinn and Johnson 1996). Salinity is constant at 36.5‰. Coral covers on the reef is about 40%; *Porites* sp. provides the reef structure and *Pocillopora* sp. is the dominant coral species (Sheppard and Salm 1988).

BAH is 360km south of BK. This study site is subject to more intense upwellings and the associated increase in nutrients during these greatly enhances the growth of macroalgae (Sheppard et al. 1992). Summer sea temperatures reach a maximum of 30°C but drop to 16-20°C during the upwelling in July and August (Rezai et al. 2004). The SE coast is not affected by the NE monsoon. The overall structure of the reefs here is similar to that of BK with similar depth profiles and coral species cover and dominance.

The comparison of closely-located reefs within the Gulf of Oman comprised three study sites: Bhandar Kayran (BK), Cemetery Bay (CB) and Bandar Jissah (BJ)

all situated within 10km of each other (Fig. 4.2). All three sites were similar in habitat, being shallow fringing coral reefs mainly composed of pocilloporid corals. All three sites experience similar temperature and salinity regimes with negligible terrigenous inputs as well as being subject to the same intensity of upwelling in the summer months (M. Clareboudt pers.obs.).

4.2.3 The impact of regional upwelling on TL calculation

Fish were collected from BK and BAH in November 2004 by spearing or by anaesthetising with a 30% clove oil and 70% alcohol mixture. Where possible the species collected were common to both sites (*Chaetodon melapterus, Istigobius decoratus, Lutjanus ehrenbergii*). However, because of the limited overlap in species distribution along the Oman coast (Randall 1995) many species were not represented at both sites; where possible, species were matched with another considered to be a functional equivalent from the same genus with similar feeding habits (Randall 1995). These pairings were as follows, *Abudefduf sordidus* and *A. vaigiensis, Apogon fluerieu* and *A. cyanosoma, Chaetodon dialeucos* and *C. nigropunctatus, Cheilodipterus arabicus* and *C. macrodon*, *Chrysiptera sheila* and *Dascyllus trimaculatus, Pomacentrus trilineatus* and *P. arabicus,* and *Scarus ghobban* and *S. persicus,* the latter in each case sampled at BK. *Pomacanthus maculosus* was present at both sites, but samples were taken during different seasons (May 2004 from BK and November from BAH), however there were no seasonal differences for species of this size (Chapter 3). Thus the data available from May 2004 from BK were used as a comparison with the same species' data from November 2004 at BAH. Replicate macroalgae and invertebrate samples of species common to both sites were also taken from the reef in the same vicinity as the fish. Algal genera included *Ahnfettia*, *Turbinaria*, *Ulva* and *Hypnea*.

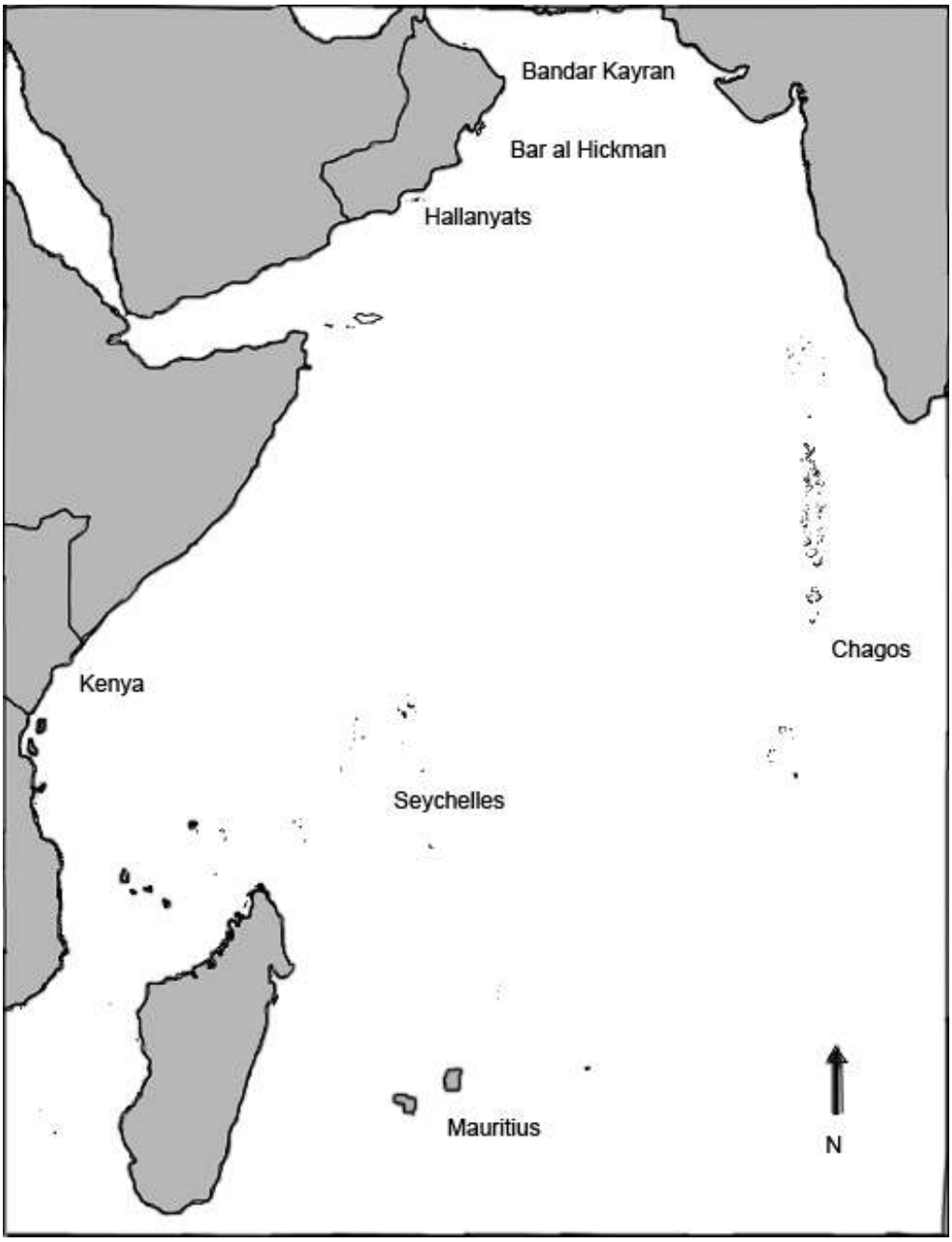


Fig. 4.1: West Indian Ocean locations of macroalgae collections

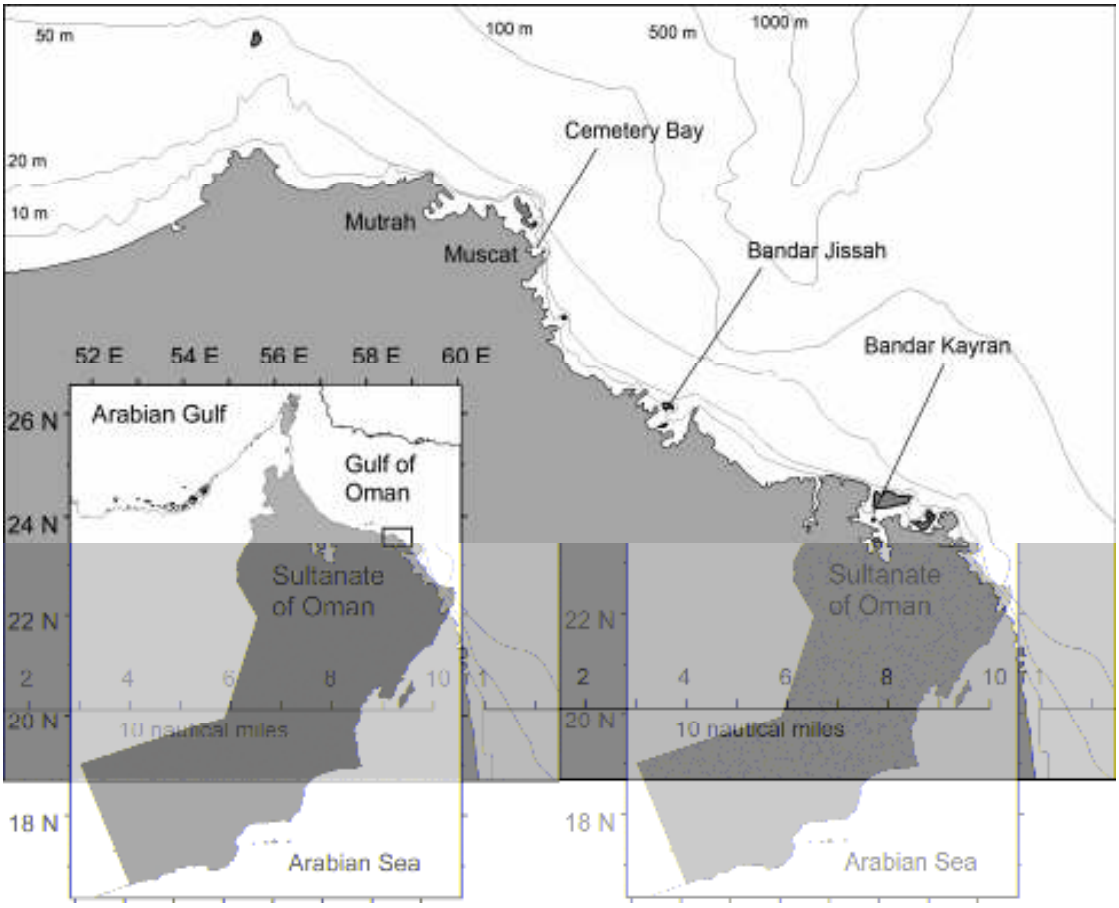


Fig. 4.2: Location of the study sites, Cemetery Bay, Bandar Jissah and Bandar Kayran in the Gulf of Oman

The invertebrates sampled encompassed a range of taxonomic groups including an echinoderm (brittlestar of the order: *Ophiuroidea* [unknown species]), a crustacean (*Phymodius monticulosus*), polychaetes (unknown species), a cnidarian (*Goniopora* sp.), a sponge (unknown species) and a mollusc (*Laevichlamys ruschenbergerii*). Three replicate zooplankton samples were collected by towing a 200μm plankton net (40 cm diameter) for 5 minutes behind a slow moving (~5 km h^{-1}) boat in water immediately adjacent to the reefs where fish and invertebrates were taken. All samples were immediately placed on ice. Upon return to the laboratory fish lengths and weights were recorded and a sample of white dorsal muscle was dissected. Invertebrates were dissected, taking muscle tissue where possible with the exception of gonad tissue from urchins. For small crustacean species and zooplankton, whole organisms were used. Algae were cleaned using forceps to remove any epiphytes from the surface of the thallus. All materials were stored frozen prior to preparation for isotope analysis. For the determination of $\delta^{15}N$ and $\delta^{13}C$ variation at a small (5km) scale, three samples each of five fish species (*Acanthurus sohal*, *Cephalopholis hemistiktos*, *Chaetodon melapterus*, *Chromis xanthopterygia* and *Pomacentrus arabicus*) were collected by spearing at each site. Stable isotope analysis was carried out as described in Chapter 2.

4.2.4 Trophic level calculation

Trophic level was calculated for each species at BK and BAH using equation 1.3. The bivalve mollusc *Laevichlamys ruschenbergerii* was selected as the baseline organism and assumed to be of TL 2.

4.2.5 Statistical analysis

Homogeneity of variance was tested with Cochran's test prior to two-way ANOVA. Length frequency distributions of fish were analysed using a Kruskal-Wallis test with the null hypothesis of no differences between sites. Two-way ANOVAs were used to test the null hypothesis that there were no significant differences in $\delta^{15}N$ and $\delta^{13}C$ within species, between sites and to quantify the variation both within and among sites. Tukey's post-hoc test was used for pair-wise comparisons of significant results.

4.3 Results

4.3.1 Variability of $\delta^{15}N$ in autotrophs on an Indian Ocean scale

Macroalgae $\delta^{15}N$ varied by 8.78‰ and $\delta^{13}C$ by 6.11‰ across the 7 locations in the West Indian Ocean (Table 4.1). The lowest $\delta^{15}N$ values were observed from Seychelles (*Padina* sp., 3.43‰) and Kenya (*Turbinaria* sp., 5.39‰) and the most enriched algae were *Ahnfettia* sp. (12.20‰), a locally abundant species at BAH, Oman. Only two genera were successfully sampled at all four locations, namely *Turbinaria* sp. and *Padina* sp.. All $\delta^{15}N$ and $\delta^{13}C$ values were normally distributed. There was no significant difference in $\delta^{15}N$ between genera (two-way ANOVA without replication $F_{1,7}$=0.65, ns) but $\delta^{15}N$ did vary between locations ($F_{3,7}$=14.2, p=0.028). Differences in $\delta^{15}N$ of algae (regardless of genera) were observed between locations ($F_{6,39}$=23.42 p>0.001). Post-hoc comparisons revealed differences between two of the sites in Oman (BK and BAH) and three of the southern sites (Seychelles, Chagos and Kenya). There were no statistical differences in $\delta^{13}C$ between locations.

4.3.2 The impact of regional upwelling on TL calculation

The $\delta^{15}N$ of fish species from BAH ranged from 15.53‰ to 18.43‰, generally higher than in fish sampled from BK (range 13.92‰ to 16.48‰ $\delta^{15}N$, Figure 4.5a). A similar pattern was found in the invertebrates common to both sites (Figure 4.5b). The $\delta^{15}N$ values of the bivalve mollusc, *L. ruschenbergerii* were 2.58‰, enriched at BAH compared to those at BK (Fig 4.5b). Using *L. ruschenbergerii* as a baseline, all samples from BAH were aligned to BK data by subtracting 2.58‰ from the $\delta^{15}N$ values, hence removing any spatial variation. The 'aligned' $\delta^{15}N$ values were not statistically different between sites except for *Scarus* spp. (Table 4.2). TL for all study organisms was calculated relative to the common baseline species, *L. ruschenbergerii*, at each site and only *Scarus* spp. showed a significant difference in TL between sites (Table 4.2). There was a notable difference in $\delta^{13}C$ of corals, zooplankton and algae between the two sites (Fig. 4.5a). At BK $\delta^{13}C$ ranged from -21.78 to -7.75 ‰ whereas at BAH these source materials occupied a smaller portion of this range, -18.30 to -9.31‰ $\delta^{13}C$. There were significant differences in $\delta^{13}C$ between sites for four of the

eleven fish species or pairings, *Istigobius decoratus, Apogon* spp*., Pomacentrus* spp. *and Abudefduf* spp. (Table 4.2) and two of the six invertebrates (*Phymodius monticulosus* and sponge). There was no difference in $\delta^{13}C$ between sites for the baseline scallop, *L. ruschenbergerii*.

4.3.3 $\delta^{15}N$ and $\delta^{13}C$ variation at a small scale (5 to 10 km)

There was no difference in total length in any of the five fish species between the sites Bandar Kayran, Cemetery Bay and Bandar Jissah (Kruskal-Wallis $p>0.01$). All species had a similar pattern of apparent trophic enrichment among the three sites; the herbivore *Acanthurus sohal* had the lowest $\delta^{15}N$ value whereas the predator *Cephalopholis hemistiktos* had the highest $\delta^{15}N$ value (Fig. 4.7). The pattern of mean $\delta^{13}C$ enrichment was the same at all three sites, the greatest difference being between the coral-feeding butterflyfish *Chaetodon melapterus* (-10.39 to -12.26 $\delta^{13}C$) and the planktivore *Chromis xanthopterygia* (-16.38 to -16.80 $\delta^{13}C$). Two of the five fish species (*Abudefduf vaigiensis* and *C. hemistiktos*) showed significant variation in $\delta^{15}N$ between sites (Table 4.3, ANOVA, $p<0.005$ for both species). The mean $\delta^{15}N$ was lowest for both species at Bandar Kayran compared to the other two closely located sites. No fish species differed in $\delta^{13}C$ between sites.

Table 4.1: Nitrogen and carbon isotope values of macroalgae (plus grand mean $\delta^{15}N$ and $\delta^{13}C$ ± SD) collected from 7 sites in the West Indian Ocean between November 2004 and February 2005.

		Hypnea	*Sarconema*	*Ulva*	*Gracilaria*	*Padina*	*Turbinaria*	*Ahnfettia*	*Halimeda*	*Caulerpa*	MEAN
$\delta^{15}N$ (‰)	Bandar Kayran, Oman	9.57	9.17	8.29	9.11						9.04 ± 0.54
	Bar Al Hickman, Oman					10.75	10.62	12.20			11.19 ± 0.88
	Hallaniyat Islands, Oman					8.38	7.87				8.13 ± 0.36
	Seychelles	6.51				3.43	6.18				5.37 ± 1.69
	Mauritius			9.76			5.90				7.83 ± 2.73
	Kenya	5.80	5.40	9.71		5.47	5.39				5.52 ± 0.19
	Chagos					6.64			7.50	4.09	6.08 ± 1.77
$\delta^{13}C$ (‰)	Bandar Kayran, Oman	-13.33	-11.84	-12.1	-11.92						-12.30 ± 0.70
	Bar Al Hickman, Oman					-9.88	-9.85	-18.35			-12.69 ± 4.90
	Hallaniyat Islands, Oman					-11.61	-12.78				-12.20 ± 0.83
	Seychelles	-18.37				-7.99	-10.55				-12.30 ± 5.41
	Mauritius			-13.3			-7.24				-10.29 ± 4.32
	Kenya	-12.56	-8.46	-8.63		-8.42	-12.67				-10.15 ± 2.25
	Chagos					-9.53			-9.36	-10.32	-9.84 ± 0.68

Table 4.2: ANOVA results for spatial comparison of fish and invertebrate species pairings at Bandar Kayran and Bar Al Hickman for $\delta^{15}N$ and $\delta^{13}C$ isotopes, aligned $\delta^{15}N$ values and trophic level estimates based on the aligned $\delta^{15}N$ data. All samples were collected in November 2004.$\delta^{15}N$ values were aligned using *Laevichlamys ruschenbergerii* as a baseline.

		$\delta^{15}N$		Aligned $\delta^{15}N$		$\delta^{13}C$		TL	
Species pairing	Code	F	p	F	p	F	p	F	p
Apogon fluerieu/ A. cyanosoma	AP	91.43	0.001	5.69	0.075	8.01	0.047	5.78	0.074
Chaetodon dialeucos/C. nigropunctatus	CH	22.81	0.009	4.17	0.111	1.85	0.245	3.3	0.144
Cheilodipterus arabicus/ C. macrodon	CE	42.93	0.003	0.51	0.515	7.71	0.05	0.51	0.516
Chrysiptera sheila/Dascyllus trimaculatus	DA	23.38	0.008	0.01	0.943	4.49	0.101	0.0	0.953
Istigobius decoratus	IS	34.1	0.028	5.28	0.148	61.58	0.016	5.88	0.136
Pomacanthus maculosus	PM	11.21	0.029	0.01	0.922	2.09	0.222	0.05	0.834
Pomacentrus trilineatus/ P. arabicus	PO	37.35	0.004	0.75	0.436	32.62	0.005	0.74	0.437
Scarus ghobban/ S. Persicus	SC	5.77	0.074	17.02	0.0015	0.02	0.888	15.27	0.017
Abudefduf sordidus/A. vaigiensis	AB	0.55	0.535	4.04	0.1825	111	0.009	4.01	0.183
Lutjanus ehrenbergii	LU	6.69	0.061	1.93	0.237	0.31	0.609	1.59	0.276
Brittlestars	BR	1.13	0.348			4.03	0.115	2.72	0.174
Laevichlamys ruschenbergerii		235.71	0.004			8.66	0.099	3.6	0.198
Phymodius monticulosus	PH	1.38	0.306			22.82	0.009	0.6	0.483
Polychaete	PO	3.68	0.195			0.6	0.521	0.88	0.448
Sponge	SP	18.28	0.013			155.7	0.000	0.08	0.796

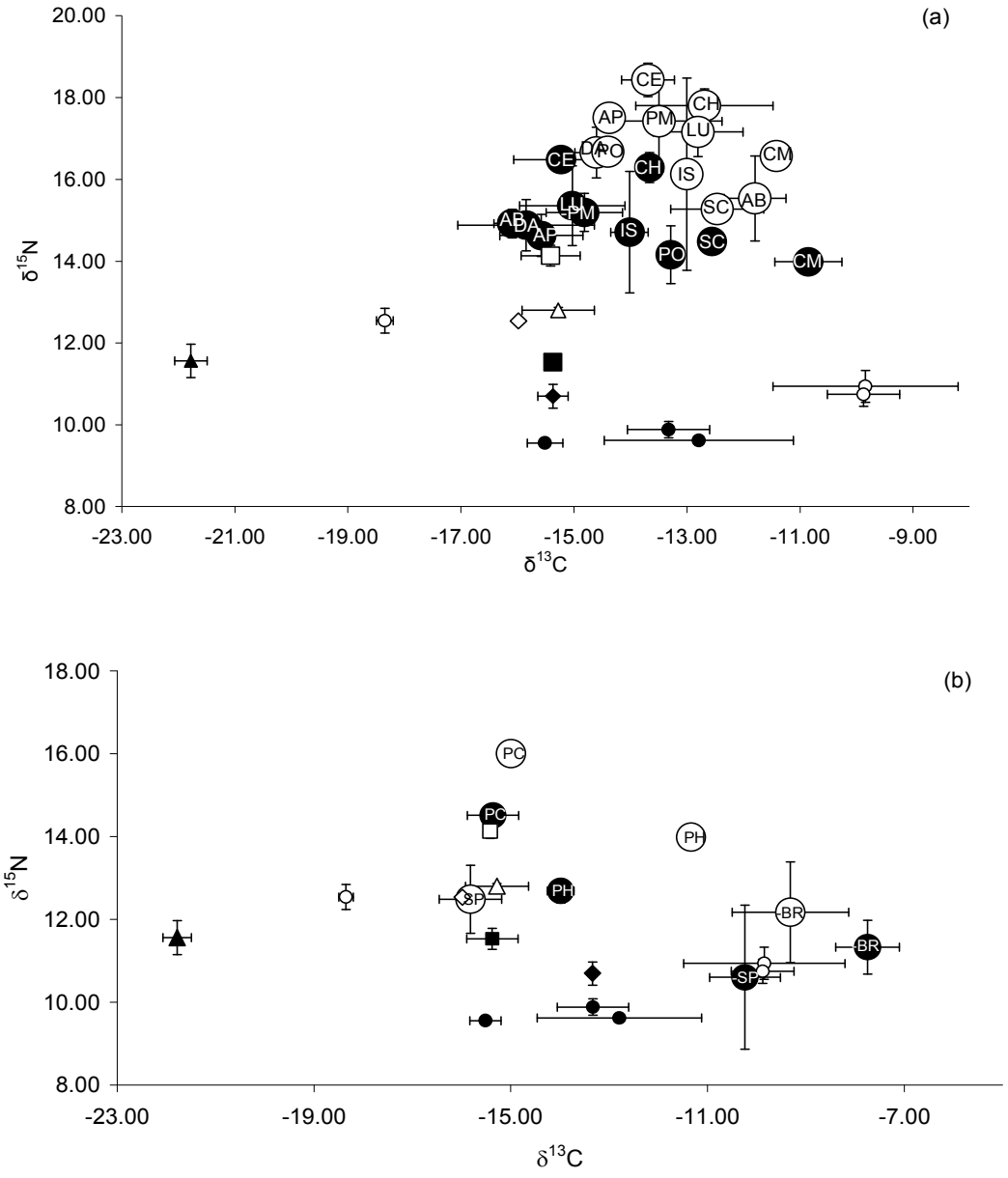


Fig 4.3: $\delta^{13}C$ and $\delta^{15}N$ bi-plots of non-aligned data for a) fish and b) invertebrates from Bandar Kayran (filled shapes) and Bar Al Hickman (empty shapes), error bars represent $\pm$ 1SD. For species codes see Table 4.2. Autotrophs are represented by different shapes. Zooplankton (triangles), coral (diamonds) and algae (small circles).

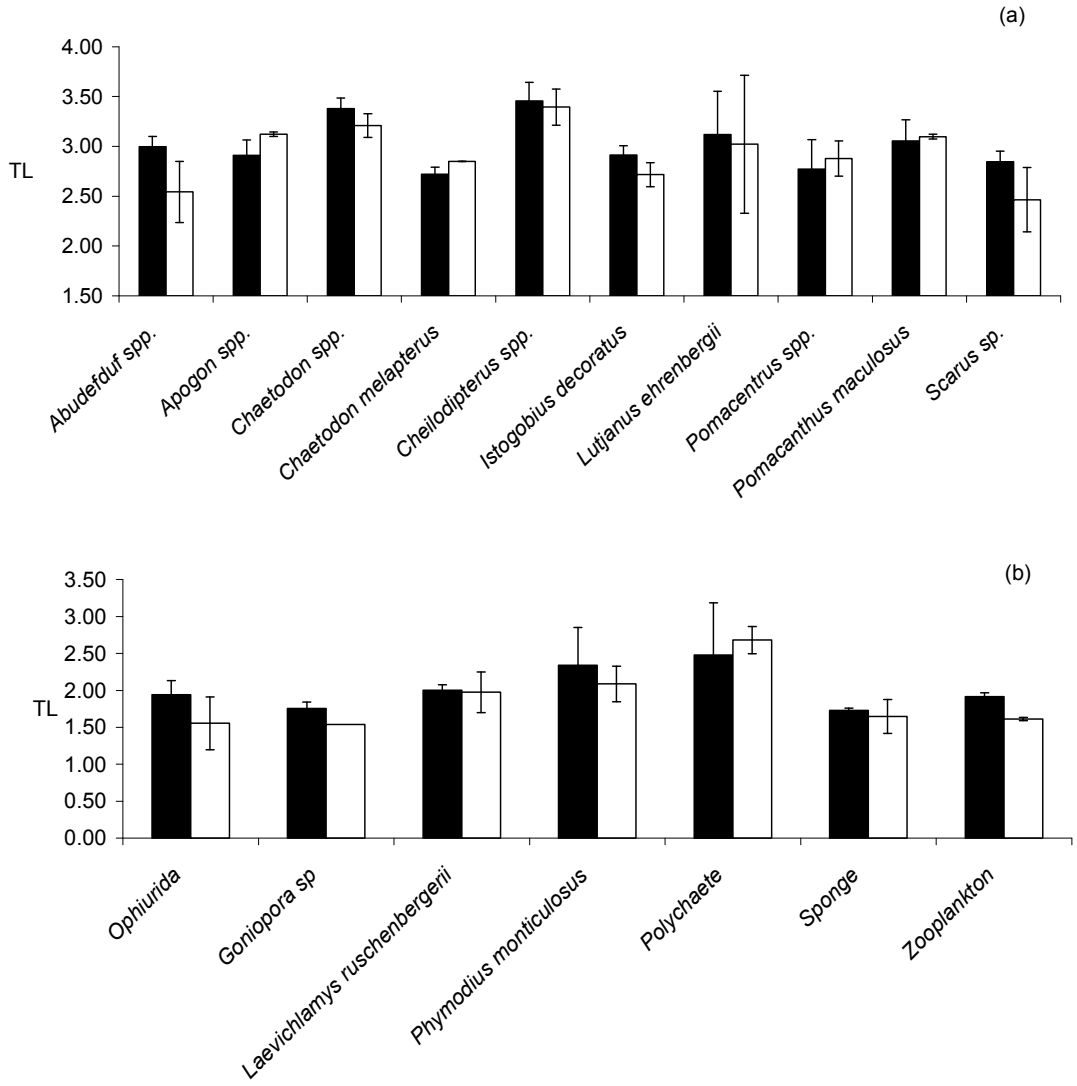


Fig 4.4: Trophic level estimates (+ 1 SD) of (a) fish species and (b) invertebrates at Bar Al Hickman (white bars) and Bandar Kayran (black bars). Trophic level was estimated based on aligned $\delta^{15}N$ values using the bivalve, *Laevichlamys ruschenbergerii* as a baseline.

Table 4.3: Results of one-way ANOVAs testing the null hypothesis of the no significant differences in $\delta^{15}N$ and $\delta^{13}C$ of muscle tissue among sites (Bandar Kayran, Cemetery Bay and Bandar Jissah) for each of the five fish species.

	$\delta^{15}N$						$\delta^{13}C$				
		df	SS	MS	F	p		df	SS	MS	F	p
A. vaigiensis	site	2	0.54	0.268	15.4	0.00	site	2	0.03	0.01	0.18	0.838
	error	6	0.1	0.017			error	6	0.46	0.08		
	total	8	0.64				total	8	0.49			
A. sohal	site	2	2.37	1.183	1.66	0.28	site	2	0.06	0.03	0.23	0.799
	error	5	3.56	0.712			error	5	0.64	0.13		
	total	7	5.93				total	7	0.7			
C. hemistiktos	site	2	2.87	1.435	12.61	0.01	site	2	1.41	0.7	0.91	0.451
	error	6	0.68	0.114			error	6	4.63	0.77		
	total	8	3.55				total	8	6.06			
C. melapterus	site	2	1.25	0.626	1.59	0.28	site	2	6.52	3.26	4.82	0.056
	error	6	2.37	0.394			error	6	4.06	0.68		
	total	8	3.62				total	8	10.6			
C. xanthopterygia	site	2	0.49	0.247	1.38	0.32	site	2	0.32	0.16	0.94	0.44
	error	6	1.08	0.179			error	6	1.03	0.17		
	total	8	1.57				total	8	1.35			

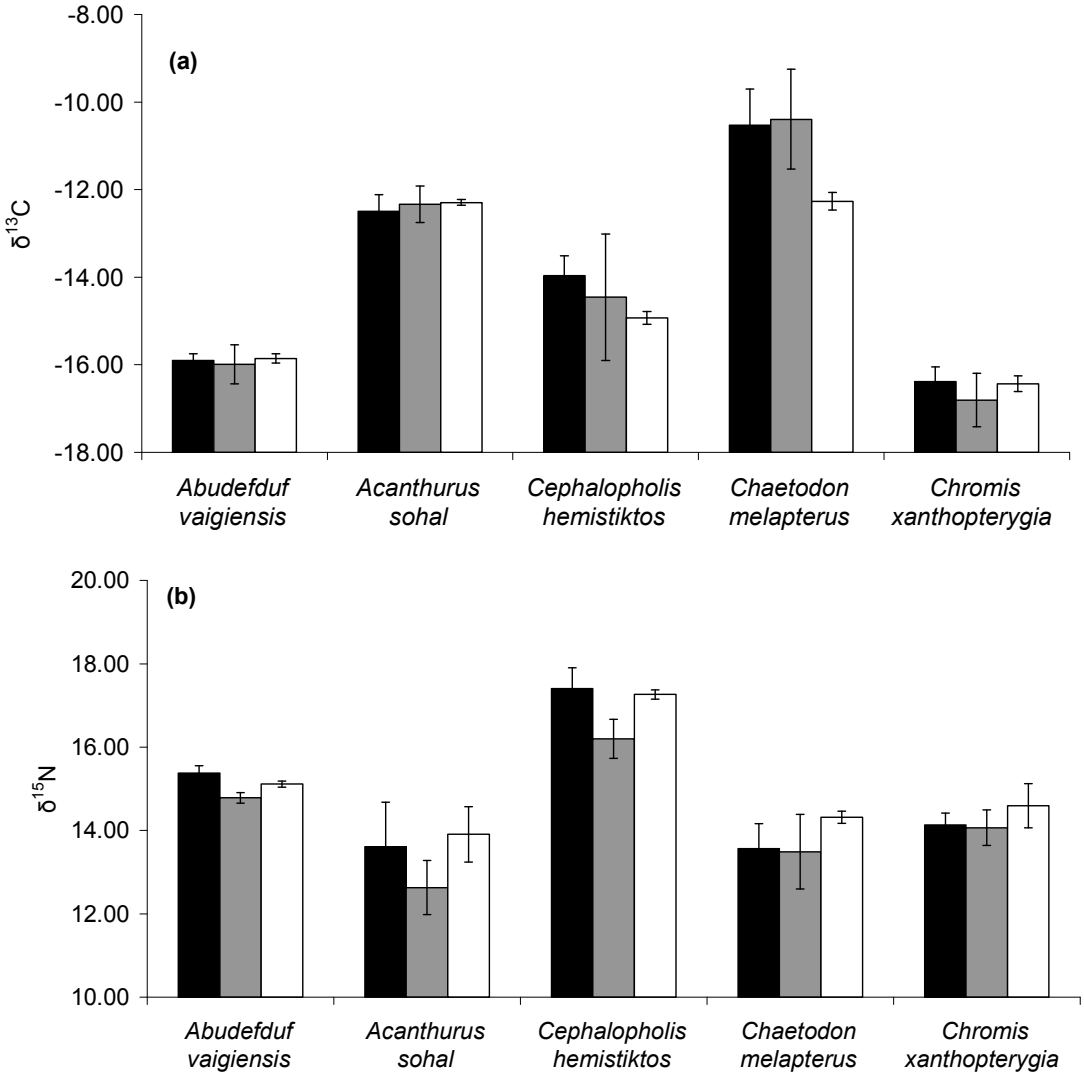


Fig. 4.5: The (a) carbon and (b) nitrogen isotope data (mean $\delta^{13}C$ and $\delta^{15}N$ $\pm$1SD) of five fish species at three reef sites in the Gulf of Oman, Bandar Kayran (black bars), Bandar Jissah (grey bars) and Cemetery Bay (white bars), each separated by 5km. All samples were collected in May 2005.

4.4 Discussion

Overall the $\delta^{15}N$ values of coral reef communities in Oman were elevated in comparison to reef communities in other geographic locations (Cocheret de la Moriniere et al. 2003, Nagelkerken and van der Velde 2004, Sammarco et al. 1999). A degree of spatial variation in stable isotope ratios was observed across all three spatial scales examined. The greatest spatial differences were noted in primary producers and over large distances (>300km). Isotopic differences were less substantial in consumer species across small distances (5-10 km). The high $\delta^{15}N$ values observed in this study can be attributed to the increased availability of enriched nitrate introduced by upwelling events in Oman. $\delta^{15}N$ values of primary producers decreased with distance from the main upwelling region on the SE Arabian coast.

Macroalgae from sites in Oman were as much as 7.5‰ enriched in ^{15}N compared to all other study sites in the West Indian Ocean. The enrichment pattern from south to north found in some seas or oceans (Mizutani et al. 1991, Takai et al. 2000, Wada and Hattori 1991) was not evident in the macroalgae sampled in the West Indian Ocean. The macroalgae in Oman and at Bar al Hickman in particular, derive their nitrogen from an enriched nitrogen source compared to macroalgae at the other Indian Ocean reef sites. Comparison of this data with previously published macroalgae $\delta^{15}N$ values throughout the world reveals a pattern in enrichment which mirrors the general nutrient pattern of the surrounding seas, where increased nutrients result in increased $\delta^{15}N$ (Table 4.4). Oligotrophic sites (Corsica and Mallorca in the Mediterranean Sea and Belize and Puerto Rico in the Caribbean Sea) are the most depleted in $\delta^{15}N$ (0-3‰) (France et al. 1998, Jennings et al. 1997, Lepoint et al. 2000, Pinnegar and Polunin 2000). The coral reef sites in the West Indian Ocean except Oman have macroalgae $\delta^{15}N$ values of a similar range to the majority of other coral reef sites (3-6‰) in the Caribbean (Abed-Navandi and Dworschak 2005, France et al. 1998, Keegan and Deniro 1988) and Indo-Pacific (Table 4.4)(Thomas et. al., unpubl., Yamamuro et al. 1995, Polunin and Heaton, unpubl.). Well-mixed temperate shelf sites (Artic, Antarctica, Southern Australia and New Zealand) again have slightly higher values (6-8‰) than those previously mentioned (Davenport and Bax 2002, Fredriksen 2003, Harrigan et al. 1989,

Hobson and Welch 1992, Moncreiff and Sullivan 2001, Rogers 2003, Smit et al. 2006). The high $\delta^{15}N$ values of macroalgae from Oman were of the same magnitude (9-12‰) as those of Christmas Island in the Pacific (Polunin and Heaton unpubl.) and of the two studies on the Chilean coast (Barrett et al. 2005, Rodriguez 2003), all of which experience upwelling (Table 4.4). These high $\delta^{15}N$ values are also comparable with those observed in the vicinity of sewage outfall areas (e.g. Gartner et al. 2002). Studies where sites were impacted by excessive land run off, experimental studies or studies that set out to monitor sites impacted by sewage were not included in the global analysis as these factors are known to impact $\delta^{15}N$ (Cohen and Fong 2005, Cole et al. 2004, Rogers 2003).

At the intermediate spatial scale, the reef communities located at Bar al Hickman and Bandar Kayran differed in species composition across some of the taxonomic groups collected for this study. The two sites, are separated by the Ras Al Hadd jet, an oceanographic feature that is capable of exporting cool, upwelled water, rich in nutrients, hundreds of kilometres offshore (Kindle and Arnone 2001, Wiggert et al. 2005)(Chapter1). The location of this feature at Ras Al Hadd, has been described as the geographic landmark with the sharpest transition zone for marine algae (Schils and Wilson 2006), which may also explain the observed differences in community structure. The ^{15}N enrichment of the biota studied at Bar Al Hickman compared to that at Bhandar Khyran is likely to be a result of the upwelling events that occur regularly along the Arabian coastline due to the southwest monsoon (Sheppard et al. 1992) (SEAWIFS data, Figs. 1.2 and 1.3). Nitrates, enriched in ^{15}N, trapped below the thermocline and returned to the surface waters during upwelling events, becomes the preferential N source for phytoplankton and macroalgal primary producers (Mullin et al. 1984). This enrichment of ^{15}N in primary producers is then propagated up the food chain. The Bandar Kayran $\delta^{15}N$ values were higher in comparison to other sites globally, but slightly lower compared to those of Bar Al Hickman, which reflects the varying influence of upwelling events at the two locations (Glynn 1993). Bhandar Kayran lies in a more protected region of coastline in the Gulf of Oman where the upwelling is less intense (Sheppard et al. 1992).

Table 4.4: Location and δ15N values of macroalgae worldwide. *Nutrient Status codes O = Oligotrophic, CR = Coral Reef, WM = Well Mixed, UP = Upwelling. NR = not reported.

Study	Site	Nutrient status *	N	E	n	Mean $\delta^{15}N$	SD
France et al (1998)	Puerto Rico, Caribbean Sea	O	18.50N	66.10W	NR	0.30	NR
Lepoint et al (2000)	Corsica, Mediterranean Sea	O	42.58N	8.75E	NR	1.80	NR
Abed-Navandi and Dworschak (2005)	Belize, Caribbean Sea	O	17.00N	88.00W	1	1.90	0.11
Pinnegar and Polunin (2000)	Corsica, Mediterranean Sea	O	42.58N	8.75E	6	2.07	0.64
Polunin and Heaton (unpubl.)	Moorea Island, French Polynesia, Pacific Ocean	CR	17.53S	149.83W	2	2.14	1.39
Yamarmuro et al (1995)	Palau, Pacific Ocean	CR	24.40S	124.00E	6	2.56	1.79
Jennings et al (1997)	Mallorca, Mediterranean Sea	O	39.56N	2.41E	3	2.74	0.55
Polunin and Heaton (unpubl.)	Kadavu (dravuni), Fiji, Pacific Ocean	CR	19.3S	178.25E	2	2.75	0.70
Thomas, Kulbeki and Polunin (unpubl.)	Nuemea, New Caledonia, Pacific Ocean	CR	20.00S	166.00E	1	3.14	NR
Keegan and DeNiro (1988)	Turks and Caicos Islands, Caribbean Sea	CR	22.20N	71.50W	4	3.70	2.24
Dunton (2001)	Antarctica, Southern Ocean	O	64.00S	64.78W	20	4.57	2.33
This study	Seychelles, Indian Ocean	CR	4.75S	55.55E	3	5.37	1.69
Fredriksen (2003)	Norway, North Sea	WM	63.15	7.45E	9	5.43	0.27
This study	Kenya, Indian Ocean	CR	4.19S	39.70E	5	5.52	0.19
This study	Chagos, Indian Ocean	CR	7.14	72.24E	3	6.08	1.77
Smit et al (2006)	Western Australia, Southern Ocean	WM	32.07S	115.67E	3	6.27	0.68
Harrigan et al (1989)	Schooner bank, Florida bay	WM	25.00N	81.00W	2	6.40	1.34
Davenport and Bax (2002)	SE Australia	WM	38.00S	149.00E	1	6.60	NR
Moncreiff and Sullivan (2001)	Gulf of Mexico, USA	WM	30.25N	88.75W	4	7.00	2.82
Hobson and Welch (1992)	Arctic	WM	75.21N	95.00W	4	7.40	1.00
Rogers (2003)	Wellington, New Zealand	WM	41.38S	174.72E	1	7.70	NR
This study	Mauritius, Indian Ocean	CR	20.18S	57.72E	2	7.83	2.7
This study	Hallanyat Islands, Oman	UP	17.57N	56.04E	2	8.13	0.36
This study	Bhandar Khyran, Oman	UP	23.49N	58.48E	4	9.04	0.54
This study	Barr al Hickman ,Oman	UP	20.35N	58.26E	3	11.19	0.88
Rodriguez (2003)	Central Chilean Coast	UP	33.58S	71.70W	9	10.82	0.95
Polunin and Heaton (unpubl.)	Christmas Island, Kiribati, Pacific Ocean	UP	1.88N	157.4W	5	11.21	0.99
Barrett (2005)	Chile	UP	28.9S	113.50W	9	12.40	0.50

Additionally the difference in the annual mean sea surface temperature of 1.5°C between the sites may influence the $\delta^{15}N$ of consumers, as tissue turnover rates can vary with temperature (Harvey et al. 2002).

The assumption of a constant rate of fractionation is acknowledged as a weakness in the calculation of trophic level using isotopic data (Post 2002). Fractionation (the step wise enrichment between diet and consumer) is influenced by a number of factors such as temperature, diet quality (in terms of C:N) and ration with considerable variation about the mean of 3.4‰ (McCutchan et al. 2003, Post 2002, Vander Zanden and Rasmussen 2001, Vanderklift and Ponsard 2003). In this analysis the exact value of trophic fractionation for each species pairing was not considered as the absolute trophic levels were not important, the analysis was simply to determine if differences in relative trophic level were likely between sites.

Despite the large differences in $\delta^{15}N$ values between sites, there were few differences in trophic level of reef organisms between Bar Al Hickman and Bandar Kayran. None of the invertebrate species and only one fish species-pairing showed a significant difference in trophic level between sites. Previous comparisons of the same species between sites have noted significant differences in $\delta^{15}N$ and trophic level of consumers, even over relatively small distances (<3 km) (Jennings et al. 1997). The evidence that the majority of organisms are of the same trophic level at both sites indicates that the higher $\delta^{15}N$ at Bar Al Hickman is due to the upwelling rather than consumers feeding differently. Very few isotope studies have focussed on the trophodynamics of reef ecosystems yet of the food webs previously described, despite high variance in the ranges of $\delta^{15}N$ and $\delta^{13}C$ between studies, there are very similar patterns in food web structure found worldwide (Keegan and Deniro 1988, Polunin and Pinnegar 2002, Polunin and Heaton unpubl., Thomas et.al. unpubl.). Using the baseline technique adopted in this analysis it is possible to investigate the differences in trophic level of coral reef species with a wide geographic distribution, regardless of the environmental setting providing vital information on niche adaptability and trophic plasticity.

The only difference in trophic level recorded was in the Scarid (parrotfish) pairing; *Scarus ghobban* at Bar al Hickman had a trophic level 0.38‰ higher than *Scarus persicus* at Bandar Kayran. There were no significant differences in the carbon values between the two species suggesting they feed on broadly comparable diets (e.g macroalgae). There is no published information for these two scarid species on their diet composition other than that they feed on a mix of algae, coral

polyps, detritus and small benthic invertebrates (Randall 1995). The difference in trophic level may be explained by one species having a higher proportion of one food item in their diet mix than the other; gut analysis would provide a more conclusive answer (Thomas and Cahoon 1993).

In marine systems enriched ^{13}C values are associated with benthic algae and seagrass and more depleted values with plankton and pelagic sources (France 1995b, Pinnegar and Polunin 2000). The source materials of the Bar Al Hickman food web reflect a more enriched benthic carbon isotope signature, while the more depleted ^{13}C suggests that the food web at Bandar Kayran has a stronger planktonic influence. Zooplankton is commonly the organism type most depleted in ^{13}C in a marine food web and this was the case at both Bar Al Hickman and Bandar Kayran. However, zooplankton of the same size class (>200μm) differed in $\delta^{13}C$ by 6.5‰ between the two sites (Fig. 4.3b), perhaps as a result of a difference in species composition. The $\delta^{13}C$ of the zooplankton diet (phytoplankton) may also differ between sites and with season as the dominance of different phytoplankton groups, namely diatoms versus dinoflagellates is known to fluctuate throughout the year and each group utilises different $\delta^{13}C$ pools (Dauby et al. 1990, Gearing et al. 1984, Wainright and Fry 1994). During November (the time of this study), the $\delta^{13}C$ of the zooplankton at Bandar Kayran was at its most depleted (see chapter 3); a time-series of zooplankton $\delta^{13}C$ was not available for Bar Al Hickman. Sponges and *Phymodius monticulosus* showed the largest differences in $\delta^{13}C$, broadly comparable with the difference observed in zooplankton and, to a lesser extent, in coral. Four of the fish pairings, *Abudefduf* spp., *Apogon* spp., *Istigobius decoratus* and *Pomacentrus* spp., differed in $\delta^{13}C$ between sites. These species are reported to feed on a variety of food types (Randall 1995) encompassing a range of $\delta^{13}C$ values. The $\delta^{13}C$ of a food source may differ between sites, as was the case with zooplankton. It is also plausible that the consumers feed on subtly different diets but at the same trophic level at each site, depending on local availability; hence they are still meeting their nutritional needs and exhibit similar $\delta^{15}N$ values.

The study sites separated by the smallest distance (5-10km) showed very little variation compared with other coastal benthic studies over similar distances min the Mediterranean (Jennings et al. 1997, Vizzini and Mazzola 2006, Vizzini et

al. 2005). Along a relatively short distance of coastline in the Gulf of Oman there was little change in the $\delta^{15}N$ and $\delta^{13}C$ of five fish species. The three sites were comparable in their environmental settings, having no obvious terrestrial inputs (land runoff is absent due to the dry climate) and are subject to a similar oceanographic regime. Variability in environmental conditions has been recognised as causing considerable spatial variability elsewhere (Jennings and Warr 2003). Similarites in the isotopic signature of taxonomic groups among sites likely reflect homogeneous environmental conditions. At each site, all five species occupied the same trophic position, defined by their $\delta^{15}N$ and $\delta^{13}C$ values. Where significant differences were observed in $\delta^{15}N$ among sites (*C. hemistiktos* and *A. vaigiensis*), it is impossible to say for certain whether this was due to a different baseline $\delta^{15}N$ signature and/or the availability and utilisation of different dietary items (Thomas and Cahoon 1993). *C. hemistiktos* is a predator, feeding primarily on crustaceans and small fish (Shpigel and Fishelson 1989) an diet component important at one site may not be so at another (Jennings et al. 1997). Similarly *A. vaigiensis* feeds predominantly on plankton and macroalgae (A. Mill unpubl. data), which are usually isotopically distinct (Jennings et al. 1997, Pinnegar and Polunin 2000). Slight differences in the availability of specific zooplantkers and/or macroalgae would see a small shift in the ratio of plankton to algae consumed by *A. vaigiensis*, which would ultimately result in different consumer signatures; this theory could be tested with isotope mixing models (Chapter 1 and Chapter 7)

4.5 Conclusions

The data support the hypothesis that nitrogen isotope ratios of producers and consumers are enriched across reef food webs in upwelled areas. The seasonal upwelling events in Oman result in considerable spatial variation in $\delta^{15}N$ in both primary producers and higher consumers. The use of a baseline organism revealed little difference in the trophic level of the same or similar species over distances > 300km. However the variability of carbon isotopes was harder to account for. Interestingly, over a distance of 360km the baseline organism showed no variability in $\delta^{13}C$ yet significant variation was apparent in some primary producers and in higher order consumers. In future studies it may be appropriate to include more than one common baseline species in order to account for several different trophic

groups (herbivores and planktivores) when assessing food webs with more than one source (benthic and pelagic). It has been suggested that this could include a filter feeder and a herbivorous gastropod (Cabana and Rasmussen 1996). In addition, any extrapolation or comparison of results over geographic distances must be carried out with caution and where possible spatial variance or environmental differences should be accounted for.

5 Explaining isotope trophic-step fractionation: why herbivores are different

5.1 Introduction

Stable isotope ratios are increasingly used to explore feeding relationships in aquatic ecosystems and there have been many attempts to develop isotope 'mixing models' to help quantify the potential importance of particular feeding linkages given the isotope signature of the consumer and potential diets (e.g., Ben-David and Schell 2001, Koch and Phillips 2002, Phillips and Koch 2002). All of these models assume that a stepwise enrichment occurs in the heavier isotope (^{13}C or ^{15}N) with each trophic level, and that this enrichment is constant (typically 3.4‰ for ^{15}N and 1‰ for ^{13}C) irrespective of the animal's biology and feeding behaviour. However, the magnitude of this per trophic-step isotope fractionation ($\Delta\delta^{15}N$ or $\Delta\delta^{13}C$) can be affected by many factors including nutritional stress, diet quality, body size, excretory mechanisms and feeding rate (Hobson and Welch 1995, Overman and Parrish 2001, Pinnegar et al. 2001, Ponsard and Averbuch 1999, Vanderklift and Ponsard 2003). While most fishes approximate the often-cited 3.4‰ $\delta^{15}N$ trophic step enrichment (see Vander Zanden and Rasmussen 2001), herbivorous fishes seem to deviate from this pattern, for reasons that remain unclear.

Polunin & Pinnegar (2002) report that the herbivorous parrotfish *Sparisoma spp.* and the Mediterranean sparid *Sarpa salpa* exhibit $\delta^{15}N$ values much higher than would be expected if they were feeding solely on plant material (based on data from Keegan and Deniro 1988, Pinnegar and Polunin 2000). Similarly, there is enrichment greater than 3.4‰ between herbivorous reef fish from French Polynesia (*Acanthurus nigrofuscus* and *Stegastes nigricans)* and the turf algae on which they feed (Table 5.1).

Several explanations have been proposed for this phenomenon, notably the possibility of 'isotopic routing' (see Gannes et al. 1997) whereby the nitrogen consumed by herbivorous fishes comes predominantly from a very minor, animal-derived component of the diet, since most marine algae are extremely poor sources of nitrogen and phosphorus (Atkinson and Smith 1983). Many herbivorous fishes are known to actively seek out animal-derived proteinaceous material to supplement

their diet (Robertson 1982) but whether or not this would alter the $\delta^{15}N$ signature of the animal sufficiently remains a matter of conjecture.

Table 5.1: Trophic level fractionation ($\Delta\delta^{15}N$) of herbivorous fish taken from published studies.

Species	$\Delta\delta^{15}N$ (‰)	Study
Sarpa salpa	4.90	Pinnegar and Polunin (2000)
Sarpa salpa	7.22	Jennings et. al. (1997)
Sparisoma spp.	4.10	Keegan and DeNiro (1988)
Stegastes	6.07	Polunin unpublished data
Stegastes nigricans	4.60	Polunin unpublished data
Acanthurus lineatus	2.79	Polunin unpublished data
Plectrogyphidodon lacrymatus	4.30	Polunin unpublished data
Acanthurus lineatus	3.77	Polunin unpublished data
Segastes (yellow)	5.25	Polunin unpublished data
Mean	4.78	

Vander Zanden and Rasmussen (2001) reviewed 35 trophic enrichment ($\Delta\delta^{15}N$) estimates to examine whether the mean or variance varied systematically with taxon, habitat or food type. Marked differences in $\Delta\delta^{15}N$ were noted between carnivores and herbivores. In particular carnivores, especially those in the wild, exhibited very tight clustering of per-trophic level fractionation values around 3.4‰, whereas herbivores exhibited hugely variable per-tropic level fractionation values ranging from –0.7‰ in the amphipod *Amphithoe valida* to +9.2‰ in the brine shrimp *Artemia salina*.

Trophic enrichment of ^{15}N is generally attributed to fractionation during amino acid deamination and transamination (Vander Zanden and Rasmussen 2001), whereby ^{14}N amine groups are preferentially removed to produce isotopically-light metabolites, leaving the remaining nitrogen pool enriched in ^{15}N (referred to as 'metabolic fractionation' Gannes et al. 1997). Alternatively, fractionation can result from isotopic discrimination during nitrogen assimilation (referred to as 'assimilative fractionation'). Metabolic fractionation is expected to be the dominant process for carnivores because the animal-derived nitrogen is biochemically more homogeneous and dominated by proteins. For herbivores, both assimilative and metabolic factors are likely to affect fractionation (Vander Zanden and Rasmussen 2001).

The food processing mode of herbivores differs from other teleost fish in that gut retention times can be very short (typically 4-5 hours Polunin et al. 1995) and gut adaptations to digest structurally complex algal material are present in many species e.g. a very long alimentary tract in most herbivorous species (Elliott and Bellwood 2003), gut fermentation in some kyphosids (Clements and Choat 1997) and very low gut pH in some pomacentrids (Lobel 1981). The absorption efficiency of essential nutrients (e.g. nitrogen, phosphorus and carbon) can be markedly lower in herbivores than in carnivorous species, even within the same family (Polunin et al. 1995). Therefore, in order to meet their bioenergetic needs herbivorous fishes tend to have higher feeding and excretion rates than carnivores.

The dynamic equilibrium model of Olive et al. (2003) indicates that food quality, feeding rate and excretion rate all influence the level of trophic fractionation. This model differs from other published models (Fry and Arnold 1982, Hesslein et al. 1993) as the steady state parameters that determine ΔN can be derived experimentally. The Olive model explains dynamics of per-trophic-level fractionation and can be used to estimate the isotope signature of a consumer at time *t* following a shift in diet. However, in most stable isotope studies the signature of the consumer (δ_a) is known and a model to estimate the isotopic value for the diet, δ_{food} (and hence $\Delta\delta^{15}N$) would be more useful. This can be achieved through a simple rearrangement of the Olive model (model parameters defined in Table 5.2):

$$\delta_{food} = \frac{((\delta_{ao}\, e^{\Omega_{in}t} - \delta_{at})\,\Omega_{in} \,/\, (e^{-\Omega_{in}t} - 1)) - Z}{\Omega_{in} q} \qquad \textbf{(5.1)}$$

The majority of fractionation studies have involved controlled feeding in the laboratory (Power et al. 2003, Vanderklift and Ponsard 2003). In the field, variability in per-trophic-level fractionation can only be studied where the consumer's diet can be well quantified and the isotope signatures of potential food items easily determined. Herbivores that graze on algal turf communities provide an opportunity in this regard, some species maintain a 'garden' of algae which they tend, manipulate and vigorously defend (Hata and Kato 2004). The diet material in the foregut of the fish species is easily distinguishable and this allows feeding preferences to be easily characterised. One damselfish and two surgeonfish were

observed in this study. *Pomacentrus arabicus*, *Acanthurus sohal* and *Zebrasoma xanthurum*, co-habit an area of high latitude reef in Oman and all feed on the same epilithic algal community. The three species differ in size and exhibit different food processing mechanisms (*Z. xanthurum* is a gut fermenting species, the other two are not).

Table 5.2: Definitions of parameters used in the model (based on Olive *et al*. (2003))

Model parameter	Units	Definition
$\Delta\delta^{15}N$	‰	Trophic step fractionation of nitrogen
δ_{food}	‰	Isotope signature of the food
δ_a	‰	Isotope signature of the consumer
R_d	%	Daily ration as a proportion of body mass consumed per day (BWD)
Ω_{in}		Ratio of mass of element ingested to that in the animal as a whole
q	%	Assimilation efficiency
z	‰	Rate of change by excretion per day
t (subscript)		Time after the diet switch
0 (subscript)		Time of the diet switch

The overall aim of this study was to determine why herbivorous fishes exhibit unusual per-trophic-level isotope enrichment patterns, and to evaluate whether a dynamic model of the fractionation process, incorporating absorption, feeding rate and excretion, could help understand observations from the field. Specific hypotheses tested include:

(i) herbivorous fishes exhibit higher $\Delta\delta^{15}N$ values because they consume more food each day than carnivorous fishes,

(ii) herbivorous fishes exhibit higher $\Delta\delta^{15}N$ values because they are less efficient at absorbing nitrogen from their food,

(iii) herbivorous fishes exhibit higher $\Delta\delta^{15}N$ values because they excrete more nitrogen per day compared to carnivorous fishes.

5.2 Materials and methods

Samples were collected during August and November 2004 and February 2005 from Bandar Kayran, Greater Muscat, Oman (23°31'N, 58°43'E). Three specimens of the herbivorous fish species *Acanthurus sohal*, *Zebrasoma xanthurum*,

Pomacentrus arabicus were collected by spearing during each sampling trip, with the exception of *P. arabicus* where only one specimen was collected in February 2005. Fish were killed and immediately placed on ice until they were processed in the laboratory (max. 5 hours). All fish species were collected after 11:00h local time to ensure a full digestive tract. Five potential dietary algae species (*Hypnea pannosa, Sarconema filiforme*, *Gracilaria* sp., *Ulva lactuca* and *Dictyopteris* sp.) were collected between 2 and 7m depth for isotope analysis. A minimum of three replicate samples were collected for each algae species. In the laboratory, fork length (FL), total length (TL) and weight (Wt) of fishes were recorded. Fresh diet material was collected from the anterior and posterior of the alimentary canal and frozen (-30ºC) either for isotope analysis or the determination of absorption efficiencies. Anterior material (the first 10% of the alimentary tract) was considered a proxy for diet and posterior material (the last 10% of the alimentary tract) a proxy for faecal material. A further sample was taken from the anterior of the gut for quantification of food items. Fish dorsal white muscle tissue was dissected from each fish frozen for stable isotope analysis. Algal materials were cleaned by hand-removing detritus, epiphytes and sediment from the samples before freezing. All frozen materials were later freeze-dried and homogenised with a mortar and pestle prior to analysis.

5.2.1 Stable isotope determination.

For each fish captured ~1mg of homogenised muscle tissue was accurately weighed into tin capsules. Algal samples were weighed into two aliquots of ~1mg and ~2mg to determine $\delta^{13}C$ and $\delta^{15}N$ separately. Samples were analysed using one of two machines, Automated Nitrogen Carbon Analysis (ANCA) 20-20 isotope ratio mass spectrometer (Scottish Crops Research Institute (SCRI), Dundee, Scotland) or a Thermo-Finnegan mass spectrometer (Scottish Universities Environmental Reactor Centre (SUERC), East Kilbride, Scotland). Internal standards and ecological samples analysed on both machines revealed slight discrepancies between the two machines but allowed data to be aligned to one machine to ensure all results were comparable (see chapter 2). Experimental precision based on the standard deviation of the internal standards was 0.2‰ for both $\delta^{15}N$ and $\delta^{13}C$ (SCRI) and 0.3‰ for $\delta^{15}N$ and 0.2‰ for $\delta^{13}C$ (SUERC).

5.2.2 Stomach contents analysis.

Two methods were used to quantify the diets of the fish depending upon the size of the fragments consumed. The stomach contents of *A. sohal* and *Z. xanthurum* were described using a line-transect method to describe the relative abundance of algal genera in the stomach. Stomach contents were laid in a transparent tray and viewed under a dissecting microscope, the extent of the transect line covered by each food category being recorded (Choat and Clements 1992). The line transect method was repeated for 5 transects for each specimen. A total of seven stomachs were analysed for *A. sohal* and five stomachs of *Z. xanthurum*. The totals for each transect were expressed as percentages to remove the effect of varying gut volume, then pooled to obtain mean percentages and variances for each fish; a pooled mean for each species was also obtained. Species-specific identification was not possible so algae were pooled by genera.

The stomachs of *P. arabicus* contained smaller algal fragments than those of the other two species, so a point intercept method was used to quantify diet. The stomach contents were laid out in a tray and algae, detritus, sediment and blue green algae were recorded where they occurred directly under predetermined points on an intercept line. 100 points were recorded and converted into a percentage to remove the effect of varying gut volume. Fragments that could be identified to genera were noted as being present in the diet. This method was repeated for the stomachs of all seven *P. arabicus* specimens sampled.

5.2.3 q and nitrogen absorption efficiency (NAE)

In the appendix to Olive et al. (2003) the authors demonstrated how *q* might be derived for a fish, given information about nitrogen absorption efficiency. Nitrogen absorption efficiency (NAE) of *A. sohal*, *Z. xanthurum* and *P. arabicus* were determined by ash determination method (Montgomery and Gerking 1980) as follows. About 100mg of dried homogenised material were weighed into a crucible and combusted in a muffle furnace at 600°C for 24 hours. Samples were then reweighed and the remaining ash expressed as a percentage of the original mass. Bulk nitrogen content of the food, fish and faeces was obtained as the area of N peak from mass spectrometry calibrated versus tryptophan (r^2=0.999).

$$Corrected\ Faecal\ Nitrogen\ (\%) = \left[\frac{\%\ ash\ in\ food}{\%\ ash\ in\ faeces}\right] \times \%\ nitrogen\ in\ faeces \qquad \textbf{(5.2)}$$

$$Nitrogen\ Absorption\ Efficiency\ (\%) = \left[1 - \frac{corrected\ faecal\ nitrogen\ (\%)}{\%\ nitrogen\ in\ food}\right] \times 100 \qquad \textbf{(5.3)}$$

Calculated NAE estimates were compared with 41 other NAE estimates of herbivorous fish (Appendix B).

5.2.4 Ω_{in} and daily ration (R_d)

Olive et al. (2003) described Ω_{in} as the 'dimensionless ratio of the mass of an element (e.g. nitrogen) in the ingested food in relation to the mass of the element in the animal as a whole'. It is closely related to the daily ration (R_d) of the animal, expressed as the weight of food ingested per day as a proportion of the animal's body mass. It is possible to determine the R_d of herbivorous fishes by determining the mean bite size and the number of bites taken in a day. It was not possible to do this for the species in this study, however bite size and counts have been made for similar grazing species elsewhere (Table 5.3). R_d values were selected from the literature, where possible from fishes in the same genus (i.e. *Acanthurus*, *Zebrasoma* and *Pomacentrus*). In order to calculate Ω_{in} from R_d it was necessary to know something about the nitrogen content of the consumer and its food, these data were obtained by mass spectrometry. The wet-weight R_d percentage was converted to dry weight and hence the amount of nitrogen consumed per day, Ω_{in}, calculated using Equation 5.4

$$\Omega_{in} = \frac{dry\ \ weight\ \ food\ \ (g) \times N_{food}}{N_a} \times \ NAE \qquad \textbf{(5.4)}$$

Where N_{food} is the percentage nitrogen content of the food and N_a is the nitrogen content of the consumer.

Table 5.3: Feeding rates R_d (%BWD) of tropical reef associated herbivorous fish species from literature.

Species	Location	Rd	Reference

Stegastes fuscus	Brazil	13.03	Ferriera et al (1998)
Sparisoma atomorium	Brazil	24.94	Ferriera et al (1998)
Acanthurus bahianus	Brazil	23.26	Ferriera et al (1998)
Stegastes nigricans	French Polynesia	30.3	Polunin et al (1995)
Ctenochaetus striatus	French Polynesia	31.1	Polunin et al (1995)
Acanthurus nigrofuscus	French Polynesia	24.2	Polunin et al (1995)
Scarus sordidus	French Polynesia	36.0	Polunin et al (1995)
Zebrasoma scopas	French Polynesia	31.8	Polunin et al (1995)
Stegastes apicalis (summer)	GBR	20.5	Klumpp and Polunin (1989)
Stegastes apicalis (winter)	GBR	11.1	Klumpp and Polunin (1989)
Atrosalarius sp.	GBR	8.5	Klumpp and Polunin (1989)
Mean		20.8	

5.2.5 Z, isotope discrimination associated with excretion

Z is the instantaneous rate of change in the isotope signature of an animal (in δ units per day) due to excretion. This term is very difficult to measure experimentally, although Olive et al. (2003) show how Z might be estimated as the instantaneous rate of ^{15}N enrichment at the onset of starvation. Following the onset of starvation (when Ω_{in} is zero), animals become progressively enriched in ^{15}N with time, due to continued export of depleted (high in ^{14}N) excretory products.

It was not possible to conduct starvation experiments for every fish in this study and thus an estimate for Z was determined using data from existing laboratory feeding experiments (e.g. Harvey et al. 2002). Given a known isotope signature of the consumer at time *t* (δ_a), together with known values for Ω_{in} and *q*, it is possible to re-arrange Equation 5.1 to solve for Z:

$$Z = \left(\frac{\delta_{at} - \delta_{a0} e^{\Omega_{in} t}}{e^{-\Omega_{in} t} - 1} \right) \Omega_{in} - \left(\Omega_{in} q \delta_{food} \right) \qquad \textbf{(5.5)}$$

For each data point an estimate of Z may be derived, with the arithmetic mean providing an overall estimate given all the available data. This procedure was attempted for nine sets of existing data (Table 5.3). There were no suitable diet switch experiments to derive data for herbivorous fish so the mean value of 0.1429 was used to express Z in the model for all three fish species.

Table 5.4: Calculation of isotope discrimination associated with excretion, Z, using data from diet switch experiments on fish

Species	Diet	Mean weight (g)	R_d	Ω_{in}	q	δ_{food}	δ_a	Z	Study
Rhinogobius sp.	Aquatic insects	0.41	0.072	0.03	0.9481	4.8	13.8	0.475	Maruyama *et al*. (2001)
Salvelinus namaycush	Pellet	125	0.02	0.013	0.9251	9.51	10.23	0.079	Harvey *et al.* (2002)
Coregonus nasus	Pellet	26	0.012	0.041	0.948	9.7	11.6	0.053	Hesslein *et al.* (1993)
Dicentrarchus labrax	Sandeels	40	0.04	0.028	0.9251	12.16	17.83	0.131	Sweeting (2004)
Dicentrarchus labrax	Dab	47	0.04	0.046	0.9251	13.87	17.48	0.1447	Sweeting (2004)
Dicentrarchus labrax	Sandeels	33.1	0.032	0.026	0.9251	13.16	16.41	0.14	Barnes (2006)
Fundulus heteroclitus	Tuna	1-2	0.04	0.026	0.9251	15.6	7.0	0.047	Logan *et al.* (2006)
Fundulus heteroclitus	Tuna	1-2	0.03	0.019	0.9251	15.6	7.0	0.083	Logan *et al.* (2006)
Chromis chromis	Plankton	10.25	0.046	0.023	0.9251	3.365	6.657	0.133	Pinnegar (2000)
Mean								0.1429	

5.3 Results

5.3.1 Stable isotope analysis

There was no significant difference in stable isotope signature of any fish species across seasons (GLM *A. Sohal* $F_{2, 7}$ =2.17, p=0.209, *Z. xanthurum* $F_{2, 7}$ =2.74, p=1.43, *P. arabicus* $F_{2, 6}$ =1.43, p=0.340). Therefore, fish from all seasons were pooled at species level to obtain mean $\delta^{13}C$ and $\delta^{15}N$ values (Fig. 5.1). The algae showed greater variability in $\delta^{13}C$ than the three fish species. An empirical estimate of $\Delta\delta^{15}N$ (Table 5.5) was calculated by subtracting the mean isotopic signature of the algae (Table 5.6) from the mean isotopic signature of each fish species.

Table 5.5: Mean length, $\delta^{15}N$ and $\Delta\delta^{15}N$ (calculated by subtracting the mean $\delta^{15}N$ of algae from the $\delta^{15}N$ of the animal) of three herbivorous fish.

Species	Mean FL (mm)	n	$\delta^{15}N$	$\Delta\delta^{15}N$
Acanthurus sohal	296	9	13.44 ± 0.65	4.92
Zebrasoma xanthurum	205	9	13.04 ± 0.60	4.52
Pomacentrus arabicus	117	7	14.19 ± 0.90	5.67

Table 5.6: $\delta^{15}N$ (mean ± SD) and %N (mean ± SD) content for 7 algae genera.

Code	Genus	$\delta^{15}N$	%N
A1	*Dictyopterus*	8.54 ± 0.12	4.28 ± 0.11
A2	*Gracilaria*	9.00 ± 0.22	1.95 ± 0.33
A3	*Hypnea*	8.37 ± 0.26	4.34 ± 0.11
A4	*Sarconema*	8.38 ± 0.82	2.86 ± 0.52
A5	*Ulva*	8.59 ± 0.38	3.65 ± 0.32
A6	*Ptercladia*	9.64 ± 0.09	3.24 ± 0.45
A7	*Turbinaria*	7.08 ± 0.21	1.43 ± 0.19
	MEAN	8.51 ± 0.30	3.11 ± 0.29

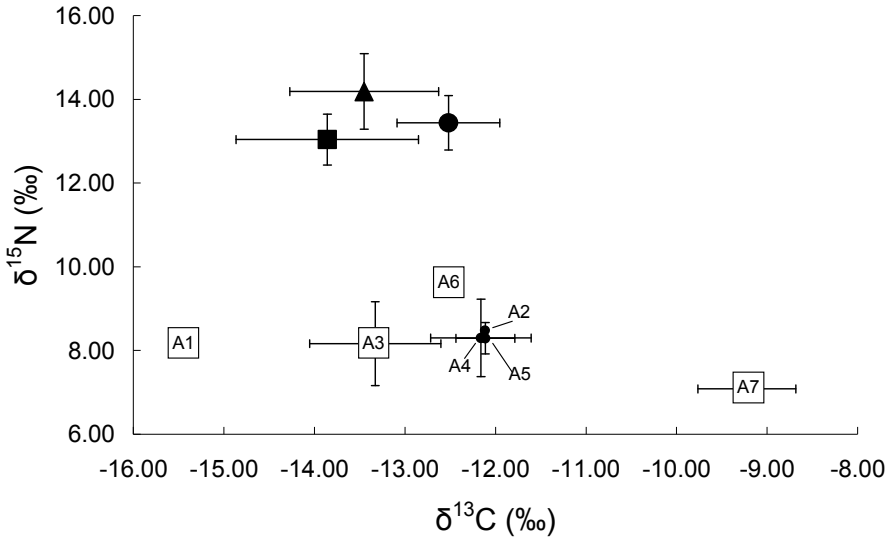


Fig. 5.1: $\delta^{13}C$ and $\delta^{15}N$ isotope plot of three herbivorous fish, *Pomacentrus arabicus* (triangle), *Acanthurus sohal* (circle) and *Zebrasoma xanthurum* (square) and algae. Algae genera in squares A1, *Dictyopterus*; A2, *Gracilaria*; A3, *Hypnea*; A4, *Sarconema*; A5, *Ulva*; A6, *Pteracladia A7, Turbinaria.* Error bars represent $\pm$ 1SD.

5.3.2 Stomach contents analysis

The fish consumed over 30 different species of algae, the dominant dietary algae genera differing among fish species: *Hypnea* spp and *Phylophora* spp. were most frequent in *A. sohal,* while *Feldmannia* spp. and *Pterocladia* spp. were dominant in *Z. xanthurum* (Table 5.7). *P. arabicus* had a diet that was 35% detritus and 60 % algae.

Table 5.7: Dominant dietary algae. Mean percentage content (± SD) of each genera consumed by *A. sohal* and *Z. xanthurum*, presence in *P. arabicus* diet is indicated by * Genera in bold are the selected algae for analysis.

Dietary Item	*Acanthurus sohal*	*Zebrasoma xanthurum*	*Pomacentrus arabicus*
Ceramium sp.		1.88 ± 1.06	
Champia sp.	1.07 ± 0.52	1.83 ± 1.83	
Chlorodesmis sp.		11.80 ± 5.42	
Cladophora sp.		4.69 ± 1.88	*
***Dictyopteris* sp.**	2.03 ± 1.02		
Dictyota sp.	11.76 ± 2.16	0.31 ± 0.31	*
Enteromorpha sp.		10.49 ± 5.74	
Feldmannia sp.		16.69 ± 8.38	*
Gelidiopsis sp.	1.06 ± 0.63	2.45 ± 2.45	*
***Gracilaria* sp.**	5.34 ± 2.45		
***Hypnea* sp.**	24.58 ± 4.38	4.99 ± 2.18	*
Lobophora sp.	5.39 ± 2.44		*
Lomentaria sp.	5.24 ± 1.69	0.94 ± 0.94	
Phylophora sp.	22.69 ± 6.53		
Polysiphonia sp.		6.71 ± 2.93	*
***Pterocladia* sp.**	2.13 ± 1.39	17.27 ± 10.77	*
***Sarconema* sp.**			*
Other < 1%	3.67	0.31	
Detritus	1.55 ± 0.69	8.97 ± 4.50	*
Sediment		4.56 ± 4.10	*
Unidentified	13.50 ± 3.03	6.12 ± 6.12	

5.3.3 Absorption efficiencies and *q*

Total and elemental absorption efficiencies for the three fish species compare well with values taken from the literature (Table 5.8 and Appendix B). *Z. xanthurum* and *A. sohal* had measured NAE values of 67.07 and 72.28 respectively and these were used in the model. There was insufficient sample size to conduct ash

analysis for *P. arabicus*, thus the mean literature NAE of 67.63 was used for this species (Table 5.8 and Appendix B).

Table 5.8: Input parameters and results of fractionation model for three herbivorous species and one planktivore. (* taken from Pinnegar (2000))

Species	Weight (g)	Rd	$\delta^{15}N$	q	Ω_{in}	Z	δ_{food}	Δn
Acanthurus sohal	500.00	20	13.09	0.7228	0.021	0.141	9.03	4.41
Zebrasoma xanthurum	250.00	21	13.16	0.6707	0.018	0.141	8.74	4.30
Pomacentrus arabicus	60.00	17	14.62	0.6763	0.017	0.141	8.73	5.68
*Chromis chromis**	20.26	4.68	6.90	0.93	0.031	0.110	3.64	3.26

5.3.4 Model outputs

The model predicted δ_{food} to be 9.03‰ for *A. sohal*, 8.74‰ for *Z. xanthurum* and 8.73‰ for *P. arabicus* (Table 5.8), giving $\Delta\delta^{15}N$ values of 4.41, 4.30 and 5.68 respectively. These are much closer to the empirically derived $\Delta\delta^{15}N$ estimates than to the usually accepted value of 3.4‰.

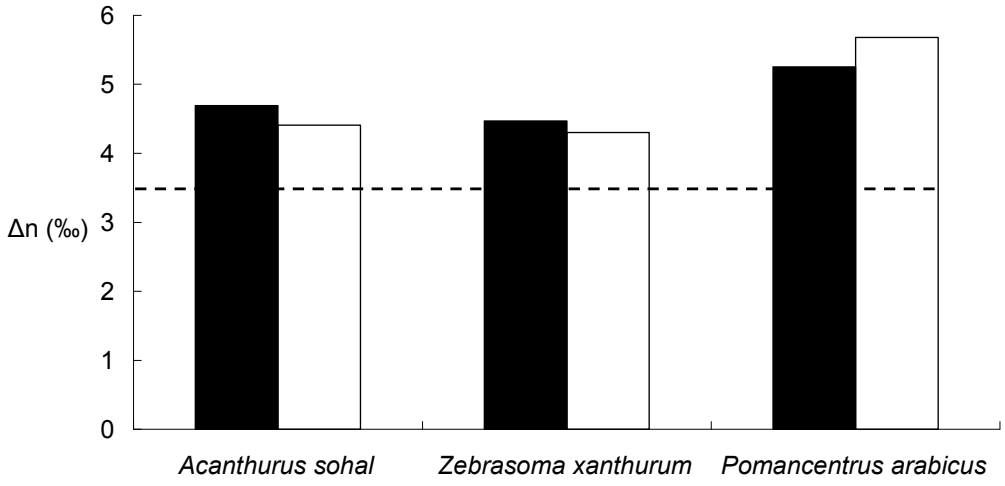


Fig. 5.2: $\Delta\delta^{15}N$ values for fish species determined by empirical measurement (filled bars) and model output (open bars). Dashed line represents the current accepted fractionation value of around 3.4‰.

5.3.5 Model sensitivity

In order to determine how feeding rate, absorption efficiency and excretion rate influence model estimation of δ_{food}, sensitivity analysis was conducted whereby values of Ω_{in}, q and Z were varied systematically and the impact on δ_{food} noted (Fig. 5.3). To compare how this may differ between a herbivorous fish and a carnivorous fish, this analysis was repeated for C*hromis chromis*, a small planktivorous fish from the Mediterranean. All parameters were taken from Pinnegar (2000) shown here in Table 5.8.

5.3.6 Daily ration

Small changes in low levels of daily ration had the greatest impact on δ_{food}. When the consumption rate was greater than 30% BWD, A. sohal showed little change in δ_{food}; in comparison *C. chromis* showed little change once BWD >7% (Fig. 5.3). When R_d <30% BWD the model predicted very different values for δ_{food} with only a little increase in R_d for *A. sohal*, similar effects but to a lesser degree were seen for *C. chromis* at BWD values < 7 % (Fig. 5.3).

5.3.7 Absorption efficiency

There was relatively little change in δ_{food} when q was 0.5-1.0 (50 – 100% efficiency; Fig. 5.2). At lower values of q there was a dramatic decrease in δ_{food}, with values becoming negative for values of q less than 0.35 for the herbivore and less than 0.47 for the carnivorous fish.

5.3.8 Excretion rate

There appeared to be a linear change in δ_{food} with Z for both species (Fig. 5.2). However, the rate at which Z influenced δ_{food} was reduced in the carnivore, between the values 0.1 and 0.2 Z δ_{food} changed by ~4‰ for *C. chromis* and by ~8‰ for *A. sohal*.

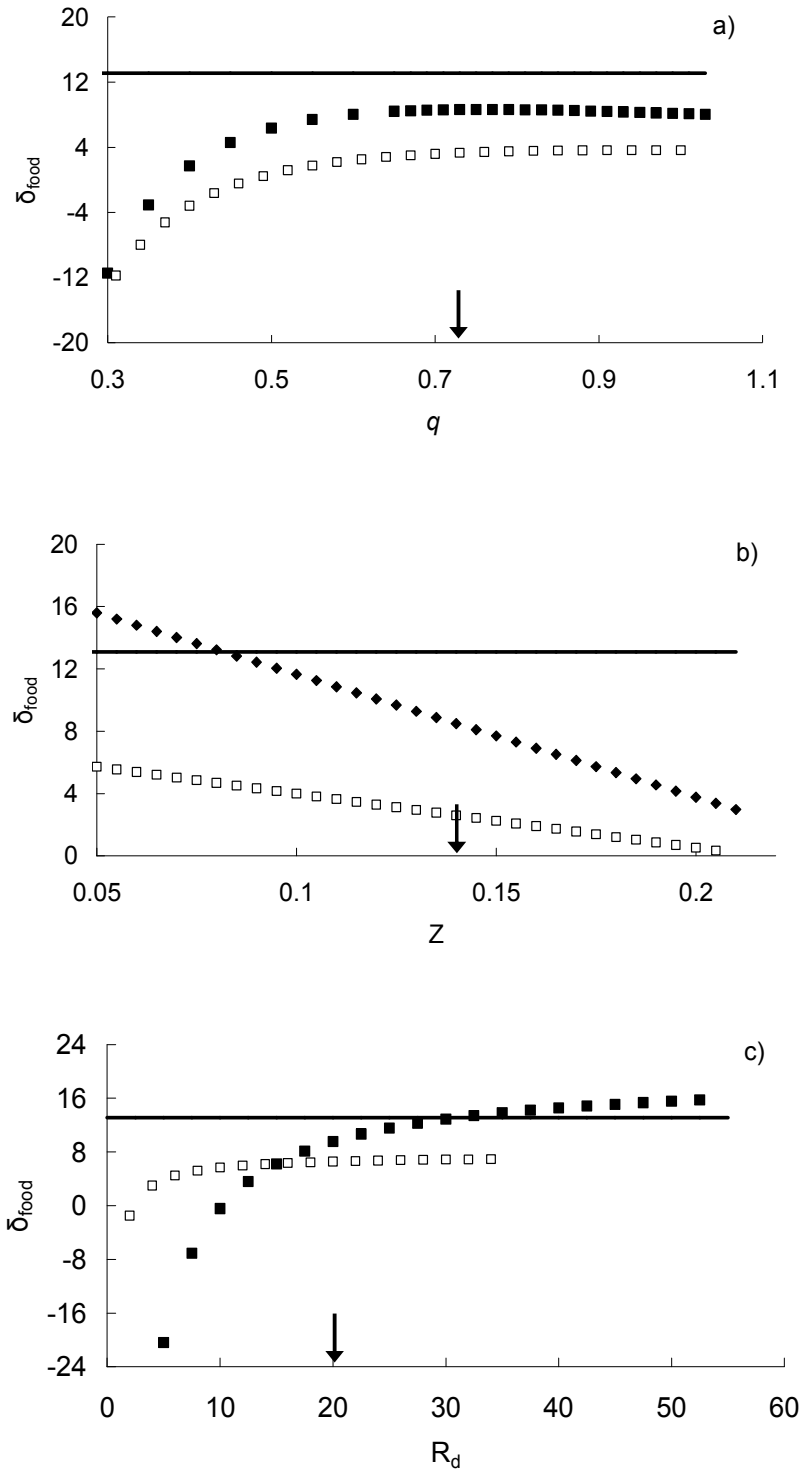


Fig. 5.3: Sensitivity of δ_{food} to varying model parameters a) Z b) q c) R_d for *Acanthurus sohal* (filled squares) and *Chromis chromis* (open squares), default parameters as in Table 5.8

5.4 Discussion

In their natural settings, herbivorous fish trophic-step $^{15}N/^{14}N$ fractionation was significantly higher than 3.4%. This contrasts with the studies that show herbivore trophic fractionation to be lower than the commonly-cited $\Delta\delta^{15}N$ of 3.4‰ (McCutchan et al. 2003, Owens 1987, Vanderklift and Ponsard 2003). Previous studies have attributed high $\Delta\delta^{15}N$ exhibited by animals consuming low quality diets (high C: N ratio) to unknown animal material in the diet (assuming the diet contained more protein than was observed Pinnegar and Polunin 2000) or assumed that the animal was undergoing nutritional stress (Adams and Sterner 2000). The wild animals in this study were evidently in good condition and visual inspection revealed no additional protein among materials in the diet other than from the algae. However by using a dynamic model, incorporating absorption, feeding rate and excretion, we repeatedly predicted higher $\Delta\delta^{15}N$ values for herbivorous fish. Previous fractionation models have used bioenergetics to determine δ_{food} with $\Delta\delta^{15}N$ based on literature values (Harvey et al. 2002). This is the first model to our knowledge to calculate both δ_{food} and $\Delta\delta^{15}N$.

Herbivorous fish consume around 20% of their body weight per day compared to only 3-4% for carnivorous fish. In this model, food consumption rate was incorporated into the term Ω_{in}, along with N content of the diet and the absorption efficiency, q. The increased R_d in herbivores would contribute to a greater $\Delta\delta^{15}N$ if the %N of the diet and q were not much smaller than those of carnivores. However, these factors are related such that to meet the bioenergetic needs of an animal feeding on a low-N food it is necessary to have a high R_d (Choat et al. 2002, Choat et al. 2004, Fris and Horn 1993). Conversely, an organism with an N-rich diet will not feed as much; Ω_{in} of herbivorous and carnivorous species may thus be broadly similar, corresponding with the dietary requirements of herbivores and carnivores being significantly different (Pandian and Marian 1985). The R_d values used in the model were taken where possible from species of the same genus with similar feeding habits because a slight change in R_d can result in a significantly different prediction for δ_{food} (Fig. 5.3). R_d may also be affected by a change in temperature as fish can alter their metabolic rates to suit their environment (Klumpp and McKinnon 1989), which may lead to seasonal variations

in the observed $\Delta\delta^{15}N$. NAE calculations for *A. sohal* and *Z. xanthurum* were similar to those of other herbivorous fish from the literature. The ash marker method (Montgomery and Gerking 1980) used to determine NAE may however lead to some inaccuracies as it is based on the assumption that ash is indigestible and that all organic matter is absorbed by the fish. Herbivorous species with gizzard-like stomachs, (e.g. *Z. xanthurum*) have previously been found to have negative assimilation efficiencies for some macronutrients, thought to be a result of high levels of inorganic materials retained in their guts (Crossman et al. 2005). The gut material of *Z. xanthurum* used for ash analysis in this study was taken from the immediate anterior and posterior of the intestine to minimise excess inorganics not present in the diet material. Sediment or inorganic matter was present in the diet (Table 5.6), however the mean NAE obtained from ash analysis was within the published range. NAE has been positively correlated with the N content of food (Pandian and Marian 1985), hence, in the model, if N content of the food were to decrease, a decrease in NAE would be expected. Body weight, food ration and temperature significantly influence absorption (Pandian and Marian 1985), yet NAE may vary with the size of the fish (Lassuy 1984); this was not the case in this study. When q in the model was below the value of 0.5 (50% efficiency), the predicted value for δ_{food} would decrease significantly. The q parameter was found to influence the difference between δ_a and δ_{food} in a way opposite to Olive et al. (2003) whereby when $q<1$ the isotopic ratio for the animal would be depleted relative to the isotopic ratio of the food (Olive et al. 2003). Fish in this study had a q value of >0.5.

Stomach contents analysis showed *Feldmannia*, *Phylophora* and *Enteromorpha* to be among the most dominant algal genera but these were not analysed for $\delta^{15}N$ and N% content due to cost constraints. Similarly it was not possible to obtain $\delta^{15}N$ value for the detritus fraction of the *P. arabicus* diet. The omission of these dietary components may have lead to errors in empirical estimations of $\Delta\delta^{15}N$. However macroalgae $\delta^{15}N$ varied little across genera and since their nitrogen source was the same it is unlikely the seven genera used would have significantly biased the mean value. The accuracy of these estimates could be improved by further analysis of all genera and weighting the contribution of each by their relative importance in the diet.

If accurate values are to be applied to trophic fractionation, controlling processes must be well understood. Our sensitivity analysis has highlighted that the value of Z is an important factor in determining the level trophic fractionation; however Z has not yet been measured directly. Z has been estimated from diet switch experiments where the study animals were not in equilibrium with their diet. All of the diet switch species were carnivorous, hence the mean Z value used in the model would be appropriate for carnivores. Whether herbivores would be more accurately described with a significantly different Z value remains to be tested. There may be a differences in Z between herbivores and carnivores as much more ingested N appears to be released as dissolved waste in carnivores than herbivores (Polunin and Koike 1987) and Z is therefore likely to be higher in herbivores. High nitrogen-use efficiency, whereby only a small portion of the ingested N is excreted, is an adaptation in herbivores to deal with low N intake and has been suggested to contribute to low $\Delta\delta^{15}N$ values (Vanderklift and Ponsard 2003). This may be the case for aphids and certain detritivores (Vanderklift and Ponsard 2003) but does not seem to be the case for herbivorous fish as they are known to exhibit high fractionation values (Table 5.1). Differential nitrogen excretion has previously been suggested as a factor contributing to variance in $\Delta\delta^{15}N$ (Minagawa and Wada 1984, Ponsard and Averbuch 1999, Vanderklift and Ponsard 2003) but so far researchers have failed to reach a consensus view. Excretion rate measurements in a range of species of differing trophic groups would potentially further our understanding of how these processes affect fractionation.

A single mean $\Delta\delta^{15}N$ may seem useful and convenient in application to food web studies, especially to determine TL. However, by applying one value to determine δ_{food} simply reflects the consumer signatures offset by 3.4‰. This approach may lead to the misinterpretation of the relative importance of potential food sources of a consumer. As the Olive model takes into account nutritional functionality, it has the potential to be used for a range of consumers in a food web giving more accurate $\Delta\delta^{15}N$ values for other feeding guilds, (e.g. planktivores, piscivores and ominivores). The model output - the value for δ_{food} - could be used within isotope 'mixing models' (e.g. Phillips 2001) to determine the different proportions that contribute to the diet mixture (Koch and Phillips 2002, Lubetkin

and Simenstad 2004). Trophic guild $\Delta\delta^{15}N$ values will allow researchers to more accurately describe food web interactions in natural settings.

6 Coexistence in a food web: Using stable isotope data to explore trophic niche segregation and feeding patterns relating to body size.

6.1 Introduction

The mechanisms that support and maintain high levels of species diversity and coexistence in coral reef ecosystems have been a matter of contention in theoretical ecology (Bellwood et al. 2006, Dobzhansky 1950, Liem 1984, Sale 1977). Reefs ecosystems commonly have a high level of redundancy within functional groups but it is not clear how these ecosystems can support such high species diversity (Paulay 1997). Trophic niche theory suggests that species that occur in densely packed stable ecosystems will tend to occupy separate trophic niches and have a high level of specialisation to avoid competition for food (Kohn and Nybakken 1975, Roughgarden 1974). Much of the theoretical literature is based on the assumption that populations are in equilibrium with the resources they utilise and differ enough in their use of the resources to coexist (Hutchinson 1961). Trophic specialisation has been shown for some coexisting reef species (Gladfelter and Johnson 1983, Klumpp and Polunin 1989, Kohn and Nybakken 1975), however the majority of reef fish species are either generalist feeders or non obligate specialists and the null hypothesis of no differences has to be considered (Sale 1977). High versatility in diet and feeding mechanisms allows generalists to be more resilient to changing conditions than specialists (Bellwood et al. 2006). In an environmentally dynamic ecosystem, a high level of feeding plasticity may allow coexistence to occur without any trophic niche segregation as has been found in some cichlid species (Genner et al. 1999).

Methods to quantify trophic niche breadth and the utilisation of particular food resources by fish have been based on feeding observations and stomach contents data (Gladfelter and Johnson 1983, Pratchett 2005, Ross 1986). The increased popularity of carbon and nitrogen stable isotope analysis to determine feeding interactions in a range of ecosystems is a reflection of the benefits this technique offers over traditional methods such as gut contents analysis (Pinnegar and Polunin 2000, Post 2002). Using the biochemical technique to analyse muscle

tissue and potential food sources it is possible to distinguish where in the food web consumers derive their energy (Pinnegar and Polunin 2000, Vander Zanden and Rasmussen 1999). Trophic position can be inferred from $\delta^{15}N$ data as a consumer is typically enriched by ~3.4‰ relative to its diet (Minagawa and Wada 1984, Post 2002). Dietary sources can be traced using $\delta^{13}C$ as it is generally more conserved, with a typical trophic fractionation of ~1‰ (Owens 1987, Pinnegar and Polunin 2000). Planktonic sources tend to be depleted in ^{13}C, while benthic sources are more enriched. In fish the isotopic signature of muscle tissue typically reflects assimilation over a feeding period of several months to years providing better temporal diet information than stomach contents (Hesslein et al. 1993, Maruyama et al. 2001). In addition it is extremely laborious to obtain gut contents data across a complete food web and results can be biased by empty stomachs (especially in piscivorous fish), indistinguishable food items through partial digestion and temporal 'diet snapshots' (Deb 1997, Hyslop 1980).

In fish, white muscle is the most commonly used tissue for stable isotope analysis on the basis that it has very little inherent variance (Pinnegar and Polunin 1999). Therefore, if individuals in a population feed on the same diet (either a single source or on the same proportions of multiple sources) there will be little variation among their $\delta^{13}C$ and $\delta^{15}N$ signatures (Bearhop et al. 2004). In contrast, a population of generalist feeders (where individuals have differing diets) will have a high degree of variance about the population $\delta^{13}C$ and $\delta^{15}N$ means (Gu et al. 1997). Hence, among-individual variance in $\delta^{13}C$ and $\delta^{15}N$ of muscle tissue can be used as an indicator of omnivory or specialisation at the population level (Bearhop et al. 2004, Bolnick et al. 2002, France 1997, Sweeting et al. 2005). Significant differences in mean isotopic signatures between species implies that there are differences in the source materials utilised or that although species rely on the same ultimate sources, they are feeding at different trophic levels (Bearhop et al. 2004, Genner et al. 1999). In theory variance in mean $\delta^{15}N$ and $\delta^{13}C$ can be used as a descriptor for trophic niche width (Bearhop et al. 2004, Sweeting et al. 2005). When partitioning of diet materials is not evident, or there is overlap in isotopic variance, coexistence may depend on inter-specific competition for food (Genner et al. 1999) or through partitioning another resource such as space or time.

In many cases body size alone is a good indicator of trophic niche as size encapsulates many aspects of the ecology of a species (Karpouzi and Stergiou 2003, Woodward et al. 2005). Predators tend to be one to three times larger than their prey, and where feeding habits are size dependant in this way a size structure is created in a food web (Cohen et al. 1993). Individuals of a species will change in size, by up to five orders of magnitude throughout their life (Cushing 1975), hence the diet of a single species is likely to vary with life history stage (Cocheret de la Moriniere et al. 2003, Renones et al. 2002). Optimal foraging theory suggests that, given a choice of prey items, as fish with a large gape size will have, larger prey item will be more favourable (MacArthur and Pianka 1966). Generally, as fish increase in size they feed on increasingly higher trophic levels (Jennings et al. 2002a) and in several fish species $\delta^{15}N$ has been observed to increase with body size or age (Badalamenti et al. 2002, Davenport and Bax 2002, Hobson and Welch 1995, Jennings et al. 2002b, Le Loc'h and Hily 2005, Overman and Parrish 2001, Wainright et al. 1993). This may result from larger animals being able to consume larger prey items that are more enriched in $\delta^{15}N$ (Davenport and Bax 2002, Renones et al. 2002) and/or from ontogenetic change in feeding habits and hence in the type of prey items consumed, e.g. from invertebrates to fish (Badalamenti et al. 2002, Renones et al. 2002). In theory, if an organism feeds on the same diet throughout its life there should be no change in $\delta^{15}N$ (Ponsard and Averbuch 1999). However, walleye salmon (*Stizostedion vitreum*) that were fed a constant diet had an unexplained enrichment in $\delta^{15}N$ as they grew (Overman and Parrish 2001). Contrary to this Vander Zanden et al (2000) found that trophic position (measured from $\delta^{15}N$ data) did not increase with increased body size in wild freshwater lake trout (*Salvelinus namaycush*). The effects of omnivory, defined here as feeding on more than one trophic level, and trophic variability may mask any relationships between body size and trophic position (Vander Zanden et al. 2000).

Coral reefs are typically surrounded by oligotrophic seas; however, in Oman the coral reefs are unusual as the oceanographic regime of the coastal waters drives seasonal changes in primary productivity altering the relative abundance of available food sources (Chapter 4). This presents a study site of a dynamic nature with food sources that may fluctuate seasonally (Chapter 3) and inter-annually to explore the extent of niche overlap in the food web and relative importance of

species having specialist or generalist feeding patterns. A wide range of coral reef fish families and species were available for analysis allowing comparisons of feeding patterns and trophic niches among and between families and trophic guilds possible.

The overall aim of this study was to describe the general trophic linkages of the reef food web at Bandar Kayran, Oman, using stable carbon and nitrogen isotope data, with the following specific hypotheses:

(i) Where closely related species coexist they will occupy different trophic niches avoiding intra-genera competition for food resources.

(ii) Within species larger individuals will have higher $\delta^{15}N$ values as they will select higher trophic level prey items than their smaller counterparts.

(iii) Feeding variability will be more prevalent in higher trophic level species, which will hence show more variation among $\delta^{15}N$ and $\delta^{13}C$ values than lower trophic level species.

6.2 Materials and Methods

All organisms were collected from a shallow reef at Bandar Kayran, Oman during May and June 2004 and May and June 2005. At least three replicates of each fish species were collected, by spearing for larger individuals and by anaesthetising with a 70% alcohol: 30% clove oil mixture for smaller species. Where possible individuals collected were at least 60% of the species L_{max} (Randall 1995), an exception to this were a few less abundant species that were sampled opportunistically (Table 6.1). Seven species namely, *Abudefduf vaigiensis, Acanthurus sohal*, *Cephalopholis hemistiktos*, *Cheilodipterus novemstriatus*, *Chromis xanthopterygia, Scolopsis ghanam* and *Parupeneus margaritatus* were sampled over a wide range of sizes to encompass the maximum length variation possible to assess changes in $\delta^{15}N$ and $\delta^{13}C$ with size. To provide data on potential diet items replicate samples of invertebrates (brittle stars, crabs, amphipods, sponge, urchins and coral) and macroalgae (of the genera, *Hypnea*, *Sarconema*, *Turbinaria*

and *Ulva*) were collected by hand from the reef using SCUBA for isotope analysis. A small bulb dropper was used to collect detritus samples from the reef flat by suction. Zooplankton samples were collected in two size classes by towing 500µm and 200µm plankton nets (40 cm diameter) for a five minute period behind a slow moving (~5 km hr^{-1}) boat in water immediately adjacent to the reefs where fish and invertebrates were taken.

All samples were immediately placed on ice. Upon return to the laboratory fish fork lengths (mm) and weights (g) were recorded and a sample of white dorsal muscle was dissected for C and N isotope analyses. Stomach contents were retained for immediate stomach contents analysis. For analyses of the invertebrates muscle tissue was dissected where possible, with the exception of urchins where gonad tissue was used. For small crustacean species and zooplankton, whole organisms were used and where organisms were small in size several replicates were included in each sample. Coral tissue was removed from the skeleton using a small water-jet and the resultant mucus/coral/water mixture was then centrifuged to concentrate sufficient coral tissue for isotope analysis. Macroalgae samples were cleaned using forceps to remove any epiphytes from the surface of the thallus. All materials were stored frozen prior to preparation for isotope analysis.

6.2.1 Stomach Contents Analysis

Qualitative stomach contents analysis was carried out for each fish collected by emptied the stomach contents into a petri-dish and examined using a dissecting microscope. Due to the small number of replicate samples of each species (in some case only n=3) it was not possible to assess the quantity of the stomach thoroughly. Identifiable items were recorded as present and described in detail. Where possible an attempt to quantify the contents was made, either through point counts or by estimating percentage volume.

6.2.2 Stable isotope analysis

Stable isotope analysis was carried out on all samples as described in Chapter 2.

6.2.3 Statistical analysis

Statistical analysis was limited due to the small replicate sample sizes within species. For this reason the 2-dimensional stable isotope data were treated as spatial data and a K-nearest neighbour randomisation test (Rosing et al. 1998) was performed to identify significant differences in $\delta^{15}N$ and $\delta^{13}C$ between species. This test has been shown to have good statistical power on small sample sizes.

To examine relationships between length and $\delta^{15}N$ and $\delta^{13}C$ it was necessary to transform the length data to log length to normalise the data. Linear regression was then performed using the statistical package R (R Development Core 2005).

6.2.4 Point count data

Abundance data were collected in May 2005 using a point count method where the observer (ACM) snorkelled over a 7m radius area for a 10 minute period. Species observed were counted and their total length was estimated. Roving species were counted in the first two minutes then not included (to avoid resampling). Cryptic and small (<50mm) species were not included. Five replicate point counts were surveyed on the reef and the data averaged across replicates. These data were combined with isotopic data to analyse trends of $\delta^{15}N$, mean length and abundance across the whole community.

6.2.5 Intra-specific variability

Using the method of France (1997) the intra-specific variability of fish at different trophic levels was assessed using the standard deviation and absolute ranges of $\delta^{15}N$ values. The aim of this was to create a measure of the magnitude of intra-specific diet breadth of each trophic level. The variability about the mean for each fish species was tabulated per unit ‰ values. For example, the grouper, *Cephalopholis hemistiktos* the standard deviation of $\delta^{15}N$ was 0.22 for each individual fish within the range 16.8-17.4‰. So the SD of 0.22 would be tabulated for of the trophic positions 16.8, 16.9 17.10…etc…17.4‰. This procedure was repeated for absolute ranges of isotopic values, whereby for the grouper the variance of the range, 0.6‰, was tabulated for each of the $\delta^{15}N$ values 16.8, 16.9 17.10…etc…17.4‰. The variability values for each species in the food web were

then grouped into trophic categories of 3‰ intervals and averaged to determine the overall variation within the trophic category. Invertebrates and autotrophs were not included in this analysis.

6.3 Results

Of the basal components sampled in the food-web macroalgae had the lowest $\delta^{15}N$ values (means by genera ranged from 6.16 to 8.17‰) and spanned a wide range of $\delta^{13}C$ values (means from -9.60 to -15.5‰) (Fig 6.1c). Detritus was the most ^{13}C enriched item (-6.43‰) and had a similar mean $\delta^{15}N$ value (10.6‰) to both the coral groups (*Hexacorallia* mean 10.1‰ and *Octocorallia* mean 10.6‰) and the annual mean zooplankton value (10.8‰).

There were no clear taxonomic trends in $\delta^{15}N$ and $\delta^{13}C$ of the invertebrates sampled on the reef (Fig. 6.1b). Mean $\delta^{13}C$ ranged from -16.5‰ (sponge) to -7.9‰ (brittlestar) and mean $\delta^{15}N$ from 8.7‰ (shrimps) to 12.5‰ (brittlestar).

Fish species showed taxonomic segregation based on $\delta^{13}C$ and $\delta^{15}N$ values with pomacentrid species having the most depleted $\delta^{13}C$ (Fig 6.1a). The remainder of the food web could be categorised based on feeding guilds as herbivorous species had lower $\delta^{15}N$ than invertivores and predatory species. Fish were further subdivided into five groupings for trophic niche analysis these groups were the three families Pomacentridae, Chaetodontidae, and Apogonidae, plus Acanthuridae and Scaridae were grouped together to form a herbivore group and the remaining fish made up the fifth grouping ‘other predators’.

6.3.1 Stomach content analysis

A high proportion of stomachs were found empty, especially for predatory species. The dominant dietary items were recorded for species where reliable observations were made (i.e. where consumed items were not too digested and were identifiable) (Table 6.1).

6.3.2 Pomacentridae

There was a 3‰ difference in mean $\delta^{15}N$ signatures between the uppermost and lowermost species in this family. *Pomacentrus arabicus* had distinctly different

diet preferences from the other pomacentrid species; the mean $\delta^{13}C$ value reflects a greater dominance of macroalgae in the diet, which is more enriched in ^{13}C than plankton. These differences were only statistically different between *P. arabicus* and *Dascyllus trimaculatus* (K-nearest neighbour distance test $p<0.02$, Table 6.2). The other species showed a general pattern of a linear increase in $\delta^{13}C$ with increasing $\delta^{15}N$ (Fig 6.2a). *Amphiprion clarkii* had the highest $\delta^{15}N$ (15.7‰) and $\delta^{13}C$ (-15.6‰) values which were significantly different to *Pomacentrus leptus* which had the lowest $\delta^{15}N$ and second lowest $\delta^{13}C$ values (K-nearest neighbour distance test $p<0.02$). These two animals also varied in length by 60mm. There were no trends in $\delta^{15}N$ with body size across the whole family (Fig. 6.2c); *A. clarkii* was of intermediate body size yet had the highest mean $\delta^{15}N$, the largest pomacentrid, *Dascyllus trimaculatus,* had a slightly lower mean $\delta^{15}N$ of 15.1‰. *P. leptus* and *D. marginatus* were of a similar body size yet there was a 1.1‰ difference in mean $\delta^{15}N$. *Chromis flavaxilla* had a very similar $\delta^{15}N$ to that of *C. xanthurum* despite being on average 80mm smaller. The stomach contents data supported the isotopic variance. *P. arabicus* had only consumed algae and *Chromis xanthopterygia* and *A. vaigiensis* had only consumed copepods. *Amphiprion clarkii* appeared to have a different diet, with fish eggs and worms present as well as zooplankton. *Dascyllus trimaculatus* had a more mixed diet with some algae strands and other small crustaceans as well as zooplankton.

Table 6.1: Species inventory including number of samples collected and overall range of fork lengths sampled. Mean fork length is also expressed as a percentage of maximum length quoted from (Randall 1995). An indication of dietary items consumed (Diet snapshot) was obtained from opportunistic gut contents observations.

Species		Fork length range (mm)			Randall L_{max}	Lmean expressed as % of L_{max}	Diet snapshot
Abudefduf vaigiensis	3	140	-	140	180	78	Zooplankton
Acanthopagrus bifasciatus	3	210	-	245	500	47	Bivalves, gastropods
Acanthurus sohal	11	115	-	420	400	65	Algae fragments
Amphiprion clarki	3	80	-	310	140	74	Fish eggs, zooplankton, worms
Apogon cyanosoma	3	34	-	50	80	50	Small shrimp
Carangoides ferdau	3	375	-	405	350	112	Crabs, Alpheus shrimp, fish
Cephalopholis hemistiktos	9	200	-	395	350	94	Small fish, coral crabs, Alpheus shrimps, squat shrimps
Chaetodon collare	3	160	-	315	160	134	Bryazoans, nematode, fish eggs, small shrimps, coral mucus,
Chaetodon melapterus	6	125	-	146	130	95	Coral mucus and polyps, occasional amphipod
Chaetodon nigropunctatus	4	105	-	112	140	78	Coral polyps and mucus, algae, nematodes
Cheilodipterus macrodon	6	155	-	210	250	78	Fish, polychaete
Cheilodipterus novemstriatus	5	36	-	51	80	54	Small coral crab
Chromis flavaxilla	3	55	-	55	72	76	
Chromis xanthopterygia	3	110	-	120	115	98	Copepods, diatoms
Dascyllus marginatus	3	40	-	40	60	67	
Dascyllus trimaculatus	3	145	-	165	140	112	Copepods, small crustaceans, occasional algae
Epinephelus stoliczkae	3	240	-	300	380	72	Small shrimps, coral crab, mantis shrimp, hermit crab, small fish
Lutjanus ehrenbergii	6	200	-	360	350	80	Small fish, crab
Parupeneus margaritatus	5	125	-	177	230	68	Crabs, polchaete, nematode, amphipod, gastropod, small fish
Pomacanthus maculosus	3	125	-	330	500	41	Fine algal strands, Gracilaria, Hypnea, sponges, tunicates, crab
Pomacentrus leptus	3	33	-	60	68	71	
Pomacentrus arabicus	5	140	-	175	145	102	Algae fragments
Rhinecanthos assasi	3	175	-	270	300	76	Bivalve
Scarus ferruginous	3	420	-	530	410	120	Very digested/undistinguishable
Scarus persicus	3	500	-	560	500	105	Very digested/undistinguishable
Scolopsis ghanam	10	100	-	330	180	102	Small fish, crustaceans
Zebrasoma xanthurum	6	120	-	215	220	81	algae fragments

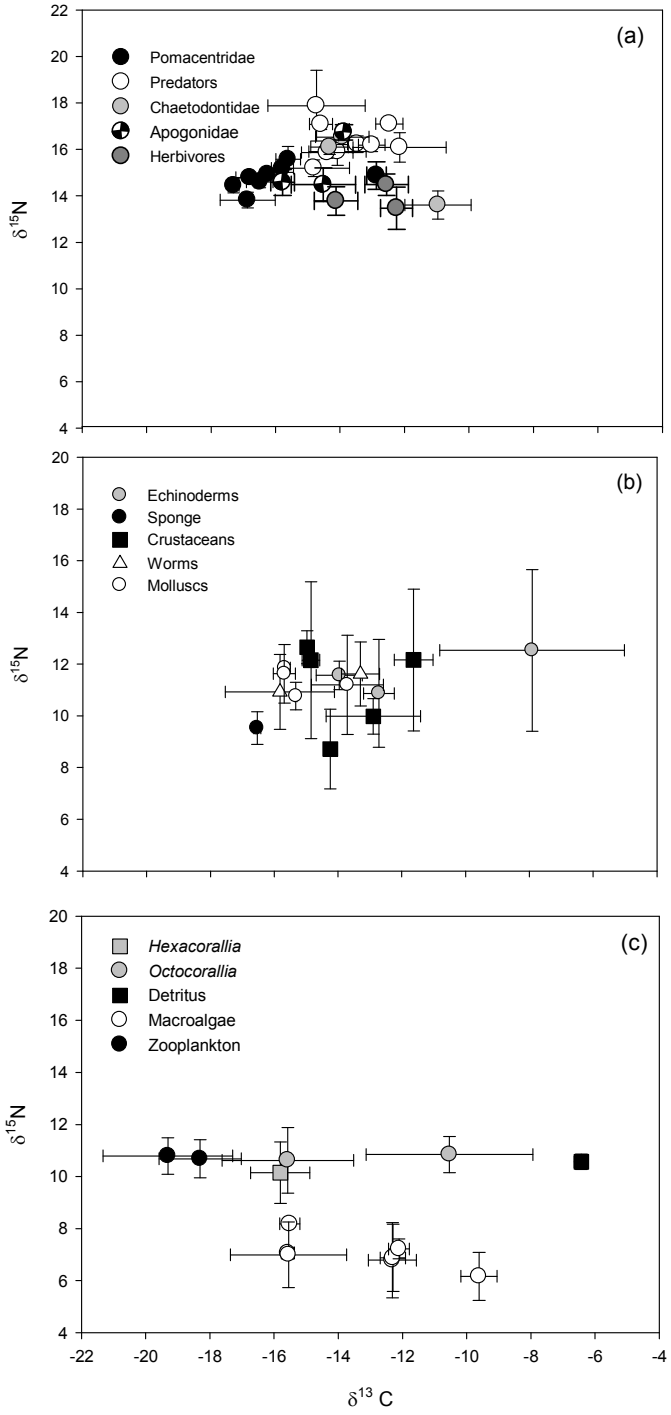


Fig. 6.1: Stable isotope ($\delta^{15}N$ and $\delta^{13}C$) bi-plots for (a) fish species (b) invertebrate species and (c) autotrophs and source materials collected from the reef ecosystem at Bandar Kayran in May/June 2004 and 2005. Error bars represent $\pm$ 1SD. Within each plot species are grouped into family or functional groups.

6.3.3 Chaetodontidae

The most common chaetodontid, *Chaetodon melapterus*, had a significantly lower $\delta^{15}N$ and higher $\delta^{13}C$ than both *C. collare* and *C. nigropunctatus* (K-nearest neighbour distance test p=0.012 and p=0.004, Fig. 6.3). *C. melapterus* and *C. nigropunctatus* were very similar in size (Table 6.1) whereas *C. collare* was on average double the length. The differences in stable isotope signatures were supported by feeding observations. *C. melapterus* was found to have 90% mucus and coral tissue with only a few individual amphipods present in the gut. Gut contents reveal the other two species had a much lower presence of coral tissue in their diet with crustaceans, nematodes and polychaete worms being more common.

6.3.4 Apogonidae

The largest fish apogonid was *Cheilodipterus macrodon*, which had similar $\delta^{15}N$ to the groupers and other apex predators, and fish and polychaetes were found in the stomach. *Cheilodipterus novemstriatus* and *Apogon cyanosoma* are much smaller species in this family (mean 49.5mm and 40mm respectively) and had significantly lower $\delta^{15}N$ than *C. macrodon* (K-nearest neighbour randomisation test, p=0.003 and p=0.0012 respectively, Fig 6.4). Despite being similar in size there was no similarity in the $\delta^{13}C$ values of these species indicating that they fed on different prey items; *A. cyanosoma* had a more planktonic diet than *C. novemstriatus*. Small shrimps and coral crabs were found in the stomachs of *A. cyanosoma* and *C. novemstriatus* respectively.

6.3.5 Herbivores

A. sohal and *Z. xanthurum* had similar $\delta^{15}N$ values but were separated on the $\delta^{13}C$ scale, with *A. sohal* being significantly more enriched (K-nearest neighbour randomisation test, p<0.001, Fig 6.2). *Scarus persicus* had a higher $\delta^{15}N$ than the acanthurids but had similar $\delta^{13}C$ to *A. sohal* (K-nearest neighbour randomisation test, p=0.281, Fig 6.2).

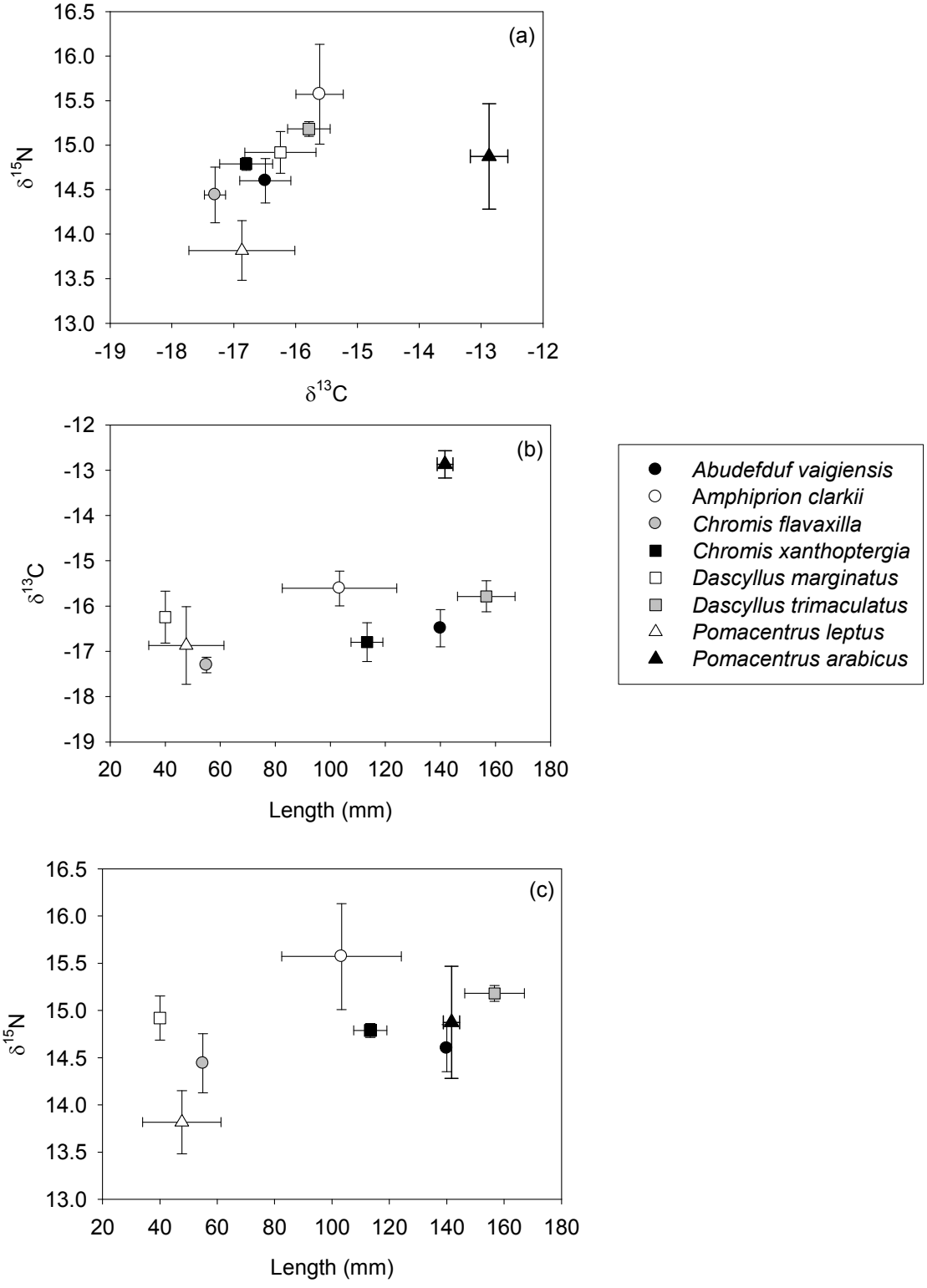


Fig. 6.2: Carbon and nitrogen stable isotope plots for 8 pomacentrid fishes (a) Bi-isotope plot (mean $\delta^{15}N$ and $\delta^{13}C \pm 1SD$) (b) $\delta^{13}C$ and body size (fork length) and (c) $\delta^{15}N$ and body size (fork length); from the reef ecosystem at Bandar Kayran. Samples collected in May/June 2004 and 2005.

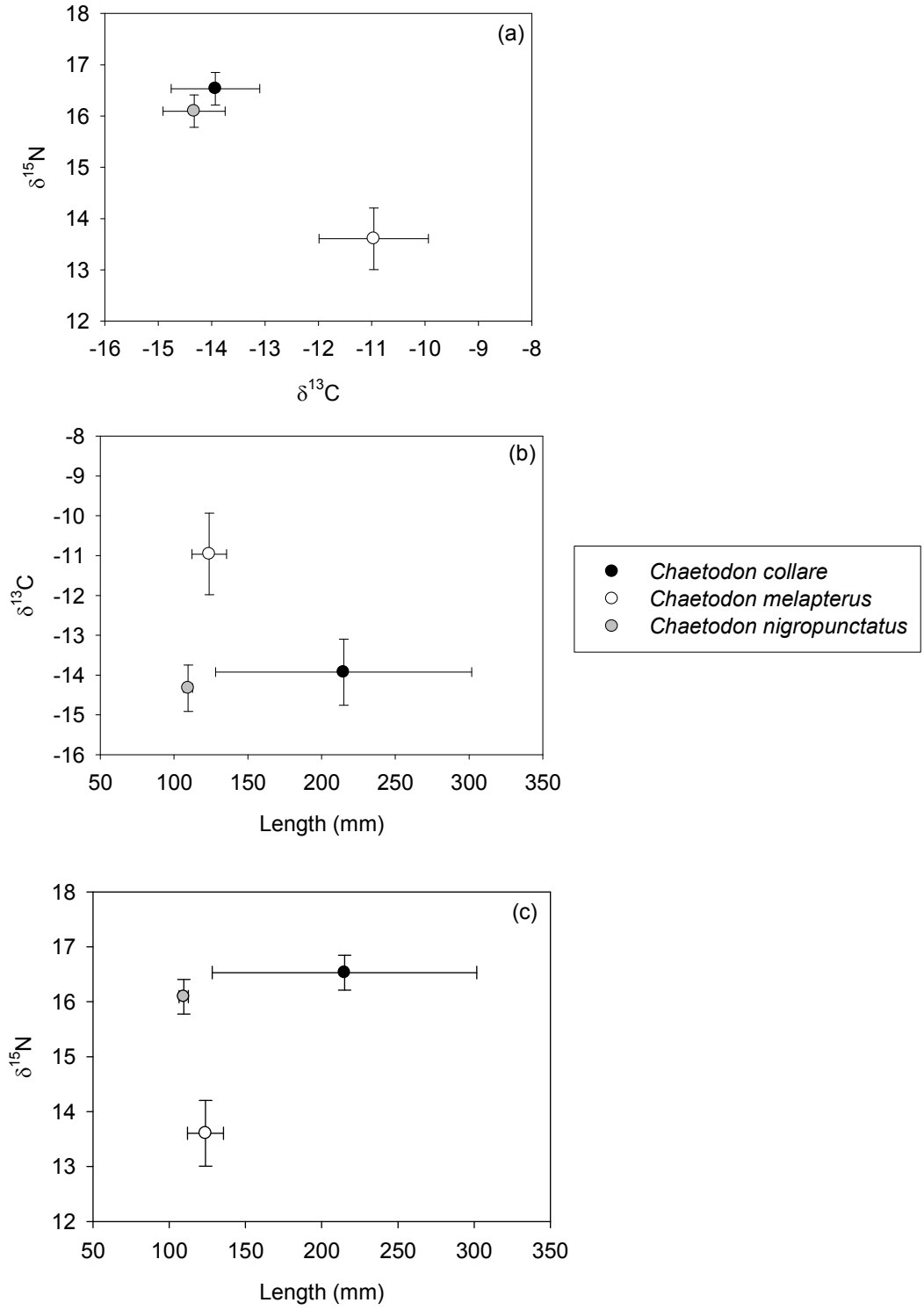


Fig. 6.3: Carbon and nitrogen stable isotope plots for 3 chaetodontid fishes (a) Bi-isotope plot (mean $\delta^{15}N$ and $\delta^{13}C \pm 1SD$) (b) $\delta^{13}C$ and body size (fork length) and (c) $\delta^{15}N$ and body size (fork length); from the reef ecosystem at Bandar Kayran. Samples collected in May/June 2004 and 2005.

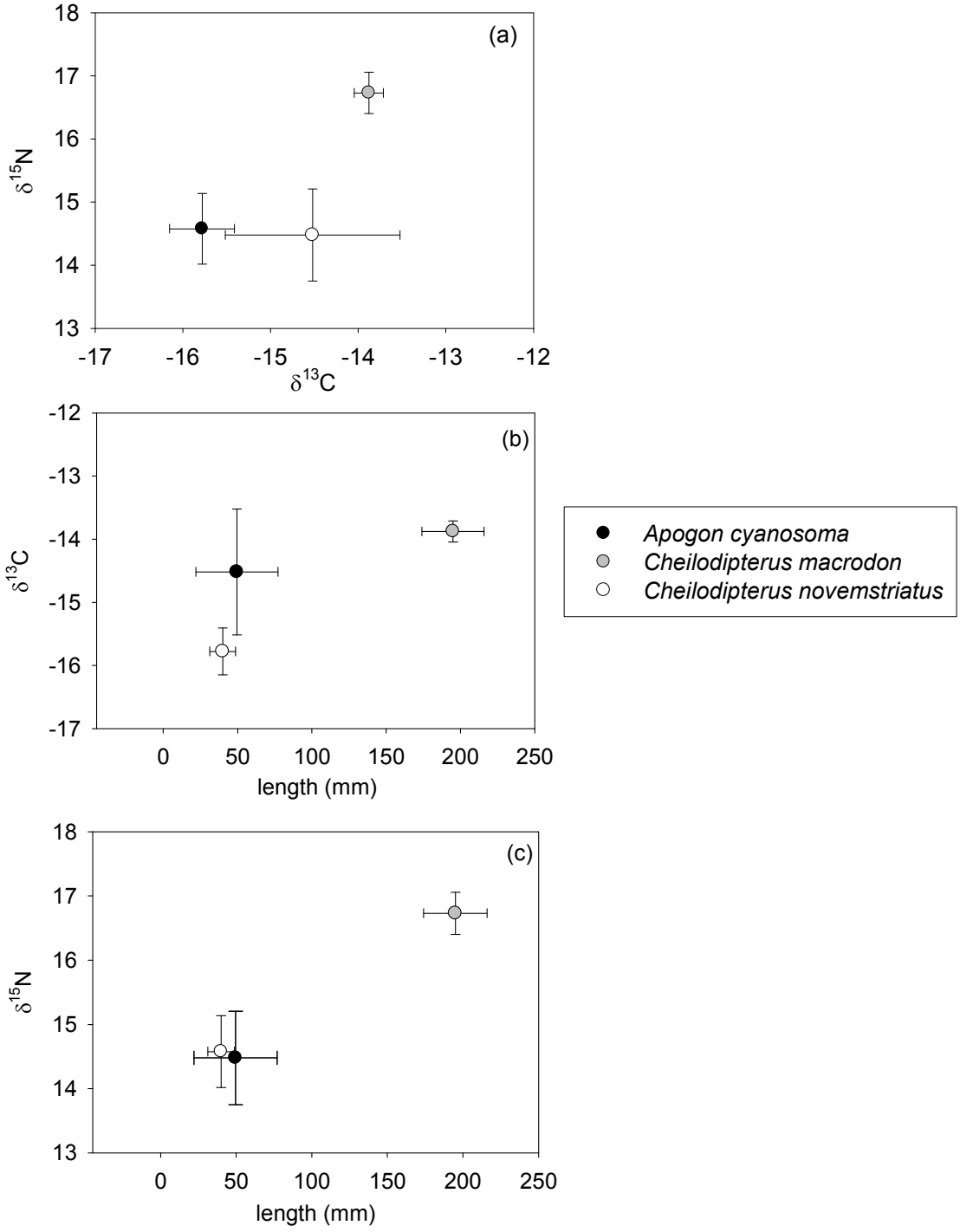


Fig. 6.4 : Carbon and nitrogen stable isotope plots for 3 Apogonidae fishes (a) Bi-isotope plot (mean $\delta^{15}N$ and $\delta^{13}C \pm 1SD$) (b) $\delta^{13}C$ and body size (fork length) and (c) $\delta^{15}N$ and body size (fork length); from the reef ecosystem at Bandar Kayran. Samples collected in May/June 2004 and 2005.

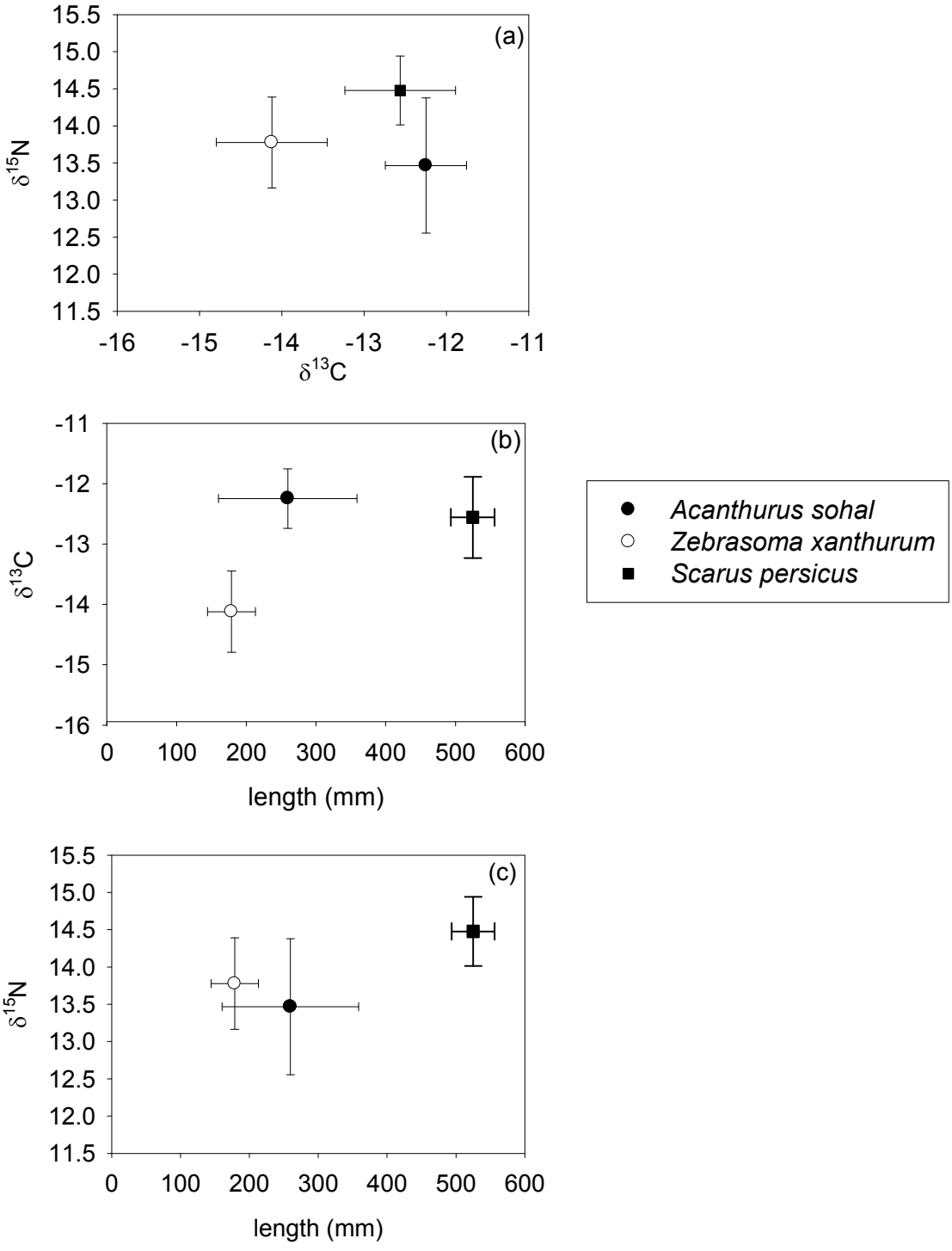


Fig. 6.5: Carbon and nitrogen stable isotope plots for 3 herbivorous fishes (a) Bi-isotope plot (mean $\delta^{15}N$ and $\delta^{13}C \pm 1SD$) (b) $\delta^{13}C$ and body size (fork length) and (c) $\delta^{15}N$ and body size (fork length); from the reef ecosystem at Bandar Kayran. Samples collected in May/June 2004 and 2005.

Detailed stomach contents analysis of *A. sohal* and *Z. xanthurum* (Chapter 5) revealed no animal components in the diet of either species. *A. sohal* feeds on fleshier algae than *Z. xanthurum* which appears to focus its diet on finer algae strands. There we no distinguishable dietary items found in the gut of the parrotfish (*Scarus persicus*).

6.3.6 Other Predators

The predator group was composed of 8 species which ranged in mean $\delta^{15}N$ from 15‰ (*Pomacanthus maculosus*) to 17.8‰ (*Carangoides ferdau*) and in $\delta^{13}C$ from -14.9‰ (*P. maculosus*) to -12.2‰ (*Epinephelus stoliczkae*). *C. ferdau* had the most variable carbon and nitrogen signatures (Fig. 6.6). *Parupeneus marginatus* and *Cephalopholis hemistiktos* had similar mean $\delta^{15}N$ signatures but very different $\delta^{13}C$ signatures but were not statistically distinguishable (K nearest neighbour randomisation test p=1.0). *P. marginatus* had a mean $\delta^{13}C$ of -14.4‰ indicating a more benthic diet than that of *C. hemistiktos* which was ^{13}C depleted reflecting a more planktonic dietary source. These two species also had much lower standard deviations than *C. ferdau*, suggesting less feeding variability among individuals. The remaining species in this group had a high level of dietary similarity, the mean $\delta^{15}N$ values clustered about 16‰ and standard deviations overlapped. *Scolopsis ghanam* and *Cephalopholis hemistiktos* showed the least similarity statistically among the species in this group (Table 6.2). There were no obvious patterns in $\delta^{15}N$ or $\delta^{13}C$ based on size for any of the animals in this group (Fig 6.6, b and c). Most of the species in this group had a mix of small fish and benthic or coral dwelling crustaceans and bivalves in their stomachs, apart from *R. assai* and *P. maculosus* which had no evidence of fish in their diet.

6.3.7 Abundance

The most abundant fish family at Bandar Kayran was the pomacentrids with *C. flavaxilla* and *C. xanthopterygia* being the most abundant species by numbers (Fig. 6.7). Other planktivores also ranked high in relative abundance followed by some of the more piscivorous species such as *Scolopsis ghanam* and *Parupeneus marginatus*. *Chaetodon melapterus* was the most common butterfly fish observed on the reef and the smaller species of apogonid were also more abundant than the

larger *C. macrodon*. The abundance of the herbivore *Acanthurus sohal* appeared low in the visual census surveys, however they were more common in the surf zone to the edge of the reef where the macroalgae coverage was denser.

6.3.8 Intra-specific variability

Among the 118 individuals in 27 species, the total range in $\delta^{15}N$ values was 12.50-17.43‰. There were similar patterns in fish variability measured as ranges and as standard deviations with fish of lower trophic level (smaller $\delta^{15}N$ values) showing more variability than fish of higher $\delta^{15}N$ value (Fig. 6.8).

6.3.9 Size related changes in diet

Of the seven species examined for size-related changes in diet, four species (*A. sohal*, *C. hemistiktos*, *P. margaritatus* and *S. ghanam*) had significant correlations of $\delta^{15}N$ with length and two species (*C. novemstriatus* and *P. margaritatus*) had significant positive correlations of $\delta^{13}C$ with length (Fig 6.9 a-n and Table 6.2). *P. margaritatus* and *S. ghanam* had a large range of $\delta^{15}N$ values among individuals of short fork lengths but became less variable at greater lengths. This pattern was not observed in all fish species; *C. hemistiktos* and *A. sohal* had a similar spread of $\delta^{15}N$ values across all lengths. There were no clear patterns of $\delta^{13}C$ or $\delta^{15}N$ changes with length for *Chromis xanthopterygia* or *Abudefduf vaigiensis*.

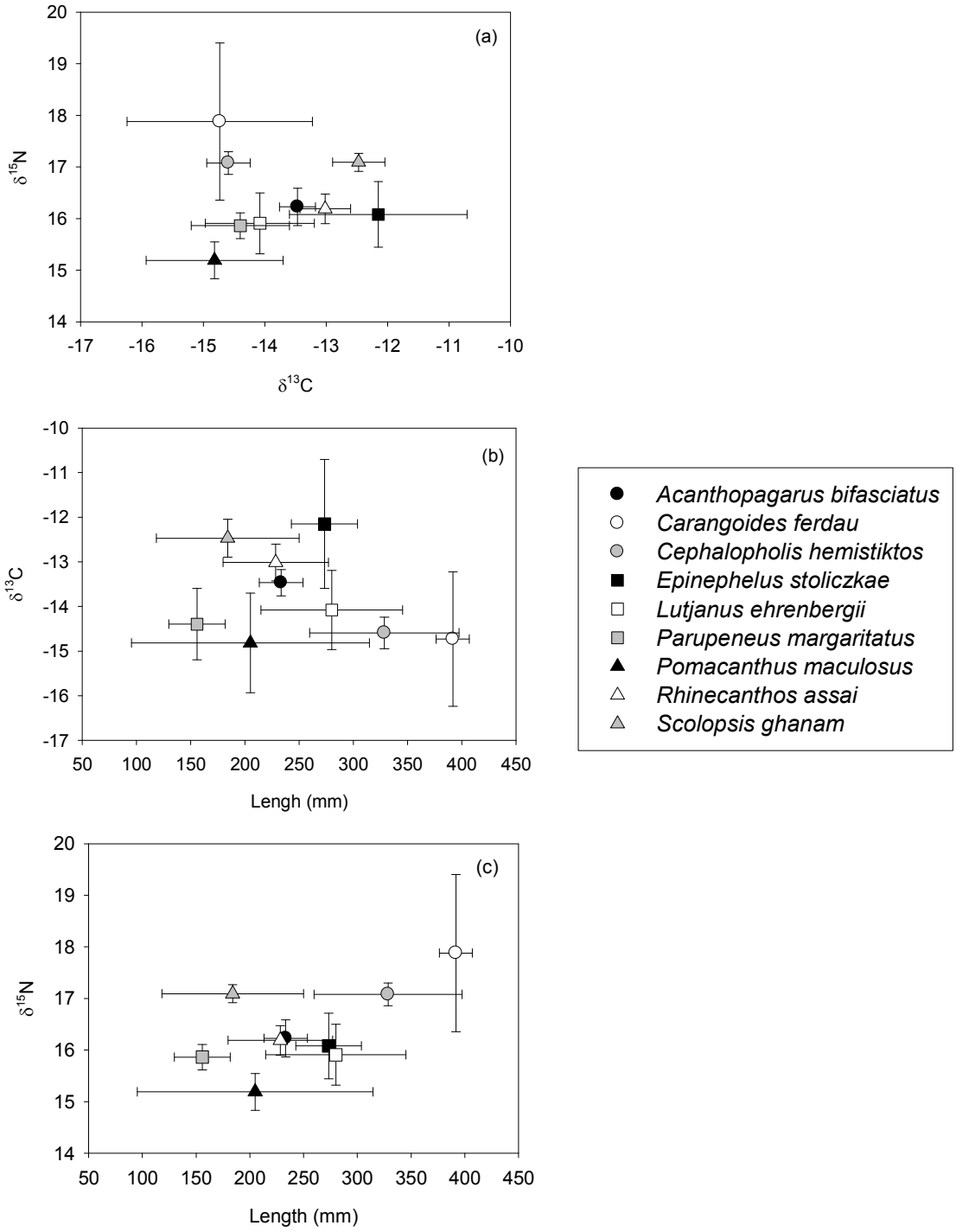


Fig. 6.6: Carbon and nitrogen stable isotope plots for 9 predatory fishes (a) Bi-isotope plot (mean $\delta^{15}N$ and $\delta^{13}C \pm 1SD$) (b) $\delta^{13}C$ and body size (fork length) and (c) $\delta^{15}N$ and body size (fork length); from the reef ecosystem at Bandar Kayran. Samples collected in May/June 2004 and 2005.

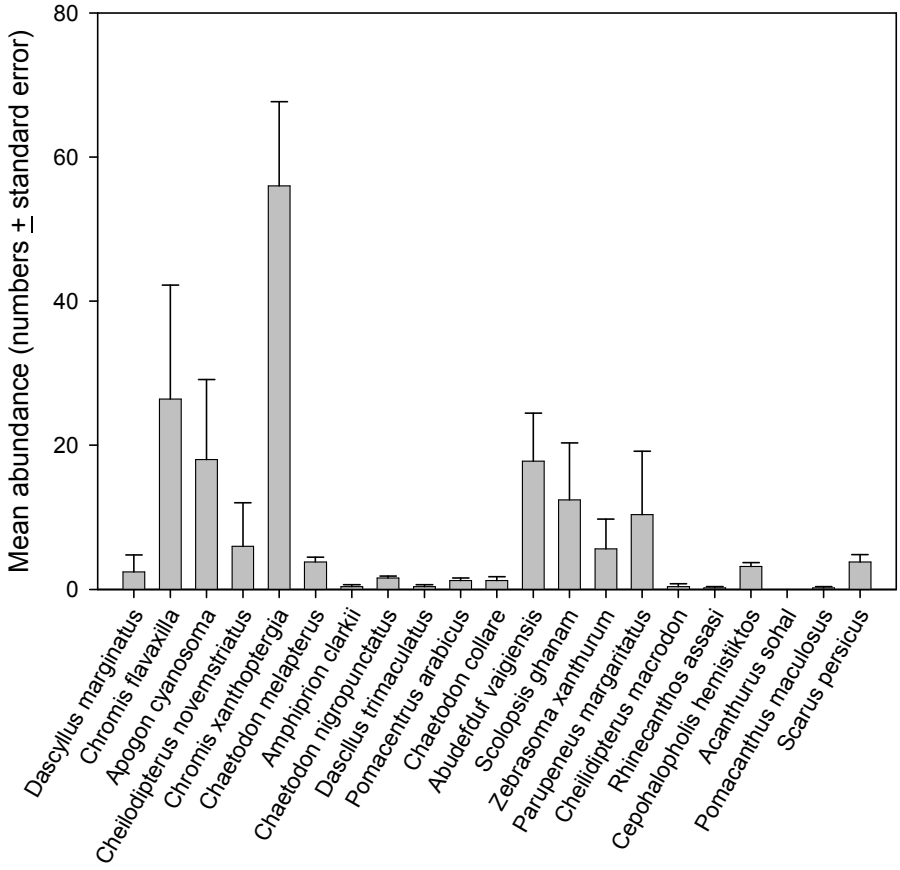


Fig 6.7: Relative abundance (numbers ± standard error) of the most common reef fish species at Bandar Kayran based on visual census surveys by snorkelling (average values taken over 5 transect replicates. The rarer species on the reef are not shown.

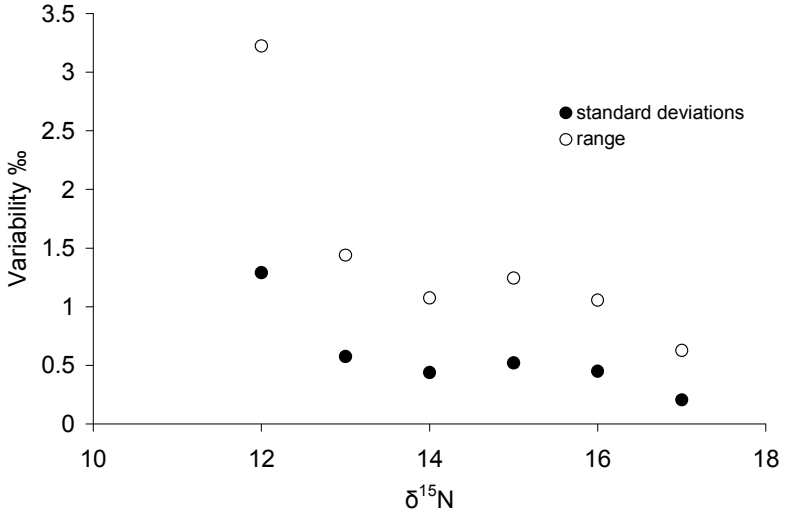


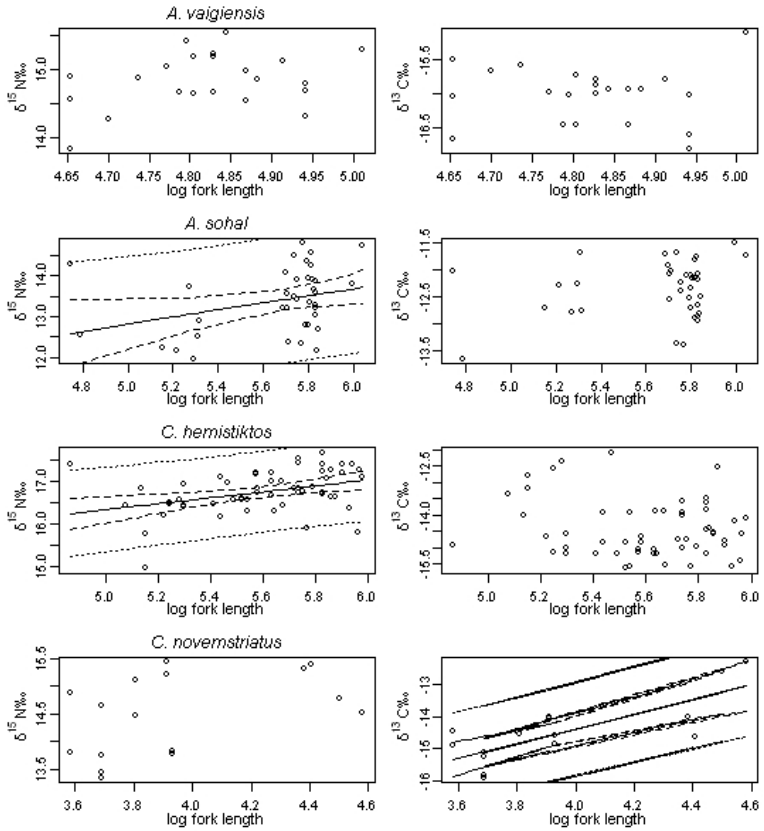
Fig. 6.8: Intra-species variability in $\delta^{15}N$, measured by standard deviation and absolute range of values for all fish species, with respect to actual $\delta^{15}N$ representing trophic position in the food web.

Table 6.2: K-nearest neighbour randomisation test statistic matrix of isotopic data of fish species at Bandar Kayran. The lower part of the matrix gives the pairwise p values and in the upper section * indicates significance at p<0.05.The boxed values group species by functional or family.

	Pomacentridae								Predators										Herbivores			Chaetodontidae			Apogonidae		
	Abudefduf vaigiensis	*Amphiprion clarki*	*Chromis flavaxilla*	*Chromis xanthopterygia*	*Dascyllus marginatus*	*Dascyllus trimaculatus*	*Pomacentrus leptus*	*Pomacentrus arabicus*	*Acanthopagrus bifasciatus*	*Carangoides ferdau*	*Cephalopholis hemistiktos*	*Epinephelus stoliczkae*	*Lutjanus ehrenbergii*	*Parupeneus margaritatus*	*Pomacanthus maculosus*	*Rhinecanthos assasi*	*Scolopsis ghanam*	*Scarus persicus*	*Acanthurus sohal*	*Zebrasoma xanthurum*	*Chaetodon collare*	*Chaetodon melapterus*	*Chaetodon nigropunctatus*	*Apogon cyanosoma*	*Cheilodipterus macrodon*	*Cheilodipterus novemstriatus*
Abudefduf vaigiensis											*		*	*			*		*	*		*	*		*	*
Amphiprion clarki	0.303						*				*						*		*	*		*			*	*
Chromis flavaxilla	1.000	0.100									*		*	*			*		*	*		*	*		*	*
Chromis xanthopterygia	1.000	0.199	1.000								*		*	*			*		*	*		*	*		*	*
Dascyllus marginatus	1.000	0.292	0.572	1.000					*		*	*	*	*		*	*	*	*	*	*	*	*		*	
Dascyllus trimaculatus	1.000	1.000	0.056	1.000	1.000		*	*	*		*	*	*	*		*	*	*	*	*	*	*	*		*	*
Pomacentrus leptus	0.137	0.017	0.145	0.142	0.050	0.020				*	*			*			*		*			*		*	*	*
Pomacentrus arabicus	0.298	0.102	1.000	0.595	0.113	0.030	0.137				*		*	*			*		*	*		*	*		*	*
Acanthopagrus bifasciatus	0.098	0.097	0.096	0.097	0.030	0.030	0.302	0.101									*		*	*		*				*
Carangoides ferdau	0.200	0.406	0.099	0.390	0.138	0.143	0.034	0.098	0.102		*		*	*			*		*	*		*	*		*	*
Cephalopholis hemistiktos	0.004	0.005	0.006	0.004	0.002	0.002	0.001	0.004	0.132	0.005		*	*		*	*		*	*	*		*		*		*
Epinephelus stoliczkae	0.096	0.101	0.099	0.098	0.031	0.029	0.335	0.096	0.394	0.200	0.045						*		*	*		*				*
Lutjanus ehrenbergii	0.011	0.094	0.010	0.012	0.008	0.019	0.171	0.009	0.929	0.011	0.024	0.062					*		*	*		*		*		*
Parupeneus margaritatus	0.017	0.180	0.017	0.017	0.008	0.035	0.022	0.019	0.928	0.037	1.000	0.075	0.911				*	*	*	*		*		*		*
Pomacanthus maculosus	0.302	0.405	0.099	0.198	0.113	0.143	0.110	0.103	0.205	0.298	0.004	0.198	0.225	0.558			*		*	*		*		*	*	*
Rhinecanthos assasi	0.099	0.098	0.097	0.107	0.027	0.030	0.300	0.097	1.000	0.101	0.005	0.704	0.751	0.181	0.097				*	*		*				*
Scolopsis ghanam	0.004	0.004	0.003	0.003	0.001	0.001	0.000	0.004	0.020	0.004	0.068	0.023	0.000	0.001	0.005	0.193		*	*	*	*	*	*	*		*
Scarus persicus	0.100	0.098	0.101	0.099	0.028	0.029	0.629	0.097	0.098	0.099	0.006	0.102	0.057	0.016	0.095	0.101	0.003			*			*		*	*
Acanthurus sohal	0.003	0.003	0.002	0.003	0.001	0.001	0.030	0.003	0.002	0.004	0.000	0.007	0.000	0.000	0.003	0.004	0.000	0.281		*	*		*	*	*	*
Zebrasoma xanthurum	0.013	0.012	0.013	0.013	0.005	0.008	0.092	0.011	0.012	0.011	0.000	0.013	0.005	0.007	0.049	0.012	0.000	0.024	0.000		*	*	*	*	*	
Chaetodon collare	0.106	0.108	0.097	0.100	0.029	0.029	0.090	0.102	0.609	0.201	0.411	0.402	0.725	0.284	0.198	0.601	0.004	0.096	0.003	0.011		*				*
Chaetodon melapterus	0.014	0.013	0.011	0.013	0.004	0.005	0.025	0.013	0.012	0.012	0.000	0.026	0.002	0.002	0.014	0.012	0.000	0.068	0.079	0.002	0.012		*	*	*	*
Chaetodon nigropunctatus	0.026	0.119	0.026	0.028	0.029	0.027	0.055	0.030	1.000	0.029	1.000	0.144	0.887	1.000	0.253	0.119	0.002	0.029	0.001	0.006	1.000	0.004		*		*
Apogon cyanosoma	1.000	0.597	0.197	1.000	1.000	1.000	0.035	0.100	0.099	0.099	0.005	0.100	0.012	0.033	0.396	0.098	0.005	0.098	0.003	0.036	0.104	0.012	0.030		*	
Cheilodipterus macrodon	0.011	0.010	0.013	0.010	0.004	0.004	0.002	0.013	1.000	0.013	1.000	0.085	0.818	1.000	0.013	0.504	1.000	0.011	0.000	0.002	1.000	0.002	1.000	0.013		*
Cheilodipterus novemstriatus	0.036	0.035	0.015	0.011	0.058	0.264	0.002	0.011	0.012	0.013	0.000	0.012	0.006	0.005	0.035	0.010	0.000	0.013	0.000	0.548	0.010	0.002	0.005	0.071	0.003	

Table 6.3: Traits contributing to the trophic niches of fish species at Bandar Kayran

Species	n	Group	Niche overlap within taxon	Overlap outwith taxon	Guild	Feeding habitat	Abundance	Length
Apogon cyanosoma	3	Apogonidae	Med	Med	planktivore	mid water/ noctornal	high	80
Cheilodipterus macrodon	6	Apogonidae	Low	High	piscivore	nocturnal	med	250
Cheilodipterus novemstriatus	5	Apogonidae	Low	Low	planktovore/omnivore	nocturnal	med	80
Chaetodon collare	3	Chaetodontidae	High	High	planktivore/corallivore	mid water	med	160
Chaetodon melapterus	6	Chaetodontidae	Low	Very Low	corallivore	reef crest	med	130
Chaetodon nigropunctatus	4	Chaetodontidae	Low	Med	omnivore	reef crest	med	140
Acanthurus sohal	11	Herbivore	Low	Low	herbivore	reef crest	low	400
Scarus persicus	3	Herbivore	Med	Low	herbivore	reef crest	med	500
Zebrasoma xanthurum	6	Herbivore	Low	Low	herbivore	reef crest	med	220
Abudefduf vaigiensis	3	Pomacentridae	High	High	omnivore	mid water	high	180
Acanthopagrus bifasciatus	3	Pomacentridae	High	High	piscivore	predator	low	500
Amphiprion clarki	3	Pomacentridae	High	Med	omnivore/planktivore	mid water	med	140
Chromis flavaxilla	3	Pomacentridae	High	Med	planktivore	coral heads	v. high	72
Chromis xanthopterygia	3	Pomacentridae	High	Med	planktivore	mid water	v. high	115
Dascyllus marginatus	3	Pomacentridae	High	Low	planktivore	coral heads	med	60
Dascyllus trimaculatus	3	Pomacentridae	Med	Low	planktivore	benthic/reef crest	low	140
Pomacentrus leptus	3	Pomacentridae	High	Low	planktivore	coral heads	N/A	68
Pomacentrus arabicus	5	Pomacentridae	Med	Low	herbivore	reef crest	med	145
Carangoides ferdau	3	Predator	High	High	piscivore	predator	low	350
Cephalopholis hemistiktos	9	Predator	Low	Low	piscivore/invertivore	predator	med	350
Epinephelus stoliczkae	3	Predator	High	High	piscivore	predator	low	380
Lutjanus ehrenbergii	6	Predator	High	High	invertivore	predator/benthic	low	350
Parupeneus margaritatus	5	Predator	High	High	omnivore	benthic	high	230
Pomacanthus maculosus	3	Predator	High	High	herbivore/invertivore	benthic	low	500
Rhinecanthos assasi	3	Predator	High	High	invertivore	benthic	low	300
Scolopsis ghanam	10	Predator	Low	Low	invertivore	benthic/predator	high	180



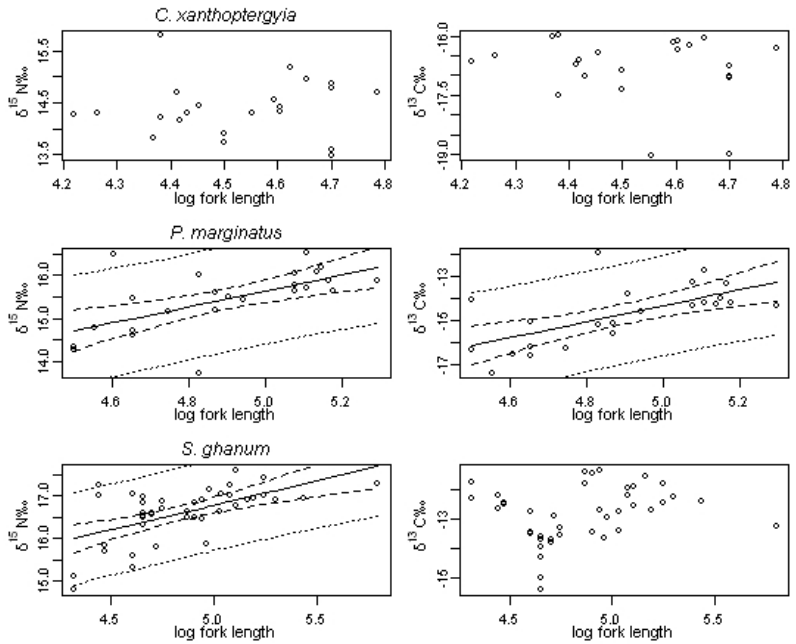


Fig. 6.9: Regression analysis of $\delta^{13}C$ and $\delta^{15}N$ and length for seven fish species All length data was log transformed prior to analysis. Confidence intervals (95%) are represented by dashed lines and prediction limits by dotted lines.

Table 6.4: Regression statistics for 7 species analysed for changes in $\delta^{15}N$ and $\delta^{13}C$ with length. R^2 and p values are only shown for significant results. Length data were log transformed to normalise prior to analysis.

Species	$\delta^{13}C$				$\delta^{15}N$			
	r^2	p	Slope	Intercept	r^2	p	Slope	Intercept
Abudefduf vaigiensis			-15.51	-0.1034			8.05	1.4095
Acanthurus sohal			-14.6351	0.4022	0.11	0.451	8.45	0.8725
Cephalopholis hemistiktos			-10.977	-0.618	0.14	0.004	12.7916	0.7075
Cheilodipterus novemstriatus	0.6	0.0004	-23.65	2.316			10.599	0.984
Chromis xanthopterygia			-13.235	-0.7901			3.59	0.17773
Parupeneus margaritatus	0.143	0.0007	-32.6436	3.66	0.38	0.001252	0.3583	1.8565
Scolopsis ghanam			-16.1704	0.6840	0.33	0.00006	11.025	1.1524

6.4 Discussion

Overall stable isotope data provided a good description of consumers' approximate trophic position and diet sources. The spread of nitrogen isotopic data confirmed that the food web had between 3 and 4 trophic levels, typical of many aquatic systems (Arias-Gonzalez et al. 1998, Vander Zanden et al. 1997). There were multiple sources to the food web with consumers deriving energy from a range of planktonic and benthic inputs. $\delta^{13}C$ was particularly useful in distinguishing between various diet sources, both among and between guilds. The use of variance of isotopic data has been shown to be useful in measuring diet breadth (Bearhop et al. 2004, Layman et al. 2007) and here the K- nearest neighbour randomization test provided a measure of trophic niche overlap.

The majority of the reef fish at Bandar Kayran had some dietary overlap with other, often closely related species, suggesting that trophic niche specialisation was rare and that generalist feeding behaviour was common. Trophic niche specialisation, although frequently referred to in theoretical texts, has seldom been demonstrated in the marine environment (Sale 1977). A notable exception is the genus of poisonous cone snail *Conus* which shows high levels of specialisation at species level (Kohn and Nybakken 1975). In Oman some diet segregation was noted between different trophic grouping/families, for example the majority of the species in the pomacentrid guild were clearly deriving their food from a more depleted $\delta^{13}C$ source (plankton) then other consumers in the food-web (Fig 6.1). However, within the pomacentrid family there was considerable isotopic overlap among species, suggesting that no single species had a clearly defined niche (Fig. 6.2). A specialised diet is not necessarily favourable in a dynamic environment, where resource availability is forever changing if a species solely relies on a single resource and that resource disappears then the consumer would be forced to switch diets or starve (Valiela 1984). In an upwelling environment, such as Oman, there may be a seasonal influence on the availability of food sources. This would promote trophic generalism and hence species coexistence may not be based on competitive processes (Connell 1978) hence, trophic niches might not be separate (Genner et al. 1999).

The carbon and nitrogen isotope analysis revealed that there was some diet segregation within the chaetodontids (butterflyfish). There were significant differences between the most abundant chaetodontid species at Bandar Kayran, *C. melapterus,* which had the lowest $\delta^{15}N$ and highest $\delta^{13}C$, and the other two chaetodontid species examined. Thus *C. melapterus* feeds at a lower trophic level and on dietary items which are less planktivorous in origin than those of the other two species. Stomach contents showed a high proportion of coral polyps in *C. melapterus*, while *Chaetodon collare* and *C. nigropunctatus* were feeding on a greater range of items with benthic invertebrates such as crustaceans and nematodes being common. *Chaetodon melapterus* may be classed as a facultative coral feeder whereas *C. collare* and C. *nigropunctatus* are generalists (Harmelin-Vivien and Bouchon-Navaro 1983).

In the Apogonidae, there was also little evidence of dietary overlap, based on the isotope data. The largest fish sampled in this family, *Cheilodipterus macrodon*, had a $\delta^{15}N$ similar to groupers and other apex predators. The low variance ($\pm$0.32 standard deviation) in mean $\delta^{15}N$ suggests that *C. macrodon* was relatively specialised in its feeding strategy, supporting research in other regions showing that this species is primarily piscivorous (Barnett et al. 2006). The two other species sampled from this family, *A. cyanosoma* and *C. novemstriatus,* were of a similar body size, and based on $\delta^{15}N$ values were of a similar trophic level but they are unlikely to have similar diets as they had distinctly different $\delta^{13}C$ values. Partitioning of food resources was also seen in the acanthurids *A. sohal* and *Z. xanthurum* which were of similar trophic level and body size but had significantly different carbon signatures.

When isotopic evidence of trophic niche partitioning was limited, as in the Pomacentridae and 'other predators' groups, other mechanisms may be contributing to the maintenance of diversity on the reef. These include habitat or temporal partitioning, intense competition or a limited population size (Darnaude et al. 2001, Nagelkerken et al. 2006, Sale 1991b). The pomacentrid guild had the most depleted ^{13}C of all the fish in the community reflecting their general reliance on planktonic food sources. There was high overlap in isotopic variance among some species however the 3‰ difference in $\delta^{15}N$ and $\delta^{13}C$ across all species indicated that there

was some diversity of feeding within the guild. *A. clarki* and *D. trimaculatus* were the most enriched in $\delta^{13}C$ and had distinctly different diets from the two most ^{13}C depleted fish, *P. leptus* and *C. flavaxilla. A. clarki* and *D trimaculatus* had macroalgae and benthic invertebrates present in their diet, and while no stomach data were collected for *P. leptus* and *C. flavaxilla* the $\delta^{13}C$ values suggest they feed on a more planktonic food source. The remaining species are generally classed as being planktivorous yet there may be some size-based selection of items occurring. Large size classes of zooplankton were more enriched in both $\delta^{15}N$ and $\delta^{13}C$ than smaller size classes so fish that preferentially select larger zooplankton will be more enriched than those that consume small zooplankton.

Apex predators tend to be less site-attached, and rove in order to find food, so distinct habitat partitioning with other site-attached predators may be limited. The stable isotope analysis indicated a high level of dietary overlap among species in two groups *Epinephelus stoliczkae, Rhinecanthos assasi*, and *Acanthopagrus bifasciatus* and among *Lutjanus ehrenbergii, Parupeneus margaritatus* and *Acanthopagrus bifasciatus*. Apart from *P. margaritatus* these species are all relatively rare on the reef (Fig. 7.5). The two most abundant species in this group, *Cephalopholis hemistiktos* and *Scolopsis ghanam,* had low isotopic variation among the individuals sampled, indicating a high degree of specialisation or feeding similarity among individuals, which is common for abundant or dominant species in some families (Jennings et al. 2002b, Pratchett 2005, Sale 1991a). Trophic generalists tend to be the subordinate species in any inter-specific competition and will vary their diet depending on the presence of competitors in the ecosystem (Beaudoin et al. 1999).

An increase in $\delta^{15}N$ with size was observed in *C. hemistiktos, P. margaritatus*, *S. ghanam* and *A. sohal* and supports similar findings in a range of other fish species (Badalamenti et al. 2002, Hobson and Welch 1995, Le Loc'h and Hily 2005, Wainright et al. 1993). The level of trophic fractionation seems not to vary in European sea bass (*Dicentrarchus labrax*)(Barnes 2006), so assuming this is true for other fish species, there are two possible explanations for $\delta^{15}N$ increasing with size. Either consumers select larger individuals of the prey species that are higher in $\delta^{15}N$ or the consumers switch prey items to species that are themselves

more ^{15}N enriched. In theory larger fish will have larger mouths and will be able to expand their diet to encompass progressively larger prey items (Karpouzi and Stergiou 2003, Scharf et al. 2000), however larger prey items may not always be of a higher trophic level (Gu et al. 1997).

Fish at different life history stages will have different nutritional needs and it is likely that a dietary switch with body size reflects a change in nutritional requirements. Ontogenetic diet changes are common and can be identified with isotopes when there is a shift in $\delta^{13}C$ along with $\delta^{15}N$ (Hentschel 1998, Renones et al. 2002, Wainright et al. 1993). Isotope analysis revealed such an ontogenetic change in *C. hemistiktos*, a species which was also found to switch diet with size in the Red Sea based on dietary analysis (Shpigel and Fishelson 1989).

Similarly some herbivorous species may have a carnivorous feeding stage as juveniles, in order to meet their nitrogen requirements (Horn 1989). There were too few juvenile acanthurids present on the reef to test this but theoretically a diet switch from carnivorous to herbivorous material would cause a decline in $\delta^{15}N$ with increasing body size. *A. sohal* had a positive relationship between length and $\delta^{15}N$ in this study yet the macroalgae diet of this species was constant, indicating that another process e.g. food processing rates (see chapter 5) may be controlling enrichment of ^{15}N. However the situation with herbivore $\delta^{15}N$ and size remains unclear as Cocheret de la Morniniere et al (2003) found no clear pattern in herbivores surveyed in mangrove and bay habitats.

Omnivory should increase with trophic position (France 1997, Lindeman 1942) but in this study intra-specific variation in $\delta^{15}N$ across all reef fish sampled declined with increasing trophic level. Despite France (1997) analysing a larger data set covering a wider range of $\delta^{15}N$ values than the present study, the interpretation of isotopic variability in terms of omnivory is not robust. Large variations in $\delta^{15}N$ may occur in species where individuals all specialise on different prey items and low variation may be observed where intra-specific feeding is wide ranging in $\delta^{15}N$ yet similar among all species sampled (Bearhop et al. 2004, Sweeting et al. 2005). The analysis by France (1997) can therefore only be used to describe intra-specific variation and not as a measure of omnivory nor feeding specialisation of species *per se*.

At the individual species level *S. ghanam* and *P. margaritatus* had a greater range of $\delta^{15}N$ and $\delta^{13}C$ values in smaller individuals than in larger fish. While it cannot be concluded that the smaller individuals were omnivorous as the $\delta^{15}N$ values did not span more that 3‰ (Ponsard and Arditi 2001), there was a greater diversity of feeding among the smaller individuals than the larger individuals. This pattern has also been observed in other fish with ontogenetic feeding patterns, especially in fish that are generalist feeders when juveniles then become primarily piscivorous as adults (Badalamenti et al. 2002, McCarthy et al. 2004, Power et al. 2002). The higher levels of feeding plasticity observed at lower trophic levels may be a result of prey being smaller and less mobile than those of their piscivorous counterparts (Gu et al. 1997) (taken from Baeudoin 1999) although this view is contradictory to that of France (France 1997). Given the considerable evidence for some species switching their diets at different stages in their life-history, the data here support the suggestion that such species should be represented in food-webs by 'ontospecies' (Deb 1997). Here the juvenile and adults of the same species would be entered separately in the food-web as they will be competing with different species for food resources at different stages in their life time (Deb 1997).

The present analysis suggests that size alone is a poor a predictor of $\delta^{15}N$ in reef fish as species of a similar size can vary in $\delta^{15}N$ by up to 4‰. The largest fish surveyed was *Scarus persicus*, a herbivore with an intermediate $\delta^{15}N$ value. There have been similar findings in other reef communities where $\delta^{15}N$ increases with body size across carnivorous fishes (Haemulidae and Lutjanidae) but not for herbivorous fishes (Acanthuridae and Scaridae) (Cocheret de la Moriniere et al. 2003). Large bodied herbivores are rare in temperate communities where size and $\delta^{15}N$ have shown a strong positive relationship at the community level (Jennings et al. 2001).

The use of stable isotopes in food web ecology to describe resource use and feeding interactions may be improved with the use of further tracers (e.g. sulphur) and isotope mixing models (Fry 1988, Lubetkin and Simenstad 2004, Phillips and Gregg 2003). A current limitation with stable isotope analysis is that it often is only possible to characterise specific dietary items into relatively broad categories, inherently masking any finer scale specialisation (Polunin et al. 2001). Isotopic

variance is emerging as a useful further insight into feeding ecology of populations and communities (Layman et al. 2007). In theory, the degree of diet specialisation of a species can be more accurately described using the within-individual and between-individual variances in isotopic ratios. A generalist population would be classified by high intra-individual and low inter-individual variance while a specialist population would have both low intra-individual and low inter-individual variance. This theory remains to be tested but this could be done using animals where a tissue with a high turnover rate such as feathers, blood or vibrissae can be sampled through time.

7 Using multi-source isotope mixing models to explore variance in trophic fractionation in marine consumers.

7.1 Introduction

Food-webs are the most generic models of ecosystems and are useful descriptors of interactions that underpin their structure and function. To be effective specific knowledge is required of the feeding habits of individual, or populations of, consumers. Isotopic data can be useful in this regard providing a time-integrated measure of the assimilated diet material (Post 2002). Detailed information can be obtained about the trophic position of consumers and about the ultimate autotrophic sources that support their nutritional needs. However, when a consumer feeds on multiple food sources it becomes more difficult to make inferences from only dual isotope data (Peterson et al. 1986). The consumer isotope signature, minus any trophic fractionation, should comprise a 'weighted mixture' reflecting the relative contributions of the different dietary item's signatures thus proportions of these can be determined using mathematical 'mixing models' such as those of Kwak and Zedler (Kwak and Zedler 1997) and Phillips (Phillips 2001)

Mixing models consist of a series of linear equations that conserve mass balance to estimate potential source contributions in a mixture (Chapter 1, Equations 1.5-1.7). The mixture and sources must initially be aligned by applying a fractionation factor (to either the consumer or food sources) to account for enrichment per trophic level and to bring the mixture within the solution space (see Fig. 1 b Phillips and Gregg 2001). Linear models can be solved to give a unique solution for n tracers and n+1 sources. Hence, a dual-isotope linear model (e.g. using $\delta^{13}C$ and $\delta^{15}N$) can partition up to three food sources to give a unique solution (Kwak and Zedler 1997, Phillips 2001). Mixing models work best when there is high isotopic separation between the sources and the variances are low (Phillips and Gregg 2001).

To increase the resolution of potential diet items, more tracers are required. After carbon and nitrogen, sulphur is often the element in marine studies (Connolly et al. 2004). Marine sources tend to have wide ranging $^{34}S/^{32}S$ ratios (Fry et al. 1982) and the inclusion of sulphur isotopes in dietary analysis has been beneficial in

distinguishing between potential sources of nutriment (Connolly et al. 2004, Peterson et al. 1986).

Three isotope (CNS) mixing models have been successful in describing diet composition for terrestrial consumers (e.g. wolves and bears) where the number of possible dietary items is small (Ben-David et al. 1997a, Ben-David et al. 1997b, Phillips 2001). However the usefulness of these models in more complex marine ecosystems is limited as the number of sources (potential diet items) is often much greater than the number of available isotope tracers. In an attempt to overcome these limitations, three separate linear model routines, IsoSource (Phillips and Gregg 2003), SOURCE and STEP (Lubetkin and Simenstad 2004), have been developed to provide formalised procedures for application to isotope data where the sources outnumber the tracers. All three routines use similar linear equations to mixing models for *n* isotope tracers and *n*+1 sources (Phillips 2001) but adjusted for >*n*+1 sources. For example, using m sources;

$$f_1 + f_2 + f_3 + ...f_m = 1 \quad (7.1)$$

$$f_1 t_1 + f_2 t_2 + f_3 t_3 + ...f_m t_m = C_t \quad (7.2)$$

$$f_1 u_1 + f_2 u_2 + f_3 u_3 + ...f_m u_m = C_u \quad (7.3)$$

$$f_1 v_1 + f_2 v_2 + f_3 v_3 + ...f_m v_m = C_v \quad (7.4)$$

where f_1 to f_m are the proportions of each of the *m* potential foods to contribute to the consumers diet (the proportions must fall between 0 and 1). The isotope tracers are *t*, *u* and *v*; and C_t, C_u and C_v are the consumer isotopic values adjusted for fractioantion. However, because it is possible for the number of sources to out-number the tracers by more than one, there is no single, unique solution. Instead the equations can be solved to give the ranges within which the dietary proportions are feasible and still conserve mass balance, the mean and standard deviation can then be calculated.

IsoSource

IsoSource was developed to 'describe a general method for determining the distribution of all possible source contributions where the number of sources precludes a unique solution' (Phillips and Gregg 2003). IsoSource solves Equations 7.1-7.4 on an incremental basis, by slightly altering the proportions of each source in turn. In doing so it calculates the isotope signature that would be achieved for all possible combinations of source proportions (i.e. those that sum to 100%). Those signatures that equal the observed signature (or are within a preset tolerance) are considered feasible and the diet proportions are retained to calculate the range and arithmetic means of possible diet contributions. The IsoSource model runs as a Visual Basic program and the mixture signature has to be corrected using appropriate fractionation factors.

SOURCE and STEP

The SOURCE and STEP models were created to 'Utilise information about different tracers simultaneously to quantify food web relationships' (Lubetkin and Simenstad 2004). Both models solve Equations 7.1-7.4 using Gaussian elimination (the removal of one source at a time for each equation, then repeating omitting a different source, until all sources have been omitted) to give the outer possible limits, or 'corner point' solutions for each source item and trophic level (Lubetkin and Simenstad 2004). The SOURCE routine describes a consumer's uptake (direct and indirect) of autotrophic sources and, unlike the IsoSource model, calculates an additional estimate for trophic level (TL) by including a fractionation value for each isotope. STEP is a mixing model that determines a consumers direct dietary preferences and can be applied to any number of sources and consumers and for a variable number of tracers. The model incorporates a user-defined per trophic step isotopic fractionation for each isotope.

The linear model approach assumes that all organic sources available to the consumers are known and the elements are mixed and partitioned equally from all sources (Kwak and Zedler 1997, Moncreiff and Sullivan 2001, Peterson and Howarth 1987, Peterson et al. 1986). Dietary routing (see Gannes et al. 1997) and ingestion of source material without assimilation are assumed to have negligible effects (Ben-David and Schell 2001). In addition, the sources must be isotopically

distinct, this is measured using the K-Nearest Neighbour Distance statistic (K-NND, see Methods) (Lubetkin and Simenstad 2004, Rosing et al. 1998).

It is recognised that uncertainty in the fractionation value of any of the isotopes can cause errors in the estimation of partitioning among food sources (Focken and Becker 1998, McCutchan et al. 2003). Indeed if trophic fractionation positions the consumers isotope signature out with the mixing space created by the diet items then the midels will not be able to return any diet estimates. Therefore it is important that an appropriate consumer-specific fractionation is applied for each isotope (Lubetkin and Simenstad 2004, Phillips and Gregg 2003). Fractionation factors for $\delta^{13}C$, $\delta^{15}N$ and $\delta^{34}S$ are commonly cited as being 0-1‰ 3.4‰, and 0‰ respectively (McCutchan et al. 2003, Peterson et al. 1985, Post 2002, Sweeting et al. 2007). However it is yet to be established how deviations between the assumed and actual fractionation of a consumer will affect model performance.

Several studies have previously tested the IsoSource, SOURCE and STEP models and have found them to compare well with stomach contents or expected values (Benstead et al. 2006, Hindell 2006, Lubetkin and Simenstad 2004, Phillips and Gregg 2003). However, it is also not known how the three models compare in their ability to predict source contribution or diet proportions or if one model performs significantly better than the others.

The aim of this study was to use a range of multi-source mixing models and isotopic data from a range of marine organisms to explore variability in trophic fractionation factors. In doing this three main issues were addressed.

i) Variability in trophic-step fractionation. To determine realistic ranges of fractionation values where feasible diet solutions were possible for different marine consumers the fractionation of all three isotopes (carbon, nitrogen and sulphur) were altered in turn using the STEP routine. The variability of possible diet contributions within these feasible diets was also examined by altering the fractionation values applied to all three isotopes.

ii) The number of tracers. The benefits of having an additional isotope tracer was tested using the SOURCE and STEP routines by comparing the relative

contributions of potential sources to the diets of consumers using two ($\delta^{13}C$ and $\delta^{15}N$) and three ($\delta^{13}C$, $\delta^{15}N$ and $\delta^{34}S$) tracers.

iii) Mathematical approach. The models SOURCE and IsoSource aim to achieve the same objective using two separate mathematical routines. To determine how well these approaches compare the means and ranges of autotrophic source contributions were estimated for the same consumers with both models. The STEP and IsoSource models were also compared in a similar manner with respect to predicted diet proportions for selected consumers.

7.2 Methods

7.2.1 Data

The fish, invertebrate and autotroph isotopic data used in this chapter was a subset of the data described previously in Chapter 6. Sample collection and analysis methods were as described in section 6.3. The species selected for model analysis represented different functional components of the reef food web and include eight fish species (*Abudefduf vaigiensis*, *Acanthurus sohal*, *Apogon cyanosoma*, *Cephalopholis hemistiktos*, *Chaetodon melapterus*, *Cheilodipterus novemstriatus*, *Chromis xanthopterygia* and *Parupeneus marginatus*), two coral commensal crustacean species (*Trapezia cymodoce* (crab) and *Alpheus* sp. (shrimp)), zooplankton samples (200µm) one bivalve (*Laevichlamys ruschenbergerii*), two coral genera (*Pocillopora* and Octocoral) and marine plants (macroalgae of the genera *Sarconema*, *Ulva*, *Dictyota*, *Graclaria*, *Hypnea* and the mangrove plant, *Avicennia marina).* Phytoplankton were not sampled therefore it was necessary to estimate a suitable value. Based on the annual mean isotope signature of 200µm zooplankton (-21‰, $\delta^{13}C$; 10.22, $\delta^{15}N$; 20.8‰, $\delta^{34}S$) the isotope specific fractionations (0.7‰ for carbon, 3.4‰ for nitrogen (Post 2002) and 1.5‰ for sulphur (Trust and Fry 1992)) were applied resulting in a approximate phytoplankton signature of -18.14‰, $\delta^{13}C$; 7.59‰, $\delta^{15}N$; 17.47‰, $\delta^{34}S$.

7.2.2 Sulphur isotope analysis

Three samples of each species were analysed for S^{34}/S^{32} ratios using and EA-IRMS at Iso-Analytical Limited, Cheshire, UK. There were insufficient sample

sizes for triplicate analysis of some algae genera (*Ulva*, *Dictyota*, *Gracilaria*); in these cases only a single sample was analsyed. The results were reported calibrated to the ^{34}S isotope standard V-CDT (troilite of the Canyon Diablo meteorite). Reference standards IAEA-S-1 (silver sulphide), IA-RO25 (barium sulphide) and IA-RO36 (barium sulphide) were run along with samples for calibration purposes and gave an analytical precision of 0.19‰. Every fifth sample was run in duplicate. The maximum difference between pairs was 0.44‰ with a mean difference of 0.1 $\pm$ 0.12‰. The mean value for these sample pairs was used in subsequent analysis.

7.3 Nearest neighbour distance

Source materials in a mixture can only be separated by stable isotopes if the tracers used show each source to be discrete. Sources that fail to meet this criteria have to be pooled. To discern which sources needed to be pooled Lubtkin and Simenstad (2004) suggested using the K-Nearest Neighbour Distance (KNND) statistic (Rosing et al. 1998) which calculates how isotopically distinct the sources are. Producers separated by >0.1 were considered distinct. Sources closer than this were combined by taking a mean of the two signatures.

7.3.1 Fractionation variability

In the STEP routine, fractionation was set at 1‰ for $\delta^{13}C$, 3.4‰ for $\delta^{15}N$ and 0 for $\delta^{34}S$ then systematically varied while values for the other two tracers remained constant. Tracers were varied in 0.2 increments within the following ranges: -1 to +3‰ for $\delta^{13}C$, 2 to 5‰ for $\delta^{15}N$ and -1 to +1 for $\delta^{34}S$. The estimated proportions of diet items were recorded for each set of tracer fractionations for each consumer species. This method was then repeated seven times with one of the following alternative tracer values; 0.5% and 1.5% for $\delta^{13}C$, 3.0‰, 4.0‰ and 4.5‰ for $\delta^{15}N$ and -1‰ and 1‰ for $\delta^{34}S$.

7.3.2 Additional tracer

In a trial to evaluate the merit of including and additional tracer consumers were given specific fractionation values. All fish species had fractionation values of 2‰ for C, 3.4‰ for N and 0.8‰ for S with the exceptions of *Parupeneus marginatus* and *Chaetodon melapterus* that had a lower S fractionation of 0‰ and

the herbivore *Acanthurus sohal* with fractionation values of 2‰ for $\delta^{13}C$, 4.5‰ for $\delta^{15}N$ and -1‰ for $\delta^{34}S$. Invertebrates had fractionation values of 1‰ for $\delta^{13}C$, 2‰ for $\delta^{15}N$ and 1‰ for $\delta^{34}S$. The trophic levels obtained within the SOURCE model were compared using two (CN) isotopes and three isotopes for each consumer. For each consumer the contributions of different potential diet items were compared using two (CN) and three (CNS) isotopes in the STEP model.

7.3.3 Model comparison

The relative contributions of different dietary items (means and ranges) to consumers signatures calculated by the Gaussian elimination and incremental methods were compared using SOURCE and IsoSource. The same fractionation values were applied to consumers as in 7.4.2. A trophic level was obtained from the SOURCE output (based on the fractionation values), this same trophic level was applied to the consumer mixtures (i.e. trophic level multiplied by the fractionation value subtracted from the consumer signature) to obtain the mixture values to be used in IsoSource.

For four consumers (*Cephalopholis hemistiktos*, *Parupeneus marginatus, Apogon cyanosoma* and *Cheilodipterus novemstriatus*) the actual diets were compared (means and ranges) using one trophic level fractionation in the STEP and IsoSource models. Due to the constraints of the IsoSource model the number of diet items was limited to ten. The diet items included in these trials were: macroalgae, zooplankton, *Apogon cyanosoma*, *Chromis xanthopterygia*, *Cheilodipterus novemstriatus*, *Alpheus* sp., *Trapezia cymodoce*, *Laevichlamys ruschenbergerii*, *Chaetodon melapterus* and *Abudefduf vaigiensis*.

7.4 Results

7.4.1 Sulphur SIA

The sulphur isotope ratios across all producers and consumers generally differed only very slightly with a very narrow maximum range of 4.68‰ (16.94-21.62‰). The most ^{34}S enriched samples were those of *Trapezia cymodoce* (mean = 20.94 $\pm$ 0.79‰) and the most depleted samples were those of mangrove (mean = 17.03 $\pm$ 0.09‰). Fish species showed an even smaller range of values with *Chromis*

xanthopterygia the most enriched (mean = 20.67 ± 0.13‰) and *Parupeneus marginatus* the most depleted (18.85 ± 0.42‰).

7.4.2 K-NND

The source materials were grouped into five distinct sources based on their isotope signatures using the K-NND statistic. Despite having a wide range of $\delta^{13}C$ values the differences among macroalgae genera were not large enough to be considered discrete using carbon and nitrogen isotopes. The inclusion of $\delta^{34}S$ allowed macroalgae to be split into two groupings (*Sarconema*/*Ulva*/*Gracilaria* and *Hypnea*/*Dictyota*) but in order to keep the number of sources equal a single marcroalgae grouping was used throughout the subsequent analysis. The mean isotope values of the grouped genera were used in the analysis.

7.4.3 Fractionation variability

The commonly cited fractionation values of 1‰ for carbon, 3.4‰ for nitrogen and 0‰ for sulphur were not suitable here for all consumers: there were no feasible diets for five of the twelve species using these values (Fig 7.1-7.3). Of the combinations of isotopes trialled the -1‰ for $\delta^{34}S$ returned possible diets for the least number of species (Fig. 7.3), however for one species, *A. sohal*, sulphur fractionation had to be negative to return a feasible diet, although this was only when carbon fractionation was positive. The invertebrate species *Laevichlamys ruschenbergerii* and *Trapezia cymodoce* only showed feasible results when sulphur fractionation was +1‰ and nitrogen fractionation was low (Fig 7.3). A wide range of nitrogen values were applicable to most consumers but only when the carbon and

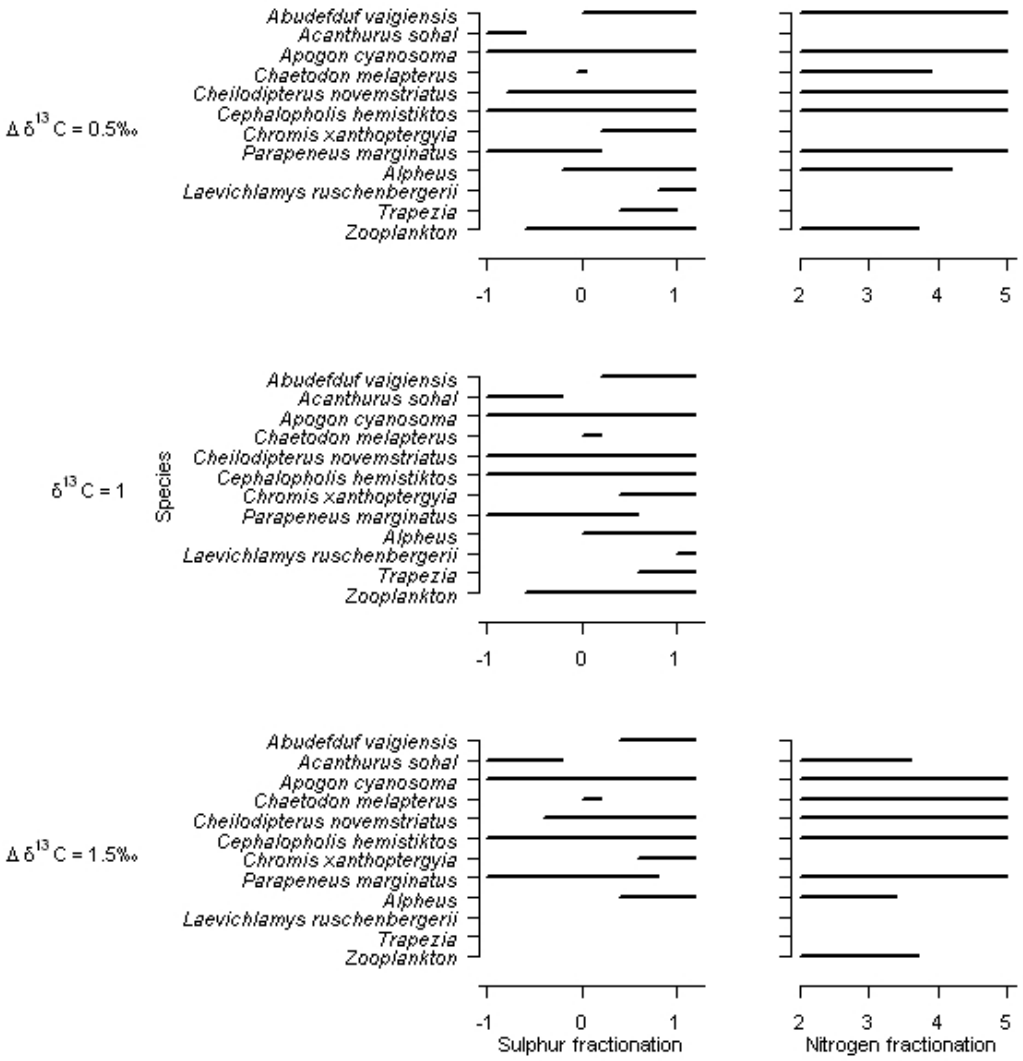


Fig. 7.1: The range of sulphur and nitrogen fractionation values over which feasible diets could be obtained using the STEP model and when carbon fractionation was set at 0.5, 1 and 1.5‰. Default fractionation values were 0‰ for $\delta^{34}S$ and 3.4‰ for $\delta^{15}N$.

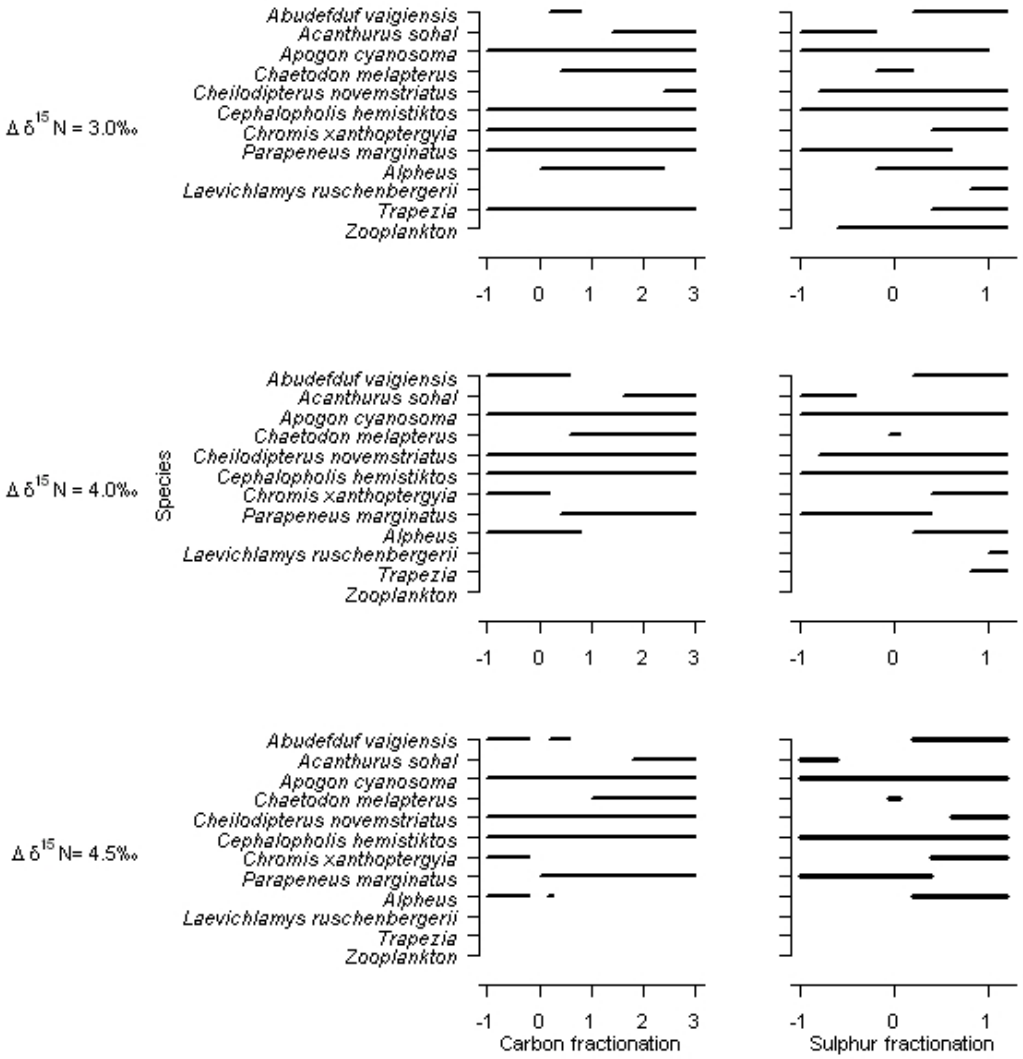


Fig. 7.2: The range of carbon and sulphur fractionation values over which feasible diets could be obtained using the STEP model and when nitrogen fractionation was set at 3.0, 4.0 and 4.5‰. Default fractionation values were 1‰ for $\delta^{13}C$ and 0‰ for $\delta^{34}S$.

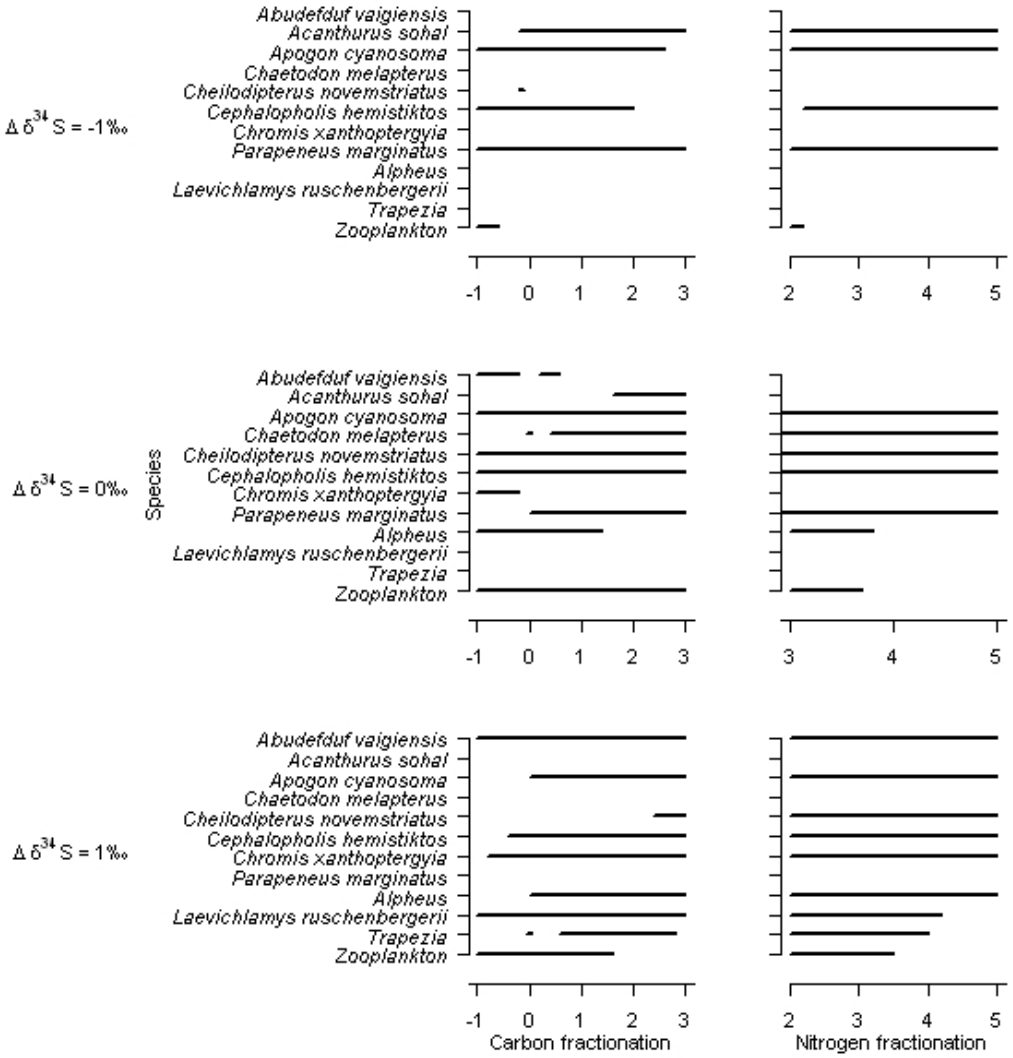


Fig. 7.3: The range of sulphur and nitrogen fractionation values over which feasible diets could be obtained using the STEP model and when carbon fractionation was set at -1, 0 and 1‰. Default fractionation values of 0‰ for $\delta^{34}S$ and 3.4‰ for $\delta^{15}N$.

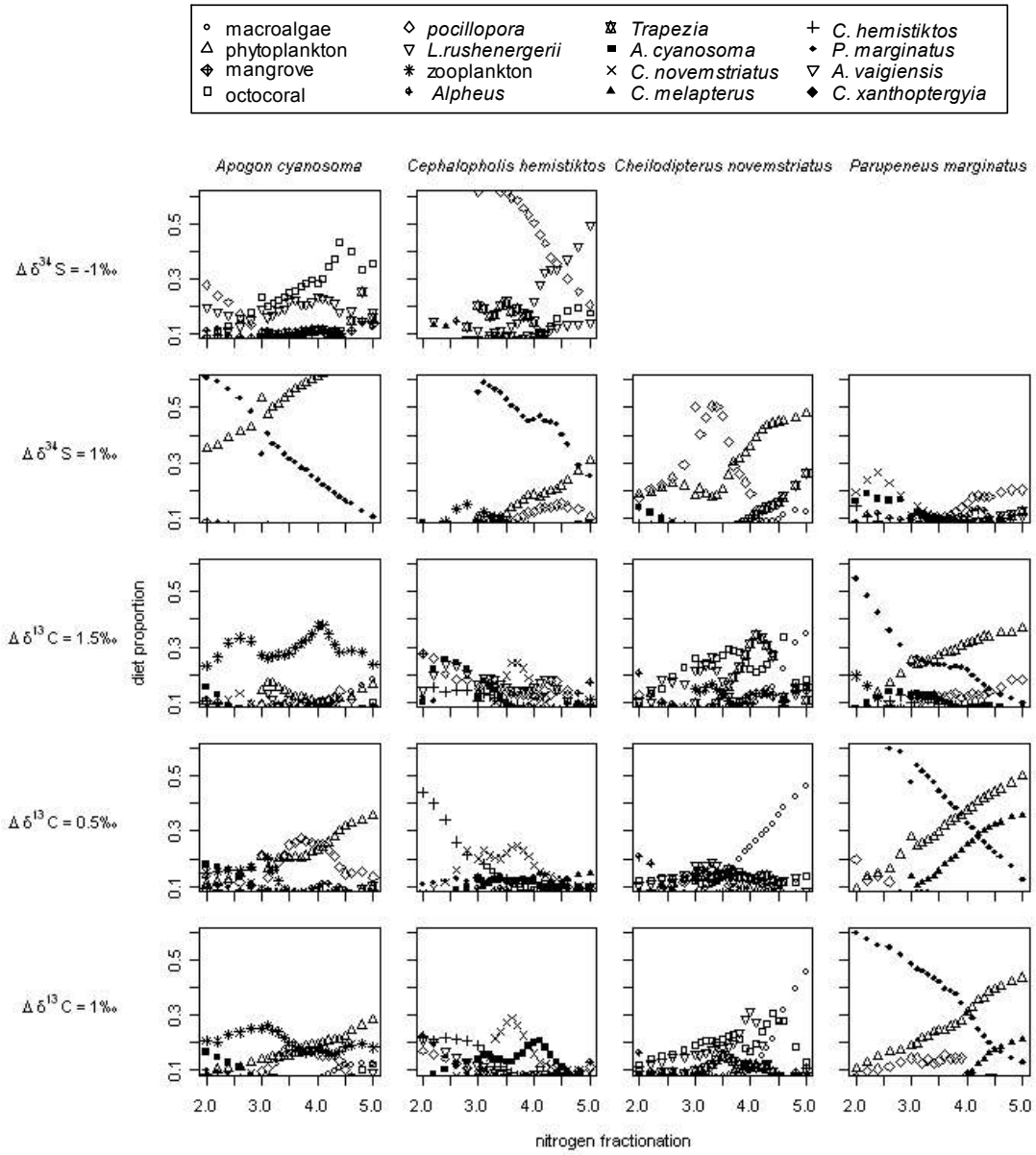


Fig. 7.4: Predicted diet proportions of four marine consumers as estimated by the STEP model (Lubetkin and Simenstad 2004) across a range of nitrogen fractionation values for 'default' (1‰ $\delta^{13}C$ and 0‰ $\delta^{34}S$) and two alternative carbon (0.5 and 1.5‰) and sulphur (-1 and 1‰) fractionations values.

sulphur fractionations were specific values. Not all consumers showed distinct fractionation ranges, and feasible diet solutions were obtained across all of the trialled fractionation values for *Cephalopholis hemistiktos*, *Apogon cyanosoma* and *Cheilodipterus novemstriatus*. The relative contributions of different diet components of these species and of *Cheilodipterus novemstriatus* varied considerably as each isotope's fractionation factor was altered (Fig.7.4). *Cephalopholis hemistiktos* changed from a diet dominated by *Pocillopora* and *Trapezia* cymode ($\delta^{34}S$ = -1‰ Fig 7.4) to a diet composed of equal proportions of *Apogon cyanosoma* and *Cheilodipterus novemstriatus* ($\delta^{34}S$ = 0‰). Some of these isotopically-feasible diet solutions that the models provided included biologically unlikely diets, such as an 80% contribution from the goatfish *Parupeneus marginatus,* a predator of equal trophic level ($\delta^{34}S$ = +1‰ Fig 7.4). The possible diets (Fig. 7.4) were those predicted when only the nitrogen fractionation was varied; there were many more possible diet combinations for all species when $\delta^{34}S$ and $\delta^{13}C$ were varied incrementally over a range of $\delta^{15}N$ values.

7.4.4 Additional tracers

There were only small differences in mean trophic level across all consumers when calculated using two isotopes (CN) compared to three isotopes (CNS) (Fig. 7.5). The mean predicted TL for *L. ruschenbergerii* and zooplankton fell below one when using both two and three isotopes suggesting that the fractionation values applied were too large for these consumers.

The mean diet contributions differed for all four consumers when predicted using three isotopes compared with two isotopes in the STEP model. Using carbon and nitrogen isotopes only, phytoplankton was predicted to contribute 5% to the diet of *Apogon cyanosoma*, this value increased to >30% when sulphur signatures were taken into account (Fig. 7.6). The mean proportion of zooplankton in the diet of *A cyanosoma* decreased from 30% to 20% with inclusion of the sulphur isotope values. *Cheilodipterus novemstriatus* was predicted to have a decrease in overall plankton contributions from 40% with carbon and nitrogen isotopes to less than 5% with three isotopes (Fig. 7.6c). *L. ruschenbergerii* and octocoral proportions, both increased when sulphur was included. Changes in the diet of *P. marginatus* were less clear as carbon and nitrogen isotopes predicted a fairly even contribution from

all diet items, the addition of sulphur increased the proportions of *A. cyanosoma* and phytoplankton and reduced the variability of the smaller diet proportions (*C. novemstriatus* and *C. xanthopterygia*) (Fig. 7.6d). For *C. hemistiktos* the most dominant dietary item changed from *C. xanthopterygia* (20%) with two isotopes to *A. cyanosoma* (30%) with all three isotopes (Fig. 7.6b).

7.4.5 Model comparison

For most consumer species SOURCE and IsoSource predicted very similar mean estimates for the proportional contribution of autotrophic sources. The SOURCE model generally gave larger standard deviations and wider feasible ranges than the incremental increase model IsoSource (Fig. 7.7); this was particularly evident for the invertebrates *Alpheus* sp. (Fig. 7.7i) and *L. ruschenbergerii* (Fig. 7.7j). Phytoplankton was estimated to account for over 70% of inputs to the diet of *Apogon cyanosoma* as estimated by both models, the ranges and standard deviations were minimal in both cases (Fig. 7.7c). The source contributions to the diet of *Parupeneus marginatus* were more ambiguous as both models showed wide ranges and large standard deviations across all five sources indicating several possible combinations could result in this consumer's isotopic signature, and no single combination could be clearly defined by either model (Fig 7.7h). The mean proportion estimates differed considerably for *C. novemstriatus* with the SOURCE model giving wider possible ranges for octocoral, *Pocillopora* and phytoplankton, and higher mean values for *Pocillopora* and phytoplankton than the IsoSource model (Fig.7.7f). SOURCE predicted considerably larger possible diet ranges than IsoSource.

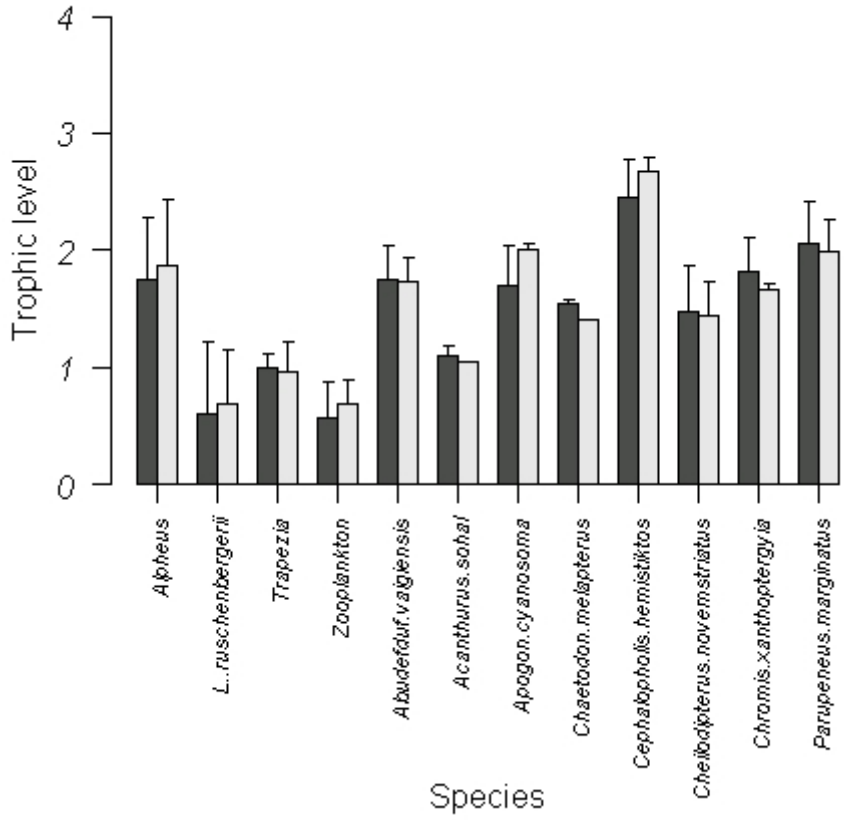


Fig. 7.5: The estimated trophic level (± 1 S.D.) of 12 marine consumers estimated by the SOURCE model using two ($\delta^{13}C$ and $\delta^{15}N$; dark bars) and three ($\delta^{13}C$, $\delta^{15}N$ and $\delta^{34}S$: light bars) isotope tracers.

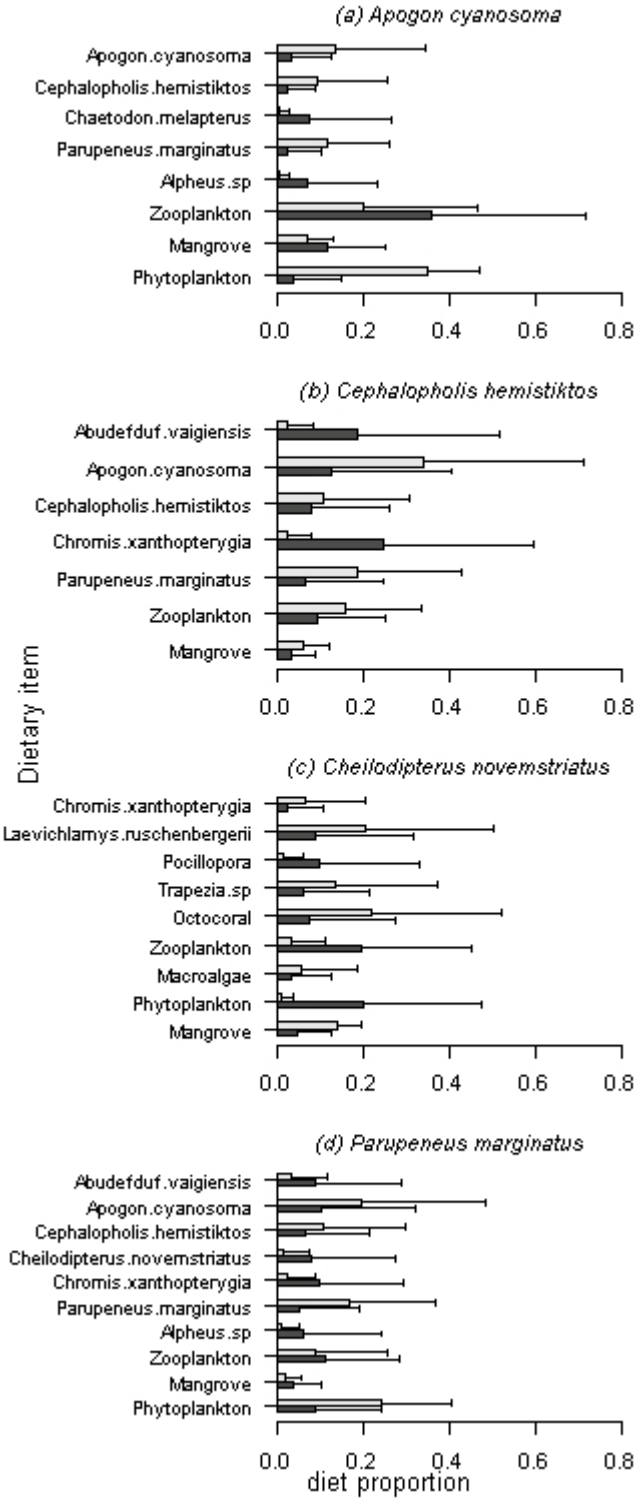


Figure 7.6: Diet proportions that contributed >0.05% of diet for a) *A. cyanosoma* b) *C. hemistiktos* c) *C. novemstriatus* and d) *P. marginatus* as predicted by STEP using two (CN- dark bars) and three (CNS -light bars) isotope tracers.

Similarly, the STEP routine predicted mean diet proportions that compared well with IsoSource when a per trophic step fractionation was applied (Fig.7.8). There were some differences between the consumers. Both models predicted small ranges and SD's for the diet of *C. hemistiktos*, indicating that it was dominated by *A. cyanosoma* and zooplankton (Fig. 7.8a). *C. novemstriatus* and *A. cyanosoma* had slightly wider ranges and SD's for the mean estimates; however both models predicted a diet dominated by zooplankton (Fig. 7.8b and c). The STEP model was less able to define the diet for *P. marginatus* where there were large ranges (up to 20% for most dietary items) within which possible diets could be obtained; the mean estimate however was very similar to that of IsoSource predicting a diet composed of *A. cyanosoma* and zooplankton (Fig. 7.8d). The IsoSource model had consistently much narrower ranges, indicating more confidence in the mean estimates.

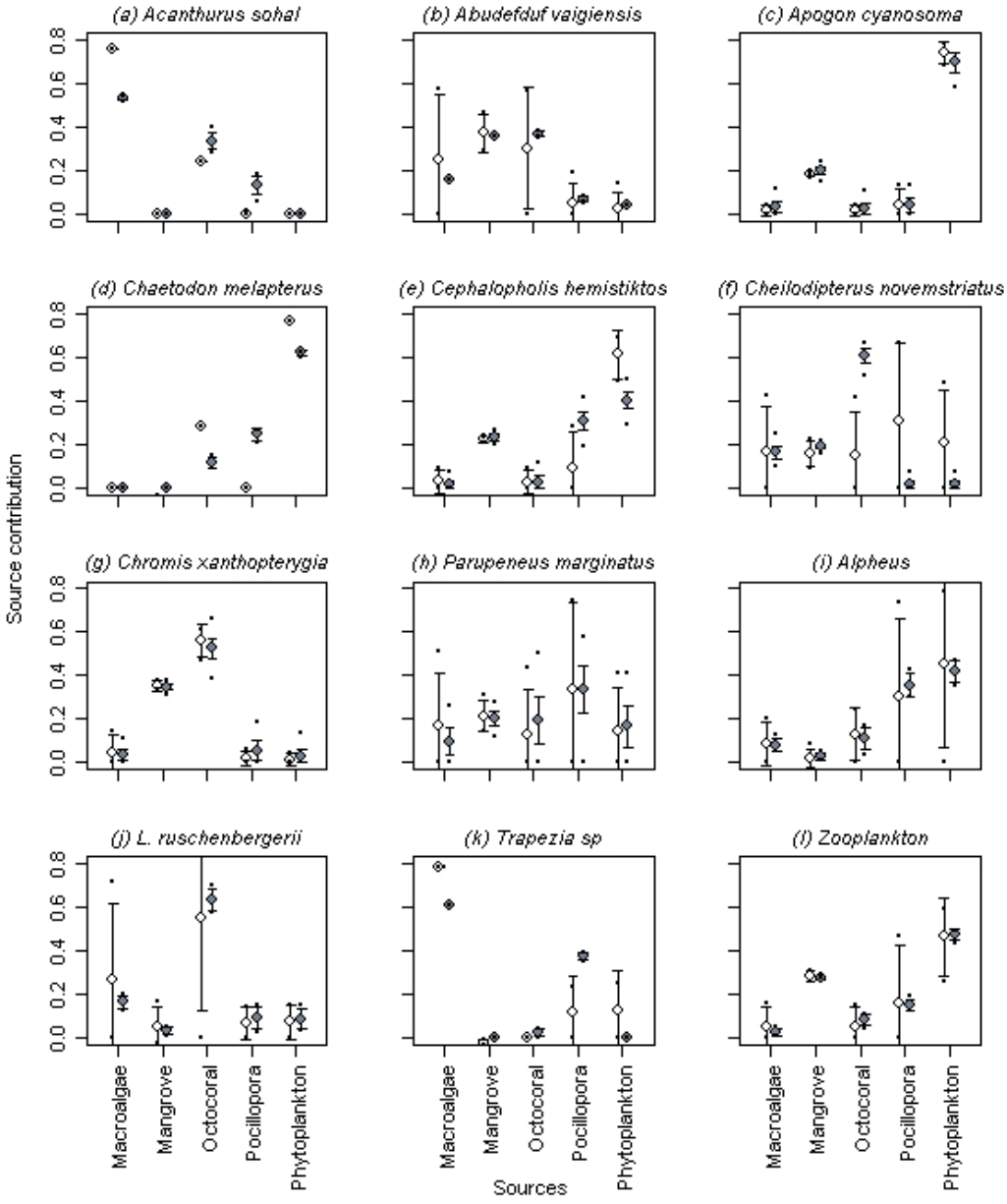


Fig. 7.7: Comparison of mean source contributions (± 1 S.D.) of 12 marine consumers predicted using SOURCE (open circles) and IsoSource (grey circles). Minimum and maximum feasible ranges are represented by filled circles for each diet item.

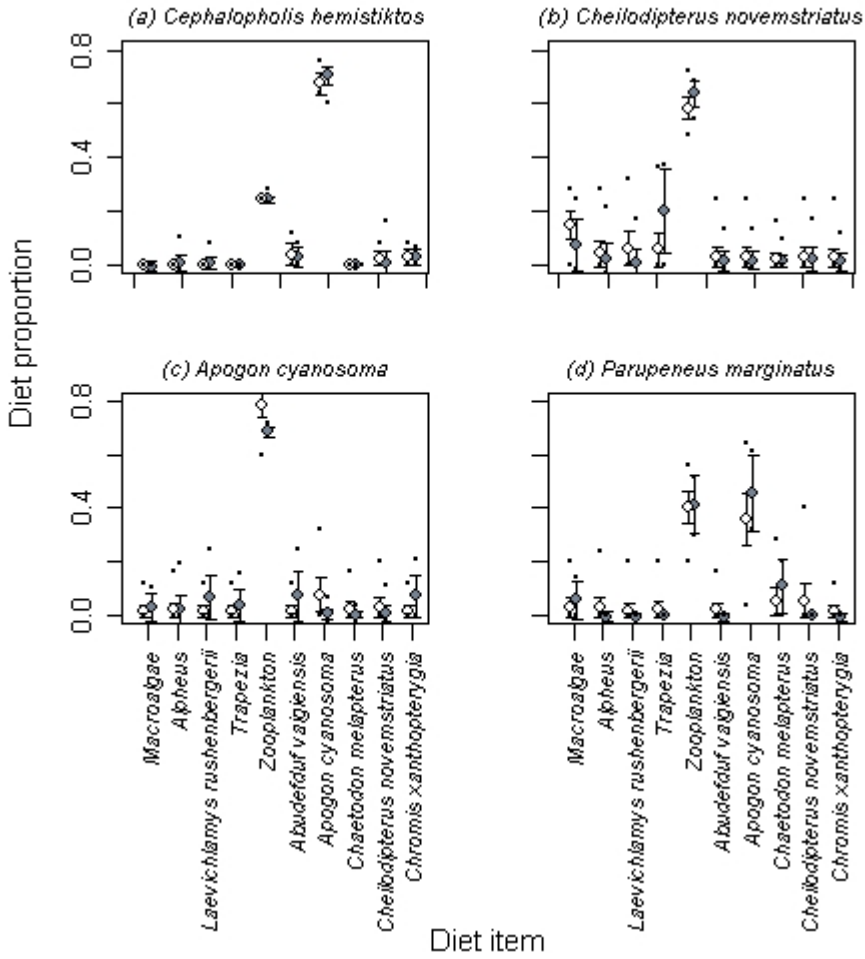


Fig. 7.8: Comparison of mean diet proportions (± 1 S.D.) of 4 marine consumers predicted using STEP (open circles) and IsoSource (grey circles), minimum and maximum feasible ranges are represented by filled circles for each diet item.

7.5 Discussion

Mixing models that can cope with a large number of sources or diet items are an asset to the interpretation of isotopic data from complex ecosystems. Despite not always providing a unique solution, these models can estimate ranges within which diet contributions are possible. However, the fractionation values, number of tracers and the model methodology have been shown to influence the reliability of the model outputs.

The use of accurate fractionation values for all three isotopes was very important in the models; small changes in any one value were found to have implications for the relative contribution of sources to the diet. Fractionation of all isotopes between consumers and their diet is widely reported as being variable and a number of factors that may influence fractionation have been highlighted (McCutchan et al. 2003, Post 2002, Vander Zanden and Rasmussen 2001, Vanderklift and Ponsard 2003). A single fractionation value for each of the three isotopes was not applicable to all consumers in this food-web. Ideally, species-specific isotope fractionations would be known from diet analysis or feeding studies and applied in mixing models (e.g. Szepanski et al. 1999). When species-specific fractionations are not known, they are often assumed and applied over a range of consumers. Incorrect fractionation values may result in unsolvable models, the omission of important dietary components or the overemphasis of unimportant components (Benstead et al. 2006, Lubetkin and Simenstad 2004).

In fish, a trophic-step fractionation value of 3.4‰ for $\delta^{15}N$ of muscle tissue is robust in most cases (Post 2002, Sweeting et al. 2007) yet the STEP model predicted no possible diet for *Acanthurus sohal* if $\Delta\delta^{15}N$ was 3.4‰. This was anticipated following empirical and model calculation of nitrogen fractionation for *A. sohal* (chapter 5). The variability of carbon and sulphur isotope fractionation is less well defined but generally reported to be small (Barnes et al. 2006, McCutchan et al. 2003, Peterson et al. 1985, Post 2002). A small trophic-step fractionation of 0.8‰ will result in a total shift of 2.4‰ for a fourth level consumer; therefore small fractionations are important when assessing the relative importance of different autotrophic source materials using mixing models. Peterson et al (1985) found

feeding experiments to give positive $\delta^{34}S$ shifts, yet field observations (Peterson et al. 1986) and more recent feeding experiments (Barnes 2006) have showed negative shifts. Twenty years on from the first review of sulphur isotope fractionation in aquatic ecology, there are still insufficient data available to establish a reliable trophic transfer estimate (McCutchan et al. 2003, Peterson et al. 1985). It would appear that sulphur fractionation might be very variable in the reef food-web studied here, many fish did not have possible diet combinations when $\Delta\delta^{34}S = 0‰$. A $\Delta\delta^{34}S$ of 0.8‰ was found to be applicable to a number of fish species; however the herbivore *A. sohal* was an exception, requiring a negative value to give a feasible diet. Negative $\Delta\delta^{34}S$ values have been reported for other species with poor quality (low C:N) diets (McCutchan et al. 2003).

Some fish species (e.g. *P. marginatus* and *C. melapterus*) had very distinct fractionation ranges within which feasible diets could be estimated by the STEP model. It was more difficult to interpret the correct fractionation values for species that had feasible diets over a wide range of fractionations. The value for each isotope was important as large changes in the predicted diet items were observed as the fractionation for each individual isotope was manipulated. For example, the diet of *A. cyanosoma* could have been dominated by zooplankton, octocoral or *P. marginatus* depending upon the fractionation factors applied. The fractionation values applied to most consumers (2‰ for C, 3.4‰ for N and 0.8‰ for S) were appropriate based on feasible diets and recommendations for fish muscle tissue (Barnes 2006, Sweeting et al. 2007). A slightly different $\Delta\delta^{34}S$ was applied to *P. marginatus* and *C. melapterus* as no feasible diet could be predicted with a $\delta^{34}S$ value of 0.8‰. The lower fractionation values of 1‰ for $\delta^{13}C$, 2‰ for $\delta^{15}N$ and 1‰ for $\delta^{34}S$ are more applicable for invertebrates based on previous studies (Barnes 2006, McCutchan et al. 2003).

Unknown per trophic step fractionations will increase the uncertainty inherent in mixing models as the range of possibilities that could contribute to the consumer mixture increases. When the fractionations are known (in this case *A. sohal*), the number of feasible diet possibilities is limited, making predictions of proportions more robust. The assumed fractionation values applied have

implications for the subsequent predicted diet proportions being biologically correct.

The range of sulphur isotope ratios across producers in the coral community was limited (16.94‰ to 20.93‰) compared to producers in other aquatic environments e.g. seagrass beds ($\delta^{34}S$ from 7.4‰ to 20.4 ‰) (Moncreiff and Sullivan 2001) and saltmarsh ecosystems (-20‰ to 27‰) (Peterson et al. 1986). Sulphur and carbon isotopes showed that mangrove material played a very minor part in the food web, despite the close proximity (few hundred metres from the reef). The limited transfer of mangrove materials may be attributed to the poor nutritional quality of the decayed leaf litter (Benstead et al. 2006) and the limited extent of the mangrove at the location.

Carbon and sulphur isotopes are generally better at distinguishing between producers than carbon and nitrogen (Connolly et al. 2004, Kwak and Zedler 1997). Using carbon and sulphur isotopes in mixing models, previous studies have indicated a wider spread of producer isotope signatures e.g. Benstead et al (2006) estimated a 5‰ difference in $\delta^{34}S$ and $\delta^{13}C$ data for each source. Producers by nature tend to be of a similar trophic level and hence have very similar $\delta^{15}N$ signatures; most of the separation between sources will therefore be based on carbon when only using $\delta^{13}C$ and $\delta^{15}N$. This problem was evident for the macroalgae studied here, which have an inherently high variability in carbon ratios across species (Raven et al. 2002). All macroalgae genera had to be grouped together to satisfy the K-NND statistic with only two tracers but with the addition of sulphur isotopes macroalgae were divisabe into two groups. However, the models would only be able to distinguish between these macroalgae groups if the sulphur fractionation of the consumer was correct, so a single macroalgae grouping was used for the analysis. The more distinct the groupings, the better the models can distinguish between sources (Phillips 2001). The models do not take the biology of the system into account, hence will assume that sources with similar isotopic signature are biologically similar, highlighting the importance of having isotopically distinct sources (Lubetkin and Simenstad 2004).

The inclusion of sulphur had little impact on the predicted trophic level of consumers. However there were notable differences in the possible diet composition

of the four species studied, suggesting the triangulation provided by the sulphur isotopes altered the model's ability to define diets. These changes in diet proportions were not predictable and varied for each consumer. It would be expected that the STEP estimations including all three isotope tracers would reflect the most accurate diet proportions. However this was not always clear. The utility of sulphur as an additional tracer will be enhanced as it becomes more routinely analysed and more is learnt about consumer specific fractionation of sulphur isotopes.

The SOURCE, STEP and IsoSource models set out to achieve the same aim, to estimate contributions to a mixture based upon isotopic data. The different methods of solving the linear mixing equations resulted in very similar mean estimates of source contributions. The differences in standard deviations are a direct result of the calculation method, the corner point technique used in SOURCE and STEP only returns the outer bounds of the solution space so a mean value is derived from these limited number of outer limits, resulting in a large standard deviation where there is a large solution space. The incremental technique of IsoSource calculated the mean from a large number of possible combinations hence the variance was often much lower. The similarity of the mean values calculated by the models suggests that solving for the outer range of possible solution space (SOURCE and STEP) is sufficient and searching through more possible combinations (IsoSource) is not necessary. This would be preferential for large data sets as the calculation process is simpler.

SOURCE and IsoSource aim to assess the basal contributions to higher consumers. However, the IsoSource model requires the user to have knowledge of each consumer's TL, whereas SOURCE takes the user input fractionation information to estimate a TL value for each consumer. In the model comparison, the TL of each species was predicted by the SOURCE data and applied to the consumer mixtures used in the IsoSource model. For these TLs, both models predicted similar mean source contributions for each consumer. *C. hemistiktos*, *A. cyanosoma*, *A. Sohal* and *C. xanthopterygia* all had relatively constrained source contributions predicted by both models, again suggesting that it is not necessary to use the more complicated incremental model. However, SOURCE frequently predicted wide

ranges, sometimes outside those predicted by IsoSource. Wide ranges provide little information about an item's contribution as any of the values within that range may be the correct solution (Benstead et al. 2006). *C. novemstriatus* was predicted by IsoSource to have a mean contribution from octocoral of 60% and with much lower contributions from all other sources, SOURCE predicted a mean octocoral contribution of <20% and higher means for three of the other sources each with ranges spanning between 40 and 60%, indicating that this model could not define within these ranges what the correct diet proportions were. It was not clear when the ranges predicted by SOURCE fell out-with the ranges predicted by IsoSource which model was correct (see Fig. 1.7f : *C. novemstriatus*).

STEP and IsoSource had similar abilities to predict constrained or diffuse solutions of specific dietary contributions for most species. For example, both models indicated ~60% contribution from zooplankton for *A. cyanosoma*. There was a high degree of certainty about this as both models predicted very small ranges. Neither model could predict a specific diet for *P. marginatus*, instead suggesting wide ranges (0 -20%) for all diet components. The 'true' consumer mixture is likely to lie in the middle of a 'cloud' of solution-space, surrounded by diet items that need not necessarily contribute to the mixture, but could equally be important (see Fig 6C Phillips and Gregg 2003). The species that have a diffuse solution tend to be species regarded as generalists and the predicted uncertainty about the diet is attributable to multiple items of similar isotopic values contributing to the consumer mixture.

At present model preference depends on the user's requirements. This analysis has shown that for describing a consumer's specific diet, IsoSource has a higher level of accuracy, however for analysis of basal inputs to a full food web SOURCE may be equally as applicable, as the calculated means are very similar but the computational speed of SOURCE is greater. The values given to trophic step fractionation are important to both models as they both need to account for this for each isotope. IsoSource has a simple to use interface; yet data can not be read or imported from other files easily, and also analysis is limited to only ten potential diet items and only a single consumer. In general the SOURCE and STEP models allow more flexibility, however at present they require some knowledge of the R or

S-plus programming language. Any number of dietary items could be included, and multiple consumers can be analysed at once. The incorporation of fractionation into these models is beneficial as an estimate of TL can be obtained, unlike IsoSource where some prior knowledge of TL is required to determine basal contributions. The models also differ in their error allowance, or the proportion of mass balance conservation. Mass balance tolerance would ideally be 100% but to allow borderline cases or to simulate biological variability, both models allow slight deviations from 100% conservation. The SOURCE and STEP models do this by allowing possible diet contributions to sum to the biologically unfeasible levels of -5% and 105% (these values can be altered by the user), as long as they result in the correct diet mixture. The IsoSource mass balance tolerance levels allow the isotope signature of the mixture to be predicted from differing source contributions to lie within a user defined range (‰) of the actual consumer signature. Using this method the proportional contribution of all sources will sum to 100%. The mass balance tolerance is recommended to be 0.5 × source increment × maximum difference between sources, to avoid missing any possible combinations with the incremental change (Phillips and Gregg 2003). To encompass inherent ecological variability it may be more appropriate to set this value higher or similarly if there is a high magnitude of the error in measurement of the sources or mixture. However, setting a wider mass balance tolerance will introduce more variability into the output (see figure 7A of Phillips and Gregg 2003) resulting in a greater uncertainty about the mean, as is seen in the SOURCE and STEP models.

7.5.1 Conclusions

Overall this analysis shows that assumptions must be made when applying mixing models to complex systems. It is clearly very important to use appropriate fractionation values for all isotopes used, especially as it is likely that different fractionation values will be necessary for different consumers.

In a complex ecosystem with a large number of potential sources, many will not be isotopically distinct. The use of multiple tracers will reduce isotopic similarity however, if sulphur isotopes are to become routinely used in food-web studies the cost of analysis will have to decrease and experimental and field

observations of trophic fractionation will be necessary to further our understanding of the variability in sulphur fractionation.

The specific model chosen for food web analysis depends on the question being asked. IsoSource may be best choice to accurately determine the diets of individual consumers while SOURCE and STEP are more flexible given larger data sets and will give estimates of TL with predictions of source contributions. In the future it may be possible to combine the methods to include the incremental methodology of IsoSource with the TL estimations variable fractionation inputs currently available in the SOURCE and STEP routines.

8 Thesis synthesis

8.1 Overview

This thesis had two core research themes: the trophodynamics of the coral communities in Oman and the use of stable isotopes as food web description tools. These themes were developed with reference to the seasonal upwelling which occurs in the region and its influence on both the local food-web, and the application and suitability of stable isotope techniques in such a dynamic system.

The findings of this thesis contribute significantly to the current knowledge of reef communities in Oman. Previously research in the region has involved oceanographic description of the upwelling (Currie et al. 1973), commercial fish species (Claereboudt et al. 2005, McIlwain et al. 2005, Valinassab et al. 2006) and compilation of species lists of reef fish (Coles and Tarr 1990, Randall 1995, Randall and Hoover 1995) and corals (Coles 1997, Coles and McCain 1990, Sheppard and Salm 1988). This study builds on these research areas by describing specific food web interactions within the marginal reef communities of Bandar Kayran, in relation to the unusual oceanographic setting in which they occur.

Stable isotopes techniques have become a popular technique in ecological studies in the last 10-20 years have been applied to a wide range of situations including migration studies and anthropogenic manipulations of ecosystems. This thesis specifically focussed on their use in dietary analysis and the interpretation of food webs assuming a per-trophic-step fractionation (Chapters 5 and 7), isotopic variance (Chapter 6) and isotope mixing models as tools (Chapter 7). When interpreting isotopic data the generic assumptions are often made that the system being studied is in a state of equilibrium (little or no temporal variance) and that a single per-trophic-step fractionation value (often cited as 3.4‰ for $\delta^{15}N$, ~1‰ for carbon) is appropriate for all consumers; both of which were challenged in this thesis.

8.2 The coral community food web of Bandar Kayran

The use of stable isotope data allowed detailed descriptions of feeding relationships within the food web, $\delta^{15}N$ being used to infer trophic level (Post 2002) and $\delta^{13}C$ providing information about the ultimate carbon sources utilised (Michener and Schell 1994). The food web at Bandar Kayran had between 3 and 4 trophic levels, based on the range of $\delta^{15}N$ data (5.8 to 17.8‰), which is typical of most aquatic systems. The available source materials covered a wide range of $\delta^{13}C$ values, from ^{13}C depleted mangroves (*Avicennia marina*, $\delta^{13}C$ = -28.15‰) to the enriched values of detritus ($\delta^{13}C$ = -6.4‰). Carbon and sulphur data suggested that the mangroves within the inner bay at Bandar Kayran contributed very little, if anything, to the reef food web, but at the other end of the carbon spectrum detritus may be an important diet component for some organisms, e.g. brittle stars.

The species with the highest trophic positions at Bandar Kayran were the grouper, *Cephalopholis hemistiktos*, the bream *Scolopsis ghanam* and the nocturnal cardinal fish, *Cheilodipterus macrodon*, indicating that these are top predators on the reef. However, both stomach content observations (Chapter 6) and isotopic mixing models (Chapter 7) showed that these species are not solely piscivorous and will also feed on benthic invertebrates. The $\delta^{13}C$ values of these species were intermediate (~ -12 to -15‰) suggesting that the food-web supporting them consists of both benthic and planktonic inputs.

The most abundant fishes on the reef were planktivorous damselfish (*Chromis xanthopterygia* and *Chromis flavaxilla*) and apogonids (*Apogon cyanosoma*). These small fish play an important role in incorporating pelagic plankton derived nutrients into the reef food web (Pinnegar 2000). This may occur directly as these fish were part of the diet of reef predators (*Cephalopholis hemistiktos*, Chapter 7) but also indirectly, through faeces consumed by other reef organisms (Robertson 1982). Other consumers were less dependent on the planktonic influences and relied on macroalgae (*Acanthurus sohal*, *Zebrasoma xanthurum* and *Pomacentrus arabicus*) or benthic invertebrates (*Epinephelus stoliczkae* and *Acanthopagrus bifasciatus*) as food sources. Feeding variability in some species was high with both isotopes and stomach content analysis revealing differences from one individual to another. The results of the size based $\delta^{15}N$

analysis in chapter six of this thesis were contrary to the theory of Lindeman (1942) and the findings of France (1997) and indicated that intra-specific feeding variability was more common at lower trophic positions.

8.3 Isotopic variance and multi-source mixing models as a food web description tools

Food webs and specific predator-prey interactions have been described with dual isotope data for over 30 years, however the use of isotopic variance to describe trophic niches is a relatively new development (Bearhop et al. 2004, Bolnick et al. 2002, Sweeting et al. 2005). Variance of stable isotope data can be used as a descriptor of intra-specific feeding variability and to define relative feeding niche-widths of species. The K-nearest neighbour randomisation (K-NND) test was use to test how isotopically distinct species were, and hence if there was trophic niche overlap (Chapter 6 Layman et al. 2007, Rosing et al. 1998). Where there was overlap among species there is the potential for trophic redundancy within the food web.

Despite the majority of coral growth in Oman being marginal, there is a high biomass and diversity of reef associated fish (Randall 1995). The processes that allow the coexistence of large numbers of species within reef systems are still largely unknown. The isotope data and the K-NND analysis provided a test that has the potential to reveal differences in trophic niches among species, and which may be one way in which species are partitioned. In the Bandar Kayran food web K-NND analysis showed that there was trophic niche overlap among a number of species, particularly within the pomacentrid family. Some species exhibited a high degree of overlap of trophic niche with most other species in the food web; these included *Chaetodon collare*, *Rhinecanthus assai* and *Pomacanthus maculosus* suggesting that if any one of these species were absent from the food web there would be no overall loss of trophic function (Layman et al. 2007). Here, other processes such as habitat differentiation and abundance may be important in allowing coexistence of these species on the reef.

A few species had distinct isotopic signatures to all other species in the food web. For example, the herbivore *Acanthurus sohal* only had significant trophic

overlap with a much less abundant scarid (*Scarus persicus*) and the coral feeding butterfly fish *Chaetodon melapterus*, suggesting its specific diet of macroalgae was relatively unique within the food web. *C. melapterus* also showed very limited overlap with other species suggesting its trophic habits were specialist.

Variance of isotopic signatures also has the potential to be a useful descriptor of feeding habits within populations, particularity where re-sampling (of fin clippings or scales) of individuals through time is possible in order to distinguish between inter-individual and inter-population variance. This may be of particular use on reefs to determine differences among guilds, for example the chaetodontids where a number of diet sources are available and it is not known whether species are obligate or facultative specialists (Pratchett 2005). At the community level, isotopic variance and other metrics of isotope data such as overall trophic diversity can be used to compare among communities or ecosystems through space and time (Layman et al. 2007).

Until recently, using isotope mixing-models to determine specific contributions of different dietary signatures to a consumer's signature had been limited by the number of available isotopic tracers. Chapter 7 reviewed three new modelling approaches (SOURCE, STEP and IsoSource) that allow the partitioning of multiple sources, using as few as two isotopes (Lubetkin and Simenstad 2004, Phillips and Gregg 2003). All three models trialled were found to give very similar diet estimations and differed only slightly in their accuracy and precision. These models aid the interpretation of isotopic data from complex systems whereas previously inferences from isotopic data were largely descriptive. The most appropriate model to use will depend on the size of dataset and the question being answered. Due to an iterative calculation process, IsoSource will provide narrower suggested diet ranges than STEP for individual consumers. SOURCE and STEP however can estimate the contributions to multiple consumers (i.e. entire food webs) simultaneously. These new, quantitative, methods of interpreting isotopic data add to the current utility of stable isotopes as simple food-web descriptors

8.4 Temporal and spatial variability in isotopic data

Previously it has not been documented how the dynamic nature of the surrounding seas, specifically the seasonal changes driven by upwelling events, influence the trophic relationships of coral reef communities in Oman. In this study it was found that in general the $\delta^{15}N$ signatures of marine biota were ~3-6‰ higher at sites in Oman in relation to similar coral-associated habitats around the globe (Chapter 4). Patterns of macroalgae ^{15}N enrichment among seven locations within the West Indian Ocean reflected local nutrient enrichment, regardless of genera, with the most enriched values occurring within the Omani upwelling region. The enriched ^{15}N values of organisms in Oman are thought to be derived from the 'new' nitrate that is upwelled to surface waters during the summer months (Chapter 1 and 3).

In Chapter 3 of this thesis it was shown how the pattern of upwelling events along the Omani coastline can be detected in both zooplankton and macroalgae that are enriched in ^{15}N and depleted in ^{13}C isotopes. Pelagic fish species feeding solely on a zooplankton food chain (*Herklotsichthys quadrimaculatus* and *Sardinella gibbosa*) had variable $\delta^{15}N$ and $\delta^{13}C$ signatures with season, reflecting the change in isotopic signature at the base of the food web. On the reef a few low trophic level organisms (*Chromis xanthopterygia* and some invertebrates) showed seasonal variability of $\delta^{13}C$ and $\delta^{15}N$ but this was generally attenuated by higher trophic level species. No temporal change in an isotope signature of a consumer implies that it is feeding constantly on the same materials (or the same isotope signature) or that turnover is slow. One species of higher trophic level that did show seasonal variability in $\delta^{15}N$ was *Cephalopholis hemistiktos*, this was either as a result of a seasonal change in diet or as a result of the diet changing it's signature on a seasonal basis, it was not possible in the study to determine which (Chapter 3).

The majority of organisms analysed in this study were fully grown adults so any turnover of muscle tissue will be due to metabolism not growth and is likely to occur over a time scale ranging from many months to years (Hesslein et al. 1993). Liver has a faster turnover rate than muscle, however in the 5 species (*Parupeneus marginatus*, *Dascyllus trimaculatus*, *Scolopsis ghanam*, *Chaetodon nigropunctatus and Chaetodon melapterus*) that liver tissue was also sampled (over three seasons)

there was no greater change observed than in the muscle tissue. None of these species had particularly strong planktonic signatures (depleted ^{13}C) where a stronger seasonal variability may have been expected. It is likely that the turnover rate of the tissues sampled (muscle and liver) were insufficient for the resolution of temporal change. As muscle tissue $\delta^{15}N$ did not vary significantly with season; this tissue was considered suitable for use in the determination of trophic level and in mixing models

It is unwise to use organisms that vary seasonally in their isotope signature as baselines from which to calculate higher trophic levels (Post 2002). Zooplankton in this study was a particularly unsuitable baseline because fast turnover rates meant their $\delta^{15}N$ and $\delta^{13}C$ strongly reflected the upwelling events in Oman and would have given variable trophic level estimates depending upon the time of sampling. Suitable baseline species have been suggested to include primary consumers with slower turnover rates and a longer life history such as bivalves and gastropods (Vander Zanden and Rasmussen 1999) that attenuate any seasonal variability. In chapter 3 the bivalve *Barbatia decussata* was used as a baseline, as it showed no change in $\delta^{15}N$ and $\delta^{13}C$ with season.

Spatial differences in $\delta^{15}N$ over small distances (5-10km) have been observed in marine ecosystems elsewhere (Deudero et al. 2004, Jennings et al. 1997, Vizzini et al. 2005) but were not evident on a small scale in the Gulf of Oman (Chapter 4). The fish species examined from the coral communities at Bandar Jissah, Bandar Kayran and Cemetery Bay did not differ in carbon or nitrogen isotopic signatures in tissues of long (muscle) or short (liver) turnover rates suggesting that the food webs of these coral communities at these three sites to not differ significantly.

Enrichment of ^{15}N was found to vary over greater spatial distances within Oman (Chapter 4). The coral reefs at Bar Al Hickman, on the Arabian Sea coast of Oman, have very rarely been visited by researchers. This is an area with great potential for further research, as many rare and little known species are found there (Sheppard et al. 2000). Fish and invertebrates from these reefs provided new information about these communities. Fish species (or their close relations) at Bar al Hickman showed a higher level of ^{15}N enrichment compared to those in the Gulf of

Oman collected during the same month. The use of a primary consumer (*Laevichlamys ruschenbergerii*) as a baseline, assumed to be of trophic level 2 at both sites, demonstrated that fish here were of approximately the same trophic level as those at Bandar Kayran. It cannot however be assumed that the magnitude of any seasonal variability of isotope signatures in producers and consumers is the same at Bar al Hickman as at Bandar Kayran. It is possible that the greater intensity of the upwelling on the Arabian Sea coast results in a greater magnitude of isotopic variability, but it was not possible to return to this remote area on a seasonal basis to test this hypothesis. The overall range in $\delta^{13}C$ values at Bar Al Hickman was smaller than at Bandar Kayran, suggesting that the overall range of dietary items was smaller and the potential for niche diversification at the base of the food web was lower than at Bandar Kayran (Layman et al. 2007).

8.5 The importance of trophic fractionation

The most important aspect concerning the application of stable isotope data as a food web description tool raised in this thesis was the value assumed for trophic fractionation ($\Delta\delta^{15}N$; Chapter 5 and 7). The suggestion that a single mean value may not be appropriate for all organisms is not new and many studies have tried to identify factors contributing to significant departures from this (McCutchan et al. 2003, Vander Zanden and Rasmussen 2001, Vanderklift and Ponsard 2003). Here it was shown that herbivorous fish have a $\Delta\delta^{15}N$ of ~4.5‰, a significantly higher value than the usually assumed 3.4‰ for fish and other consumers. High $\Delta\delta^{15}N$ values have previously been noted in other herbivorous fish species, but were thought to be due to dietary routing or it was assumed that there must have been unknown animal component of the diet (Pinnegar and Polunin 2000). In Chapter 5 the high $\Delta\delta^{15}N$ was explained using a model incorporating diet quality, feeding rate, assimilation efficiency and excretion rate. These physiological processes are known to be distinctly different in herbivores; they consume food of a poorer quality and have a higher feeding rate than carnivorous fish (Horn 1989). Previously models determining $\Delta\delta^{15}N$ have required knowledge of the $\delta^{15}N$ value of the food source which, in an ecological setting, is often the unknown parameter (Harvey et al. 2002). The model described in Chapter 5 (adapted from the fractionation model of Olive et al (2003)) is unique in that instead of using a $\delta^{15}N$ value of the diet

physiological parameters are modelled to predict a $\delta^{15}N$ value for the diet and hence derive a value for $\Delta\delta^{15}N$. This model is a significant advance in understanding of the processes that lead to ^{15}N enrichment and could be used to look for other systematic departures for the assumed mean of 3.4‰ for example in species that feed on relatively high or low quality (protein content/C: N ratio) diets (e.g. fish of specific guilds, e.g. corallivores).

Stable isotopes are often cited as being more useful than gut contents data for long term diet and trophic level estimations. However, the value given to $\Delta\delta^{15}N$ is fundamental to the use of isotopic data to infer trophic position and overall food chain length (Post 2002). If the $\Delta\delta^{15}N$ value used to estimate trophic level is inaccurate then the usefulness of this tool diminishes. Generic fractionation values can be useful in providing a rough estimate of overall trophic positions within a food web, as in Chapter 4 where 3.4‰ was used to compare the spatially separated food webs, but care must be taken in interpreting this information in detail. In chapter 4 absolute trophic levels were not essential as only relative trophic levels were being compared between sites.

At the individual level it is critical that appropriate fractionation values are assumed when using mixing models to estimate diets (Chapter 7). Using a value that is not suitable for the particular organism being studied will also adversely affect the estimation of direct dietary components elsewhere in the system or ultimate food web sources. When various fractionation values were trialled in the mixing model analysis in Chapter 7, the often cited fractionations of 1‰ for $\delta^{13}C$, 3.4‰ for $\delta^{15}N$ and 0‰ for $\delta^{34}S$ were found to be unsuitable for 5 out of the 12 species included. The invertebrate species were found to only have feasible diets with smaller (<3.4‰) $\Delta\delta^{15}N$ values. Sulphur fractionation was found to be particularly variable with some species requiring a positive value (e.g. *Abudefduf vaigiensis* and most invertebrates) for a feasible diet estimate and others requiring a negative value (*Acanthurus sohal*). Five species had no feasible diet solutions where sulphur fractionation was 0‰. There was no identifiable pattern as to which species require positive or negative values for this element, although previously it has been suggested that as with $\Delta\delta^{15}N$. $\Delta\delta^{34}S$ can vary with diet quality, but there are currently too few data to be conclusive (McCutchan et al. 2003). Often in models

$\Delta\delta^{15}N$ ($\Delta\delta^{13}C$ or $\Delta\delta^{34}S$) are not empirically measured, as the diet is not known. The model analysis here highlights the need to acknowledge that there is error associated with assuming particular fractionation values.

8.6 A note on methodological procedures

With the recent increase in the volume of isotopic data reported in the ecological literature it is timely that preparation techniques are being called into question with several authors highlighting the need for standardising preparation procedures (Jacob et al. 2005, Jardine and Cunjak 2005, Sweeting et al. 2004). The use of multiple machines for the isotopic analyses in this thesis, posed an unforeseen problem associated with machine bias (Chapter 2). It is expected that the calibration methods trialled herein for IRMS render results obtained from different laboratories comparable; hence inter-machine calibrations are rarely undertaken when interpreting results in comparison to other studies. Through further analysis it was determined that this was not a unique problem and that the accuracy and precision of different mass spectrometers can result in readings from any two instruments being as different as 2.1‰ for $\delta^{15}N$ (Appendix A). This underlying machine bias is frequently overlooked, yet may influence the interpretation of isotopic data where data originate from multiple instruments, particularly in meta-analyses. This simple study underlines the importance of including internal standards within sample runs and acknowledging or accounting for any associated error.

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9 Mass spectrometer bias in stable isotope analysis Introduction

Isotope ratio mass spectrometry (IRMS) is a commonly utilised tool in ecological research. However, concern has been raised over the accuracy and reporting of errors by authors who are not familiar with stable isotope methodology, in some cases making the interpretation of the data questionable (Jardine and Cunjak 2005, Werner and Brand 2001). The most commonly used isotopes in ecological studies, carbon ($^{12}C/^{13}C$) and nitrogen ($^{14}N/^{15}N$), have a wide range of ecological applications including tracking migration, diet/feeding interactions, environmental tracers and pollution detection (Hobson 1999, Hobson and Wassenaar 1999, Owens 1987). A range of recent papers have discussed sample processing methodology (Carabel et al. 2006, Feuchtmayr and Grey 2003, Jacob et al. 2005, Schlechtriem et al. 2003, Sweeting et al. 2004, 2006), analytical error (Jardine and Cunjak 2005) and individual versus analytical variation and experimental design (Lancaster and Waldron 2001), all with a view to increase standardisation of preparation techniques to improve comparability between studies and results. One potential source of error that has not been addressed is variation arising from the use of multiple machines.

In theory, all mass spectrometers should report the same values for biological material (within error range) as all IRMS machines are calibrated to International Atomic Energy Agency (IAEA) standards. International standards are of limited supply and IRMS laboratories are expected to maintain day to day calibration of machines by use of internal standards within a range of accepted values (Werner and Brand 2001). The use of standards in this way is thought to make comparison of results across separate machines and studies feasible (Hobson and Schell 1998). As a further check on repeatability achieved by the IRMS facility researchers are encouraged to submit blind replicates of a portion of their samples within each sample run (Jardine and Cunjak 2005). In addition, a single reference sample should be included within each run to account for variation with time if samples are analysed over multiple runs (Jardine and Cunjak 2005).

Usually a study will only use one IRMS machine to conduct analyses but situations do arise when multiple machines are used (e.g. Davenport and Bax 2002) especially when researchers are conducting analyses in the same geographic region as other authors (e.g. Deudero et al. 2004) or over an extended time period (Barnes 2006). If more than one machine is used within a single study, submitting duplicate material to be analysed on both machines allows calibration of any discrepancies in data (Pinnegar 2000, Tamelander et al. 2006) but, as multiple machine analyses are most commonly from comparisons made between new data and previously published data (e.g. Takai et al. 2000) this is not always possible. Any large scale inter-machine variability will cast doubt over the validity of meta analysis of data sets from multiple machines, a practise that is common in literature reviews (e.g. France 1997).

The aim of this paper is to illustrate the potential problems in using multiple IRMS machines when analysing ecological samples and to highlight the importance of submitting reference samples. We estimated the variation in isotopic data among multiple IRMS machines by analysing the same homogenous sample across eight machines. The implications of inter-machine variation are discussed.

9.2 Methods

The biological tissue chosen for this study was cod muscle (*Gadus morhua*). The specific material was prepared from a single fish of large body mass that was freeze dried and homogenised to a fine powder. The material has been analysed on one machine as an internal standard for over 4 years and has maintained consistent isotopic values among runs ($\delta^{15}N$ SD = 0.18 ‰, $\delta^{13}C$ = 0.11 ‰). Preserved cod muscle is known to not change in isotopic signature with time (Sweeting et al. 2004) and fulfils the criteria set out for a secondary standard material in Werner and Brand (2001). The material was sent to the eight facilities for $\delta^{13}C$ and $\delta^{15}N$ analysis in the manner in which they usually accept biological material – either pre-weighed into tin capsules or as an un-weighed homogenous sample to be prepared at the facility in question. The IRMS facilities were informed that the tissue was marine fish muscle but no indication

was given of expected isotopic signature. The IRMS machines used in this study remain anonymous and will be referred to as M1, M2 etc.

To quantify the variability in $\delta^{15}N$ and $\delta^{13}C$ we employed a one-way ANOVA with random effects (Raudenbush and Bryk 2002) using the lme library (Pinheiro and Bates 2000) for the statistical package R (R Development Core 2005). This model can be represented in linear form by a sample-level (*i*) model

$$Y_{ij} = \beta_{0j} + r_{ij} \qquad (9.1)$$

and a machine-level (*j*) model for the sample means

$$\beta_{0j} = \gamma_{00} + \mu_{0j} \qquad (9.2)$$

that yields a combined (random-effects) model

$$Y_{ij} = \gamma_{00} + \mu_{0j} + r_{ij}. \qquad (9.3)$$

This approach allowed us to account for unequal sample sizes among machines, estimate the variability of results both within and among laboratories, and make general inferences about the wider 'population' of IRMS machines. The test-statistic for differences among machines was

$$H = \Sigma n_j (\hat{Y}_{.j} - \hat{\gamma}_{00})^2 / \hat{\sigma}^2 \qquad (9.4)$$

which has a large-sample χ^2 distribution with J-1 degrees of freedom. We calculated a plausible value range for the machine means as

$$PVR = \hat{\gamma}_{00} \pm 1.96(\hat{\tau}_{00})^{1/2} \qquad (9.5)$$

where $\hat{\tau}_{00}$ is the estimated variability among machine means (Raudenbush and Byrk 2002). We also calculated the intraclass correlation, representing the variance in sample values among machines, as

$$ICC = \hat{\tau}_{00} / (\hat{\tau}_{00} + \hat{\sigma}^2) \qquad (9.6)$$

where, $\hat{\sigma}^2$ is the estimated sample-level variability (Raudenbush and Byrk 2002).

9.2.1 Results

Throughout the analysis of samples no abnormalities were reported by the laboratories (e.g. abnormality of peak size) and the data was taken as being reliable. The variability of isotopic signatures was greater among the eight different machines than within a single machine (Table 3.1, Figure 3.1). The lowest raw nitrogen isotope value reported was 14.18‰ by M6 and the highest was 18.33 ‰ by M5. Mean nitrogen values varied by 2.1‰. Four machines had very similar means (M1, M3, M7 and M8), while there were two machines with lower (M4 and M6) and two with higher (M2 and M5) values respectively (Figure 3.2a). Overall, carbon showed less variation than nitrogen with all values falling within a 1‰ range (-16.84 to -15.88‰, both values M4; Figure 3.2b).

The estimated grand mean for $\delta^{15}N$ was 15.40‰, with a standard error (SE) of 0.25 and a 95% confidence interval of (14.90, 15.90)‰. Four machines had 95th quartile ranges that did not include the grand mean. The sample-level variability, $\hat{\sigma}^2$, was 0.17 while the estimated variability among machines, $\hat{V}ar(u_{0j})$, was 0.46. We found that the differences in $\delta^{15}N$ among IRMS machines were highly significant (Table 3.2); the estimated range of plausible mean $\delta^{15}N$ values among machines was (14.05, 16.75)‰, suggesting that any two machines may routinely differ in $\delta^{15}N$ by 2.7‰. Overall, the interclass correlation estimate indicated that 74% of the variance in sample $\delta^{15}N$ values was due to differences in the machines used.

Carbon-isotope results were much more consistent among machines than for nitrogen, where the estimated grand mean of $\delta^{13}C$ was -16.47% (SE=0.13) with a (-16.59, -16.35) 95% confidence interval. Three machines had 95th quartile ranges that did not span the grand mean. Sample-level variability was low, at 0.04, and $\hat{V}ar(u_{0j})$ was lower still, at 0.02. Again the differences in $\delta^{13}C$ among machines were significant, and the estimated range of plausible $\delta^{13}C$ values for all machines was (-16.74, -16.20)‰. For carbon, approximately 35% of the sample variation could be attributed to machine-level differences.

Table 9.1: Mean (+/- 1 SD) $\delta^{15}N$ and $\delta^{13}C$ values of cod (*Gadus morhua*) muscle analysed on eight IRMS machines.

Machine	n	$\delta^{15}N$			$\delta^{13}C$		
M1	5	15.53	+	0.21	-16.63	+	0.03
M2	10	16.42	+	0.11	-16.48	+	0.08
M3	9	15.36	+	0.17	-16.26	+	0.08
M4	9	14.81	+	0.29	-16.66	+	0.30
M5	29	16.24	+	0.61	-16.37	+	0.24
M6	6	14.29	+	0.07	-16.35	+	0.06
M7	5	15.37	+	0.05	-16.57	+	0.06
M8	4	15.12	+	0.28			

Table 9.2: Results of a one-way ANOVA with random effects for (a) $\delta^{15}N$ and (b) $\delta^{13}C$ signatures of cod (*Gadus morhua*) muscle estimated from 8 IRMS machines.

(a)

Fixed effect		Coefficient	se	
Grand mean (γ_{00})		15.40	0.24	
Random effect	**Variance component**	**df**	χ^2	**p-value**
Machine mean (u0j)	0.476	7	232.83	<0.001
Sample-level variance (rij)	0.170			

(b)

Fixed effect		Coefficient	se	
Grand mean (γ_{00})		-16.47	0.13	
Random effect	**Variance component**	**df**	χ^2	**p-value**
Machine mean (u0j)	0.019	7	25.33	<0.001
Sample-level variance (rij)	0.037			

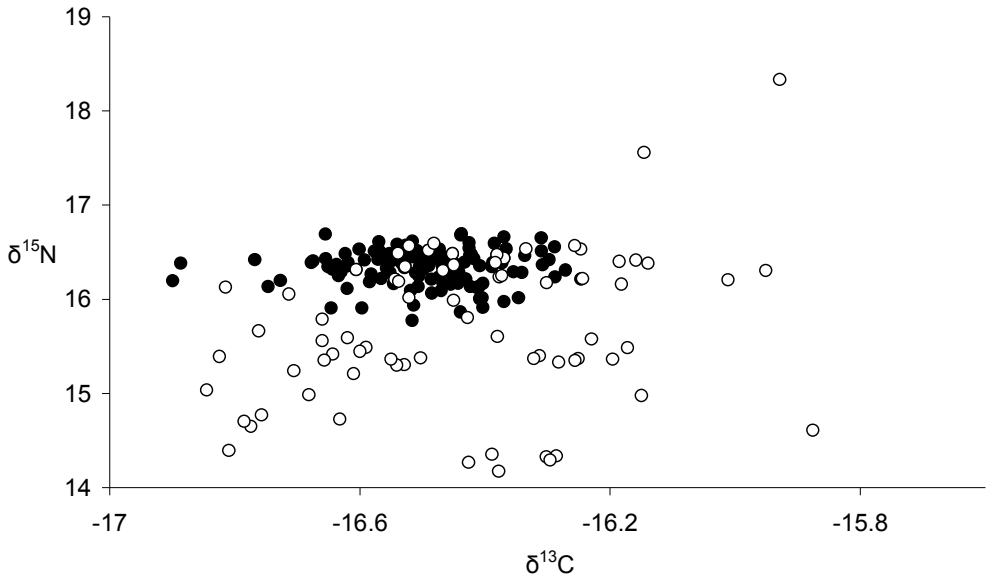
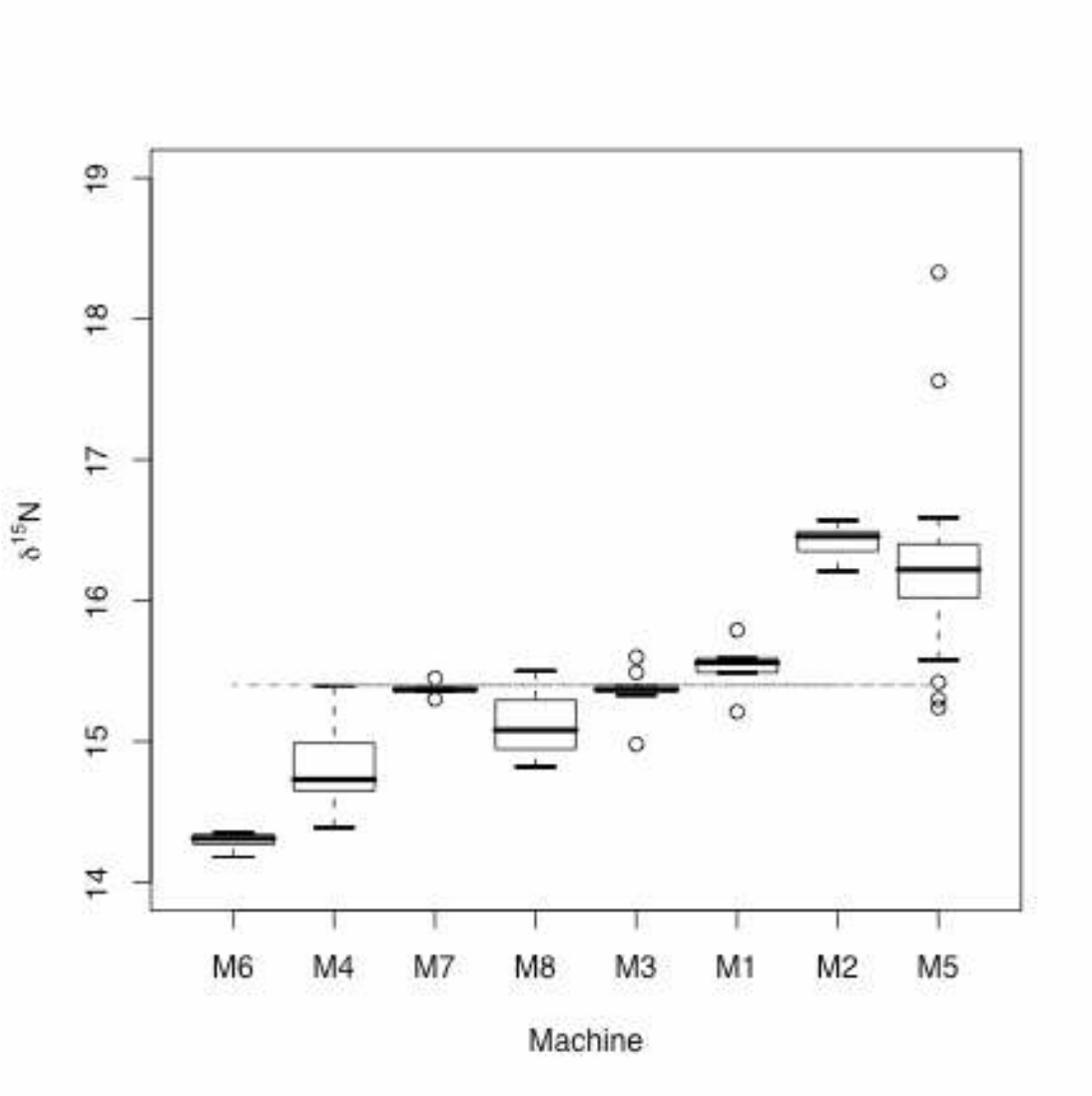


Fig. 9.1: Precision plot of $\delta^{13}C$ and $\delta^{15}N$ values for cod (*Gadus morhua*) muscle analysed on a single machine (filled squares) and multiple machines (empty squares).

(a)



(b)

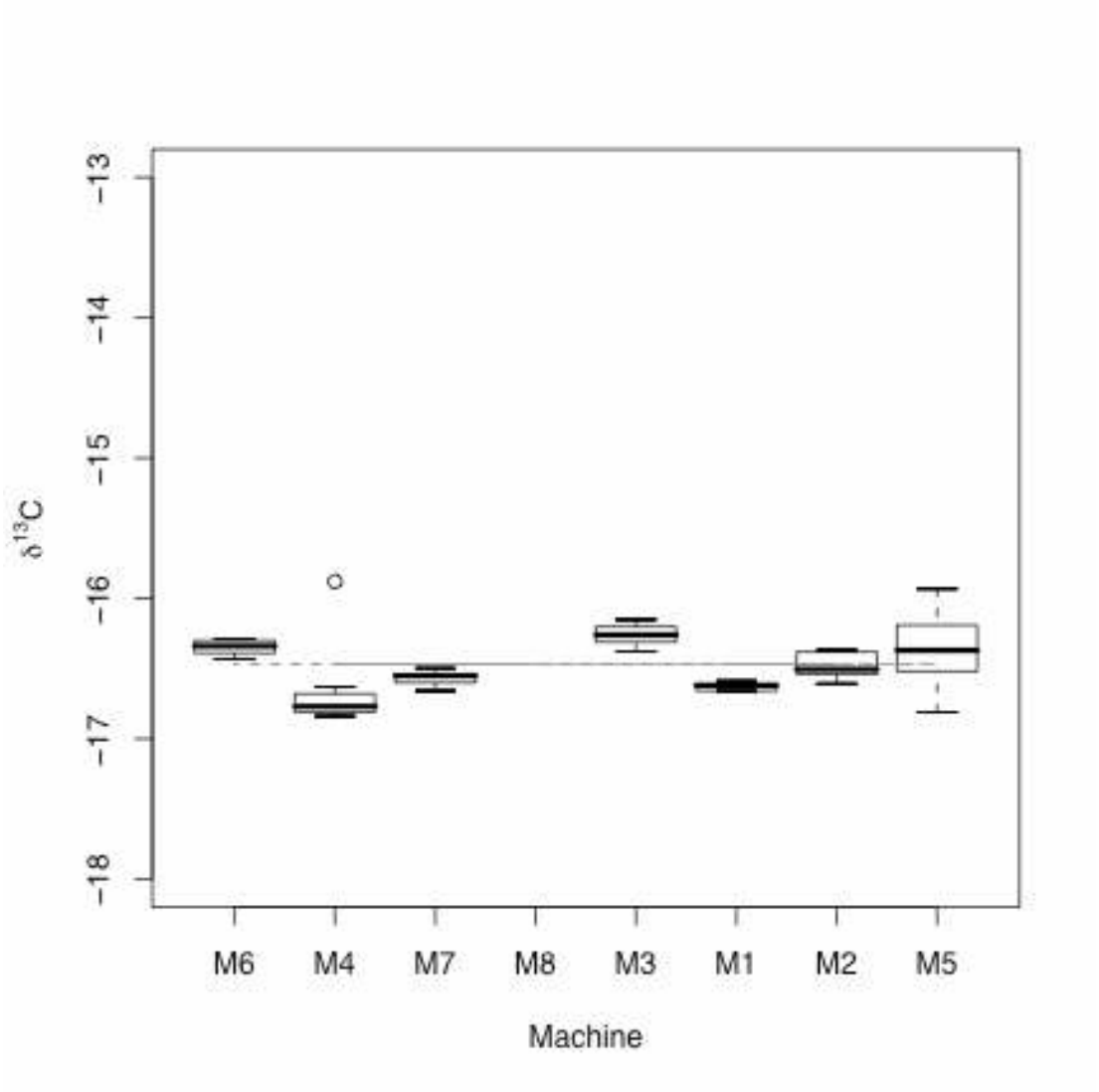


Fig. 9.2: Summary boxplots of stable-isotope values of cod (*Gadus morhua*) muscle analysed on 8 IRMS machines for (a) $\delta^{15}N$ and (b) $\delta^{13}C$. Dashed line represents estimated grand mean of each isotope estimated from among all machines.

9.3 Discussion

In theory the use of IAEA standards across machines should render data obtained from multiple machines comparable (Hobson and Schell 1998). However, this inter-machine comparison shows that the isotopic ratios of a single sample analysed on multiple machines can routinely differ in $\delta^{15}N$ by 2.7‰, a significant proportion of the commonly applied trophic fractionation estimate of 3.4‰ (Post 2002). Hence, any inter-machine variation that goes unmeasured may mask important ecological effects that may otherwise be evident in isotopic data.

Inter-machine variation is an important issue when multiple machines are used in a single study (e.g. Tamelander et al. 2006). Analyses on multiple machines are common but authors routinely fail to report whether differences between machines were measured and give no indication of having accounted for inter-machine variability (e.g. Adams and Sterner 2000, Davenport and Bax 2002, Harvey and Kitchell 2000, Jones and Waldron 2003, Lorrain et al. 2002). The validity of comparing results derived from multiple machines within a study can be confirmed with a small amount of extra effort by including a reference sample (Hobson and Schell 1998). When significant isotopic differences have been found between multiple machines, alignment of all data to one machine is necessary prior to interpretation (Tamelander et al. 2006). Ideally, a range of isotopic values will be measured on each machine to ensure differences are linear and alignment can be preformed with a simple regression (e.g. Pinnegar 2000).

Frequently studies make comparisons between new data and existing published values to aid in the interpretation of ecological significance of isotopic ratios; but the new and published data often originate from different IRMS facilities (Deudero et al. 2004, Kline et al. 1993, Kurle and Worthy 2002, Pinnegar et al. 2003). This problem could be particularly acute if studies rely on published data to infer temporal trends in the trophic status of their study species (e.g. MacNeil et al. 2005) or where food-web mixing-models (Lubetkin and Simenstad 2004) are developed using new and existing data. Current research efforts focus on the use of large spatial networks of published

isotopic values to further examine ecological effects at regional and global scales (West et al. 2006). While this direction of future research should be encouraged, the assimilation of collective data sets has the draw back of originating from multiple machines. Unless the accuracy of IRMS machines is improved as a whole, machine-level variation will cast doubt on combined results.

By necessity, reviews and meta-analyses use data obtained from multiple machines, often more than five at once (France 1995a, Pinnegar et al. 2003). Averaged over long-runs and among environments machine-level variation may be muted somewhat, but there is no simple way to account for the affect. Review papers should, at the very least, acknowledge that inter-machine variation may be a significant source of error.

It should, however, be noted that inter-machine variability does not confound the results of meta-analysis papers that report on non-absolute isotope ratios such as fractionation (McCutchan et al. 2003, Post 2002, Robbins et al. 2005, Vander Zanden and Rasmussen 2001, Vanderklift and Ponsard 2003), tissue turnover rates (Dalerum and Angerbjorn 2005, McIntyre and Flecker 2006) and calculated trophic levels (Pinnegar et al. 2003). Such values or rates are relative and remain comparable provided each estimate of fractionation, turnover or trophic level was derived from results obtained from a single machine.

While it is beyond the scope of our study to assign sources to the bias we observed among IRMS machines, we have several suggestions as where to start. Firstly, the expense of IAEA standards may lead to a reduction in their use for tuning internal standards within a given lab. If not applied regularly, IAEA standards cannot ensure the absence of standard drift in machines. Second, the model of machine used may play a role. While in theory machines from different manufacturers should achieve the same results, some models may be more reliable in precision or may be less susceptible to drift. Finally, another likely source of bias is the action of the IRMS operators themselves. Setup and maintenance of IRMS machines is notoriously difficult, where a balance between analytical accuracy, precision and timely production

of results must be developed. Ecologists rely on machine operators to inform them of any unusual or potentially unreliable results. Differences in approach to laboratory maintenance and standardization are a likely source of bias among machines. This emphasizes the importance of the individuals in stable isotope research and supports the necessity of the high levels of training they routinely attain.

9.4 Recommendations

In light of our findings we encourage researchers to conduct analyses, where possible, on a single machine and preferably without a long delay between sample runs. We advocate the recommendation of Jardine and Cunjak (2005) that the researcher should include their own sample reference within all runs. A sample reference will not only provide a timely indication of the accuracy of the machine but will also allow an independent check on variation between runs and between machines as emphasised in this study. In addition, if disparity between two machines occurs, where possible, data should be aligned by linear regression obtained over a range of isotopic values. For consistency when reporting laboratory details, multiple machines should be listed and authors should report where differences were observed and if the data were aligned. When using existing published data, the use of relative machine-independent values (e.g. fractionation, turnover rates) is essential. Where this is not possible, the error associated with machine-level variation should be acknowledged and the results should be qualified accordingly.

Appendix B

Appendix B: Literature review of NAE in herbivorous fish

Species	Family	NAE	Food	Food type	Ref
Cebidichthys violaceus	Stichaeidae	82.6	Microcladia coulteri	Algae	Horn & Neighbors (1984)
Cebidichthys violaceus	Stichaeidae	83.6	Smithora naidum	Algae	Horn & Neighbors (1984)
Cebidichthys violaceus	Stichaeidae	78.4	Gigaetina leptorhynchos	Algae	Horn & Neighbors (1984)
Cebidichthys violaceus	Stichaeidae	82.5	Iridaea flaccida	Algae	Horn & Neighbors (1984)
Cebidichthys violaceus	Stichaeidae	74.9	Porphyra perforata	Algae	Horn & Neighbors (1984)
Cebidichthys violaceus	Stichaeidae	52.8	Spomgomorpha coalita	Algae	Horn & Neighbors (1984)
Cebidichthys violaceus	Stichaeidae	71.4	Gigartina canaliculata	Algae	Horn & Neighbors (1984)
Cebidichthys violaceus	Stichaeidae	71.7	Ulva lobata	Algae	Horn & Neighbors (1984)
Holocanthus bermudensis	Pomacanthidae	85	Enteromorpha salina	Algae	Menzel (1959)
Plectroglyphidodon lacrymatus	Pomacentridae	76.5	Ingested algae	Algae	Polunin (1988)
Plectroglyphidodon lacrymatus	Pomacentridae	75.3	Ingested algae	Algae	Polunin (1988)
Plectroglyphidodon lacrymatus	Pomacentridae	91.7	Ingested algae	Algae	Polunin (1988)
Stegastes apicalis	Pomacentridae	57	Ingested algae	Algae	Klump & Polunin (1989)
Stegastes (Eupomacentrus) fuscus	Pomacentridae	57.51	Ingested algae	Algae	Ferreira et al. (1998)
Girella tricuspidata	Kyphosidae	83.5	Enteromorpha intestinalis	Algae	Anderson (1988)
Girella tricuspidata	Kyphosidae	57.6	Enteromorpha intestinalis	Algae	Anderson (1988)
Girella tricuspidata	Kyphosidae	52.86	Enteromorpha intestinalis	Algae	Anderson (1988)
Stegastes nigricans	Pomacentridae	48.7	Ingested algae	Algae	De Loma et al. (2000)
Stegastes nigricans	Pomacentridae	60.9	Ingested algae	Algae	De Loma et al. (2000)
Siganus fuscescens	Siganidae	58.95	Acanthophora spicifera	Algae	Pillans et al.(2004)
Siganus fuscescens	Siganidae	47.22	Gracilaria edulis	Algae	Pillans et al.(2004)
Siganus fuscescens	Siganidae	49.01	Zostera capricorni	Seagrass	Pillans et al.(2004)
Siganus fuscescens	Siganidae	25.8	Dictyota dichotoma	Algae	Pillans et al.(2004)
Siganus fuscescens	Siganidae	19.5	Lobophora variegata.	Algae	Pillans et al.(2004)
Hermosilla azurea	Kyphosidae	77.7	Chondracanthus canaliculatus	Algae	Sturm & Horn (1998)
Hermosilla azurea	Kyphosidae	73.7	Mazzaella leptorhnchos	Algae	Sturm & Horn (1998)
Hermosilla azurea	Kyphosidae	82.7	Ulva lobata	Algae	Sturm & Horn (1998)
Hermosilla azurea	Kyphosidae	84.5	Macrocystis pyrifera	Algae	Sturm & Horn (1998)
Hermosilla azurea	Kyphosidae	72.4	Pachydictyon coriaceum	Algae	Sturm & Horn (1998)
Hermosilla azurea	Kyphosidae	80.7	Sargassum	Algae	Sturm & Horn

			muticum		(1998)
Ctenopharyngodon idella	Cyprinidae	95.1			Carter & Brafield (1992)
Monocanthus chinensis	Monocanthidae	79.87	Microdictyon umbilicatum	Algae	Conacher et al. (1979)
Monocanthus chinensis	Monocanthidae	67.08	Podidonia australis	Seagrass	Conacher et al. (1979)
Parablennius sanguinolentus	Blenniidae	72.18	Ulva lactuca	Algae	Horn & Gibson (1990)
Parablennius sanguinolentus	Blenniidae	50.81	Ulva lactuca	Algae	Horn & Gibson (1990)
Parablennius sanguinolentus	Blenniidae	55.30	Ulva lactuca	Algae	Horn & Gibson (1990)
Parablennius sanguinolentus	Blenniidae	58.59	Ulva lactuca	Algae	Horn & Gibson (1990)
Gobius cobitis	Gobiidae	64.13	Ulva lactuca	Algae	Horn & Gibson (1990)
Gobius cobitis	Gobiidae	62.68	Ulva lactuca	Algae	Horn & Gibson (1990)
Gobius cobitis	Gobiidae	61.89	Ulva lactuca	Algae	Horn & Gibson (1990)
Gobius cobitis	Gobiidae	65.58	Ulva lactuca	Algae	Horn & Gibson (1990)