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Determining carbon and nitrogen stable isotope discrimination for marine consumers

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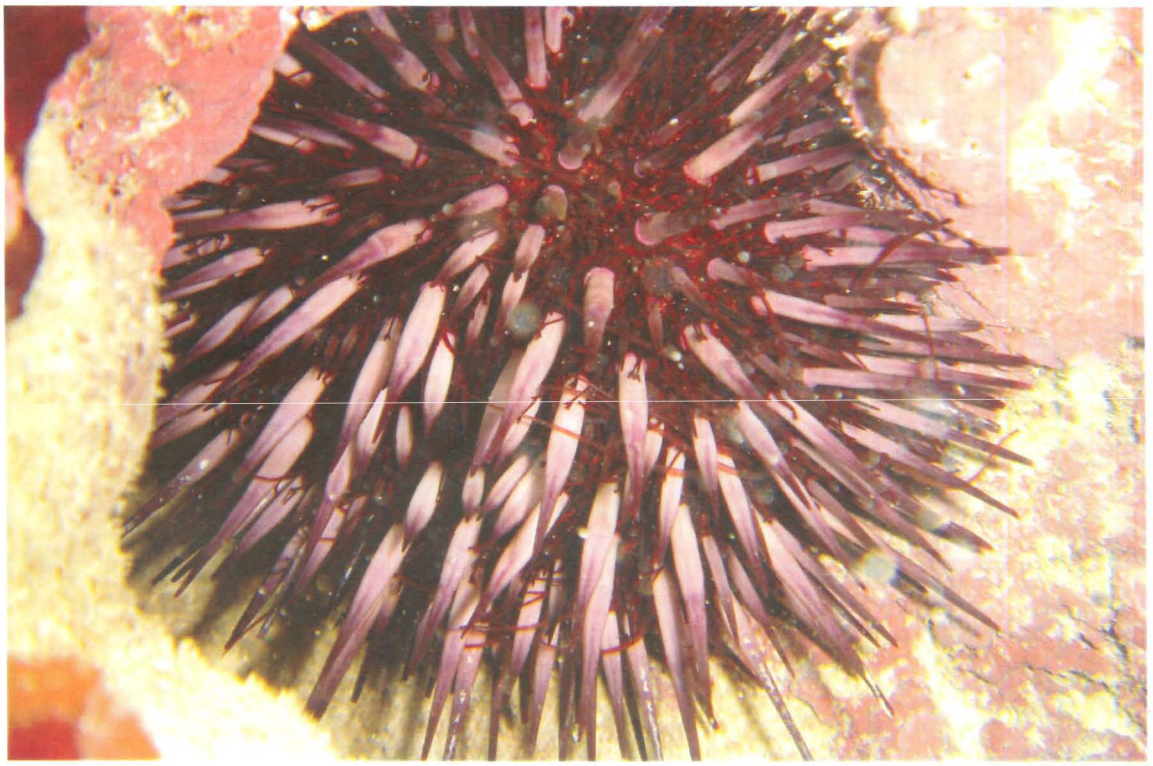
DETERMINING CARBON AND NITROGEN STABLE ISOTOPE DISCRIMINATION FOR MARINE CONSUMERS



by

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Abstract

The application of stable isotope ratios to food web studies is increasing, and the use of generalised discrimination values (0.4±1.4‰ for $\delta^{13}C$ and 3.4±1.1‰ for $\delta^{15}N$), which are being widely applied to many studies, may not be valid. The broad objective of this study was to evaluate the assumption that these discrimination values are applicable to a range of benthic marine consumers, and therefore appropriate to be used in trophic analyses using carbon (C) and nitrogen (N) stable isotopes in marine food webs.

The first aim was to determine if there were differences in discrimination values: (1) among different groups of macroalgae when fed to specific consumers; and (2) among different types of consumers when fed on the same type of algae. The feeding treatments used representatives of brown, fleshy red, calcareous red and green macroalgal food sources, which were fed to three types of consumers: the sea urchin *Heliocidaris erythrogramma*, the hermit crabs *Paguristes purpureantennatus* and *Calcinus dapsiles*, and the gastropod *Turbo torquatus*. Food sources and consumers were collected from the field, and each food source was subsequently fed to each consumer in a series of controlled aquaria experiments. Replicate individuals of each consumer were sampled at regular intervals up to 56 days after initiation of the experiments. Muscle tissue was processed and analysed using mass spectrometry to determine $\delta^{13}C$ and $\delta^{15}N$ values. In accordance with the feeding treatments, starvation treatments were also conducted.

The results showed a general trend of no change in $\delta^{13}C$ and $\delta^{15}N$ isotope ratios for the invertebrate consumers over time, and this was also the case for the starvation treatments. Observations during the experiments indicated that animals were active and consuming the algae in the feeding treatments compared to the starved invertebrates which remained relatively immobile. Constant feeding by invertebrates suggests that discrimination values derived from the experiment are likely to be realistic. Results indicated that $\delta^{13}C$ and $\delta^{15}N$ discrimination differed among groups of algae, particularly for carbon. Average $\delta^{13}C$ discrimination values were 1.56‰ for brown algae, 2.31‰ for fleshy red algae, 3.38‰ for calcareous red algae, 1.46‰ for green algae, while average $\delta^{15}N$ discrimination values were 2.60‰, 2.03‰, 2.53‰, and 2.93‰ for the respective algal groups. $\delta^{13}C$ and $\delta^{15}N$ discrimination also differed among consumers when fed on the same food source. Average $\delta^{13}C$ discrimination values were 2.67‰, 2.70‰, 2.58‰

and 1.31‰ for the urchin *H. erythrogramma*, the hermit crabs *P. purpureantennatus* and *C. dapsiles* and the gastropod *T. torquatus*, respectively, while average $\delta^{15}N$ discrimination values were 3.14‰, 3.80‰, 2.15‰ and 1.31‰ for the respective species. Thus, the results of this study indicate that different discrimination values are exhibited for different algal food sources and by different consumers under the environmental conditions within this study. Furthermore these values differed from the assumed discrimination values that are applied to many stable isotope studies.

In many isotope-based food web studies, mixing models are utilised to quantify the possible contributions of different food sources to a consumer's diet. This process has better outcomes than traditional gut content analysis, as it reflects actual assimilation of food sources rather than what material is found in the gut tract. The second aim was to compare results of the Isosource mixing model when using either assumed or experimentally-derived discrimination values to evaluate the contribution of the different macroalgal species to the natural diets of marine invertebrates. The assumed discrimination values of 0.4‰ for $\delta^{13}C$ and 3.4‰ for $\delta^{15}N$ were applied to the food source stable isotope dataset as input data for Isosource, and compared to results when the experimentally derived values were applied to the same dataset. Results from Isosource were not possible using the assumed values, due to the isotope ratios of consumers sitting outside the range of sources. However when the experimentally derived discrimination values were applied, the model ran successfully. These mixing model results indicated that brown, red and green algae all contributed to the production of consumers, but there were differences in the relative contributions of these food sources among the consumer species examined.

The current study has quantified C and N discrimination for key marine invertebrate and algal types, and particularly for C these values were found to be higher (1.46-3.38‰) than those generally applied, i.e. 0.4 ± 1.4‰. The assumed discrimination values are not applicable across all food sources or consumer groups. The consequences of applying incorrect discrimination values include preventing any sources of production to be established, or leading to erroneous conclusions about an organism's trophic level or food source.

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Cheers

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Chapter 1: Introduction

1.1 Motivation

Stable isotopes can be a powerful tool for understanding environmental, ecological and biological processes (Peterson & Fry 1987, Adams & Sterner 2000, Beaudoin et al. 2001, Polunin et al. 2001) and for modelling food web dynamics in a wide range of environments (Vander Zanden & Rasmussen 2001, Davenport & Bax 2002). Extracting and interpreting information about trophic levels from stable isotopes has been an active area of research over the last 30 years (Jepsen & Winemiller 2002). One approach is to identify important feeding links within an assemblage of consumers, then define a trophic structure through the use of isotope ratios (Jepsen & Winemiller 2002).

The use of stable isotope ratios for ecological studies requires knowledge of the isotopic shift, known as discrimination, between diet and consumer (McCutchan et al. 2003). In the absence of site and organism-specific discrimination values, interpretation of food web relationships have generally relied on a number of critical assumptions (Adams & Sterner 2000, Post 2002, McCutchan et al. 2003). The assumptions are that the stable isotope of carbon ($\delta^{13}C$) discriminates by 0.4‰ ± 1.4 SD between trophic levels, while the stable isotope of nitrogen ($\delta^{15}N$) discriminates by 3.4‰ ± 1.1 SD between trophic levels. These discrimination values are assumed to be valid for all trophic levels (Gannes et al. 1997, Fantle et al. 1999, Oelbermann & Scheu 2002) and for all consumers (Yokoyama et al. 2005).

Proper interpretation of field data demands that we validate these assumptions (Gannes et al. 1997) for all trophic levels in all ecosystems. Progress in the use and interpretation of stable isotope data in animal ecology will be best achieved if the collection of field data is accompanied by relevant laboratory experiments to evaluate isotope dynamics (Gannes et al. 1997). In recent years, there has been a call for more research to be conducted on the assumptions underlying inference from isotope analyses in general (Gannes et al. 1997, Yokoyama & Ishihi 2003), as well as specifically for lower trophic levels in the marine environment (Macko et al. 1982, Yokoyama & Ishihi 2003).

The primary objective of this study is, therefore, to evaluate common assumptions associated with trophic analysis of carbon and nitrogen stable isotopes. This will be

achieved by determining $\delta^{13}C$ and $\delta^{15}N$ discrimination values between key marine primary producers and consumers, and by conducting a sensitivity analysis on discrimination levels used in the interpretation of field data from a marine ecosystem.

1.2 Thesis Structure

This Introduction presents an overview of stable isotopes, their importance in ecological studies and key assumptions regarding their use. This section also explores the state of knowledge on benthic trophodynamics in the southwestern region of Australia. Chapter 2 describes the research approach and methods, including information on experimental treatments, isotope modelling and statistical analyses. Chapter 3 presents the results obtained from the experiments and outcomes from sensitivity analyses using a mixing model program. Chapter 4 compares and discusses the data and their implications for food web studies, in addition to providing recommendations for future research within the field.

1.3 Background

1.3.1 Stable isotopes and food web studies

Traditionally, studies of food web dynamics have often relied on stomach contents analysis or direct observations to evaluate the diets of organisms (Gurney et al. 2001). However, there are several limitations associated with these methods. For stomach content analysis it may be difficult to provide quantitative results as stomach contents may be degraded to such an extent that they cannot be accurately identified (Gurney et al. 2001). Also, some elements may be over-represented as they are harder to digest, taking longer to break down and therefore not accurately reflecting their relative abundance in the original diet. Lastly, each analysis only represents a short time period, thus many individuals must be analysed if an accurate estimation of the diet of a species is to be assessed, which is both labor intensive and time consuming (DeNiro & Epstein 1978, Pinnegar & Polunin 1999, Gurney et al. 2001). Simultaneously there are also limitations with direct observations, such as awareness (Jardine et al. 2003) and the time period of the observations may not capture the full extent and variety of a consumer's

diet (Pearson et al. 2003). Therefore, alternative methods have been used to investigate trophic position, with one of the most promising being stable isotopes.

Isotopes are atoms of the same element with a different numbers of neutrons, and stable isotopes hold their 'extra' neutron until it is removed through chemical reactions (Lajtha & Michener 1994, Dawson & Brooks 2001). The main stable isotopes used in food web research include carbon ($^{13}C/^{12}C$), nitrogen ($^{15}N/^{14}N$), and sulfur ($^{34}S/^{32}S$) (Lajtha & Michener 1994). The ratios of these stable isotopes in the tissues of a consumer are derived from assimilated food and, therefore, reflect dietary input integrated over time (Das et al. 2003), with carbon and nitrogen found to be particularly useful in food web studies. Typically, the concentration of the heavier isotopes (^{13}C and ^{15}N) in the body will be slightly higher than in the diet, as the lighter isotope (^{12}C and ^{14}N) is preferentially utilized in metabolic processes and then excreted, while the heavier isotope is retained (Focken & Becker 1998).

The abundance of an isotope is measured and reported as a deviation from a standard, creating an arbitrary but internationally accepted value that is represented as delta (δ) and reported as parts per thousand (‰) (Dawson & Brooks 2001). The value of δ is calculated as:

$$\textbf{Equation 1.} \quad \delta = \left(\frac{R_{\text{sample}}}{R_{\text{standard}}} - 1 \right) \times 1000$$

$$R = {}^{13}C/{}^{12}C \text{ or } {}^{15}N/{}^{14}N$$

where R_{sample} and $R_{standard}$ represent the ratio of heavy to light isotopes of the sample and the standard, respectively. A standard is an internationally accepted reference, but in practice internal working standards are typically used on a daily basis that are unique to a particular working laboratory (Dawson & Brooks 2001).

The strength of isotope analysis is that it is an accurate time-integrated measurement of food assimilation by an individual organism (Gurney et al. 2001). The difference in the isotopic composition of a consumer, relative to its food source, is called 'discrimination' (Gillon et al. 1998), and calculated as:

$$\textbf{Equation 2.} \quad \Delta = \delta consumer - \delta producer$$

Discrimination, in conjunction with δ values, can be used to determine 'who ate whom' in the trophic system. Note that in many articles the change in isotopic ratios between trophic levels is mistakenly called fractionation, which in fact refers to the underlying biochemical reactions that discriminate against the heavier isotope of a particular element (Eggers 2000).

Differences in physical properties of the isotopes and enzymatic fractionation between isotopes are thought to be some of the causes of the increase values with trophic level (Hansson et al. 1997). Consequently, the magnitude of the isotopic shift between trophic levels can vary among species, and among tissues within an individual (McCutchan et al. 2003). Furthermore, the ratios of stable isotopes can change between diet and consumer due to differential digestion or discrimination during assimilation and metabolic processes (DeNiro & Epstein 1978, 1981, Minagawa & Wada 1984, Oelbermann & Scheu 2002), as well as the turnover rate of the proteins in the tissue examined (Gurney et al. 2001). Discrimination and isotope ratios of algae can also differ between and within groups. The resultant differences in the discrimination levels between a consumer and the variety of food sources need to be incorporated in Equation 2 to provide accurate contributions of food sources to the consumer.

The accuracy of the primary producer/consumer discrimination for carbon and nitrogen cannot be determined from the isotope ratio between the primary consumer and primary producer collected in the field (DeNiro & Epstein 1978). This is due to the complex nature and interconnectedness of ecosystems, combined with consumers utilizing a variety of food sources. Accurately assessing the trophic position of primary consumers is often difficult as feeding strategies will adapt according to changes in food composition and availability (Gurney et al. 2001). Therefore, the isotopic signature of an individual alone is not enough to report on trophic position (Post 2002). The feeding links are complex and modified by seasonality and system productivity and these properties make it difficult to generalize about feeding relationships and to identify dominant linkages in the trophic structure (Jepsen & Winemiller 2002, Post 2002, Vizzini & Mazzola 2003, Grey et al. 2004, Vuorio et al. 2006).

Carbon and nitrogen are assumed to exhibit a standard discrimination between all trophic levels, an assumption that has greatly increased the use of C and N in food web studies (Post 2002). Carbon is assumed to have a nominal discrimination value of 0.4‰ ±1.4 SD between trophic levels (Vander Zanden & Rasmussen 2001) and has therefore

been used to determine the food source of a consumer. In comparison, nitrogen isotopes are assumed to become enriched on average by 3.4‰ ±1.1 SD with increasing trophic level (Vander Zanden & Rasmussen 2001), with discrimination used to calculate a trophic shift up the food web (Focken & Becker 1998).

Carbon

Carbon performs an important role in ecosystems. It can occur in both inorganic and organic forms, with primary sources of the former including ocean carbonate (HCO_3^-), dissolved carbon dioxide ($CO_{2(aq)}$) and carbon from animal activity (Smit 2001, Cloern et al. 2002). These sources are directly utilized by primary producers in the synthesis of organic matter. In marine systems, dissolved inorganic carbon (DIC) is a large source of carbon with levels maintained due to equilibrium exchange processes of atmospheric CO_2 with ocean carbonate (Smit 2001). The variations in the equilibrium between CO_2 and HCO_3^- can alter the ratio of carbon isotopes in dissolved inorganic matter, hence creating spatial and temporal differences in isotopic ratios (Smit 2001). These variations relate to oceanic processes, such as upwellings, and biological processes such as photosynthesis and decomposition.

The form of inorganic carbon utilized by primary producers will strongly alter their carbon ratio. Many marine macrophytes predominately use CO_2 over HCO_3^- (Lajtha & Michener 1994). This will lead to a negative carbon ratio as an increased use of CO_2 will increase the negativity of the carbon ratio (Smit 2001). This is an important feature of some marine macrophytes as it gives them a unique carbon ratio, which allows them to be singled out for specific carbon isotope research/experiments.

In the case of consumers, carbon isotopic ratios ($\delta^{13}C/\delta^{12}C$) within the tissues of consumers are related to ratios found in the material assimilated from their diet (DeNiro & Epstein 1978). As an animal incorporates dietary carbon, it has been found its isotopic composition closely reflects the isotopic composition of its diet (DeNiro & Epstein 1978). For this reason, $\delta^{13}C$ is often used to establish the source of food.

Nitrogen

Inorganic forms of nitrogen include nitrate (NO_3^-), nitrite (NO_2^-), and ammonium (NH_4^+) from animal activity (Smit 2001). Within marine systems, these forms of dissolved inorganic nitrogen are essential in maintaining benthic primary production (Gartner et al. 2002, Campbell et al. 2003). Atmospheric nitrogen is also available in a dissolved form, but can only be utilized by N_2-fixing organisms such blue-green algae (Minagawa & Wada 1984, Robinson 2001, Vuorio et al. 2006). Due to the complex and dynamic processes and interconnectedness of the oceanic system, there can be large temporal and spatial variation of abundance and forms of nitrogen isotope (Boon & Bunn 1994, Vander Zanden & Rasmussen 2001, Cloern et al. 2002, Jennings & Blanchard 2004, Yokoyama et al. 2005, Syvaranta et al. 2006).

Nitrogen isotopes have great potential in the investigation of trophic ecosystems and are largely used to help determine an organism's trophic position (Adams & Sterner 2000). This can be accomplished by using either enriched tracer experiments, or examining the levels of natural abundance (Adams & Sterner 2000). Tracer experiments use an enriched $\delta^{15}N$ that can be followed through the food web to help determine the trophic level of consumers (Hobson & Wassenaar 1999, Robinson 2001, Le Loc'h & Hily 2004, Jacob et al. 2006). However, few studies adopt this approach due to logistical issues, and natural variation in $\delta^{15}N$ is frequently used to determine the trophic structure. This approach assumes predictable discrimination between food source and consumer (Adams & Sterner 2000). The first main discrimination experiments, conducted by DeNiro and Epstein (1981), showed a range of 1.3‰ to 5.3‰ in trophic level enrichment, with an average of 3.4‰ and a standard deviation of 1.1‰. The full extent to which the variation consists of analytical or sampling errors has not yet been determined (Post 2002, Olive et al. 2003), and therefore the assumptions have been adopted without adequate testing.

1.3.2 Previous research

The use of isotopes to determine trophic levels has been widely accepted and applied by the international scientific community (Adams & Sterner 2000). Stable isotopes have

been applied in a diverse range of areas of research, from the study of trophic level and energy flow with in an ecosystem (Peterson & Fry 1987) to tracing pollution (Gartner et al. 2002) and evaluating the diets of fossil organisms (DeNiro & Epstein 1978, Hobson & Wassenaar 1999). So far, much of the research has been terrestrial-based, with studies within the marine environment still greatly lagging behind (Seminoff et al. 2006).

There has been a wide range of studies conducted on different trophic levels within terrestrial ecosystems, although the primary areas of research have been plants and mammals (Jardine et al. 2003, Vanderklift & Ponsard 2003, Yokoyama et al. 2005). Plants have been a key area of study due to their role as primary producers, with a focus on where they gain their carbon and nitrogen and how they utilize it (Lajtha & Marshall 1994). There has been a vast amount of work conducted on both C_3 or C_4 plants and their various tissues, as well as discrimination ratios between the plants and primary consumers (Raven et al. 2002, Brandes et al. 2006). This also applies for mammals, as the main research includes determining their trophic level and role within the ecosystem (Post 2002, Pearson et al. 2003, Bearhop et al. 2004, Thompson et al. 2005). Terrestrial mammals have also been used to study the effects of nutritional stress on a consumer's isotope ratio (Hobson et al. 1993, Adams & Sterner 2000), and tracer experiments to determine how source isotopes are distributed through a consumer's body (Oelbermann & Scheu 2002, McCutchan et al. 2003, Vanderklift & Ponsard 2003). Although understanding patterns of isotope discrimination is essential for the correct interpretation of field data, research is still trying to comprehend the full potential of isotopes in different trophic levels across different ecosystems (Gannes et al. 1997, Hobson & Wassenaar 1999, Oelbermann & Scheu 2002, McCutchan et al. 2003, MacNeil et al. 2006).

In marine ecosystems, a large portion of research has been devoted to nutritional ecology of marine vertebrates, including sea turtles (Seminoff et al. 2006), sea birds (Hobson et al. 1993), sharks (MacNeil et al. 2006) and cetaceans (Ruiz-Cooley et al. 2004). Across the globe evaluation of marine trophic links through the use of stable isotopes has occurred, from pelagic ecosystems off the Antarctic Peninsula (Dunton 2001, Raven et al. 2002, Jacob et al. 2006) to seagrass ecosystems in Western Australia (Smit 2001, Raven et al. 2002). Internationally, there has been a wide variety of trophic research conducted on marine mammals (Das et al. 2003) and the flow of detrital material through the food chain (Bouillon et al. 2001, Dunton 2001, Bouillon et al. 2002, Cloern et al. 2002). Nationally in Australia, a large isotope-based trophic study

was conducted by Davenport (2002) in the marine ecosystem off south-eastern Australia. The study looked at a variety of consumers and producers, ranging from primary through to quaternary, and the discrimination ratios between each level. Locally research has been conducted on seagrass (Schmidt et al. 2004), urchins (Vanderklift et al. 2006) and marine invertebrates (Crawley 2006).

However, there has also been research conducted in different marine ecosystems within Australia, including mangrove, seagrass, salt marsh and pelagic ecosystems. As mangrove ecosystems have a unique isotope value, compared to other marine ecosystems, it makes it easy to trace the flow of nutrients outside the mangroves, to the marine ecosystems (Bouillon et al. 2002, Kieckbusch et al. 2004). Similar research has been conducted on reef systems with the flow of nutrients of seagrass and detached macroalgae away from the reef systems (Lepoint et al. 2000, Hyndes & Lavery 2005, Smit et al. 2005) and is used to study the trophic impact of the movement of seagrass of reef systems (Lepoint et al. 2000). Within salt marsh systems, there has been research conducted on the carbon flow within and through the ecosystem, as well as trophic ecology studies between gastropods and crustaceans and between salts marshes and mangrove systems (Guest et al. 2004).

1.3.3 Significance and Consequences of Discrimination Values

Recent literature has indicated that the assumptions for discrimination values for $\delta^{13}C$ and $\delta^{15}N$ are not valid for all species of consumers (DeNiro & Epstein 1978, 1981, Cabana & Rasmussen 1996, Focken & Becker 1998, Vander Zanden & Rasmussen 1999, Adams & Sterner 2000, Post 2002, McCutchan et al. 2003). Given the current widespread use of stable isotopes in food web studies, it is therefore critical to quantify $\delta^{15}N$ and $\delta^{13}C$ discrimination for a wide variety of organisms.

One of the most recent critiques has argued that the mean discrimination values used disregard the large variation in trophic discrimination in lower trophic levels (Goedkoop et al. 2006). This especially applies for aquatic food webs in which discrimination of $\delta^{13}C$ and $\delta^{15}N$ can be highly variable, ranging from -0.7‰ to 9.2‰ for nitrogen, and -2.1‰ to 2.8‰ for carbon (Goedkoop et al. 2006). This variability is also highest for herbivores and detritivores near the bottom of the food web (Vander Zanden & Rasmussen 2001, Vanderklift & Ponsard 2003, Goedkoop et al. 2006).

In a feeding experiment for invertebrates fed different food sources, Goedkoop et al. (2006) demonstrated that discrimination values for $\delta^{15}N$ ranged from 0.7‰ to 2.7‰. This revealed that assimilation within invertebrates can occur with considerably lower isotopic discrimination than the assumed enrichment of 3.4‰. Field studies have shown in the case of carbon that enrichment of $\delta^{13}C$ in invertebrates relative to the average mangrove litter signal was 6.8‰ (Bouillon et al. 2002), which is similar to those obtained in temperate salt marsh ecosystems (Bouillon et al. 2002). Davenport (2002) found that δ N values between trophic levels are variable, but a difference of 3-4‰ is often found, and for carbon the same applies but with a value of 1‰. Das (2003) found that marine mammals cover a range of N and C discrimination values and conformation to the assumed values is not common. These data were then used to evaluate the trophic level of several species of marine mammals, with the outcome compared to trophic levels of the same mammals from a different study that used the assumed values of DeNiro and Epstein (1978). From this, notable differences were found between the trophic levels using the two different discrimination values.

Discrimination values play a significant role in understanding trophic structure, but even with the studies on discrimination values as detailed above, consequences of using the 'wrong' discrimination value has yet to be quantified. The questions remain, 'how accurate is the discrimination value required to be', 'how is the outcome affected if the discrimination value is too high or too low' and 'does the type of ecosystem that is studied play a part in the sensitivity of trophic analysis to varying discrimination values'? In recent, it has been considered that the assumed discrimination values of 0.4±1.4‰ for carbon and 3.4±1.1‰ for nitrogen are not correct for all consumers within all ecosystems. Thus there is a particular need for more research to be conducted into the discrimination levels in different marine ecosystems, and between the different trophic levels within marine ecosystems. Trophic level provides information on how the ecosystem works, including key species, flow of energy and nutrients and feeding relationships. An understanding of the ecosystem and the important species is essential for improved management and conservation of the ecosystem, especially the food web.

1.4 Research Plan

The purpose of this study was to examine the change in $\delta^{13}C$ and $\delta^{15}N$ of different marine primary consumers, when fed different groups of algae. The aims of this

research centred on testing the assumptions underlying the use of stable isotopes in studies for marine trophic structure. Specifically, the study aims to test whether $\delta^{13}C$ and $\delta^{15}N$ discrimination differs among different primary consumers as well as producers, and to compare results of the Isosource mixing model when using either assumed or experimentally-derived discrimination values to evaluate the contribution of different macroalgal species to the natural diets of marine invertebrates.

Two hypotheses where proposed to meet these aims:

> *Hypothesis 1:* Red, green and brown algae will have different $\delta^{15}N$ and $\delta^{13}C$ ratios and will yield different stable isotope discrimination when consumed by the same organism.
>
> *Hypothesis 2:* Consumers will have different $\delta^{15}N$ and $\delta^{13}C$ ratios and will yield different stable isotope discrimination when restricted to a single food source.

Stable isotope values of brown algae (Phaeophyta), red algae (Rhodophyta, both fleshy and calcareous) and green algae (Chlorophyta) and different types of herbivorous invertebrates, were determined during controlled feeding experiments to determine the levels of discrimination between the primary producers and consumers. The discrimination ratios yielded by the laboratory experiments were then applied to field data for local primary producers and consumers. The application of the assumed and experimentally determined discrimination values were evaluated through a mixing model analysis.

Chapter 2: Methods and Materials

This research project has incorporated two main components: stable isotope discrimination experiments, and food web evaluation using a mixing model. In the first phase of the study, which addressed Aims 1 and 2, discrimination experiments involved feeding three taxa of primary consumers with six different macroalgal food sources under controlled conditions over time. To address Aim 3, discrimination values determined from the first phase of the study were then applied to field data to establish the potential contribution of each food source to the production of three consumers using the stable isotope modelling program Isosource (Phillips 2001). These results were then compared to the results obtained from analyses using the same data, but applying the widely assumed discrimination of 0.4±1.4‰ for $\delta^{13}C$ and 3.4±1.1‰ for $\delta^{15}N$.

2.1 Discrimination values

2.1.1 Sample Collection

All primary producers (macroalgae) and consumers (herbivorous or omnivorous invertebrates) were collected by SCUBA on the eastern side of Quinns Rocks reef system (31°40'70"S, 115°40'55"E to 31°41'20"S, 115°40'81"E), Western Australia. Sampling was carried out at one location between May and August 2006 to limit the influence of spatial and temporal isotopic variability (Boon & Bunn 1994).

Algae were collected by hand, placed in calico bags and stored in seawater at ambient temperature until transferred to the laboratory for processing. In the laboratory, algae were sorted by species and cleaned to remove senescing components of the plant, epiphytic algae, detritus and other unwanted material. Material was then cut into smaller segments, mixed together to standardize the overall sample, and stored at -20°C in ziplock bags until needed.

Four species of primary consumers, namely the sea urchin *Heliocidaris erythrogramma*, the hermit crabs *Paguristes purpureantennatus* and *Calcinus dapsiles*, and the gastropod *Turbo torquatus*, were collected by hand and stored in several eskies for the journey to the laboratory. These invertebrates were then housed in aquaria until the start of the experiments. Each species was housed separately and held for 48 hours

to allow for acclimatization to the new environment, the reduction of shock and removal of unhealthy animals. The hermit crabs were scrubbed with a scourer or wire wool to remove the algae from the shell of individuals. These four invertebrate species were chosen as they were abundant, easy to collect by hand within the collection area during the sampling period, and were able to be kept in captivity.

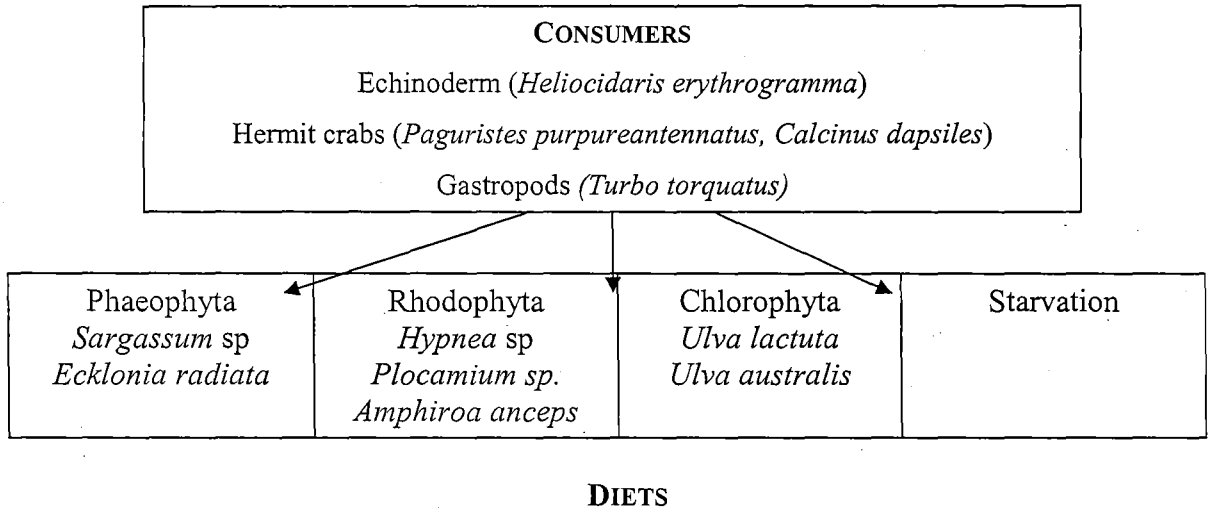

Figure 2.1 The different species of primary consumers that were fed to each species of consumer. Note that the starvation treatments did not include *Calcinus dapsiles.*

Each consumer-producer combination (Figure 2.1) required 24 individuals, which were collected during a single day. This was done to remove the influence of spatial and temporal variability on isotope signatures, and to allow all individuals of each combination to start at the same time. Due to the extensive number of individuals required and space limitations in the aquaria facility, different suites of consumer species were collected on different days to allow all of the experimental combinations to be carried out over the course of the study. The required number of individuals of each species of consumer (Table 2.1) relates to the experiments that they were participating in, with extra individuals collected and included in the experiment to allow for mortalities.

Table 2.1. Required number of individuals of each species for Hypothesis 1 and 2.

	Species	Required	Spares	Total
Echinoderm	*Heliocidaris erythrogramma*	180	54	234
Gastropod	*Turbo torquatus*	180	54	234
Hermit crab	*Paguristes purpureantennatus*	180	54	234
	Calcinus dapsiles	108	36	144
Total		648	196	846

2.1.2 Experimental Design

Seven species of macroalgae were used as food sources: two brown algae (*Ecklonia radiata* and *Sargassum* sp.), three red algae (*Hypnea* sp. and *Plocamium* sp.), of which one is coralline red algae (*Amphiroa anceps*) and two green algae (*Ulva lactuca* and *Ulva australis*). These were fed to each of the four species of consumer over a sufficient period of time (35 to 56 days) to allow carbon and nitrogen isotope ratios from the new food source to be assimilated into their muscle tissue. The 56-day period was considered sufficient for a change in the isotopic composition of the muscle tissue as the assumed half-life of carbon in mammalian tissue is 28 days (Nadon & Himmelman 2006) and invertebrates are known to have a faster isotopic turnover than mammals (Polunin et al. 2001, McIntyre & Flecker 2006). In addition to the feeding experiments, a starvation experiment was conducted for some species of consumers. Initially, all consumer-source combinations were run, but due to unexpected mortalities during the experimental period some treatments were ceased. Other treatments finished after a 35-day period, as some consumer-producer combinations had to be concluded early due to unexpected deaths of consumers.

Three individuals of each consumer species were allocated for each species of algae per time period. Sampling was carried out initially every seven days for the first 21 days, and then at day 35 and day 56. This allowed for the detection of changes in the isotope ratios of the consumers to be monitored over an extended time period. Each group of three individuals were set up as shown in Figure 2.2, with n=3 individuals per individual experimental unit (IEU).

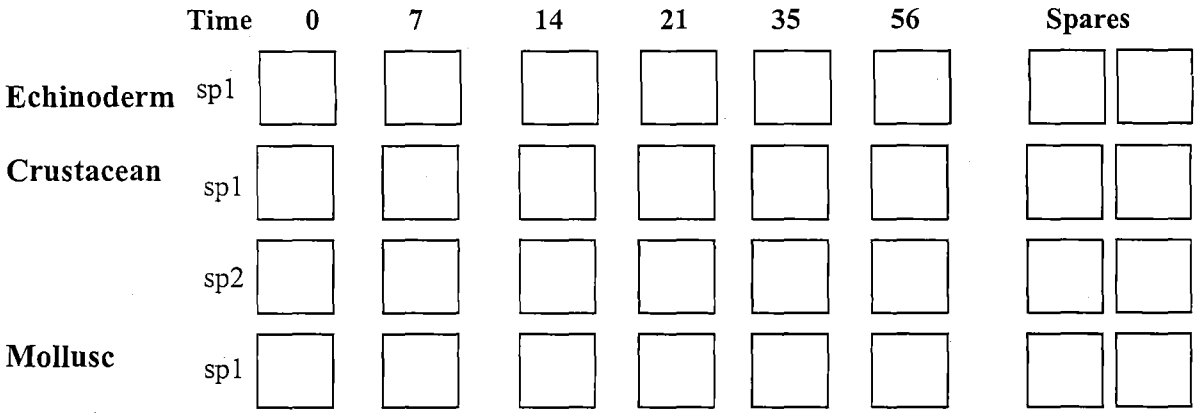

Figure 2.2 Experimental design for the combination of consumer and producer over the 56-day period.

The IEUs were plastic containers with polystyrene floats and were designed to float at water level, with mesh sides to allow for water flow, and contained three individuals in each unit (Plates 2.1 and 2.2). Each unit allowed maximum flow of water into and around the unit that provided oxygen to the animals and removed waste material (Plates 2.3 and 2.4). The aquaria setup allowed for a continuous flow of fresh saltwater into and out of the aquaria, with additional oxygenation.

Time 0 individuals were sampled after 48 hrs in the holding tanks to represent the starting point for each experiment. When sampled, all individuals from a random IEU were placed in ziplock bag and immediately transferred to a -20°C freezer to minimize stress on the animal and the subsequent effects on the muscle tissue.

2.1.3 Starvation

Starvation experiments were conducted on *H. erythrogramma, P. purpureantennatus* and *T. torquatus* using a similar design to the feeding experiments, except that the animals were not fed over the 56-day period. Starvation was not conducted on the hermit crabs *C. dapsiles* due to lack of time and individuals. The body condition was evaluated at the time of sampling for the individuals being sampled. Body condition of the *H. erythrogramma* was determined by measuring the dry weight of the internal

organs and muscle in relation to the dry weight of the exoskeleton and the Aristotle's lantern. The muscle was removed from around the Aristotle's lantern and from inside the test. The body condition of the hermit crab, *P. purpureantennatus* was determined using the dry weight of the individual in relation to the length of the carapace, whereas the gastropod *T. torquatus* was determined from the dry weight of the body in relation to the dry weight of its shell. These methods for determining body condition have been previously used on other marine invertebrates, (Steele & Mulcahy 2001)

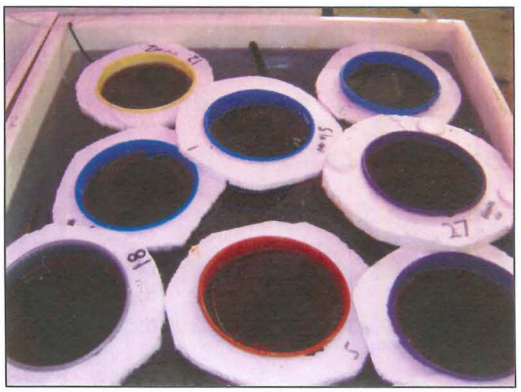

Plate 2.1 Large IEU aquaria set-up used for *H. erythrogramma*

Plate 2.2 Small IEU aquaria set-up used for *P. purpureantennatus, C. dapsiles* and *T. torquatus*

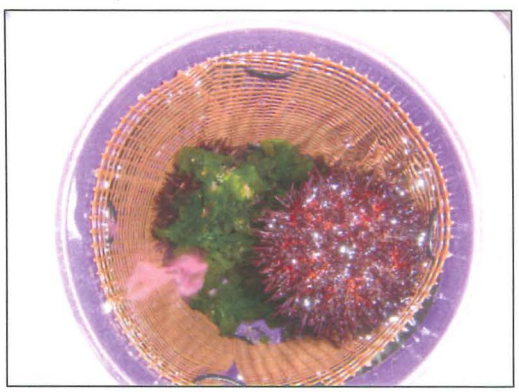

Plate 2.3 Large IEU containing *H. erythrogramma*

Plate 2.4 Small IEU containing *P. purpureantennatus*

2.1.4 Sample Processing

Invertebrate samples were defrosted, and muscle tissue was removed and washed in deionised water and then placed in individual Eppendorf tubes. The muscle tissues sampled were the Aristotle's lantern for urchin *H. erythrogramma*, foot of the gastropod for *T. torquatus,* and muscle from the legs of the hermit crab *Paguristes purpureantennatus* or entire appendages of the hermit crab *Calcinus dapsiles*. All tissue was dried at 60°C (Lajtha & Marshall 1994) for 48hrs before being ground to a fine homogeneous powder using a Retsch MM200 ballmill. The powder was stored in the Eppendorf tubes in a desiccator until being weighed into tin or silver cups.

After grinding, any samples containing inorganic carbonates (i.e. *Calcinus dapsiles* and *Amphiroa anceps*) were sub-sampled and acidified with 1.0 M hydrochloric acid (HCl) (Das et al. 2003) (Bouillon et al. 2002) until they stopped bubbling, and then re-dried. As recommended by Pinnergar and Polunin (1999), these acidified sampled were only analysed for carbon, as acidification will create significant modifications in the $^{15}N/^{14}N$ ratios resulting from HCl treatment (Bunn et al. 1995).

Samples were analysed for $\delta^{15}N$ and $\delta^{13}C$ using an ANCA-NT (Europa Scientific, Crewe, UK) interfaced with a 20/20- isotope ratio mass spectrometer (Europa Scientific, Crewe, UK). *P. purpureantennatus* and *T. torquatus* samples were weighed to 2 μg and placed in tin capsules, with dual analysis of carbon and nitrogen against 1.7 μg of fish standard. *C. dapsiles* samples were weighed to 3 μg into tin and silver capsules, with a carbon-nitrogen dual analysis against 1.5 μg fish standard. The algae and standard were weighed to 5 μg into tin capsules, with a dual analysis against bladderwrack. Plant and fish reference material, which had been previously calibrated against Vienna Peedee Belemnite (V-PDB) or Ambient Inhaler Reservoir (AIR) standard reference materials, were use to determine by comparison the $\delta^{13}C$ and $\delta^{15}N$ ratios of the samples.

2.1.5 Discrimination Values

The discrimination values were calculated for each different consumer-source combination through calculating the average of the mean values for days 21, 35 and 56, and subtracting the mean source value (Equation 2). These time intervals were chosen since little change in the isotopic ratio of the consumers over the experimental time

could be detected. Therefore, the isotopic ratio in the tissue of the consumer was stabilized relative to the diet and could be used to determine the discrimination values. While the isotope values were often similar throughout the course of the experiment, values from days 0, 7 and 14 were not used to calculate the discrimination values to ensure there was limited influence from the consumer's previous diet.

Equation 2 $\Delta = \overline{\delta \text{consumer}_{t21\text{-}56}} - \overline{\delta \text{producer}}$

where δ is ^{13}C or ^{15}N

2.1.6 Statistical Analysis

Each consumer-producer dataset of isotope ratios over time was tested for normality and homogeneity of variances, using Levene's test or the Kolmogrov-Smirnov test, respectively, to determine if the data met the assumptions of parametric statistics. If the data were normally distributed and had homogeneous variances, they were analysed using Analysis of Variance (ANOVA), followed by Tukey's post-hoc test if a significant difference was found. If the data did not meet the requirements of ANOVA, the data were analysed using the non-parametric Kruskal-Wallis test followed by a Mann-Whitney U test to determine where the significance was located within the data set. Missing data from a set of replicates were replaced with the mean from the remaining replicates (Underwood 1997).

2.2 Mixing Model

The discrimination values determined through the feeding experiments were used to determine the proportion of different producers contributing to the production of consumers based on field data collected from Quinns Rocks. The field data were used to evaluate and compare the contributions of producers to primary consumers using both the discrimination values determined from this study and the assumed discrimination values of 0.4‰ ± 1.4 S.D. for $\delta^{13}C$ and 3.4 ‰ ± 1.1 for $\delta^{15}N$ (DeNiro & Epstein 1978, 1981). The outcomes of both sets of discriminations were compared using the Isosource mixing model.

2.2.1 Mixing Model

Isosource is a program that is frequently used with $\delta^{13}C$ and $\delta^{15}N$ values to determine the relative contributions of different sources to a consumer's diet (Phillips 2001). It uses stable isotope ratios of various potential sources of a consumer's diet and the consumer's stable isotope ratio (Phillips & Gregg 2003). The program applies a discrimination level to the muscle tissue of an organism to determine the contribution of each food source in a consumer's diet.

$$\text{Isosource input} = \Delta_{consumer} + \delta_{algae}$$

Where Δ is the discrimination value applied to the data set and δ_{algae} is the isotope ratio of the algae.

As a consumer may utilise different food sources, the isotope ratio of the food sources influences the isotope ratio of the consumer. The amount a food sources influences a consumer is known as the percentage contribution of that food source to an organism's diet. Once the isotope ratios of a consumer and its food sources have been determined, a discrimination value can be used with Isosource to determine the source proportion and percentage contribution to a consumer's diet.

The program can use either the standard or experimentally determined discrimination for the calculations. The discrimination values for both the assumed levels and those determined from this study were added to the mean values of algae collected from the field. The $\delta^{13}C$ and $\delta^{15}N$ for each consumer, along with those values for the food sources, were used as input values for the Isosource mixing model.

Chapter 3: Results

In this chapter, initially the variability in $\delta^{13}C$ and $\delta^{15}N$ for natural populations of the invertebrate consumers used in the experimental studies will be examined (Section 3.1), followed by the results from experimental treatments where organisms were fed on known diets for up to 56 days to examine shifts in $\delta^{13}C$ and $\delta^{15}N$ (Section 3.2). From these, C and N discrimination values were determined (Section 3.3). These values were then applied to data collected from the field to evaluate food web relationships using the isotope mixing model program Isosource, and compared to results using the same data set but applying the assumed discrimination values applied in many studies (Section 3.4). Note that the time series data for each type of primary producer is grouped by consumer, and followed by the relevant statistical analyses.

3.1 Field Variability

The $\delta^{13}C$ and $\delta^{15}N$ values for all organisms processed at the beginning of experiments ($t = 0$) were used to provide an estimate of the natural range of field variation in these parameters. High variability in $\delta^{13}C$ was exhibited for all consumer species (Figure 3.1) with a range of 2.54‰ for the urchin *Heliocidaris erythrogramma*, 2.09‰ for the hermit *Paguristes purpureantennatus*, 3.68‰ for the hermit crab *Calcinus dapsiles*, and 1.58‰ for gastropod *Turbo torquatus*. A similar trend was seen for $\delta^{15}N$ (Figure 3.1), for which the range of values was highest for *H. erythrogramma* (1.84‰) and lowest for *C. dapsiles* (1.28‰). In terms of mean (±SE) values for $\delta^{13}C$, there was considerable overlap between *H. erythrogramma* (-16.19‰ ± 0.20) and *P. purpureantennatus* (-16.19‰ ± 0.49), and *C. dapsiles* (-16.43‰ ± 0.19), while *T. torquatus* was notably lower (-17.89‰ ±0.13). Furthermore, $\delta^{15}N$ isotope ratios were similar for *H. erythrogramma* and *C. dapsiles* (8.18‰ to 10.02‰ and 8.16‰ to 9.44‰ respectively) and intermediate to $\delta^{15}N$ isotope ratios *P. purpureantennatus* (9.23‰ to 11.03‰) and *T. torquatus* (6.72‰ to 8.48‰).

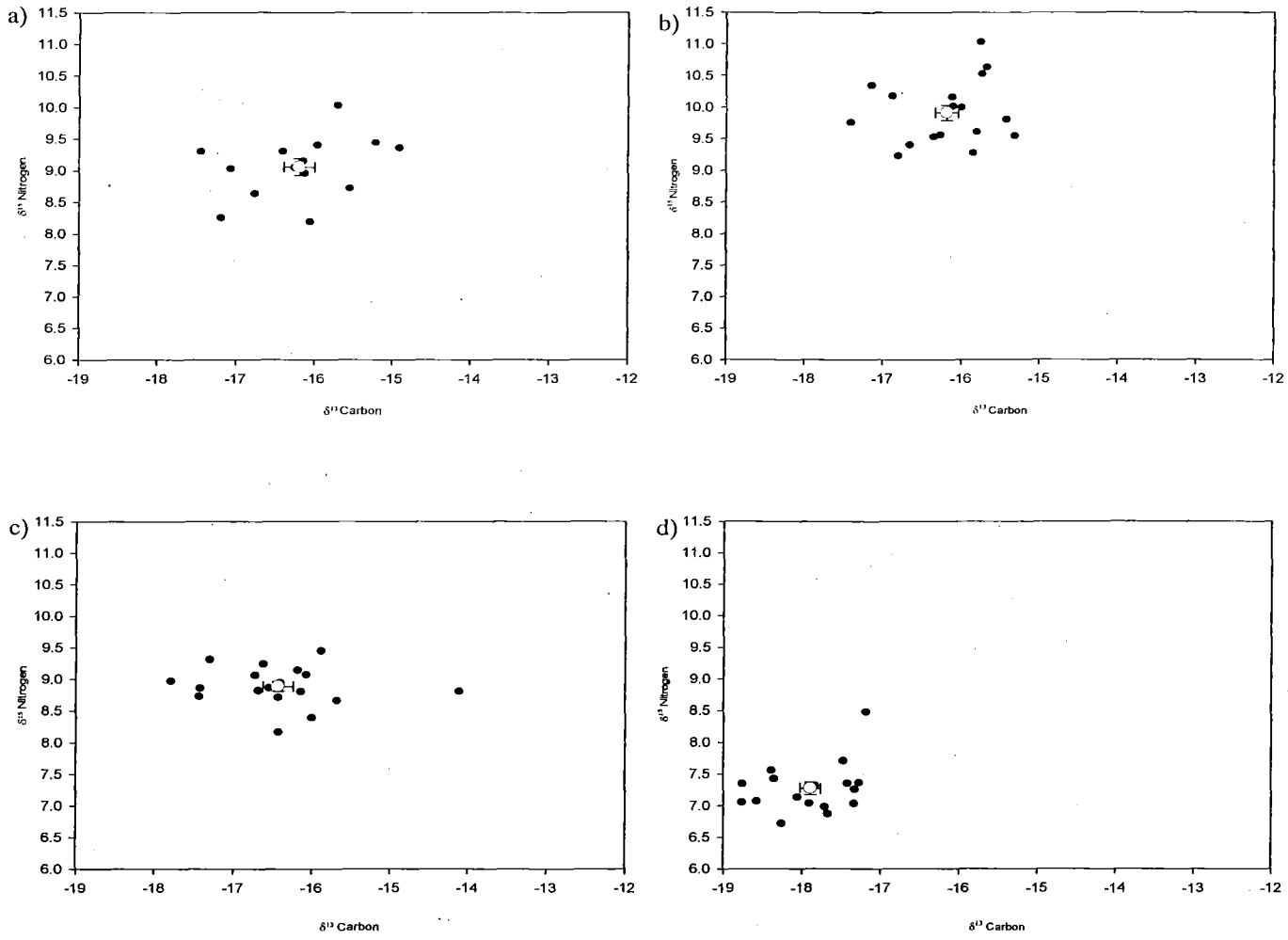

Figure 3.1 Natural field variability in 13C and 15N for a) Heliocidaris erythrogramma, b) Paguristes purpureantennatus, c) Calcinus dapsiles and d) Turbo torquatus. The open symbols indicate mean ±SE.

3.2 Feeding Treatments

3.2.1 *Heliocidaris erythrogramma*

Values of $\delta^{13}C$ and $\delta^{15}N$ showed either no change or no consistent change over time for the sea urchin *H. erythrogramma* fed on brown algae (Phaeophyta), fleshy red algae (Rhodophyta), green algae (Chlorophyta), or calcareous red algae (Figure 3.2). ANOVA or the Kruskal-Wallace test (Table 3.1) showed that only those urchins fed on *Plocamium* sp. ($p = 0.032$) and *A. anceps* ($p = 0.001$) differed over time for $\delta^{13}C$, and those fed on *Plocamium* sp. ($p = 0.003$), *Hypnea* sp. ($p = 0.021$), and *Ulva australis* ($p = 0.017$) differed over time for $\delta^{15}N$. Even where there were significant changes over time, there was no consistent shift in $\delta^{13}C$ or $\delta^{15}N$ values over the course of the experiment (Figure 3.2), with the exception of these fed on *Plocamium* sp. which decreased in $\delta^{13}C$ from t=0 to t=7 then plateaued (Figure 3.2c). In most cases, the consumer isotope value remained above the food source.

The starvation treatment showed a significant difference in the $\delta^{13}C$ ($p=0.005$), starting with a high $\delta^{13}C$ value at time = 0 but decreasing by time = 7, after which the values stabilised showing no significant difference in the $\delta^{15}N$. No trend was shown in the change in body condition (Table 3.2) for the urchin as it varied by 0.06 over 56 days (Table 3.3).

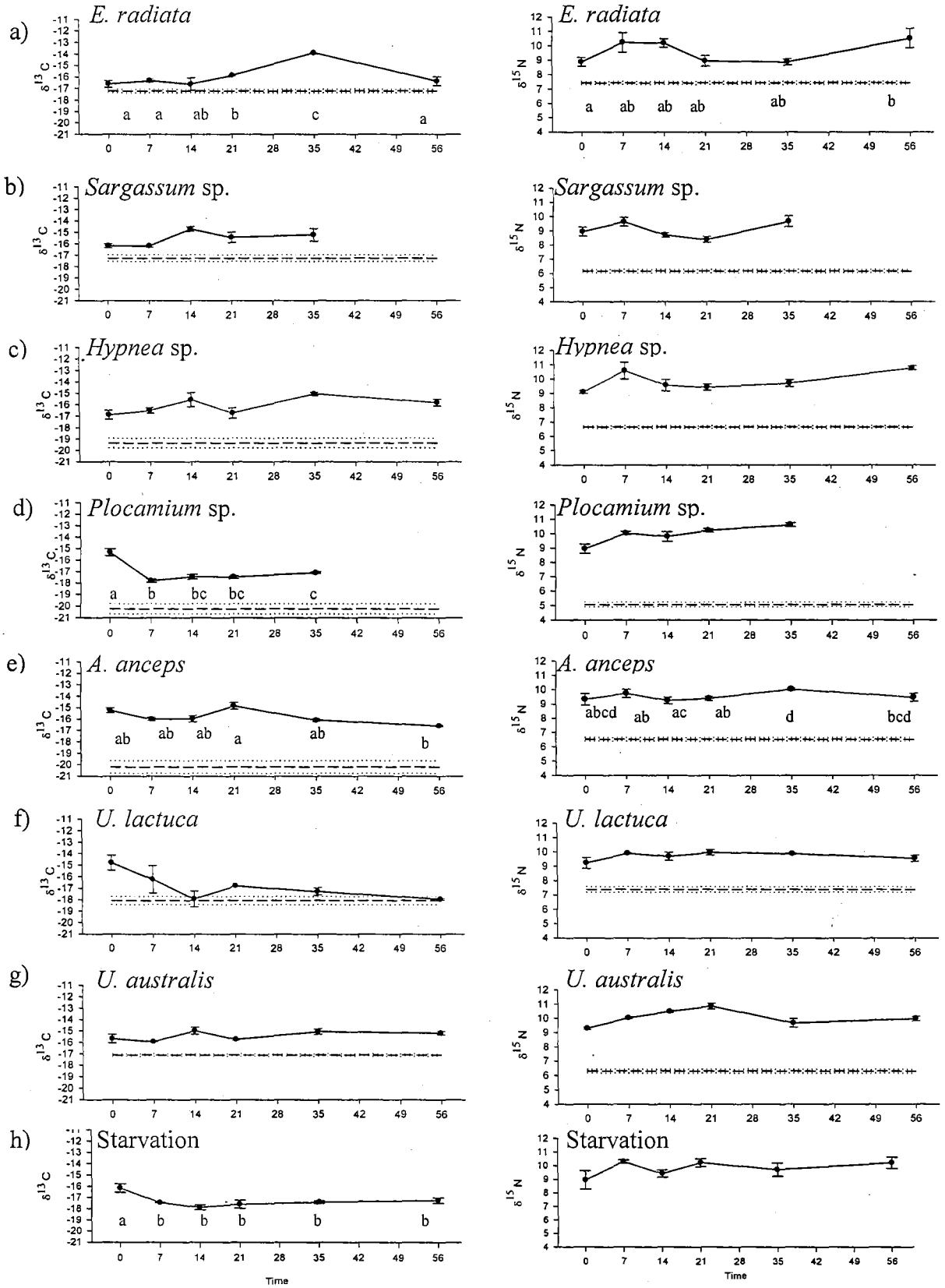

Figure 3.2 The change in carbon and nitrogen stable isotope values in muscle tissue of *Heliocidaris erythrogramma* fed on diets of a) *Ecklonia radiata*, b) *Sargassum sp.*, c) *Hypnea*, d) *Plocamium sp.*, e) *Amphiroa anceps*, f) *Ulva lactuca,* g) *Ulva australis,* and h) starvation treatment. Broken horizontal lines indicate the mean (+SE) value of source material. Values for both source and consumer are mean ± SE, n=3.

Table 3.1 Summary of One-way ANOVA and Kruskal-Wallis analysis of variance by ranks for $\delta^{13}C$ and $\delta^{15}N$ isotope values of *Heliocidaris erythrogramma* fed different diets of algae.

a) Carbon
(i) One-way ANOVA

Algae	df	Mean-Square	F	p
Sargassum sp.	4	1.242	1.535	0.265
Hypnea sp.	5	1.593	3.427	0.333
A. anceps	5	1.230	9.927	0.001
Starvation	5	1.040	6.052	0.005

(ii) Kruskal-Wallis

Algae	df	chi-square	p
E. radiata	5	10.993	0.052
U. lactuca	5	8.506	0.130
U. australis	5	9.842	0.080
Plocamium sp.	4	10.567	0.032

b) Nitrogen
(i) One-way ANOVA

Algae	df	Mean-Square	F	p
E. radiata	5	1.857	2.915	0.060
Sargassum sp.	4	0.976	1.588	0.252
Hypnea sp.	5	1.351	4.109	0.021
A. anceps	5	0.259	1.637	0.224
U. lactuca	5	0.186	1.341	0.132
Plocamium sp.	4	1.178	8.446	0.003
Starvation	5	0.890	2.016	0.148

(ii) Kruskal-Wallis

Algae	df	chi-square	p
U. australis	4	12.113	0.017

Table 3.2 Summary of One-way ANOVA and Kruskal-Wallis analysis of variance by ranks for body condition of *Heliocidaris erythrogramma.*

	df	Mean-Square	F	p
H. erythrogramma	2	0	0.261	0.775

Table 3.3 Mean ratio of change in body condition for the urchin *Heliocidaris erythrogramma* subjected to starvation treatment over different time periods, n=3 for each time period.

Time (days)	Exoskeleton (g)	Soft tissue (g)	Ratio
0	21.90	1.37	0.11
7	24.00	1.49	0.12
14	20.50	1.12	0.11
21	23.20	1.43	0.11
35	45.33	3.45	0.16
56	34.93	3.50	0.10

3.2.2 *Paguristes purpureantennatus*

Similar to *H. erythrogramma*, $\delta^{13}C$ and $\delta^{15}N$ of the hermit crab *P. purpureantennatus* did not change over time when fed on most species of primary producers (Table 3.4, Figure 3.3). There was, however, a significant difference in $\delta^{13}C$ for crabs fed on *Sargassum* sp. ($p = 0.011$) and *U. lactuca* ($p = 0.026$) and $\delta^{15}N$ for those fed on *Hypnea* sp. ($p = 0.028$) and *U. australis* ($p = 0.031$). Despite these significant differences there were no consistent directional trends in the change in $\delta^{13}C$ and $\delta^{15}N$ over time. While ANOVA indicated that *P. purpureantennatus* fed on *U. australis* showed a change in $\delta^{15}N$ over the experimental period, post-hoc tests did not reveal any significant pairwise comparisons (Figure 3.3g). In general, the $\delta^{13}C$ of the consumer muscle tissue was less negative than its food source (Figure 3.3). This trend did not apply for those on a *U. lactuca* diet, where the $\delta^{13}C$ value at day 56 was similar to the mean producer value (Figure 3.3e). The starvation experiments indicated no significant differences in either the $\delta^{13}C$ or $\delta^{15}N$ values (Figure 3.3 h). No consistent trend was shown in the body condition of these crabs over 56 days of starvation (Table 3.5 and 3.6).

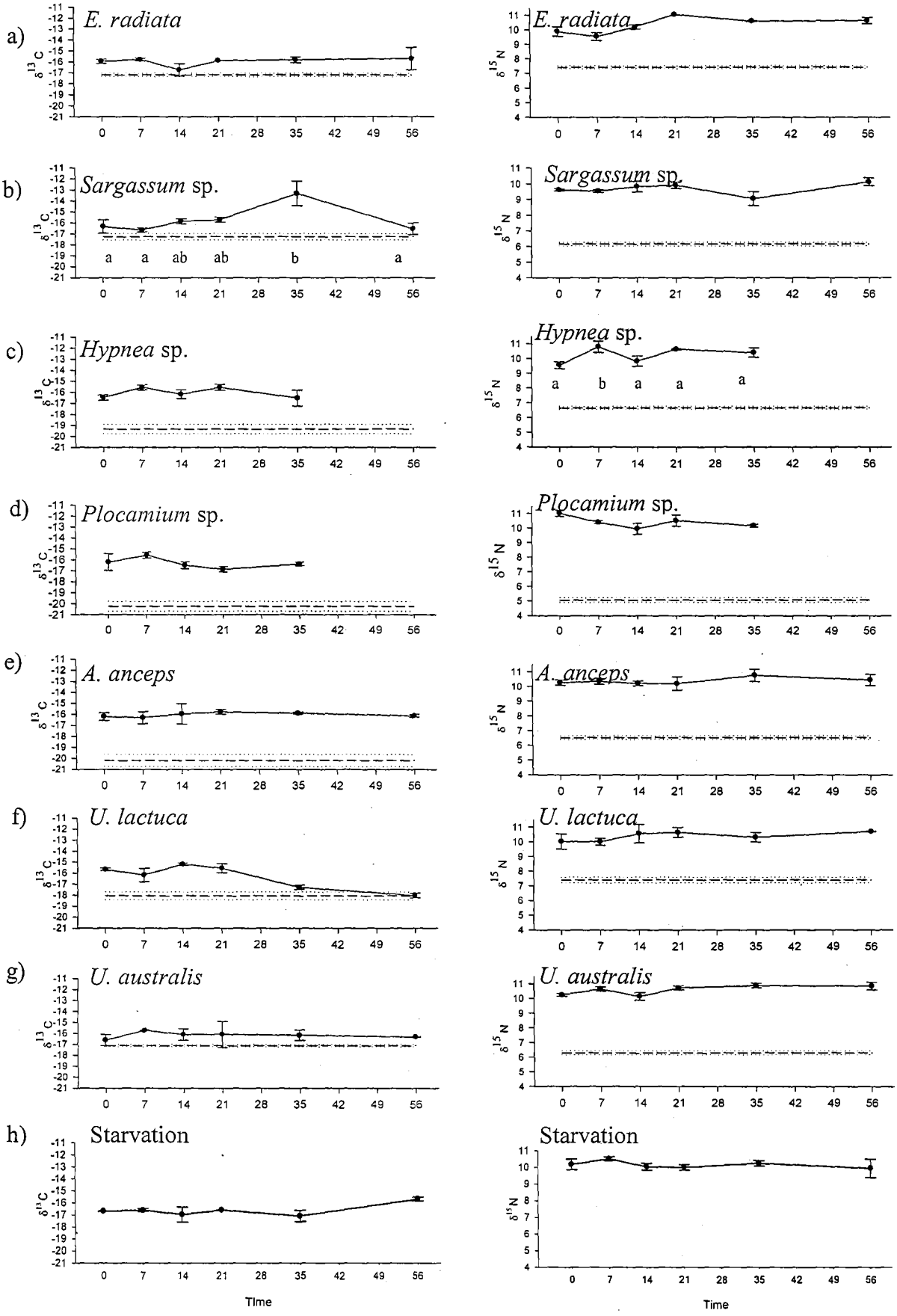

Figure 3.3 The change in carbon and nitrogen stable isotope values in muscle tissue of *Paguristes purpureantennatus* fed on diets of a) *Ecklonia radiata*, b) *Sargassum sp.*, c) *Hypnea*, d) *Plocamium sp.*, e) *Amphiroa anceps*, f) *Ulva lactuca,* g) *Ulva australis,* and h) starvation treatment. Broken horizontal lines indicate the mean (+SE) value of source material. Values for both source and consumer are mean ± SE, n=3

Table 3.4 Summary of One-way ANOVA and Kruskal-Wallis analysis of variance by ranks for $\delta^{13}C$ and $\delta^{15}N$ isotope values of *Paguristes purpureantennatus* fed different diets of algae, n=3.

a) Carbon
(i) One-way ANOVA

Algae	df	Mean-Square	F	p
E. radiata	4	0.555	0.629	0.653
Sargassum sp.	5	4.598	4.991	0.011
Hypnea sp.	4	0.681	1.369	0.304
Plocamium	4	0.685	1.317	0.329

(ii) Kruskal-Wallis

Algae	df	chi-square	p
U. lactuca	5	12.696	0.026
U. australis	5	3.469	0.628
A. anceps	5	2.529	0.772
Starvation	5	7.938	0.160

b) Nitrogen
(i) One-way ANOVA

Algae	df	Mean-Square	F	p
E. radiata	5	0.613	3.020	0.071
Sargassum sp.	5	0.415	2.155	0.128
Hypnea sp.	4	0.861	4.305	0.028
Plocamium sp.	4	0.490	2.256	0.135
A. anceps	5	0.128	0.611	0.694
U. lactuca	5	0.292	0.863	0.533
U. australis	5	0.297	3.641	0.031
Starvation	5	0.131	0.506	0.767

(ii) Kruskal-Wallis

Algae	df	chi-square	p
E. radiata	4	8.373	0.079

Table 3.5 Summary of One-way ANOVA and Kruskal-Wallis analysis of variance by ranks for body condition of *Paguristes purpureantennatus* fed different diets of algae.

	Mean	df	chi-square	p
P. purpureantennatus	1.42	2	0.654	0.721

Table 3.6 Ratio of change in body condition for *Paguristes purpureantennatus* subjected to starvation treatment over different time periods. Mean values are shown, n=3

Time (days)	Carapace (g)	Body (g)	Ratio
0	1.00	1.30	1.30
7	0.55	1.83	1.83
14	1.10	1.63	1.63
21	0.70	0.60	0.60
35	1.13	1.63	1.63
56	1.43	1.50	1.50

3.2.3 *Calcinus dapsiles*

No significant differences were observed in either $\delta^{13}C$ or $\delta^{15}N$ for the hermit crab *C. dapsiles* feeding on any type of algae over the course of the experiments (Table 3.7). Both $\delta^{13}C$ and $\delta^{15}N$ values of this consumer generally remained above the values of food sources (Figure 3.4).

The *Plocamium* sp. and starvation treatment were not applied to the hermit crab *C. dapsiles* due to limited room within the aquaria facilities and limited time. The hermit *P. purpureantennatus* had both the *Plocamium* sp. and starvation treatment applied as the representative hermit crab invertebrate. *P. purpureantennatus* was chosen as the representative hermit crab due the abundance of this species collected prior to the commencement of the treatments.

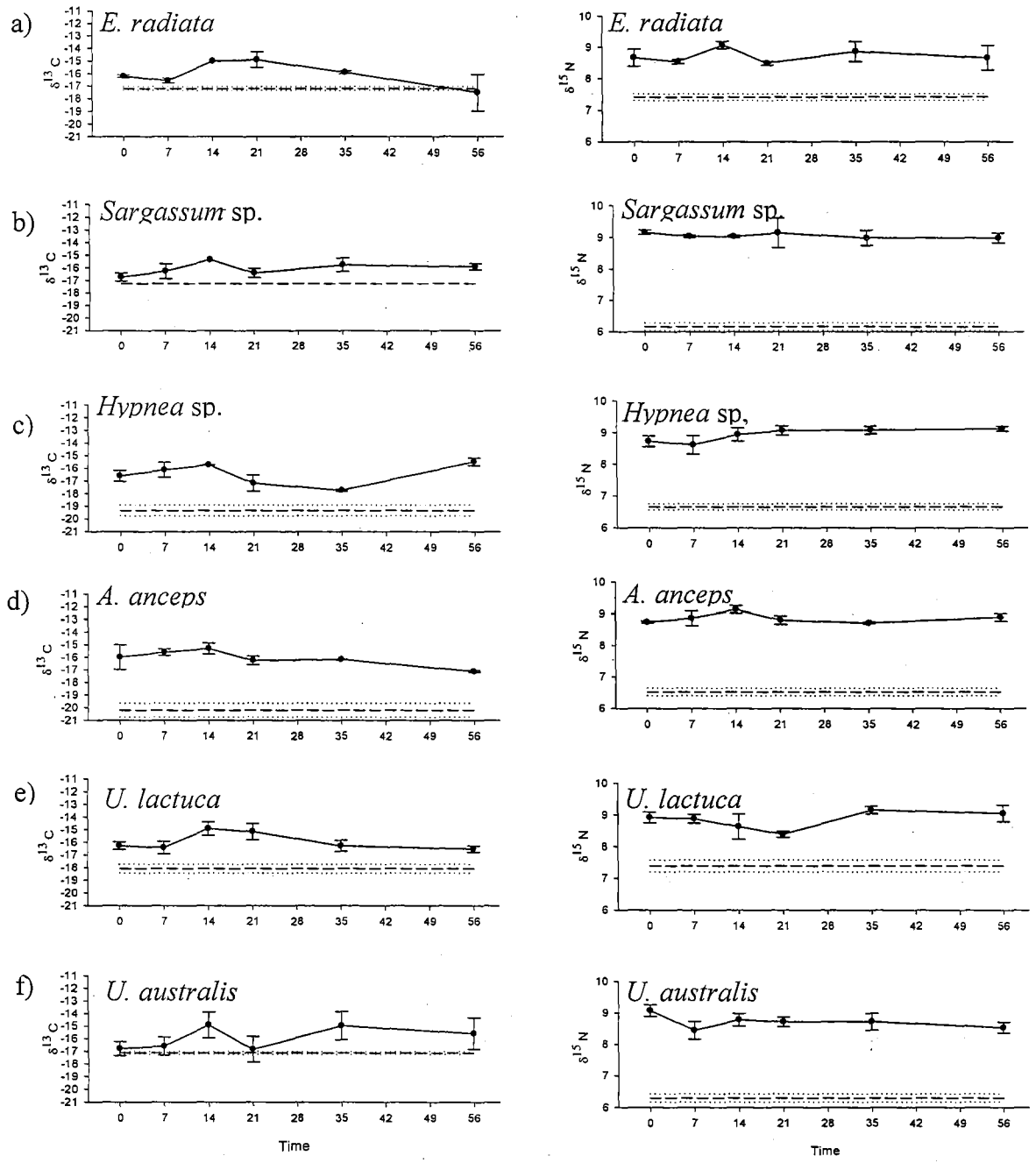

Figure 3.4 The change in carbon and nitrogen stable isotope values in muscle tissue of *Calcinus dapsiles* fed on diets of a) *Ecklonia radiata*, b) *Sargassum sp.*, c) *Hypnea*, d) *Amphora anceps*, e) *Ulva lactuca*, and f) *Ulva australis*. Broken horizontal lines indicate the mean (+SE) value of source material. Values for both source and consumer are mean ± SE, n=3.

Table 3.7 Summary of One-way ANOVA and Kruskal-Wallis analysis of variance by ranks for $\delta^{13}C$ and $\delta^{15}N$ isotope values of *Calcinus dapsiles* fed different diets of algae, n=3.

a) Carbon
(i) One-way ANOVA

Algae	df	Mean-Square	F	p
Sargassum sp.	5	0.752	1.823	0.183
U. lactuca	5	1.467	2.479	0.092
U. australis	5	2.484	1.756	0.197

(ii) Kruskal-Wallis

Algae	df	chi-square	p
E. radiata	4	8.357	0.079
Hypnea sp.	5	11.012	0.051
A. anceps	5	9.795	0.081

b) Nitrogen
(i) One-way ANOVA

Algae	df	Mean-Square	F	p
Sargassum sp.	5	0.018	0.113	0.987
U. lactuca	5	0.241	1.597	0.234
U. australis	5	0.150	1.176	0.376

(ii) Kruskal-Wallis

Algae	df	chi-square	p
E. radiata	5	4.930	0.425
Hypnea sp.	5	6.454	0.264
A. anceps	5	5.708	0.336

3.2.4 *Turbo torquatus*

For the gastropod *T. torquatus*, none of the experimental treatments resulted in a significant change in the $\delta^{13}C$ or $\delta^{15}N$ values of the muscle tissue over the experimental period (Fig. 3.5, Table 3.8). Regardless of the food source, mean $\delta^{13}C$ values were variable, but similar to those of the macroalgae. $\delta^{15}N$ values remained elevated above the food source for all types of macroalgae (Figure 3.5). Similar to the feeding experiments, $\delta^{13}C$ and $\delta^{15}N$ values of gastropods starved over the 56 day period did not change over the experiment. No clear trend was exhibited over the experimental time in the body condition of *T. torquatus* (Table 3.9), with the body condition ratio ranging between 0.05 and 0.10 (Table 3.10). Results were not collected from the other feeding treatments these organism had sustained too many deaths due to complications with the aquaria facility, and unfortunately it was too late in the honours time period to restart the treatments.

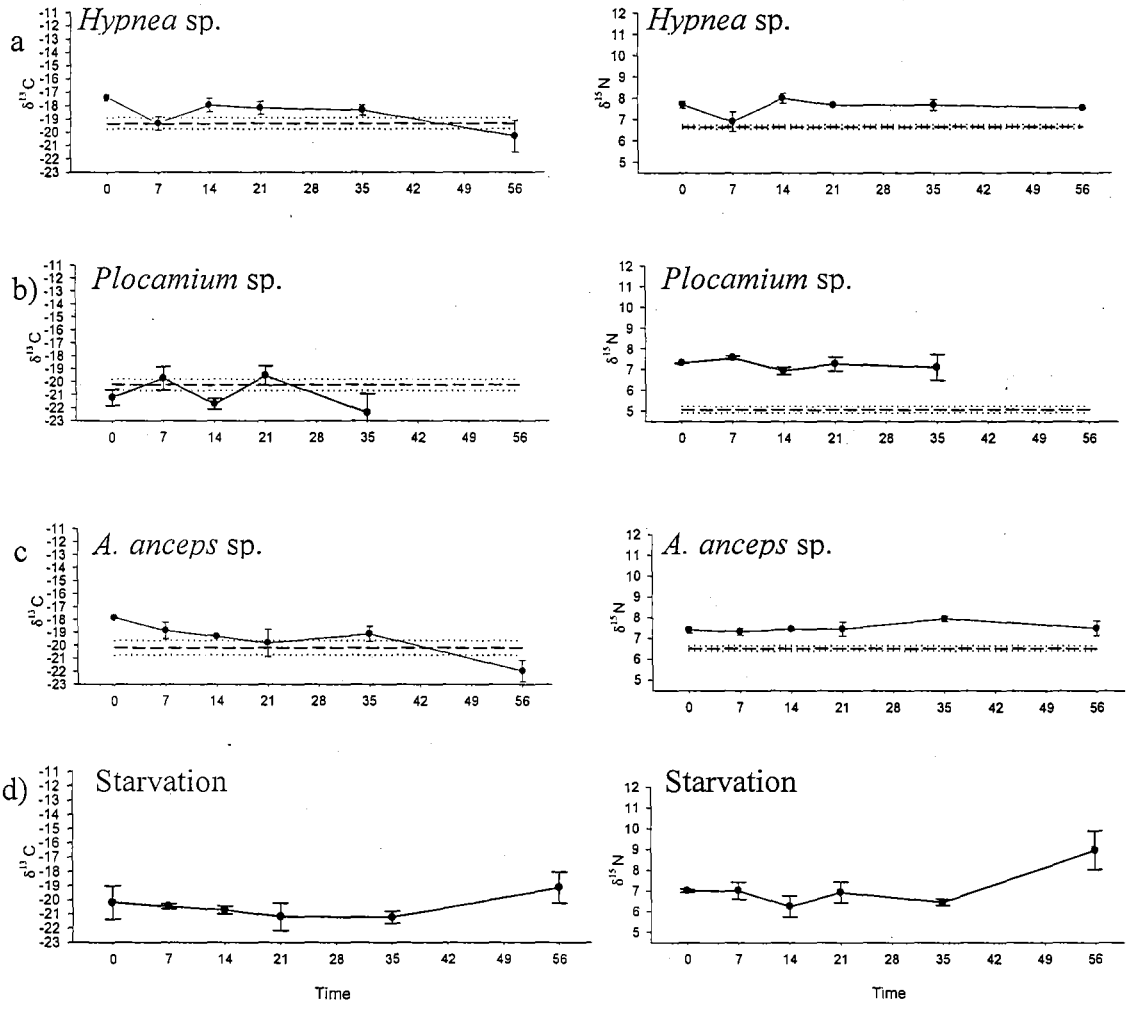

Figure 3.5. The change in carbon and nitrogen stable isotope values in muscle tissue of *Turbo torquatus* fed on diets of a) *Hypnea*, b) *Plocamium sp.*, c) *Amphiroa anceps*, d) starvation treatment. Broken horizontal lines indicate the mean (+SE) value of source material. Values for both source and consumer are mean ± SE, n=3.

Table 3.8 Summary of One-way ANOVA and Kruskal-Wallis analysis of variance by ranks for $\delta^{13}C$ and $\delta^{15}N$ isotope values of *Turbo torquatus* fed different diets of algae.

a) Carbon
(ii) Kruskal-Wallis

Algae	df	chi-square	p
Hypnea sp	4	8.813	0.117
A. anceps	5	10.240	0.069
Plocamium sp.	4	6.967	0.138
Starvation	5	5.444	0.364

b) Nitrogen
(i) One-way ANOVA

Algae	df	Mean-Square	F	p
Hypnea sp.	5	3.303	2.772	0.069
A. anceps	5	5.679	4.414	0.474
Plocamium sp.	4	4.693	1.926	0.183
Starvation	5	1.836	1.187	0.371

Table 3.9 Summary of One-way ANOVA and Kruskal-Wallis analysis of variance by ranks for body condition of *Turbo torquatus* fed different diets of algae.

	df	Mean-Square	F	p
T. torquatus	2	0	0.065	0.938

Table 3.10 Ratio of change in body condition for *Turbo torquatus* subjected to starvation treatment over different time periods. Mean and standard error are shown, n=3 for each time period.

Time (days)	Shell (g)	Body (g)	Ratio
0	89.90	3.81	0.08
7	42.23	1.44	0.07
14	71.70	3.82	0.10
21	73.40	3.06	0.08
35	87.15	2.05	0.05
56	41.433	1.40	0.06

3.3 Discrimination Values

Consumers exhibited a large difference in $\delta^{13}C$ discrimination values when fed on different groups of algae, with those fed on red algae showing higher discrimination values compared to green and brown algae regardless of consumer (Table 3.11). Fleshy (*Hypnea* sp. and *Plocamium* sp.) and calcareous species held a range of discrimination values from -0.94‰ to 4.71‰, whereas for brown algae the range was 1.08‰, and green algae 2.00‰. The two *Ulva* species had different discrimination values across all consumers. The $\delta^{15}N$ discrimination values between the groups of algae were similar between groups. The range for the discrimination values between groups was 2.10‰ for the brown algae, 4.20‰ for the red algae and 2.60‰ for the green algae, where the red algae held the highest discrimination value.

There was a range of $\delta^{13}C$ discrimination values between the algae species for all consumers. For *H. erythrogramma* the range was 2.3‰, *P. purpureantennatus* 3.3‰ and *C. dapsiles* it was 3.8‰. There was a general trend for *C. dapsiles* to have lower $\delta^{13}C$ discrimination values; whereas *H. erythrogramma* and *P. purpureantennatus* had similar $\delta^{13}C$ discrimination values across all algae species. For $\delta^{15}N$, the range in discrimination values between algae species for different consumers was slightly lower than $\delta^{13}C$ with ranges of 2.5‰ for *H. erythrogramma*, 2.5‰ for *P. purpureantennatus* and 1.5‰ for *C. dapsiles*. The general trend for $\delta^{15}N$ discrimination values between consumers was for *C. dapsiles* to have lower discrimination values and *H. erythrogramma* and *P. purpureantennatus* to have similar discrimination values across algae species. *T. torquatus* had low discrimination values for both $\delta^{13}C$ and $\delta^{15}N$, but results only include red algae.

Table 3.11 Discrimination values for different consumer-producer combinations for (i) $\delta^{13}C$ and (ii) $\delta^{15}N$ determined from the feeding experiments.

(i) $\delta^{13}C$

	H. erythrogramma	*P. purpureantennatus*	*C. dapsiles*	*T. torquatus*
E. radiata	1.52	1.11	1.10	-
Sargassum sp.	2.18	2.03	1.42	-
Hypnea sp.	3.56	3.25	2.67	0.64
Plocamium sp.	2.90	3.74	-	-0.98
A. anceps	4.39	4.26	4.71	0.14
U. lactuca	0.54	1.98	2.43	-
U. australis	1.85	0.95	0.90	-

(ii) $\delta^{15}N$

	H. erythrogramma	*P. purpureantennatus*	*C. dapsiles*	*T. torquatus*
E. radiata	2.20	2.95	1.36	-
Sargassum sp.	2.67	3.54	2.88	-
Hypnea sp.	3.22	3.63	2.39	1.08
Plocamium sp.	4.70	5.39	-	2.04
A. anceps	3.02	3.84	2.18	1.06
U. lactuca	2.37	3.18	1.41	-
U. australis	3.93	4.06	2.61	-

3.4 Mixing Model Analysis

When the typically assumed discrimination values of 0.4‰ for $\delta^{13}C$ and 3.4‰ for $\delta^{15}N$ were applied to the food sources collected from the field, no mathematically logical solutions could be found by Isosource. The $\delta^{13}C$ values ranged from -17.07‰ to -19.80‰, while the $\delta^{15}N$ values ranged from 6.50‰ to 10.25‰ (Figure 3.6a-c). The mixing model analysis requires the $\delta^{13}C$ and $\delta^{15}N$ values for the consumer to be located within the polygon created by the sources (Phillips and Gregg 2003), but the $\delta^{13}C$ values for all consumers (*H. erythrogramma*, *P. purpureantennatus* and *C. dapsiles*) were higher than any of the food sources, resulting in the $\delta^{13}C$ and $\delta^{15}N$ values being located outside the polygon created by the food sources (Figure 3.6a-c). In contrast, when the discrimination values determined from the current study (Table 3.12) were applied to the values for macroalgae, the $\delta^{13}C$ and $\delta^{15}N$ values of each consumer were located within the polygon created by the food sources (Figure 3.6 d-f). The food sources created a tighter cluster when the discrimination values from this study were applied, and the consumers generally lay in the middle of the polygon (Figure 3.6 d-f).

Results from the mixing model indicated that no single food source made large contributions to the production of *H. erythrogramma* (Figure 3.7). In fact, there was a high probability of low proportions of the variety of food sources contributing to the carbon and nitrogen content of this consumer, with a higher probability that *U. lactuca* and *Plocamium* sp. contribute slightly larger proportions than the other food sources (Figure 3.7). This also applies for *P. purpureantennatus*, which assimilates a low proportion of carbon and nitrogen from a variety of food sources, with a larger contribution from *Plocamium* sp., (Figure 3.8). In comparison, there was a high probability that *A. anceps* and to a lesser extent *Hypnea* sp. contributed at least 20% to the production of *C. dapsiles*, while the other algae contributed lower proportions to the production of this consumer (Figure 3.9).

Table 3.12 Mean discrimination values for different algal groups combined with each consumer for (i) $\delta^{13}C$ and (ii) $\delta^{15}N$ determined from the feeding experiments.

(i) $\delta^{13}C$

Phylum		*H. erythrogramma*	*P. purpureantennatus*	*C. dapsiles*	*T. torquatus*
Phaeophytes	n=2	1.85	1.57	1.26	N/A
Rhodophytes - fleshy	n=2	3.23	3.50	2.67	-0.17
Rhodophytes - calcareous	n=1	4.39	4.26	4.71	0.14
Chlorophytes	n=2	1.20	1.50	1.67	N/A

(ii) $\delta^{15}N$

Phylum		*H. erythrogramma*	*P. purpureantennatus*	*C. dapsiles*	*T. torquatus*
Phaeophytes	n=2	2.44	3.25	2.12	N/A
Rhodophytes - fleshy	n=2	3.96	4.51	2.30	1.56
Rhodophytes - calcareous	n=1	3.02	3.84	2.18	1.06
Chlorophytes	n=2	3.15	3.62	2.01	N/A

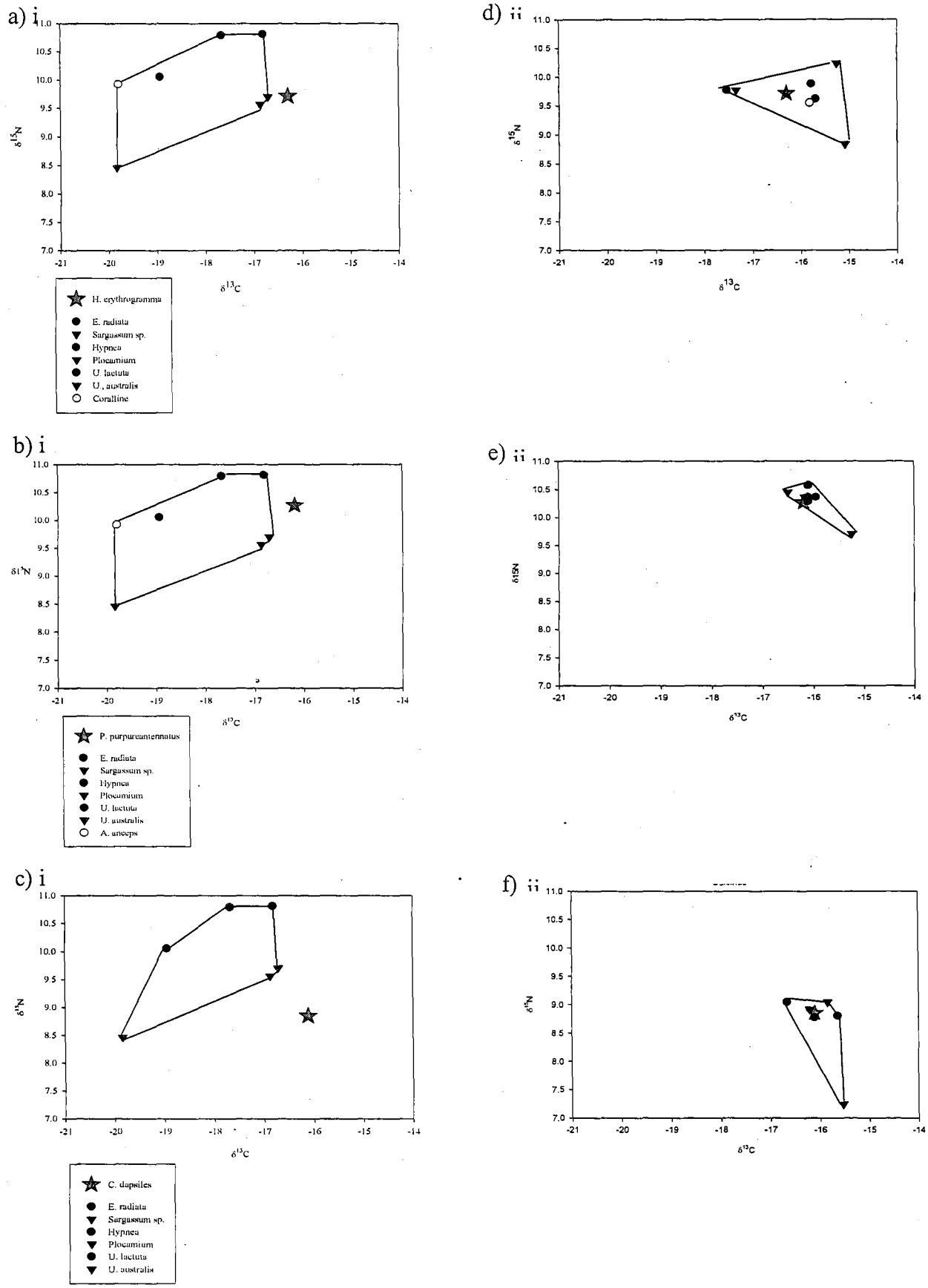

Figure 3.6 Polygon plots of $\delta^{13}C$ and $\delta^{15}N$ isotope ratios of different food sources and consumers, with a-c showing the assumed discrimination values and d-f showing the experimentally determined, calculated, discrimination values.

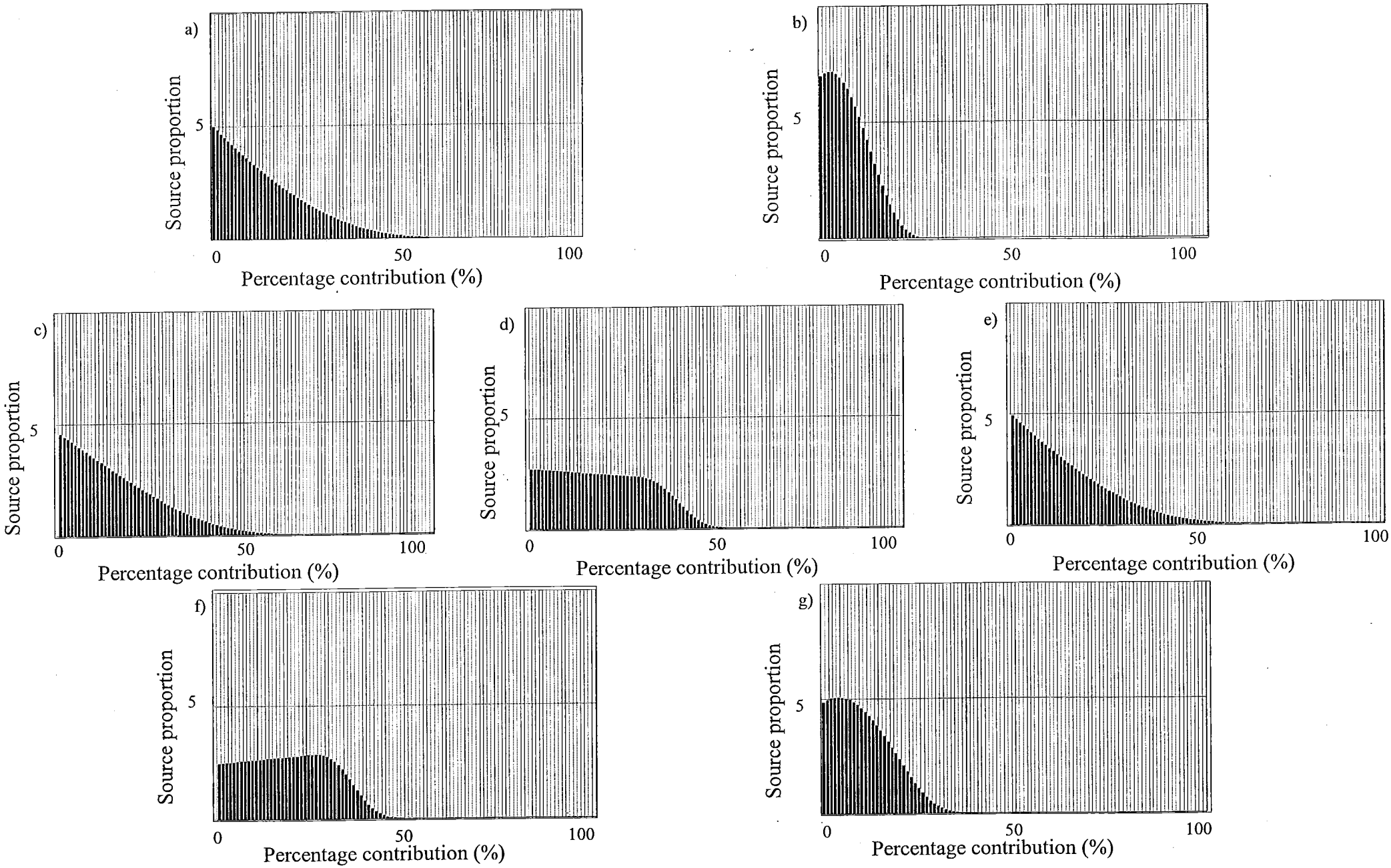

Figure 3.8 Isosource histograms for *H. erythrogramma* field data based on experimentally determined discrimination values for food sources a) *E. radiata* , b) *Sargassum* sp., c) *Hypnea* sp., d) *Plocamium* sp., e) *A. anceps*, f) *U. lactuca* and g) *U. australis*. With probability of contribution to diet on Y-axis and percentage contribution to diet on X-axis.

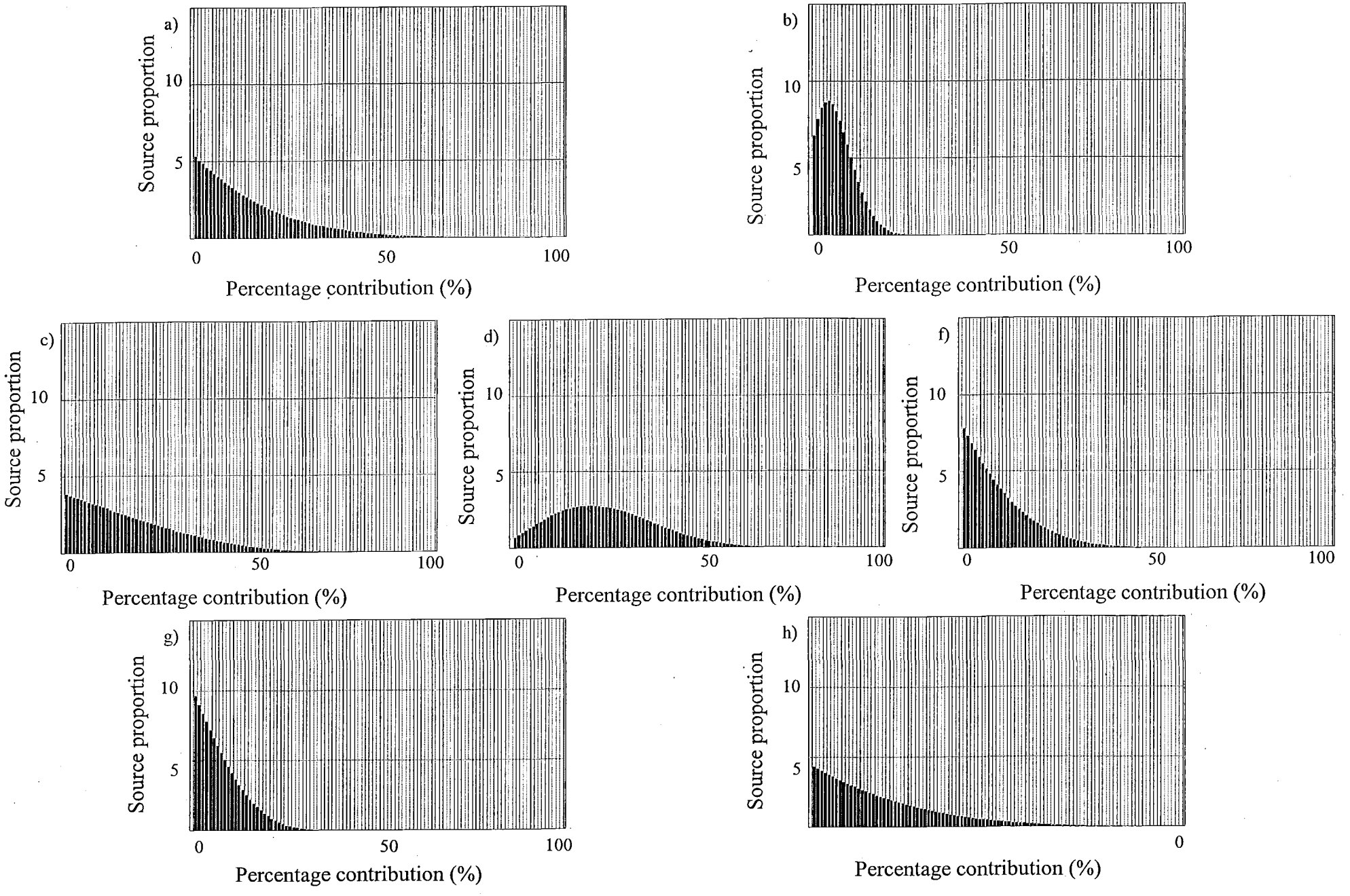

Figure 3.8 Isosource histograms for *P. purpureantennatus* field data based on experimentally determined discrimination values for food sources a) *E. radiata* , b) *Sargassum* sp., c) *Hypnea* sp., d) *Plocamium* sp., e) *A. anceps*, f) *U. lactuca* and g) *U. australis*. With probability of contribution to diet on Y-axis and percentage contribution to diet on X-axis.

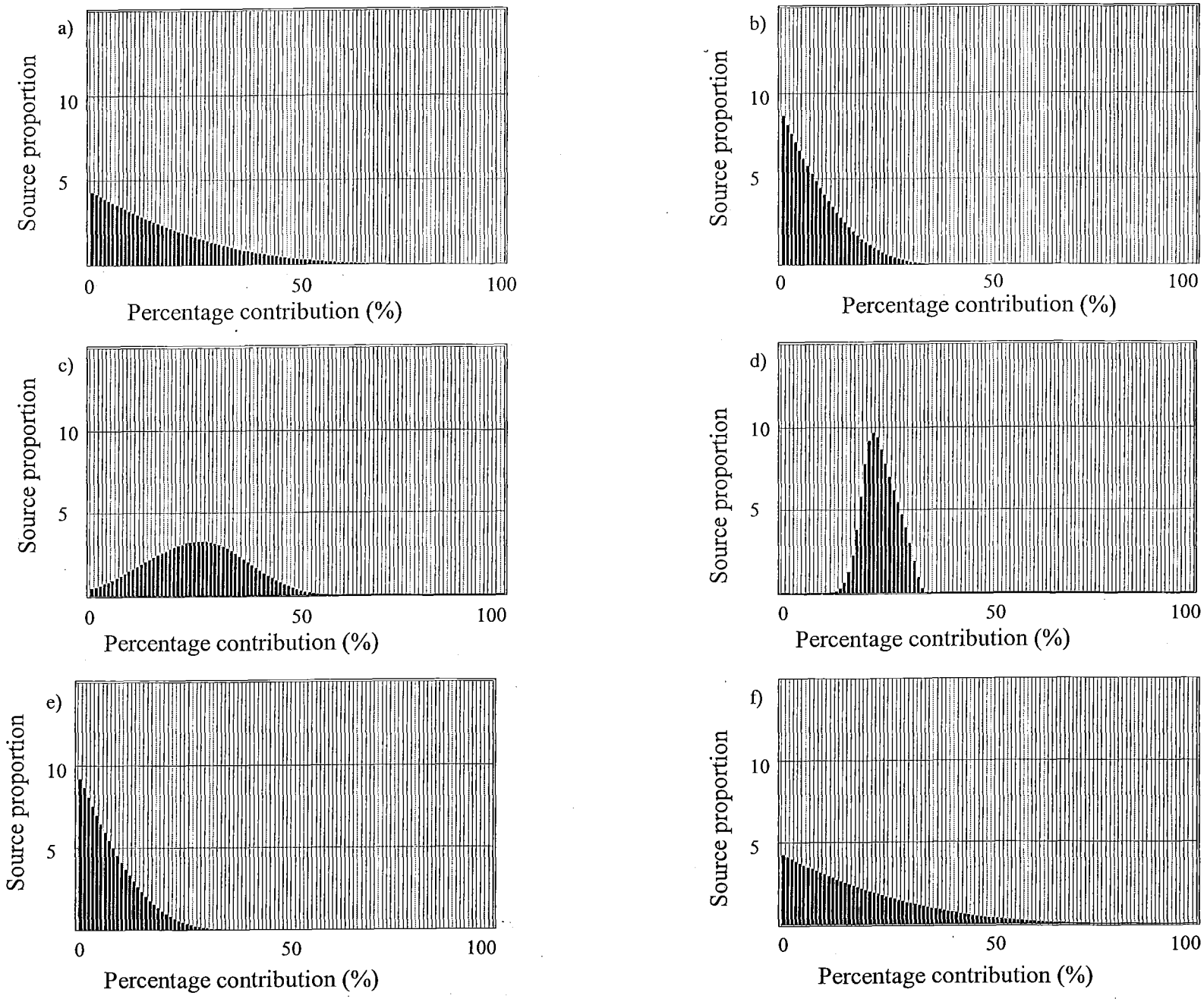

Figure 3.8 Isosource histograms for *C. dapsiles* field data based on experimentally determined discrimination values for food sources a) *E. radiata* , b) *Sargassum* sp., c) *Hypnea* sp., d) *Plocamium* sp., e) *U. lactuca* and f) *U. australis*. With probability of contribution to diet on Y-axis and percentage contribution to diet on X-axis.

Chapter 4: Discussion

When utilising carbon (C) and nitrogen (N) stable isotopes in studies of food web dynamics, the application of the correct discrimination ratio between consumer and food source is critical (Schmidt et al. 1999, Vander Zanden & Rasmussen 2001, Davis 2004). This study has quantified $\delta^{13}C$ and $\delta^{15}N$ discrimination values for a range of marine invertebrate consumers (urchin, hermit crab, and gastropod) and macroalgal food sources (brown, red and green algae). There were differences in discrimination values both between algal groups and consumer types, with the overall range for the different feeding treatments from -0.17 to 4.71‰ for $\delta^{13}C$ and 1.06 to 4.51‰ for $\delta^{15}N$. These values had a notably higher range for $\delta^{13}C$ and lower range for $\delta^{15}N$ than the 'standard' discrimination value generally applied (0.4‰ ± 1.1SD and 3.4‰ ± 1.4SD, respectively; (DeNiro & Epstein 1978, 1981). Successful application of these study-specific discrimination values to food web analysis using a mixing model (Phillips 2001, Lubetkin & Simenstad 2004) highlighted the importance of accurately quantifying these ratios for specific types of algal sources and consumers in marine trophodynamic studies.

4.1 Experimental determination of discrimination values

As in a limited number of other studies (Minagawa & Wada 1984, Jacob et al. 2005, MacNeil et al. 2006), the current study used feeding treatments to determine a change in discrimination ratios between a consumer and a source Previous work has shown that a consumer's isotope ratio can change, either increase or decrease, and subsequently stabilise from its original value over the course of the feeding experiment (Olive et al. 2003). However, this trend was not reflected in the current study, as there was either no change or no consistent change in $\delta^{13}C$ and $\delta^{15}N$ for any of the four consumers fed on specific types of algae over the duration of the feeding experiment. A possible reason for this was that the food sources selected had similar stable isotope values to those of the natural diet of the invertebrates (Vanderklift et al. 2006).

Starvation experiments were conducted to establish whether $\delta^{13}C$ and $\delta^{15}N$ values of invertebrates changed over the same duration of the feeding treatments, and establish the validity of results from those experiments. Within this study, starvation treatments

for the urchin *Heliocidaris erythrogramma*, the hermit crab *Paguristes purpureantennatus* and the gastropod *Turbo torquatus* showed no change in either isotope ratio over the treatment period, with the exception of $\delta^{13}C$ for *H. erythrogramma*. This is an unusual response as, overviews of other studies (Fantle et al. 1999, Hobson & Wassenaar 1999, Adams & Sterner 2000, Oelbermann & Scheu 2002, McCutchan et al. 2003, Olive et al. 2003, Vanderklift & Ponsard 2003, Schmidt et al. 2004) concluded that starvation can affect nitrogen and carbon enrichment. Despite the lack of change in the stable isotope values for "starved" animals in the current study, there was no indication that the invertebrates were detrimentally affected by starvation based on body condition factors. Observations suggested that invertebrates were less mobile in the starvation treatments compared to the feeding treatments, which presumably indicates a reduced metabolism in the absence of food. Since invertebrates within the feeding treatments were observed to be active and continually consuming their food source over the experimental period, the $\delta^{13}C$ and $\delta^{15}N$ values relative to those of the food sources are likely to reflect the discrimination levels exhibited by consumers fed on specific food sources. Therefore, $\delta^{13}C$ and $\delta^{15}N$ values for the invertebrates remained relatively stable when fed on selected brown, red or green algae in the experiments.

4.2 Discrimination values

Within this study, each consumer type exhibited different discrimination values when fed on either brown, red or green algae. In general, discrimination levels were higher for red algae for both $\delta^{13}C$ and $\delta^{15}N$. The average discrimination values were 2.31‰ for $\delta^{13}C$ and 3.08‰ $\delta^{15}N$ for fleshy red algae and 3.38‰ $\delta^{13}C$ and 2.53‰ $\delta^{15}N$ for calcareous red algae. Brown and green algae had similar values (1.46‰ and 1.56‰ for $\delta^{13}C$ and 2.93‰ and 2.60‰ $\delta^{15}N$, respectively). The discrimination values for $\delta^{13}C$ as determined through this study were much higher than the average assumed discrimination value of 0.4‰ that many studies apply to $\delta^{13}C$ for food web studies using stable isotopes (Beaudoin et al. 2001, Polunin et al. 2001). In comparison, the discrimination values for $\delta^{15}N$ from this study were similar to the average assumed discrimination value of 3.4‰ that is applied in many studies (Vander Zanden et al. 1996, Jennings & Blanchard 2004, Maxwell & Jennings 2006). The values obtained in the current study fall within the upper standard deviation of that provided by DeNiro & Epstein, 1981.

This study shows that the application of the assumed $\delta^{13}C$ discrimination value over an experimentally determined $\delta^{13}C$ discrimination value could create significant errors in determining the source of production of consumers, whereas the use of assumed values for $\delta^{15}N$ are less likely to produce 'no-result' conclusions regarding trophic level. The commonly applied $\delta^{13}C$ and $\delta^{15}N$ discrimination values originated from a series of laboratory experiments by DeNiro and Epstein (1979, 1981) where terrestrial organisms were fed a controlled diet over different periods of time. These values, 0.4‰ ± 1.1SD for $\delta^{13}C$ and 3.4‰ ± 1.4SD for $\delta^{15}N$, have since been utilised in food web studies in a wide variety of habitats (Adams & Sterner 2000, Vander Zanden & Rasmussen 2001, Jepsen & Winemiller 2002, Das et al. 2003, Estrada et al. 2003, McCutchan et al. 2003).

Over the last decade it has been suggested by many (Gannes et al. 1997, Fantle et al. 1999, Oelbermann & Scheu 2002) that the assumed discrimination values are not applicable to all trophic levels, all species of consumers and all species of food sources (Yokoyama et al. 2005). The assumed values were generally accepted before further research had been conducted on their validity. Currently, there has not been a quantified value that is specific for any trophic level, but only suggestions on how to determine the discrimination value and what might be influencing the difference between species and trophic level (Cabana & Rasmussen 1996, Oelbermann & Scheu 2002, Trueman et al. 2005). Recently, studies have made progress towards a better understanding of the application of discrimination values to different sources and consumers.

The differences in $\delta^{13}C$ and $\delta^{15}N$ discrimination values for different groups of algae is likely to be related to consumers metabolising and assimilating algae to different extents (Peterson & Fry 1987, Albay & Pokorny 2002, Post 2002, Smit et al. 2005). This is due to algae containing different amounts of complex carbohydrates, such as cellulose, which are harder to digest. The main factor determining each algal groups' isotope ratio is photosynthesis. In terrestrial vegetation, during photosynthesis the fractionation of $\delta^{13}C$ occurs through two different stages, diffusion through the stomata followed by carboxylation (Brandes et al. 2006, Farquhar et al. 1982). In marine algae the amount of variation in discrimination between groups is due to different carboxylation as shown through experiments (Albay & Pokorny 2002) and field data (Cloern et al. 2002, Raven et al. 2002, Mbabazi et al. 2004, Brandes et al. 2006). The $\delta^{13}C$ range for algae is determined by the amount of CO_2 and HCO_3^- that is utilised, as it can vary slightly

depending on the individual (Bouillon et al. 2002, Raven et al. 2002). The $\delta^{13}C$ discrimination between a food source and a consumer is a reflection of the isotope ratio of the food source (DeNiro & Epstein 1978, Focken & Becker 1998, Jardine et al. 2003, McCutchan et al. 2003).

$\delta^{15}N$ isotope ratios of algae are also influenced by photosynthesis, but not to the same extent as $\delta^{13}C$. Photosynthesis in relation to $\delta^{15}N$ occurs as algae require the energy provided by photosynthesis to assist in taking up inorganic nitrogen (Burris 1976, DeNiro & Epstein 1981). The present study has shown that fleshy and calcareous red algae and brown and green algae have similar discrimination of $\delta^{15}N$, ranging between 2.53 and 3.08‰. While the level of discrimination for $\delta^{15}N$ was similar amongst algal groups, it has been shown among groups of algae in other studies that such differences are determined by the amount and variety of inorganic nitrate, nitrite and ammonium assimilated by each group of macroalgae (Sweeney & Kaplan 1980).

In addition to this study showing that discrimination of $\delta^{13}C$ differs among types of algae consumed by a species of consumer. This current study has also shown that discrimination of $\delta^{13}C$ and $\delta^{15}N$ differs for different types of marine invertebrate consumers fed on the same algal food type. $\delta^{13}C$ and $\delta^{15}N$ discrimination ratios were similar for the urchin *H. erythrogramma*, and the hermit crab *P. purpureantennatus*, but were slightly lower for the hermit crab *C. dapsiles*, and far lower for the gastropod *T. torquatus*. For example, when fed on *Hypnea* sp., the discrimination of $\delta^{13}C$ was 3.56‰ for *H. erythrogramma*, 3.25‰ for *P. purpureantennatus*, 2.67‰ for *C. dapsiles* and 0.64‰ for *T. torquatus,* whereas $\delta^{15}N$ discrimination was 3.22, 3.63, 2.39 and 1.08‰ for the respective consumers. The present study has therefore demonstrated that a single discrimination value should not be applied across all types of consumers, as each type of marine invertebrate consumer discriminates for particular stable isotopes from its food differently. This is supported by Fantle (1999), also demonstrated through feeding treatments of juvenile blue crabs that $\delta^{13}C$ discrimination values can range from -2.5‰ to 0.4‰. McCutchan et al (2003) who demonstrated a difference in discrimination values between food sources when fed to a species of consumer. The range of $\delta^{13}C$ and $\delta^{15}N$ values was determined for a species of butterfly fed different food sources, and discrimination was found to vary from +0.4‰ to -2.7‰ for $\delta^{13}C$ and +0.8‰ to 5.4‰ for $\delta^{15}N$.

Vanderklift and Ponsard (2003) conducted a meta-analysis on discrimination of $\delta^{15}N$ and found that there was a difference in discrimination values between taxonomic classes. It was shown that invertebrates had a lower discrimination (2.08‰) than vertebrates (2.88‰), and birds (3.05‰) have higher discrimination than marine fish (2.96‰). It has also been demonstrated that there is a difference between trophic levels especially among lower trophic levels (Gannes et al. 1997, Vander Zanden & Rasmussen 2001, Hart & Lovvorn 2002, McCutchan et al. 2003, Hyndes & Lavery 2005). McCutchan et al (2003) also demonstrated a difference in $\delta^{15}N$ discrimination values between trophic levels for organisms raised on invertebrate diet (1.40‰±0.21‰), raised on plant/algal diets (2.20‰±0.30‰) and raised on high protein diets (2.00‰±0.65‰).

Metabolic processes and digestion can influence the discrimination between a food source and a consumer (Minagawa & Wada 1984, Sydeman et al. 1997, Focken & Becker 1998, Nyssen et al. 2002, McCutchan et al. 2003, Jacob et al. 2005, MacNeil et al. 2006) and metabolic process is dependent on the type of food consumed. Foster (1999) suggested that some food sources are easier to digest due to several factors, including chemical defences, cell wall toughness and secondary metabolites (Jardine et al. 2003, Goedkoop et al. 2006, MacNeil et al. 2006). Furthermore, particularly within algae, the amount of complex carbohydrates increase the difficulty of digestion (McCutchan et al. 2003).

4.3 Evaluation of Marine Trophodynamics using Mixing Models

Mixing models for stable isotopes are used to quantify the contribution of food sources to a consumer's diet, and particularly in determining how much of each food source is being assimilated (Phillips & Gregg 2003). The Isosource mixing model by Phillips & Gregg (2003) is commonly used in trophic analyses to determine the probability of different food sources being part of a consumer's diet (Abed-Navandi & Dworschak 2005, Behringer & Butler IV 2006). As mixing models rely on discrimination values, the application of the most accurate discrimination value is critical (McCutchan et al. 2003). An inaccurate discrimination value could place a consumer in the wrong trophic level, determine the incorrect food source/s, or may not even allow the mixing model to function (Phillips 2001, Olive et al. 2003).

To allow Isosource to function there is one main requirement, which is that the consumer's isotope ratio must fit within the range of the values for the group of food sources, once discrimination values have been applied (Phillips 2001, Olive et al. 2003). In the current study, when the assumed discrimination values of 0.4‰ for $\delta^{13}C$ and 3.4‰ for $\delta^{15}N$ were applied to the food source data, the isotope ratios of the invertebrates were located outside the algal isotope ratios (Figure. 3.12). Therefore, the application of assumed discrimination values to the source values could not yield results from the mixing model. In contrast, when the $\delta^{13}C$ and $\delta^{15}N$ species-specific discrimination values, as determined from the experiments, were applied to a data set, the isotope ratios of the invertebrates were located within the isotope ratios of the algae. This allowed Isosource to run the calculations and thus determine potential contributions of the different food sources to the range of consumers examined in the study. Thus, it was only possible to establish the potential contributions of food sources to the production of consumers by determining discrimination values through experiments.

Isosource results, using the discrimination values determined form the study, showed that in the wild all of the invertebrates were consuming a varied algal diet, although each type of consumer utilised somewhat different proportions of each source. For the urchin *H. erythrogramma*, the Isosource results showed that this species consumed a range of food sources, though greater proportions of its diet consists of *Plocamium* sp. (up to 35%) and *U. lactuca* (up to 30%). A similar trend was shown with *P. purpureantennatus*, as it also consumed small proportions of a variety of food sources with a higher proportion of its diet consisting of *Plocamium* sp (up to. 25%) The same applied to *C. dapsiles,* as a large proportion of its diet consisted of red algae.

Research by Vanderklift et al (2005) on gut contents of *H. erythrogramma* found that this organism was almost exclusively herbivorous, with a large portion (up to 55.5%) of its diet consisting of brown algae. However, through Isosource it has been shown that a greater portion of the urchin's diet consists of *Plocamium* sp (up to 35%). and *U. lactuca* (up to 30%), and smaller proportions of the other algae *E. radiata* (up to 16%), *Sargassum* sp. (up to 15%), *Hypnea* sp (up to 18%), *A. anceps* (up to 18%), and *U. australis* (up to 18%). This apparent contradiction between studies is most likely due to the difference between consumption and assimilation (McCutchan, 2003). Brown algae, especially *E. radiata*, are known to contain phlorotannins that act as herbicide

(Steinberg & Altena 1992) and may reduce an organism's ability to metabolise the food source. Whereas green algae, like *U. lactuca*, are known to be easier to digest and help maintain the immunodeficiency systems and growth rates of marine invertebrates (Wassef et al. 2001). Therefore *U. lactuca* would be easily digested and assimilated, whereas brown algae may be abundant in the digestive tract of the organism, but with notably lower metabolic assimilation. The trend of higher proportions of red algae in the diet were also seen the hermit crab results. However, unlike the urchins, there is little data on the natural diets of *Paguristes purpureantennatus,* but the genus has been shown to consume both plant (Orth et al. 2006) and animal material (Pezzuti et al. 2002).

The amount and variety of food sources each consumer is feeding on may also relate to compensatory feeding patterns where the diet of a consumer is determined by their ability to obtain a food source. The marine invertebrates within this study all had low mobility within the environment, therefore they ate the algae sources that were close at hand. It has been shown by Stachowicz (1999) that marine crabs restrict their mobility to avoid predation and remain within their refugia. If the algae sources that were close to their location had a low nutritional value or were hard to digest, then the invertebrates would consume a greater quantity of the algae to maintain their health (Stachowicz & Hay 1999, Cruz-Rivera & Hay 2001, Pum Lee et al. 2004).

4.4 Implications for Environmental Management

This study has shown that discrimination of $\delta^{13}C$ and $\delta^{15}N$ differs among groups of algae when fed to the same type of consumer, but also amongst consumers when fed on the same food source. Discrimination of $\delta^{13}C$ ranged between 1.46 and 3.38‰, while that of $\delta^{15}N$ ranged between 2.60 and 3.08‰ for different algal groups and consumers. Discrimination values determined from this study should be used for the relevant food sources and consumers in preference to discrimination values derived from an average of other types of organisms from other environments (DeNiro & Epstein 1978, 1981). A number of recent studies in stable isotope-based ecology have highlighted the importance of applying an accurate discrimination value for determination of trophic levels and food sources (Macko et al. 1982, Gannes et al. 1997, Yokoyama et al. 2005). The current study has quantified $\delta^{13}C$ and $\delta^{15}N$ discrimination for key marine invertebrate and algal types, and particularly for C these were found to be higher than the generally applied value of 0.4 ± 1.4‰.

The application of an incorrect discrimination value may lead to the placement of an organism into the wrong trophic level or incorrectly linked to a certain food source. The use of trophic levels is important in management as it shows an organism place within an ecosystem and is used to determine trophic links in a variety of ecosystems and organisms. Examples of this include the life history of a species of beetle (Tooker & Hanks 2004) to predicting trophic position of sharks (Estrada et al. 2003) and the structure of a lake ecosystem (Jepsen & Winemiller 2002). However, of recent isotopes and discrimination values have been used within the marine ecosystem to assist with management of resources. Le Loc'h (2004) has determined the impact of species exploited through fishing on the trophic interaction of the ecosystem, whereas Jennings (2004) has used isotope discrimination values to help determine compare trophic structure between commercially fished ecosystems and ecosystems that are not fished. Other studies use isotopes and discrimination to determine migratory patterns of many terrestrial and marine species (Hansson et al. 1997, Maruyama et al. 2001, Quillfeldt et al. 2005).

Owing to the impractical nature of gut contents analysis, stable isotope techniques are becoming more prevalent in food web studies that are then used to provide information for ecosystem management (Keedwell et al. 2001). While present research has indicated the importance of accurately quantified discrimination values for groups of macroalgae and invertebrate consumers, it would be logistically impossible to run discrimination experiments on all potential consumer-source combinations before conducting a large field-based trophic study. For practical purposes, it may be required to provide generalisations of $\delta^{13}C$ and $\delta^{15}N$ discrimination values for different types of marine invertebrate or algae, but at a more defined taxonomic or functional level. While not ideal, this will still go some way towards creating greater accuracy in marine trophodynamic studies than applying the overly generalised discrimination values in current widespread use (Gannes et al. 1997).

4.5 Recommendations

There is still a great need for further research into the quantification of discrimination values in stable isotopes based food web studies. Feeding treatments incorporating food sources that are either strongly enriched or depleted in $\delta^{13}C$ and $\delta^{15}N$ could further clarify the discrimination levels between a consumer and a food source, particularly in

the case where the consumers' natural stable isotope levels do not shift over time when fed on a specific food source collected from their habitat. Preferably, the food source should have a stable isotope value that is outside the natural range of the diet, but the type of source should be part of the consumer's diet. This could be achieved by artificially altering the stable isotope value of the food source through providing the algae with enriched or depleted C or N. In addition, determination of discrimination value could come from feeding treatments incorporating manufactured diets. Manufactured diets are beneficial as they have a known isotope ratio and remain stable through out the feeding treatment. This can also control the protein levels of the consumer and the between-tissue growth rates if different areas of muscle tissue were to be studied. In a meta-analysis by Vanderklift & Ponsard (2003), there was no significant difference in the discrimination of stable isotopes using manufactured and natural diets of plant or animals.

Discrimination values are the limiting factor in the application of isotopes within trophic studies. A better grasp of the mechanisms contributing to discrimination values and an understanding of what $\delta^{13}C$ and $\delta^{15}N$ discrimination values should be applied will greatly increase the interprebility of stable isotopes. The main use for stable isotopes will be trophic studies looking at organisms and their trophic level within the ecosystem. However, the addition of isotopes as a tool for environmental management may help manage many aspects of ecosystems. Main aspects would include the flow of nutrients and pollutants through the ecosystem, understanding the effects of altering populations of organisms, such as increasing or reducing commercial fisheries; or predator control for the survival of different species. If there is a possibility that an action may alter the ecosystem or food web, then an understanding of stable isotopes might enhance a manager's ability to predict the possible outcomes.

Throughout this study there were implications with the survival rates of the organisms. The urchin *H. erythrogramma,* was fragile and individuals frequently died from disease. In some cases, mass mortalities of urchins would occur within a 24 hr period. However, a second species of urchin, *Holopneustes porosissimus*, was collected for the feeding treatment and all individuals died in less than 24 hrs after collection. A second species of gastropod was also used for the feeding treatments; however this species did not survive in captivity for an adequate period of time to collect any results. At one stage throughout the feeding treatments, both species of hermit crabs had individuals that

would leave their shells overnight and die. For future studies it is recommended that a great care be take in choosing species, as some species have a lower survival rate in captivity.

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