

2015

## **Dynamics of baseline stable isotopes within a temperate coastal ecosystem: Relationships and projections using physical and biogeochemical factors**

Andrew Mackey  
*Edith Cowan University*

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**Dynamics of baseline stable isotopes within a temperate coastal ecosystem:  
relationships and projections using physical and biogeochemical factors**

**By**

**Andrew Mackey**

**Master of Science (MSc)**

**This thesis is presented in fulfilment of the requirements for the degree of Doctor of  
Philosophy**

**Faculty of Computing, Health and Science**

**Edith Cowan University**

**February 2015**

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is not included in this version of the thesis

## ABSTRACT

Measurements of carbon ( $^{13}\text{C}/^{12}\text{C}$ ;  $\delta^{13}\text{C}$ ) and nitrogen ( $^{15}\text{N}/^{14}\text{N}$ ;  $\delta^{15}\text{N}$ ) stable isotope ratios have become important tools for: estimating energy flow and trophic positions in aquatic foodwebs; comparing food webs; and aiding in the tracking of wide-ranging consumers. However, each of these applications requires accurate measurements of isotopic signatures in organisms at or near the base of the food web (e.g. autotrophs and their consumers), which act as basal reference points from which to calibrate inferences. Therefore, understanding variations in isotopic baselines, and the mechanisms leading to their variability, is crucial for food web ecology.

Using the shallow temperate reefs along the lower west coast of Australia as a test case, the broad aim of this thesis was to determine isotopic variations in a coastal food web and their relationship with surrounding environmental factors or food sources, to determine the suitability and issues for using such baselines in interpreting trophic links and positions in food webs and predicting shifts in isotopic signatures across broad spatial and temporal scales. To achieve this, I have determined the critical scales for accurately capturing baseline isotopic variation in ecologically important autotrophic and consumer taxa (e.g. macroalgae, particulate organic matter and suspension and grazing consumers), and then related isotopic variation in macroalgae to properties of their surrounding physical and biogeochemical environment. I have then used these relationships to project and forecast baseline values in space and time. Lastly, I have tested the suitability of different primary consumers, representing suspension and grazing functional groups to capture spatial and temporal isotopic variation of their respective diets, to act as invariable proxies of  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  baselines.

Isotopic variability of basal resources differed between autotrophs and consumers, and among species within these groups, and between isotopes (i.e.  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$ ), demonstrating the difficulty in capturing accurate and representative values. Spatial patterns for the  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  in the kelp *Ecklonia radiata*, the  $\delta^{15}\text{N}$  in the calcareous red alga *Amphiroa anceps*, and the  $\delta^{13}\text{C}$  in the ascidian *Herdmania momus* and the bivalve *Septifer bilocularis*, were influenced by variation at replicates (10s of m) and sites (1s of km) over regions (10s of km). Whereas, the reverse was true for  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  in the foliose red alga *Plocamium preissianum*,  $\delta^{15}\text{N}$  in *S. bilocularis*, and  $\delta^{13}\text{C}$  in the gastropod *Turbo torquatus*, with regional differences being the greatest source of influence. Temporally, patterns of  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  variation in each taxon and

trophic group were more comparable, with seasonal variation eliciting a weak or negligible effect, but monthly variation often resulting in a strong effect.

Temperature, light, water motion, and measurements of dissolved inorganic carbon, nitrogen and phosphorous correlated with spatial and temporal variation in the stable isotopes of macroalgae, but these relationships varied with taxa and isotope. Surprisingly, water temperature was the best single explanatory variable, accounting for ~50-60% of variation in the  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  of *P. preissianum*, and the  $\delta^{15}\text{N}$  of *E. radiata* and *A. anceps*. From the above relationships, spatial predictions of  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values in macroalgae showed clear latitudinal patterns, which covered a far wider range of values than temporal predictions, over a 12-month period. This illustrates the potential scale in the shift of isotopic baseline food sources over broad scales, and its implications for food web studies.

Primary consumers, particularly the bivalve *Septifer bilocularis* and the gastropod *Turbo torquatus*, generally mitigated a large proportion of the autotrophic  $\delta^{15}\text{N}$  variation and displayed relatively stable  $\delta^{15}\text{N}$  over time, and appeared to time-integrate the  $\delta^{15}\text{N}$  of their diet. Further, by conducting a controlled feeding study, I showed that *S. bilocularis* exhibited slow  $\delta^{15}\text{N}$  turnover estimates (e.g. half-life of 56 days), which from simulations, negated and “dampened” the effect of fluctuating  $\delta^{15}\text{N}$  values of food sources. This suggests that, owing to their high abundances and wide distributions, these species can be used to compare  $\delta^{15}\text{N}$  baselines over large spatial scales. However, this was not the case for  $\delta^{13}\text{C}$ , where primary consumers were as variable as those autotroph(s) they are assumed to proxy. Therefore, consistency in consumer  $\delta^{15}\text{N}$  does not necessarily equate to consistency in  $\delta^{13}\text{C}$ .

The fact that different autotrophs and consumers elicit different patterns of variation, show that sampling designs need to be compatible to the research questions of interest. Otherwise, improper allocation of sampling effort may decrease the probability of detecting ecologically important differences. Consequently, my results provide a reference from which to determine the appropriate sampling design to capture variation in ecologically important taxa, to help inform future studies. Further, the models I have developed to predict isotopic values for important autotrophic sources, and the identification of reference taxa of baseline  $\delta^{15}\text{N}$  (bivalves and gastropods), could be used by ecologists to remove a large proportion of unexplained variation, thus facilitate the interpretation of variation in stable isotopes of consumers in food webs, to help answer important questions in food web ecology.



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## List of publications included

**Mackey AP**, Hyndes GA, Carvalho MC, Eyre BD. (2015) Physical and biogeochemical correlates of spatio-temporal variation in the  $\delta^{13}\text{C}$  of marine macroalgae. *Estuarine, Coastal and Shelf Science* (In Press, Accepted Manuscript, [doi: 10.1016/j.ecss.2014.12.040](https://doi.org/10.1016/j.ecss.2014.12.040))

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**Mackey AP**, Hyndes GA. Temporal and spatial variation in the  $\delta^{15}\text{N}$  of primary consumers: inferences and implications for baseline  $\delta^{15}\text{N}$  and trophic level estimates in marine food webs. In review: To be submitted to *Limnology and Oceanography*.

**Mackey AP**, Hyndes GA. Spatial and temporal variation of  $\delta^{13}\text{C}$  in suspension-feeding and grazing marine consumers. To be submitted.

**Mackey AP**, Hyndes GA. Isotopic turnover in the marine ledge mussel (*Septifer bilocularis*), and simulations in the  $\delta^{15}\text{N}$  response of mussels (primary consumers) and fish (secondary consumers) to a dynamic kelp baseline. To be submitted.

## **Statement of contribution**

To whom it may concern,

I, Andrew Mackey, contributed to conceiving, designing and performing, the experiments, analysing the data, and writing all the manuscripts included in this thesis, as well as writing the thesis itself.

28<sup>th</sup> November, 2014

I, as supervisor, and co-author on all publications included here, endorse that this level of contribution by the candidate indicated above is appropriate.

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28<sup>th</sup> November, 2014

## LIST OF CONFERENCE PRESENTATIONS

**Mackey AP**, Hyndes GA, Carvalho MC, Eyre BD. (2013) Dynamics of  $\delta^{13}\text{C}$  in temperate reef autotrophs related to environmental data, with projections of spatial and temporal patterns. 53rd Estuarine and Coastal Sciences Conference (ECSA 53). 13-17 October 2013. Shanghai, China. Oral presentation.

**Mackey AP**, Hyndes GA, Carvalho MC, Eyre BD. (2014) Environmental correlates of isotopic variation in primary producers and consumers within a temperate coastal ecosystem. 10th International Temperate Reef Symposium (ITRS). 12-17 January 2014. Perth, Western Australia. Poster presentation.

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# CHAPTER 1. INTRODUCTION

## 1.1 Biomarkers

Food web studies over the last 30 years have seen the emergence in the use of biomarkers as techniques to examine food web structure and sources of production (DeNiro and Epstein 1977; Duggins et al. 1989; Michener and Schell 1994; Jennings et al. 2002b; Vizzini and Mazzola 2003; Boecklen et al. 2011). Of these approaches, lipid compounds and fatty acid analysis (cellular material), have been shown to be useful in tracing these dietary components through food webs (Guest et al. 2010; Hanson et al. 2010; Dethier et al. 2013), but stable isotope analysis (SIA) is the most widely used (particularly in aquatic systems), and has proven to be a powerful and flexible tool in food web ecology (del Rio et al. 2009; Boecklen et al. 2011). This is not surprising, given that it has many advantages over traditional methods. Although stomach content analysis (SCA) allows for dietary sources to be definitively identified, it is limited by the difficulty in identifying and quantifying organisms that are macerated or rapidly digested, and differentiating between ingested and assimilated material (Vander Zanden et al. 1997). Moreover, temporal integration of dietary information is often difficult to quantify using gut contents alone, as it gives only a ‘snapshot’ (e.g. recently consumed diet) of dietary prey at any given time (Bearhop et al. 2004; Rybczynski and Walters 2008). In comparison, isotopic values can be easily obtained from every organism sampled (as opposed to ‘empty guts’), are related only to assimilated material, and may represent long, medium or short-term averages of dietary information (Hill and McQuaid 2009; Boecklen et al. 2011).

Stable isotopes are atoms of the same element that are identical in chemical and physical properties, but differ by mass related to different numbers of neutrons. For example, carbon has two stable isotopes, with  $^{12}\text{C}$  (e.g. six protons and six neutrons) representing 98.89%, and  $^{13}\text{C}$  (e.g. six protons and seven neutrons) representing 1.11% of their natural abundance (Greenwood and Earnshaw 1997). The stable isotopic ‘signature’ of an organism is quantified as part per mill (‰) and prefixed by the delta ( $\delta$ ) notation to indicate its ratio against a recognised standard. The most commonly used stable isotopes in trophic ecology are  $\delta^{13}\text{C}$  (e.g.  $^{13}\text{C}/^{12}\text{C}$ ),  $\delta^{15}\text{N}$  (e.g.  $^{15}\text{N}/^{14}\text{N}$ ) and to a lesser extent  $\delta^{34}\text{S}$  (e.g.  $^{34}\text{S}/^{32}\text{S}/^{33}\text{S}$ ), with their ratios in the tissues of consumers reflecting those of their diet in a broadly predictable manner. Typically,  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  in consumers are heavier than their dietary components, as lighter isotopes are either preferentially excreted ( $^{15}\text{N}$ ) or respired ( $^{13}\text{C}$ ) (Olive et al. 2003). This net

increase, termed trophic enrichment, and denoted by the  $\Delta$  symbol, is relatively small in  $\delta^{13}\text{C}$  ( $\sim 0\text{-}1\text{‰}$ ), but distinct in  $\delta^{15}\text{N}$  ( $\sim 2\text{-}5\text{‰}$ ) (DeNiro and Epstein 1977; Post 2002; Vanderklift and Ponsard 2003). As food webs are supported by primary producers, these autotrophs represent the first trophic step or baseline reference points from which isotopic data in food webs can be calibrated.

Combined,  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  provide complementary data that help determine the structure of food webs. Conveniently,  $\delta^{13}\text{C}$  values of autotrophs often fall into broadly distinct groups representing different functional groups with different modes of photosynthesis (e.g. C3, C4 and CAM displaying values  $-34$  to  $-22$ ,  $-16$  to  $-10$ , and  $-20$  to  $-10$ , respectively) (O'Leary 1988). As  $\delta^{13}\text{C}$  is relatively conserved through the food web, but varies across functional groups at the base of the food web, it can be used to discriminate among sources (Simenstad and Wissmar 1985; Vizzini et al. 2002; Melville and Connolly 2003; Hanson et al. 2010). In comparison,  $\delta^{15}\text{N}$  displays a distinct increase through each trophic transfer, and so is used to discriminate among trophic levels (Jennings et al. 2002a; Pinnegar and Jennings 2002). Consequently, the single or dual use of  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  can be used to reconstruct diets (Parnell et al. 2010; Dethier et al. 2013), characterise consumers' trophic niche (Bearhop et al. 2004; Sweeting et al. 2005; Jaeger et al. 2010), and quantify complex food webs (Davenport and Bax 2002; Jennings et al. 2002b), as well as trace sources of pollution (Gartner et al. 2002; Gorman et al. 2009) and track migratory patterns and residency times of animals (Hobson 1999; Graham et al. 2010). For example, simply plotting the mean values of species in  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  bi-plots can be used to infer food web structure and trophic links by way of relative positions in the bi-plot space (see Layman et al. 2007), while the variance of values can be used as a measure of trophic niche width (see Bearhop et al. 2004). More quantitatively, mathematical mixing-models are used to estimate the proportions of dietary components to the isotopes of consumers (see Parnell et al. 2013). As human sources of nitrogen (e.g. sewage effluent, fertilizers) are significantly enriched in  $\delta^{15}\text{N}$  relative to seawater,  $\delta^{15}\text{N}$  can be a measure of pollution (Cabana and Rasmussen 1996). In comparison, tracing the movements of animals may be possible if variation in the stable isotopes at the base of food webs follows broad geographic patterns (see Hobson 1999).

## 1.2 Assumptions and limitation of SIA

Although SIA has many advantages over traditional trophic methods, it often relies on the assumptions that baseline (autotrophic) values are relatively constant (see Boecklen et al. 2011; Hyndes et al. 2013; Dethier et al. 2013), and that these values change consistently and predictably from diet to consumer (e.g.  $\Delta$ ). While mean  $\Delta$  across multiple trophic transfers may equal  $\sim 0.5\text{‰}$  for  $\delta^{13}\text{C}$  and  $\sim 3\text{‰}$  for  $\delta^{15}\text{N}$  across a single step (Post 2002),  $\Delta$  can vary by  $> 6\text{‰}$  (DeNiro and Epstein 1977; Michener and Schell 1994; Post 2002), and can differ among functional consumer groups (Vanderklift and Ponsard 2003). Undoubtedly, variation in consumer  $\Delta$  could mislead ecologists in their conclusions. However, numerous studies are attempting to either explain the causes of variability in  $\Delta$  (Hobson and Clark 1992; Hobson et al. 1993; Vanderklift and Ponsard 2003), or identify more accurate estimates of  $\Delta$  for specific animal groups, based on taxonomy, physiology or processes of excretion/respiration and dietary guild (Vanderklift and Ponsard 2003). Moreover, recent advances in mixing-models incorporate variability associated with  $\Delta$  (Parnell et al. 2010; Parnell et al. 2013). Yet, the issue of baseline variation is often overlooked.

Autotrophs represent the first reference points from which isotopic data in food webs can be calibrated, and so aquatic consumers may be assigned a trophic level or linked to different primary sources against these baselines. For example,  $\delta^{13}\text{C}$  is clearly separated between seagrasses, macroalgae and phytoplankton (Farquhar 1989; Raven et al. 2002), which has proved useful in proportioning their relative trophic role in food webs (Lepoint et al. 2000; Melville and Connolly 2003; Hill et al. 2008). Yet, there is considerable overlap in  $\delta^{13}\text{C}$  among species and taxa within these groups (Hanson et al. 2010), and accurate estimates of  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  are complicated by intraspecific variation, which can be significant, even in individuals metres apart (e.g. Vanderklift and Wernberg 2010). Differences in isotopic values in autotrophs depend largely on the  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  of the available nutrient sources (Johnson et al. 2006; Cornelisen et al. 2007; Campbell and Fourqurean 2011), and the physiochemical processes that influence their fractionation and uptake, e.g. light, temperature, salinity and hydrodynamics (Cornelisen et al. 2007; Umezawa et al. 2007). Therefore, highly dynamic systems will likely result in highly dynamic  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  baselines. Despite these concerns, studies often obtain isotopic signatures of autotrophs and their consumers from a discrete point in time and over a limited geographical range (see Boon and Bunn 1994; Miller and Page 2012), or worse still,

values from the literature are used instead as proxies (Boecklen and Yarnes 2011). A major concern is that spatial and temporal variation may mask or distort subsequent interpretations.

Since the dynamics of  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  in autotrophs are strongly influenced by their surrounding physical and biogeochemical environment, these variables may account for much of the  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  variations. Linking these variables with  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  of autotrophs represents a convenient method for predicting baseline isotopic variation and has proved successful for phytoplankton (Rau et al. 1989) and phytoplankton consumers, as baseline proxies (Cabana and Rasmussen 1996; Post 2002; Gustafson et al. 2007). Consequently, for phytoplankton and their known consumers, these differences or “isoscapes” have been used to correct for baseline variation across geographic scales, and compare multiple systems (Jennings and Warr 2003; Barnes et al. 2009; MacKenzie et al. 2011). In terms of macrophytes, much of the work examining the influence of physical and biogeochemical factors on  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  has been biased towards species in freshwater (Black et al. 1981; Finlay 2004) and estuarine environments (Cornelisen et al. 2007b), or seagrasses in marine systems (see Raven et al. 2002). Because macroalgae differ from seagrasses as they lack connective tissue, leaves, and roots, and have unique biochemical functions and pathways to obtain nutrients directly from the water column (Cooper and McRoy 1988; Kennedy 2011), variability in their  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  is likely to be influenced by different processes.

### **1.3 Stable isotope variation in temperate reef systems**

Over temperate coastal reefs, macroalgae dominate primary production in abundance and biomass, and form an extremely diverse group of autotrophs with contrasting taxonomy, morphology and physiology (Keith et al. 2013) and an important baseline food source in food webs in a range of ecosystems (Steneck et al. 2003). Intraspecific variation in  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  in macroalgae can vary by 10‰, depending on site and season (Raven et al. 2002; Hyndes et al. 2013). Even over relatively small spatial and temporal scales, there are many reported instances of isotopic variation exceeding values assumed for trophic enrichment (e.g. ~0.5‰,  $\delta^{13}\text{C}$  and ~3‰,  $\delta^{15}\text{N}$ ) (Guest et al. 2010; Hyndes et al. 2013). This has led to several studies that describe the scope and scale of isotopic variation in macroalgae over coastal temperate systems (Guest et al. 2010; Hanson et al. 2010; Vanderklift and Wernberg 2010). For example, Guest et al. (2010) showed that the isotopes of macroalgae do not necessarily increase with spatial scale,

as variation among sites can be greater than that of regions. To complicate matters further, even when sampling occurs at the same time and place, patterns are often not consistent among species or taxa (Hyndes et al. 2013; Dethier et al. 2013). Although these studies provide a valuable resource that helps inform ecologists of appropriate sampling designs, they do not address the underlying factors that influence ‘shifting baselines’, which remains poorly understood.

Temperate coastal reef systems are highly variable in space, due to uneven depths and levels of hydrodynamic forces and proximity to terrestrial outputs, and in time, through seasonal shifts in a range of environmental factors, e.g. light, temperature and nutrients. Therefore, these environmental factors may account for much of the spatial and temporal isotopic variation in autotrophs, and could be used to predict baseline isotopic patterns in macroalgae. Correcting for baseline  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  (in trophically important species), could simplify broad-scale comparisons of trophic structure in these physically dynamic systems (e.g. energy pathways and trophic level). Furthermore, local environmental data could be used as tools to help direct appropriate sampling designs to accurately capture isotopic values in those species. For example, if spatial  $\delta^{13}\text{C}$  variation was found to be closely linked to one environmental factor, ecologists could use knowledge of the spatial variability in that factor to estimate isotopic variation in other regions, and so inform appropriate sampling designs.

#### **1.4 Primary consumers as baseline proxies**

While methods to predict isotopic values in autotrophs have enormous value, they have limitations too. Although phytoplankton are sampled in bulk, macrophytes are sampled individually. Macrophytes are highly diverse (particularly macroalgae) with contrasting taxonomy, morphology and physiology, and thus are likely to have different physiological/isotopic responses to environmental conditions, with different spatial and temporal patterns (Raven et al. 2002). Therefore, measuring the production-weighted mean of isotopic values of autotrophs, as would be required to estimate the mean isotopic values at the base of food webs, is methodically challenging (see Jennings and Warr 2003). Another approach is to use primary consumers with variable and non-specialist diets, where their isotopic values represent an assimilated-weighted mean of their dietary sources. The advantage is that, while autotrophs have isotopic values that are highly variable in space and time, making

it difficult to gain accurate and representative values, consumers time-integrate the spatial and temporal variation of their diet. This level of time-integration relates to the biomass and metabolic rate of the consumer (Sweeting et al. 2005; Hill and McQuaid 2009). Since the highly metabolic tissue rapidly turns over the assimilated isotopic composition of the consumer's diet, the assimilated stable isotopes of small short-lived species (e.g. zooplankton), or highly metabolic tissue in larger longer-lived species (e.g. blood plasma and liver) often reflect the short-term variations in their diet. In contrast, the assimilated stable isotopes of less-active muscle tissue from larger longer-lived consumers reflect a longer term mean (Boecklen et al. 2011). Therefore, the stable isotopes of long-lived and slow growing primary consumers may absorb variation, and track energy flow at the base of the food web, and so provide a general and relative reference from which to apportion trophic position and primary sources supporting consumers.

## **1.5 Aims**

It is difficult to make accurate conclusions regarding food-web structure, fate of baseline food sources, and shifts in trophic levels and dietary specialisation without an understanding of what drives variation in isotopic signatures among baseline food sources and their consumers in marine systems. Therefore, the broad aim of this research was to evaluate the “basal” (e.g. primary producers/consumers) isotopic variations in a temperate coastal reef food web and their relationship with surrounding environmental factors or food sources, to determine the suitability and issues for using such baselines in interpreting trophic links and positions in food webs and predicting shifts in isotopic signatures across broad spatial and temporal scales.

In achieving this broad aim, I used the resultant dataset to:

1. Examine spatial and temporal variation in the stable isotopes of ecologically important and widespread macroalgae, suspended particulate organic matter, and suspension-feeding and grazing primary-consumers, to determine critical scales of variation among taxa (Chapter 2, 3, 4 and 5);
2. Relate variations in macroalgae to factors of their surrounding physical and biogeochemical environment, and use any relationships to project (in space) and forecast (in time) isotopic patterns that may account for baseline variation in temperate coastal regions (Chapters 2 and 3);

3. Evaluate the suitability of different taxa of primary consumers, representing suspension and grazer functional groups, as baseline proxies of primary source  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  (Chapter 4, 5 and 6); and
4. Describe the propagation of temporally dynamic baselines across multiple trophic levels and the potential effect of temporal disparity on defining trophic relationships in food webs (Chapter 6).

The study was undertaken in the coastal reef system in temperate Western Australia. A characteristic feature of the coastline in south-western Australia is the presence of limestone reefs running parallel to the coastline (Sanderson 2000) that hosts a diverse assemblage of macroalgae, which are food for grazers and suspension feeders (Andrew 1999). Due to the Leeuwin Current that prevents the development of major upwellings, the region is considered oligotrophic (Hanson et al. 2005). Consequently, picoplankton that are able to utilize the low levels of nutrients through their high surface to area ratio represent >85% of phytoplankton biomass (Hawks, 2006). However, phytoplankton is only a small contributor to the suspended particulate organic matter pool, with detrital macroalgae the major component. Although the small kelp *Ecklonia radiata* dominates the biomass of macroalgae in the region (Wernberg et al. 2010), differences in environmental conditions (e.g., physical disturbances, sedimentation, and light availability) create a mosaic of patches with high species richness and diversity (Kendrick et al., 1999).

The reefs in the study region vary in their exposure to different levels of oceanic swells and therefore water velocity (Sanderson 2000) and their proximity to terrestrial outputs and human population density. The climate is Mediterranean and seasonally variable, with winter characterised by frequent storms and seawater temperatures of  $\sim 17^\circ$ , and late summer to early autumn characterised by calmer conditions and water temperatures rising to  $\sim 24.5^\circ$  (Smale and Wernberg 2009; de Bettignies et al. 2013). The dynamic nature of the region as a whole, make it ideal to relate spatial and temporal isotopic variation in primary sources with their physical and biogeochemical environment, and identify different species of primary consumer (from different functional groups) to represent time-integrated proxies of primary source isotopes. Accordingly, I extrapolate these regional relationships to develop predictive models of isotopic patterns in foodwebs across temperate Australia and New Zealand. These research outcomes facilitate the interpretation of variation in the stable isotopes in marine food webs.

I achieve the aim and objectives through five central chapters (Chapters 2 – 6), which are in manuscript format to facilitate publication in peer-reviewed journals. I use the terms ‘we’ and ‘our’ in these chapters as they contain co-author(s). Due to the related themes of each chapter, there is some degree of repetition across them, particularly within introduction and methods sections.



## Chapter 2. Physical and biogeochemical correlates of spatio-temporal variation in the $\delta^{13}\text{C}$ of marine macroalgae

### 2.1 Abstract

Carbon isotope ratios ( $^{13}\text{C}/^{12}\text{C}$ ) can be used to trace sources of production supporting food chains, as  $\delta^{13}\text{C}$  undergoes relatively small and predictable increases ( $\sim 0.5\text{‰}$ ) through each trophic level. However, for this technique to be precise, variation in  $\delta^{13}\text{C}$  signatures of different sources of production (baseline sources) must be clearly defined and distinct from each other. Yet  $\delta^{13}\text{C}$  in the primary producers of marine systems are highly variable over space and time, due to the complexity of physical and biogeochemical processes that drive  $\delta^{13}\text{C}$  variation at the base of these foodwebs. We measured spatial and temporal variation in the  $\delta^{13}\text{C}$  of two species of macroalgae that are important dietary components of grazers over temperate reefs: the small kelp *Ecklonia radiata*; and the red alga *Plocamium preissianum*, and related any variation to a suite of physical and biogeochemical variables. Patterns in  $\delta^{13}\text{C}$  variation, over different spatial (10s m to 100 km) and temporal scales (weeks to seasons), differed greatly between taxa, but these were partly explained by the  $\delta^{13}\text{C}$  of dissolved inorganic carbon (DIC) and light. However, while the  $\delta^{13}\text{C}$  in *E. radiata* was not related to water temperature, a highly significant proportion of the spatio-temporal variation in  $\delta^{13}\text{C}$  of *P. preissianum* was explained by temperature alone. Accordingly, we applied this relationship to project (across temperate Australasia) and forecast (in time, south-western Australia) patterns in *P. preissianum*  $\delta^{13}\text{C}$ . The mean projected  $\delta^{13}\text{C}$  for *P. preissianum* in the study region varied by only  $\sim 1\text{‰}$  over a 12-month period, compared to  $\sim 3\text{‰}$  over 2000 km. This illustrates the potential scale in the shift of  $\delta^{13}\text{C}$  in baseline food sources over broad scales, and its implications to food web studies. While we show that those relationships differ across taxonomic groups, we recommend developing models to explain variability in  $\delta^{13}\text{C}$  of other baseline sources to facilitate the interpretation of variation in  $\delta^{13}\text{C}$  of consumers in food webs, particularly where data for baselines are absent over broad scales.

## 2.2 Introduction

Carbon isotope ratios of  $^{13}\text{C}/^{12}\text{C}$  (expressed as  $\delta^{13}\text{C}$ ) can be used to trace sources of production supporting food chains, as  $\delta^{13}\text{C}$  is generally considered to undergo relatively small and predictable increases ( $\sim 0.5\text{‰}$ ) through each trophic level (Olive et al. 2003). Combined with  $\delta^{15}\text{N}$  (e.g.  $^{15}\text{N}/^{14}\text{N}$ ), which often reflect shifts in trophic position (Jennings et al. 2008),  $\delta^{13}\text{C}$  has been used to determine the structure of food webs, e.g. rainforests (McGlynn et al., 2009), and infer diet and trophic niche of consumers (Bearhop et al. 2004). However, this often relies on the premise that the  $\delta^{13}\text{C}$  between sources of production form groups that display marked differences in their  $\delta^{13}\text{C}$  (O’Leary 1988). Unfortunately, taxa within these groups often have highly varied and/or overlapping  $\delta^{13}\text{C}$  signatures (Hanson et al. 2010). These variations are often not fully considered, despite having been recognised as a problem for foodweb ecologists for many years (Stephenson et al. 1984; Fenton and Ritz 1989), which has been highlighted in recent literature (e.g. Cloern et al. 2002; Guest et al. 2010; Hyndes et al. 2013; Dethier et al. 2013). Such spatial and temporal variation may mask or distort subsequent interpretations and our ability to gain an accurate understanding of food webs, trace the movement of animals between habitats, or develop predictions of  $\delta^{13}\text{C}$ .

Broad predictions of  $\delta^{13}\text{C}$  variation in autotrophs are developing rapidly for terrestrial systems over temporal and particularly spatial scales. This has led to the development of predictive modelling of plant- $\delta^{13}\text{C}$  patterns across time (e.g. Kodama et al. 2008; Marron et al. 2008) and landscapes or “isoscapes” (Hobson et al. 2012; Powell et al. 2012). These have helped identify the geographic patterns of migratory or wide-ranging species, e.g. insects (Miller et al. 2011), birds (Hobson et al. 2012) and primates (Crowley 2012). However, these broad predictions are only just emerging for marine environments, due to the complexity of physical and biogeochemical processes that drive  $\delta^{13}\text{C}$  variation at the base of these food webs.

In marine systems, macroalgae form an extremely diverse group of primary producers with contrasting taxonomy, morphology and physiology (Carvalho and Eyre 2011; Keith et al. 2013) and provide an important baseline food source in food webs in a range of ecosystems (Steneck et al. 2003). Furthermore, considerable  $\delta^{13}\text{C}$  variation has been reported over temperate reefs between individuals of the same species depending on site and season (Dethier et al. 2013). As the dynamics in the  $\delta^{13}\text{C}$  of macroalgae are strongly influenced by the physiological mechanisms by which dissolved nutrients are assimilated (Raven et al. 2002), the surrounding physical and biogeochemical environment may therefore account for spatial and temporal

variations in  $\delta^{13}\text{C}$ . Moreover, these physiological responses will likely vary between taxa with different physical adaptations and life history traits (Raven et al. 2002), whereby different sources of production may be distinguished from each other.

Studies from estuaries or under controlled laboratory conditions have shown that an increase in irradiance produces a higher demand for carbon, and in some taxa the energy required to assimilate relatively  $\delta^{13}\text{C}$  enriched  $\text{HCO}_3^-$  over relatively depleted  $\text{CO}_2$  (Cornelisen et al. 2007). Water motion and salinity regulate the diffusive boundary layer, which determines the mode of carbon assimilation and level of fractionation, whereas temperature controls metabolism and photosynthesis (Booth and Beardall 1991; Cornelisen et al. 2007). As the variables of temperature, salinity, light and depth are widely available and commonly recorded (through extensive oceanographic and remote sensing data), linking these variables with autotroph- $\delta^{13}\text{C}$  could represent a convenient method for making broad predictions of spatial and temporal variations in marine environments. Furthermore, such relationships could be used to predict the response of  $\delta^{13}\text{C}$  in marine autotrophs to continued increases in seawater temperatures (e.g. Wernberg et al. 2011) and  $\text{CO}_2$  (e.g. Doney et al. 2009), and so adjust for such differences when comparing past, present and future  $\delta^{13}\text{C}$  data from temperate systems.

Research describing  $\delta^{13}\text{C}$  in marine macroalgae has primarily been focused on spatial variation (e.g. Guest et al. 2010; Vanderklift and Wernberg 2010). And those that have combined both spatial and temporal scales are often limited to only a few sampling periods (e.g. Hyndes et al. 2013; Dethier et al. 2013). Furthermore, few studies have attempted to link  $\delta^{13}\text{C}$  in marine macrophytes with their physical and biogeochemical environment. In situ environmental variables have, however, been correlated with the  $\delta^{13}\text{C}$  of phytoplankton (Rau et al. 1982; Lara et al. 2010), and even their consumers. Barnes et al. (2009) showed spatial variation in  $\delta^{13}\text{C}$  of filter-feeding scallops was strongly correlated with temperature, which was thought to be propagated through their phytoplankton diet. As a result, they were able to predict up to 79% of the spatial  $\delta^{13}\text{C}$  variation in species of fish, using a single variable model of bottom temperature. In another study by MacKenzie et al. (2011), the effect of sea surface temperature (SST) was indirectly linked to the  $\delta^{13}\text{C}$  in Atlantic salmon (again through phytoplankton) and used to determine the geographic locations of feeding grounds. The case for macroalgae is, however, very different to phytoplankton. Phytoplankton are sampled in bulk, giving a production weighted mean of  $\delta^{13}\text{C}$ , whereas macroalgae are sampled individually, and  $\delta^{13}\text{C}$  among taxa that occupy the same habitats, can have very different spatial (Vanderklift et al 2010) and temporal patterns (Dethier et al, 2013). This suggests that differences exist among

taxa in the way the environmental factors interact with their physiological mechanisms for capturing and storing carbon. Understanding these  $\delta^{13}\text{C}$ /environmental relationships could provide a better understanding of shifts in  $\delta^{13}\text{C}$  of baseline sources to more accurately interpret  $\delta^{13}\text{C}$  values in food web studies (Barnes et al. 2009).

In this study, we describe  $\delta^{13}\text{C}$  in two taxonomically contrasting macroalgae on temperate subtidal reefs over various spatial (10s m to 100 km), and temporal (weekly to seasonal) scales over a 13-month period. The kelp *Ecklonia radiata* is abundant along the southern coasts of Australia and New Zealand, and supports food webs in that region (Guest et al. 2010; Vanderklift and Wernberg 2010). Similarly, the red alga *Plocamium preissianum* represents foliose red algae, which are abundant and support food webs in temperate reef and seagrass systems (McClanahan 2008; Shepherd and Edgar 2013). Secondly, we analysed spatial and temporal  $\delta^{13}\text{C}$  variations against a suite of physical and biogeochemical variables, including those where relationships could be conveniently applied to extensive oceanographic and remote sensed data. We then used our subsequent models to project (in space) and forecast (in time) patterns in  $\delta^{13}\text{C}$  to determine if environmental variables could be used for predictions in  $\delta^{13}\text{C}$  and whether these could facilitate interpretation of food web data and generation of hypotheses.

## **2.3 Materials and methods**

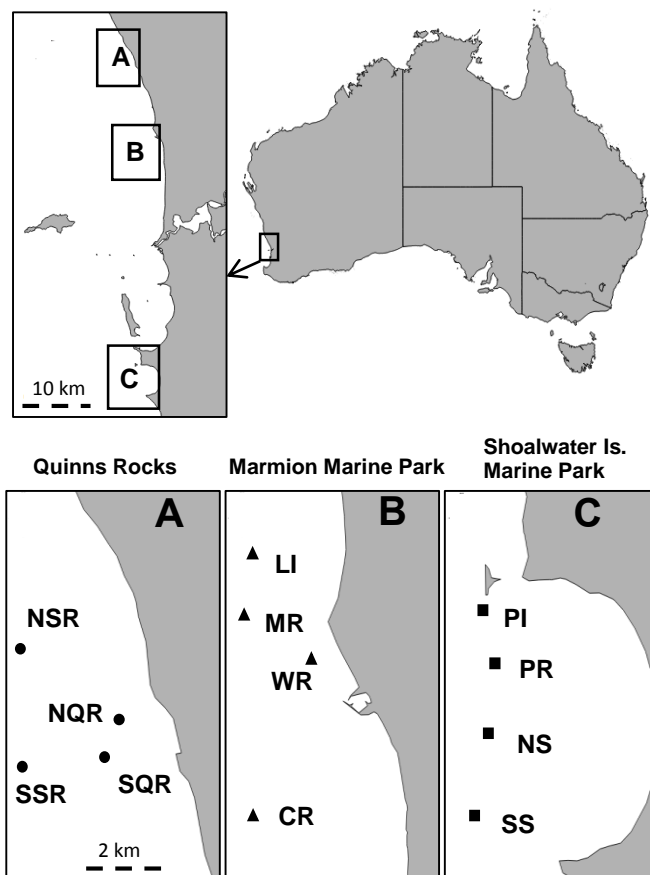
### **Study area**

Our study was conducted over subtidal reefs along the lower west coast of Australia. A characteristic feature of this coastline is the presence of limestone reefs running parallel to the shore that are exposed to prevailing south-westerly oceanic swells (Sanderson 2000). These reefs dissipate wave energy, forming gradients of water velocity from high-energy offshore reefs to protected inshore reefs. The region is also considered oligotrophic due to the Leeuwin Current that prevents the development of any major upwellings (Pearce and Griffiths 1991). The climate is Mediterranean with seawater temperatures ranging from 17°C in winter rising to 24.5°C in late summer (Smale and Wernberg 2009).

## Sampling design

Our spatial sampling was conducted on reefs in early autumn 2013 (18<sup>th</sup> - 20<sup>th</sup> March), and encompassed three spatial scales. These included three regions Quinn's Rocks (31°41'09'S, 115°40'26'E), Marmion Marine Park (31°49'13'S, 115°43'01'E) and Shoalwater Islands Marine Park (32°19'43'S, 115°41'23'E), four sites within each region, and five replicates within each site (Fig.1). Each region was separated by 10s of km (regional scale), while sites were spaced > 1 km apart (site scale), and replicates taken 10s of m apart (replicate scale). At each site, and from a depth of between 5 and 7 m, we collected by hand using SCUBA, replicate samples of two taxonomically contrasting macroalgae: the small kelp *Ecklonia radiata* (of the order Laminariales) and the red alga *Plocamium preissianum* (of the order Plocamiales). Macroalgae were collected as whole, and only mature individuals were taken (~55 cm *E. radiata*, ~20 cm *P. preissianum*), as young and old plants can vary significantly in their  $\delta^{13}\text{C}$  (Fredriksen 2003).

Our temporal sampling followed the same protocol but from just two sites within Marmion Marine Park (Little Island (LI) and Marmion Reef (MR), Fig 1). At these sites, we sampled every month over 13-months, with two additional samples collected one to two weeks apart in April and August/September 2013.



**Figure 1.** Locations of reef sites within each region of south-western Australia (from north to south). (A) Quinns Rocks: north Scarpie Reef (NSR), north Quinns Rocks (NQR), south Quinns Rocks (SQR), south Scarpie Reef (SSR). (B) Marmion Marine Park: Little Island (LI), Marmion Reef (MR), Wanneroo Reef (WR), Centaur Reef (CR). (C) Shoalwater Islands Marine Park: Penguin Island (PI), Passage Rocks (PR), north Sisters (NS), and south Sisters (SS).

## Biogeochemical data

Continuous measurements of light (as relative Lux,  $Lx/m^2$ ), temperature ( $^{\circ}C$ ) and water velocity (relative.  $m.sec^{-2}$ ) were recorded from each site throughout the study using Hobo<sup>TM</sup> data loggers (Onset computer Corporation, Bourne, Massachusetts, USA). Loggers were mounted on the flat surface of bisected buoys, which were attached to the reef (depth 5-7 m) via a 1 m tether, and oriented towards the surface. All loggers were tested and calibrated before deployment to eliminate discrepancies among them. Loggers deployed spatially (across the 12 sites) and temporally (over 13-months) were fitted and recorded data two weeks prior to the first sampling event. Two sets of loggers from our spatial design were lost (site NSR in Quinn's Rocks and site NS in Shoalwater Islands Marine Park), and were thus excluded from the

analyses. At corresponding sites and times as macroalgae, subsurface (~4 m) seawater samples were collected for salinity, and concentrations ( $\text{mg C L}^{-1}$ ) and  $\delta^{13}\text{C}$  (‰) of dissolved organic carbon (DOC) and inorganic carbon (DIC) using a messenger-activated water bottle. Duplicate subsamples (50 ml) were immediately passed through 0.2  $\mu\text{m}$  precombusted filters into vials containing saturated  $\text{HgCl}_2$  and kept chilled prior to analysis.

### **Sample processing and stable isotope analysis**

All macroalgae were measured (length mm) and areas chosen for analysis were cleaned, using a scalpel if necessary, to remove any epibionts or other contaminants. A 4 cm section of the stipe was removed from the thallus of *E. radiata*, whereas an entire section of thallus was used for *P. preissianum*. Material was dried at 60°C and homogenised to a powder using a ball and mill. All macroalgae samples were then weighed dry (~2.3 mg) into tin capsules (8 x 6 mm), then analysed for  $\delta^{13}\text{C}$  and %C using a Thermo Finnigan Flash EA 112 interfaced via a Thermo ConFlo III with a Thermo Delta V Plus IRMS. Ratios  $^{13}\text{C}/^{12}\text{C}$  are expressed as delta ( $\delta$ ) relative to the international carbon standard Pee Dee Belemnite. DOC and DIC concentrations and  $\delta^{13}\text{C}$  were measured, as described by Oakes and Eyre (2014), via continuous flow wet-oxidation isotope ratio mass spectrometry using an Aurora 1030W total organic carbon analyser coupled to a Thermo Delta V Plus Isotope Ratio Mass Spectrometer (IRMS). Sodium bicarbonate (DIC) and glucose (DOC) of known isotope composition dissolved in helium-purged milli-Q were used for drift correction and to verify concentrations and  $\delta^{13}\text{C}$  values. Reproducibility for DOC and DIC, respectively, was  $\pm 0.3 \text{ mg L}^{-1}$  and  $\pm 0.4 \text{ mg L}^{-1}$  (concentrations) and  $\pm 0.08\text{‰}$  and  $\pm 0.10\text{‰}$  ( $\delta^{13}\text{C}$ ).

### **Data analysis**

Our initial analysis examined how  $\delta^{13}\text{C}$  in each taxa varied across different scales of space and time. The magnitude of variation in  $\delta^{13}\text{C}$  at each spatial and temporal scale (i.e. to determine the variance components) was determined using the permutational analysis of variance (PERMANOVA+) package in PRIMER v6 (Plymouth Routines in Multivariate Research), based on Euclidian distances. A PERMANOVA for each taxon was conducted using three different designs. The patterns in spatial variation were separated into two factors, namely region and site nested in region. The patterns in temporal variation were separated into: (a) a broad temporal design with month nested in season, including a spatial factor (site) nested in

month; and (b) a contracted temporal design with weeks nested into months. Variance component tests are recognised as the best estimates of the contribution of a given factor to variability in the response variable (Graham and Edwards 2001).

### **Environmental variables**

Our physical and biogeochemical variables (here after termed environmental variables) included temperature and water velocity (rel. m.sec<sup>-2</sup>) (from data loggers attached to the reef ~5 m depth), and mean values over the previous 30-days (prior to a sampling event) were linked to the  $\delta^{13}\text{C}$  signatures. For light, mean daily lux (Lx/m<sup>2</sup>) from only the first 10 days after deployment or sampling (when sensors were cleaned) were used in the analysis due to fouling. Data on the concentrations and  $\delta^{13}\text{C}\%$  of DOC and DIC, and salinity, were based on the time of sampling. For *E. radiata* only, a monthly estimate of growth (mg.g<sup>-1</sup>.day<sup>-1</sup>) over 2-years from Marmion Marine Park; taken from Kirkman (1989) was added to the dataset as a potential correlate. We applied principle component analysis (PRIMER v6) on normalised environmental data to identify patterns between sites, regions, months and seasons.

Multiple linear regression analyses were used to select predictor variables, from all physical and biogeochemical variables, that accounted for spatial, temporal and combined spatial and temporal variability in  $\delta^{13}\text{C}$  for each production source, using distance-based linear models (DistLM) in PERMANOVA+ (add-on in PRIMER v6). DistLM does not assume normality of data distributions as hypotheses are tested by permutations (Anderson 2001). It also controls for Type I and II errors that may occur through multiple testing using conventional (parametric) data (Ernst 2004). A “step-wise” procedure using the “Bayesian Information Criterion” (BIC) was used to determine models that best fit the data with fewest predictor variables. We included marginal tests to identify individual variables (e.g. independent of other variables) that could be used to predict  $\delta^{13}\text{C}$ . We do not suggest particular variables or models are necessarily causative. These may be variables involved indirectly or simply acting as proxies for other underlying causes. Conventional multiple and simple linear regressions were conducted to derive regression equations for the models identified in our DistLM’s (where assumptions of linear regression were met).

We projected isoscapes (for summer and winter) and produced monthly forecasts for  $\delta^{13}\text{C}$  in *P. preissianum* using our linear model (for the combined spatial and temporal dataset) with



corresponding oceanographic data from the World Ocean Atlas 2009 (NOAA, [www.nodc.noaa.gov/OC5/WOA09](http://www.nodc.noaa.gov/OC5/WOA09)). The World Ocean Atlas 2009 is a set of objectively analysed ( $1^\circ$ grid) fields of *in situ* oceanographic data. Isoscapes were created using Ocean Data View (ODV) version 4 (Schlitzer 2002), and are displayed as colour-shaded maps of contoured data, setup through the Data Interpolating Variational Analysis (DIVA) feature in ODV. We confined our isoscapes to only the ranges of oceanographic data experienced in our own spatial and temporal datasets, and the known distribution for *P. preissianum*, limiting our projections to temperate Australasia. Our temporal forecast model of  $\delta^{13}\text{C}$  variation in *P. preissianum*, representing variation from the spatial margins of our temporal sampling region (e.g. south-western Australia), are simply plotted as predicted  $\delta^{13}\text{C}$  (‰) ( $\pm\text{SE}$ ) for each month.

## 2.3 Results

### Spatial and temporal $\delta^{13}\text{C}$ variation

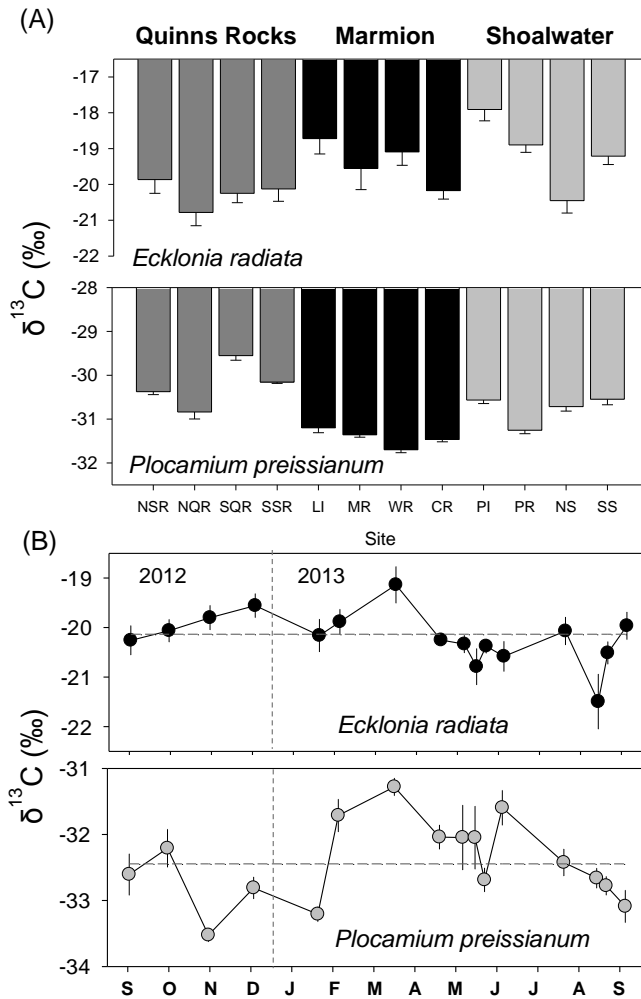
*E. radiata* displayed significant variation in  $\delta^{13}\text{C}$  across sites ( $p = 0.01$ ), but not regions ( $p = 0.13$ ), with the greatest source of variation at the replicate (residual, 50%) and site (33%) levels (Table 1, Figure 2A). Mean  $\delta^{13}\text{C}$  ranged from -20.8 to -17.9‰ across all sites and regions. In terms of broader temporal variation over 13-months of sampling at reefs in Marmion Marine Park,  $\delta^{13}\text{C}$  of *E. radiata* varied significantly across months ( $p = 0.04$ ) but not across seasons ( $p = 0.17$ ) (Table 1, Figure 2B). However, the greatest source of variation occurred at the replicate (residual) level (70%) and then at the month level (17%). The mean  $\delta^{13}\text{C}$  ranged from -21.6 to -19.1‰, with the values being slightly more enriched (relative to the overall temporal mean) from November to March, and slightly depleted from April to August (Figure 2B). At the finer temporal scale,  $\delta^{13}\text{C}$  of *E. radiata* varied significantly across weeks within months, but not between months, while the greatest source of variation was again at the replicate (residual) level (79%) (Table 1).

The spatial variation in *P. preissianum* displayed significant variation across sites ( $p = 0.01$ ) and regions ( $p = 0.01$ ), with the greatest source of variation at the region (45%) followed by replicate (34%) and site (21%) levels (Table 1, Figure 2A). Mean  $\delta^{13}\text{C}$  ranged from -29.5 to -31.7‰ across all sites and regions. In terms of broader temporal variation,  $\delta^{13}\text{C}$  of *P. preissianum* varied significantly across months ( $p = 0.01$ ) but not across seasons, while the

greatest source of variation occurred at the replicate (45%) followed by month (32%) level. Mean  $\delta^{13}\text{C}$  ranged from -31.3 to -33.5‰, and values were at their most enriched between February and May, and most depleted between November and January. At the finer temporal scale,  $\delta^{13}\text{C}$  variation was not significant for either weeks within months (although close to significance,  $p = 0.06$ ) or between months, reflecting the greatest source of variation among replicates (59%) (Table 1, Figure 2B).

**Table 1.** Variance components estimates (and % variance) for the  $\delta^{13}\text{C}$  in macroalgae from: (A) a spatial design, with reefs nested in location; (B) a broad temporal design, with months nested in seasons and sites nested in month; and (C) a constricted temporal design, with weeks nested in month. Results were obtained from Permutational analysis of variance (PERMANOVA) based on Euclidian distance. Negative values are treated as zero.

<i>Ecklonia radiata</i>					
Design	Factors	df	MS	P	Var. (%)
A	Region	2	7.08	0.13	0.22 (17.1)
	Site	4	2.74	0.01	0.42 (33.1)
	Residuals	48	0.63		0.63 (49.8)
B	Season	3	3.53	0.17	0.05 (5.7)
	Month	8	1.91	0.04	0.15 (16.5)
	Site	12	0.65	0.53	-0.01 (0)
	Residuals	112	0.70		0.70 (69.8)
C	Month	1	0.41	0.72	-0.05 (0)
	Week	4	1.29	0.05	0.13 (20.8)
	Residuals	34	0.49		0.49 (79.2)
<i>Plocamium preissianum</i>					
Design	Factors	df	MS	P	Var. (%)
A	Region	2	6.49	0.01	0.29 (44.9)
	Site	9	0.89	0.01	0.14 (21.3)
	Residuals	48	0.22		0.22 (33.9)
B	Season	3	5.32	0.35	0.03(4.0)
	Month	8	4.29	0.01	0.28 (32.3)
	Site	12	1.28	0.01	0.16 (18.6)
	Residuals	113	0.39		0.39 (45.0)
C	Month	1	4.84	0.13	0.21 (27.1)
	Week	4	1.12	0.06	0.11 (14.0)
	Residuals	33	0.46		0.46 (59.0)

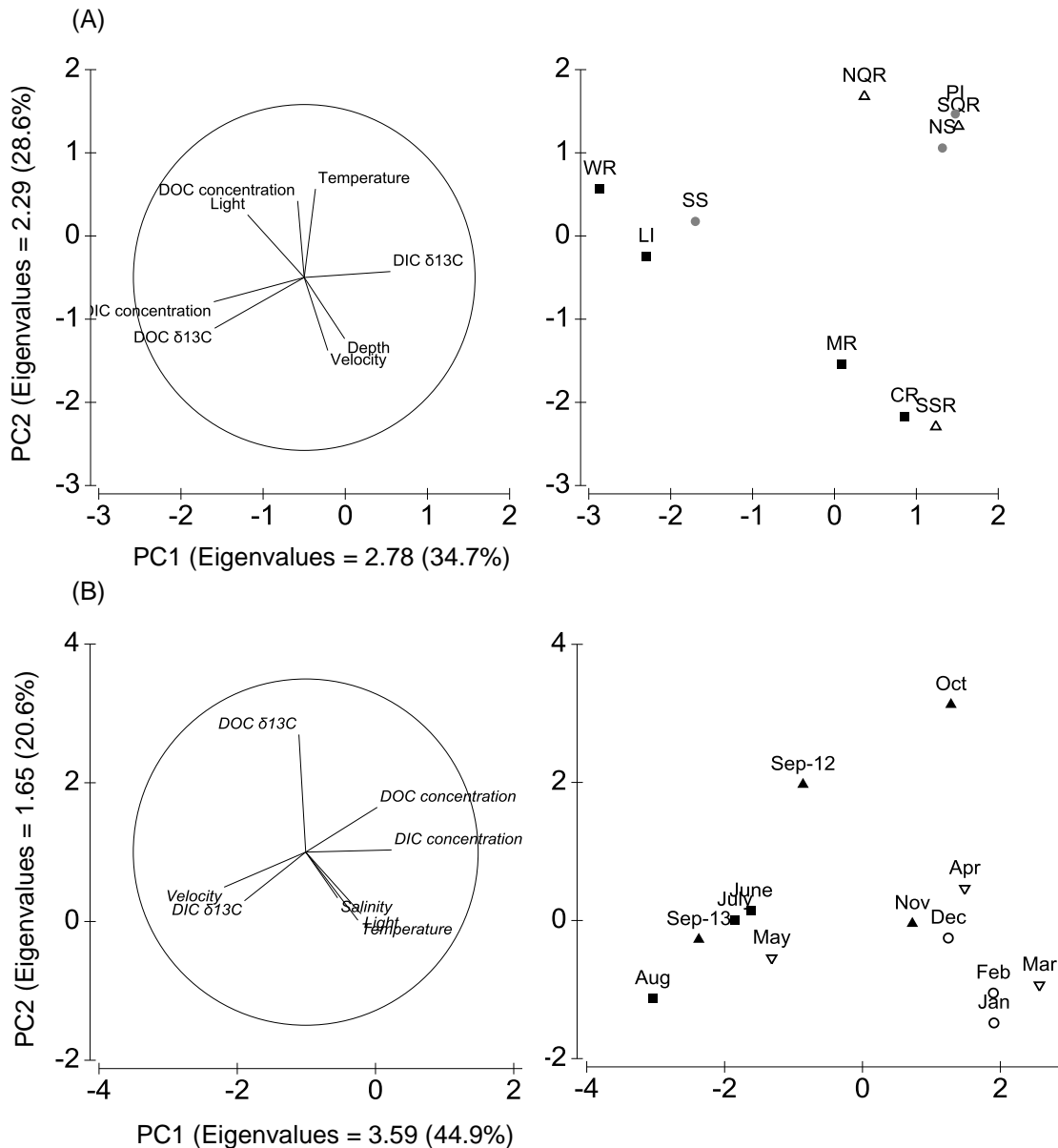


**Figure 2.** Means ( $\pm$ SE) of  $\delta^{13}\text{C}$  in the brown alga *Ecklonia radiata* and red alga *Plocamium preissianum* at: (A) four sites within each region (see Fig 1 for locations of sites); and (B) over 13-months from Marmion Marine Park, where sampling points are spread by Julian Day, but ticks labelled by month. Dashed lines represent mean  $\delta^{13}\text{C}$  in each source.

### Principal component analysis of environmental data

The spatial environmental data fell into distinctive groups of sites, irrespective of region (Figure 3A). The first axis distinguished WR, LI (Marmion) and SS (Shoalwater) from the remaining sites, where there was a depletion in  $\delta^{13}\text{C}$ -DIC, but an increase in DIC concentration and an enrichment in  $\delta^{13}\text{C}$ -DOC. The second axis distinguished three groups, with MR, CR (Marmion) and SSR (Shoalwater) experiencing cooler temperatures and higher water velocities relative to NQR, SQR (Quinns Rocks), PI and PR (Shoalwater). The sites WR, LI (Marmion) and SS (Shoalwater) were at an intermediate level. The temporal environmental data were less

defined, but the first axis showed a division between the months of May to August (and Sep, 2013) with higher water velocities and decreased DIC concentration relative to the months of Nov to March (Figure 3B). The second axis distinguished Sep, 2012 and October from the remaining months by way of an enrichment in  $\delta^{13}\text{C}$ -DOC.



**Figure 3.** Principle component analysis (PCA) using normalised means of environmental data measured at: (A) 10 sites across three regions in south-western Australia (see Fig 1 for locations of sites); and (B) over 13-months from within Marmion Marine Park from September 2012 to September 2013. Months follow austral seasons, e.g. autumn (March – May), winter (June – August), spring (September – November), and summer (December – February). Eigenvalues for each principle component are given in their respective axis, with the circle overlay scaled to the maximum effect size. Variables (left panels) and sites/dates (right panels) are separated for clarity.

## Independent environmental correlates of $\delta^{13}\text{C}$

Based on marginal tests from DistLM, significant spatial correlations between production sources were highly disparate. *E. radiata*  $\delta^{13}\text{C}$  was not correlated with any variables, whereas *P. preissianum*  $\delta^{13}\text{C}$  showed significant correlations with DIC  $\delta^{13}\text{C}$  (positive) and DOC  $\delta^{13}\text{C}$  (negative) (Table 2). For the temporal dataset, *E. radiata* showed correlations with water velocity and DIC  $\delta^{13}\text{C}$  (both negative,  $p < 0.05$ ), and highly significant correlations with DOC and DIC concentration (both positive,  $p < 0.01$ ) (Table 2). *P. preissianum* was correlated with DIC  $\delta^{13}\text{C}$  (positive,  $p < 0.05$ ), and highly significantly correlated with temperature (positive,  $p < 0.01$ ). For the combined spatial and temporal datasets, *E. radiata* positively correlated with light, and negatively with DIC  $\delta^{13}\text{C}$  ( $p < 0.05$ ), and highly significantly correlated with DIC concentration (negative,  $p < 0.01$ ). *P. preissianum* was significantly correlated with light ( $p < 0.05$ ), and highly significantly correlated with temperature (positive,  $p < 0.01$ ).

**Table 2.** Correlations between the  $\delta^{13}\text{C}$  in macroalgae and independent environmental variables. Results are separated by spatial, temporal and combined spatial and temporal datasets, and were obtained from Marginal tests within distance based linear models (DistLM) based on Euclidian distance. Only significant results are displayed.

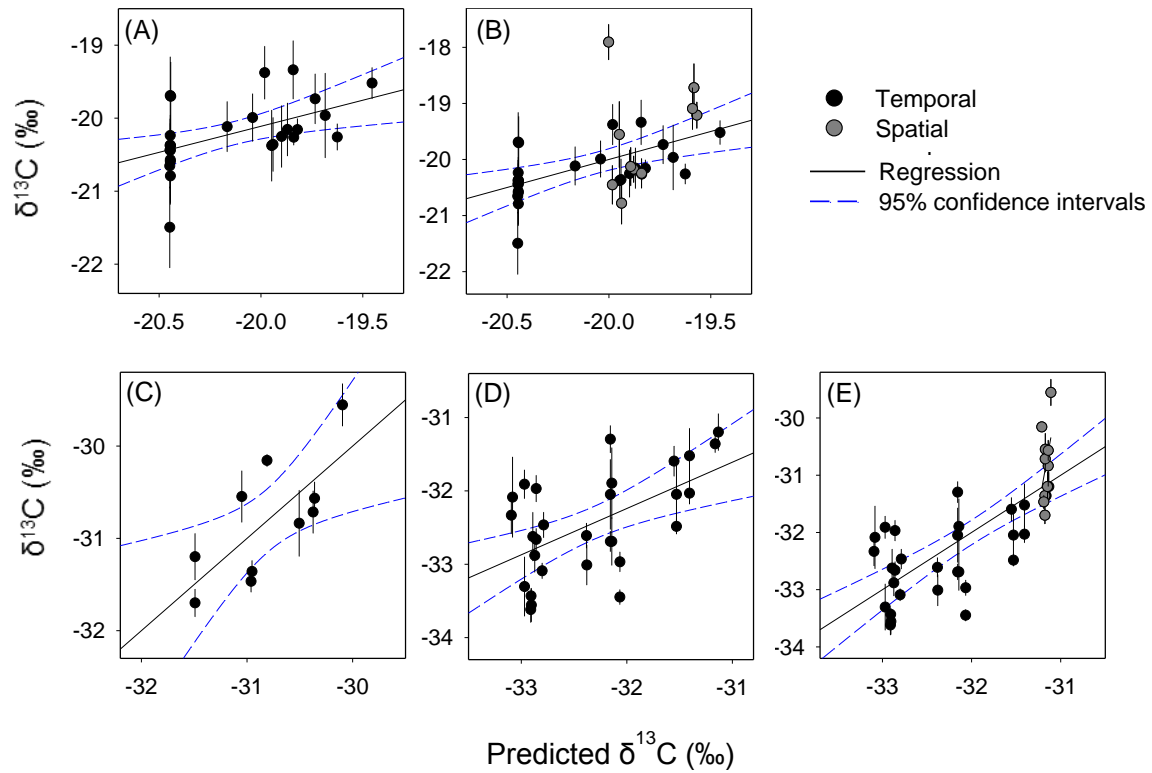
<i>Ecklonia radiata</i> ( $R^2$ )			
Variables	Spatial	Temporal	Combined
Velocity (rel. m.sec <sup>-2</sup> )		0.16	
Light (Lx/m <sup>2</sup> )			0.12
DIC/ $\delta^{13}\text{C}$ (‰)		0.22	0.12
DIC con (mg C L <sup>-1</sup> )		0.33	0.25
DOC con (mg C L <sup>-1</sup> )		0.23	
<i>Plocamium preissianum</i> ( $R^2$ )			
Variables	Spatial	Temporal	Combined
Light (Lx/m <sup>2</sup> )			0.18
Temperature (°C)		0.32	0.55
DIC/ $\delta^{13}\text{C}$ (‰)	0.46	0.15	
DOC/ $\delta^{13}\text{C}$ (‰)	0.53		

## Environmental models of $\delta^{13}\text{C}$ in macroalgae

We carried out DistLM to identify the best combination of variables in explaining  $\delta^{13}\text{C}$  in each production source, so as to avoid possible Type I and II errors in conducting multiple analyses (Table 3a). Using these models, we applied conventional multiple and simple linear regressions to derive equations that could be used to predict  $\delta^{13}\text{C}$  based on these relationships (that yielded identical  $r^2$  values as those from each DistLM) (Table 3b). Our spatial analysis for  $\delta^{13}\text{C}$  in *E. radiata* did not return any significant models explaining variability, whereas the model output for the temporal dataset yielded the combination of DIC  $\delta^{13}\text{C}$  and concentration (Adj.  $R^2 = 0.35$ , Figure 4A, regression equation in Table 3). The combined dataset returned DIC concentration alone but predicted less of the  $\delta^{13}\text{C}$  variation (Adj.  $R^2 = 0.23$ ). In comparison, a far greater amount of variation in  $\delta^{13}\text{C}$  for *P. preissianum* was explained in each dataset. The spatial model returned a single explanatory variable, DOC  $\delta^{13}\text{C}$  (Adj.  $R^2 = 0.47$ ). The temporal dataset yielded a significant model combining light, temperature and DIC  $\delta^{13}\text{C}$  (Adj.  $R^2 = 0.51$ ), whilst the combined dataset identified temperature alone in significantly explaining  $\delta^{13}\text{C}$  variation (Adj.  $R^2 = 0.55$ , Figure 4, Table 3).

**Table 3.** Results of (A) distance based linear models (DistLM), using Bayesian Information Criterion (BIC) (Schwartz 1978), to identify the physical and biogeochemical variables that best explain variation in the  $\delta^{13}\text{C}$  in macroalgae; and (B) regression models based on the identified models in A, including the more conservative adjusted  $r^2$  values. Different models were used for spatial, temporal and combined spatial and temporal designs.

(A)	Selected model			
Design	<i>Ecklonia radiata</i>	Adj. $r^2$ (%)	$p$	BIC
Temporal	$\delta^{13}\text{C}$ -DIC, and DIC con.	35.1	0.001	-42.0
Combined	DIC con.	23.1	0.002	-36.5
	<i>Plocamium preissianum</i>			
Spatial	$\delta^{13}\text{C}$ -DOC	47.2	0.019	-12.6
Temporal	$\delta^{13}\text{C}$ -DIC, temperature, and light	50.6	0.001	-31.8
Combined	Temperature	55.4	0.001	-23.3
(B)	Regression equation			
Design	<i>Ecklonia radiata</i>	Adj. $r^2$ (%)	$p$	SE
Temporal	$\delta^{13}\text{C} = -20.1 - 0.41 \times \delta^{13}\text{C}\text{-DIC} + 0.02 \times \text{DIC con.}$	35.1	0.001	0.43
Combined	$\delta^{13}\text{C} = -20.5 + 0.03 \times \text{DIC con.}$	23.1	0.002	0.57
	<i>Plocamium preissianum</i>			
Spatial	$\delta^{13}\text{C} = -36.0 + 0.21 \times \delta^{13}\text{C}\text{-DOC}$	47.2	0.016	0.48
Temporal	$\delta^{13}\text{C} = -35.8 - 0.63 \times \delta^{13}\text{C}\text{-DIC} + 0.2 \times \text{temperature} - 0.002 \times$	50.6	0.001	0.49
Combined	$\delta^{13}\text{C} = -38.4 + 0.3 \times \text{temperature}$	55.4	0.001	0.66

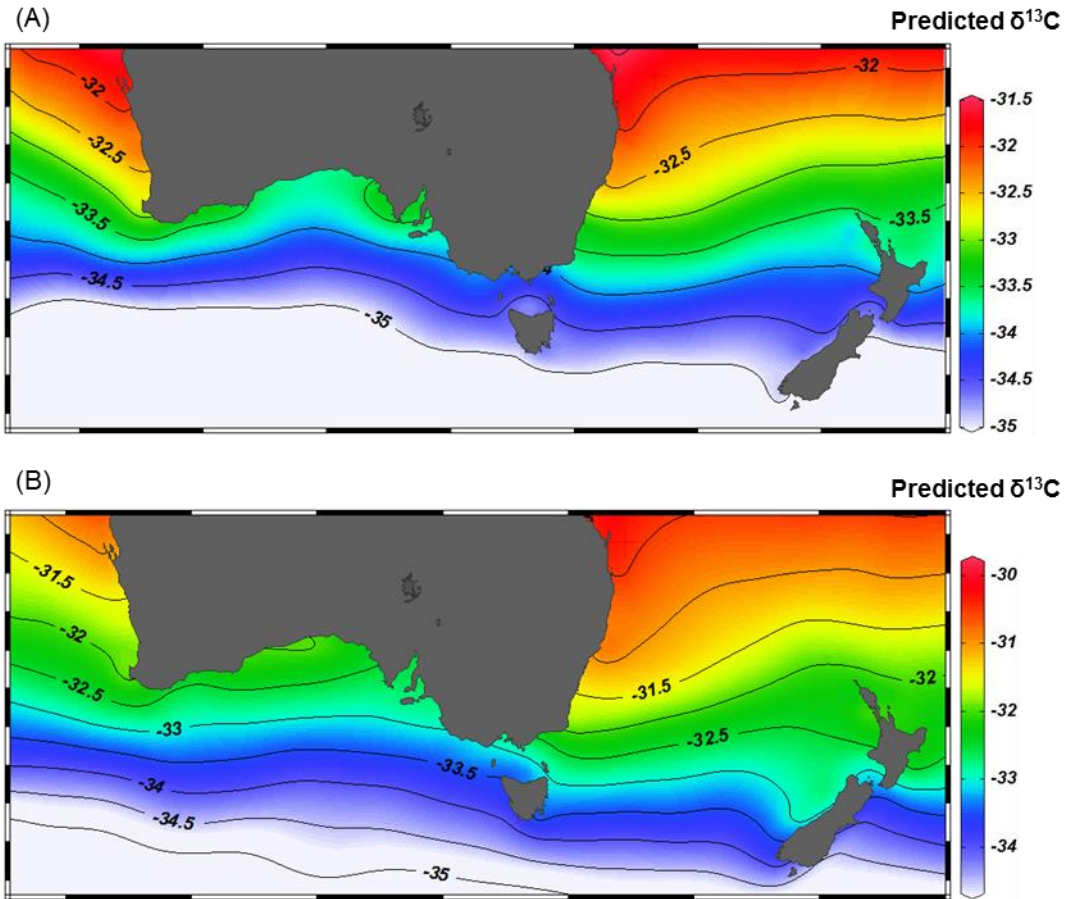


**Figure 4.** Relationship between  $\delta^{13}\text{C}$  in the brown alga *Ecklonia radiata* and red alga *Plocamium preissianum* (from spatial, temporal and combined datasets) vs. predicted ratios from simple or multiple regressions. **E. radiata:** (A) (temporal) vs.  $\delta^{13}\text{C}$ -DIC, DIC con; (B) (combined) vs. DIC con. **P. preissianum:** (C) (spatial) vs.  $\delta^{13}\text{C}$ -DOC; (D) (temporal) vs. temperature,  $\delta^{13}\text{C}$ -DIC, light; (E) (combined) vs. temperature. Dashed lines represent 95% confidence intervals.

### Spatial projections and temporal forecasts

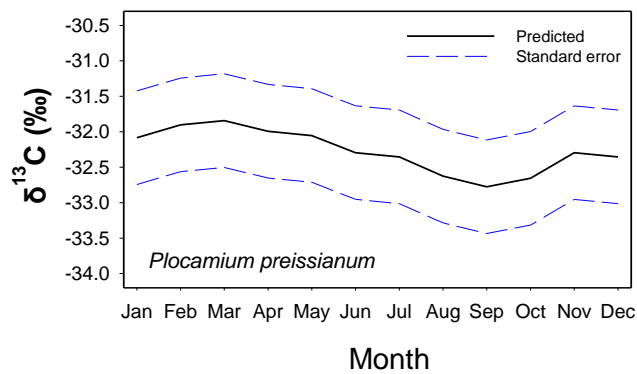
Predicted  $\delta^{13}\text{C}$  for *P. preissianum* was calculated for temperate Australasia by applying our combined spatial and temporal regression model to mean seasonal temperature data for summer and winter (at a 5 m depth horizon) from the World Ocean Atlas 2009 (NOAA) (Figure 5). *E. radiata* was excluded from this analysis as its predictive models included variables that were not readily obtainable. For *P. preissianum*,  $\delta^{13}\text{C}$  isoscapes show contours following clear latitudinal patterns of relatively depleted values in the south (e.g. around the South Island of New Zealand and Tasmania), and becoming progressively enriched towards the north for both summer and winter. The coasts of mid to south western and mid to north eastern Australia are occupied by a relatively narrow  $\delta^{13}\text{C}$  range (between -31.0 to -32.5 in summer and -32 to -33.5 in winter).





**Figure 5.** Predicted variation in the  $\delta^{13}\text{C}$  of the red alga *Plocamium preissianum* across temperate Australasia (at a 5 m depth horizon) for (A) winter; and (B) summer. Contours are extended away from coastlines to clearly illustrate latitudinal patterns. Ratios based on a significant linear relationship with temperature (standard error = 0.66‰).

A monthly forecast of *P. preissianum*  $\delta^{13}\text{C}$  was calculated by applying our model to mean SST data for south-western Australia and is therefore restricted to our study region (Figure 6). Our temporal forecast for  $\delta^{13}\text{C}$  in *P. preissianum* exhibited more enriched values in the late Austral summer and early autumn (e.g. from January to May) and relatively depleted values through winter and spring.



**Figure 6.** A predicted forecast of temporal variation in the  $\delta^{13}\text{C}$  of the red alga *Plocamium preissianum* for south-western Australia. Ratios based on a significant linear relationships with temperature (standard error = 0.66‰).

We looked for further evidence of a temperature/ $\delta^{13}\text{C}$  effect in *P. preissianum*, but this was limited to a single record. Using temperature (17.3°C) data taken from the region and month of sampling (Coobowie Bay, South Australia, July 19997 (Raven et al., 2002) the model predicted a value of -33.20 ( $\pm 0.83$ ), compared to the actual value of -33.74 (a difference of 0.54). As this single analysis was extremely limited, we focused on another species of the same genus, *Plocamium cartilagineum*, for which the greatest amount of  $\delta^{13}\text{C}$  data was available, and is referred to in the discussion.

## 2.4 Discussion

### Spatial and temporal $\delta^{13}\text{C}$ variation in macroalgae

Our observed spatial patterns for  $\delta^{13}\text{C}$  in *E. radiata* follow those found by other studies of kelp (e.g. Simenstad et al. 1993; Guest et al. 2010; Vanderklift and Wernberg 2010), where  $\delta^{13}\text{C}$  variation was at least as significant among sites (e.g. between 100 to 1000 m) as among regions (100s km apart), and variation among replicates was high. In the red alga *P. preissianum*, variation was also significant among sites, but contrasted with the brown alga *E. radiata* at the regional scale, where the greatest source of variation was found. Significant variation between equivalent scales (as our regions) have been shown in other Rhodophyta, e.g. *Neorhodomela* (Dethier et al. 2013), *Metagoniolithon* (Hyndes et al., 2013), and in particular *Rhodymenia sonderi* where variance was significant and variance components were high at both regional

and site scales (Vanderklift and Wernberg 2010). However, other Rhodophyta have not shown these  $\delta^{13}\text{C}$  patterns, e.g. *Amphiroa* and *Laurencia* (Hyndes et al. 2013).

Our examination of temporal variability in the  $\delta^{13}\text{C}$  *E. radiata* fell within a similar range to those of our spatial dataset, and seasonal variation was not significant, but both monthly and weekly variation was, despite residuals being the greatest source of variation. Similarly,  $\delta^{13}\text{C}$  variation in *P. preissianum* occurred among months but not among seasons. These results, however, may be deceptive and an effect of how we partition seasons. For example, our principal component analysis of the environmental data showed two distinctive periods, from the start of winter to mid-spring (e.g. May to September) and from late spring to early autumn (e.g. November to March). Regardless, our results show temporal variation in  $\delta^{13}\text{C}$  for two divergent macroalgae species. Our results concur with other studies of marine macroalgae, where substantial and significant  $\delta^{13}\text{C}$  variation has been shown for a range of taxa, among months (Wefer and Killingley 1986; Hill et al. 2008), seasons (Vizzini and Mazzola 2003; Dethier et al. 2013), and among years (Hill et al. 2006).

Inferences about the sources of energy supporting food webs through  $\delta^{13}\text{C}$  analyses can be undermined if spatial and temporal variation is unaccounted for. The high spatial and temporal variation, and the divergence in the sources of variability among taxa, may mask or distort subsequent interpretations and our ability to gain an accurate understanding of food webs, and the sources of energy supporting consumers. However, by using  $\delta^{13}\text{C}$  variation to our advantage, by linking variation to factors of the surrounding physical and biogeochemical environment, we show that in some taxa, spatial and temporal patterns can be broadly predicted.

## **Environmental correlates**

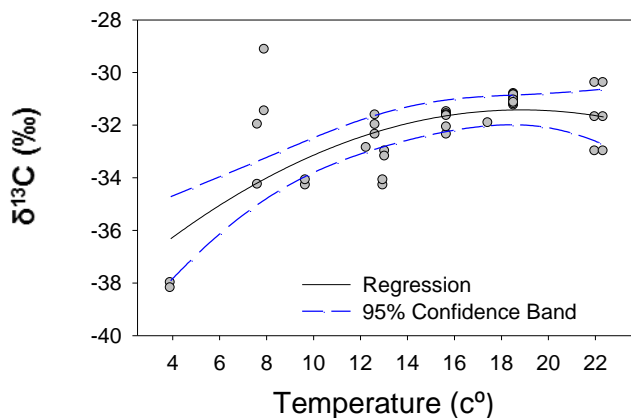
Among the different forms of DIC, only  $\text{HCO}_3^-$  (relatively  $\delta^{13}\text{C}$ -enriched) and  $\text{CO}_2$  (relatively  $\delta^{13}\text{C}$ -depleted) can be assimilated by macroalgae through photosynthesis (Raven et al. 2002). However, *P. preissianum* is within a group of taxa that are thought to lack the ability to absorb  $\text{HCO}_3^-$ . Therefore, the relationship with DIC- $\delta^{13}\text{C}$  must be the result of  $\delta^{13}\text{C}$  fluctuations in the  $\text{CO}_2$  component of the DIC pool. The correlation between DOC concentrations and  $\delta^{13}\text{C}$  in *E. radiata* is interesting, as the release of DOC by kelps has been shown to follow growth and photosynthetic rates (Wada et al. 2007), and therefore may be the result of *E. radiata* altering

the DOC of the local environment. The strong correlation between  $\delta^{13}\text{C}$ -DOC and  $\delta^{13}\text{C}$  in *P. preissianum* (Adj.  $r^2 = 0.47$ ) suggests that *P. preissianum* is assimilating dissolved organic carbon. DOC can be important to the growth of phytoplankton by interacting with bioavailable iron (Lee et al. 2009), and may initiate a change in cell metabolism to benefit from high DOC concentrations (Znachor and Nedoma 2009). Smith and Penhale (1980) found that uptake of DOC in a seagrass (e.g. *Zostera* sp.) and its epiphytes to be linear with no saturation point, whereas, Oakes et al. (2011) provided evidence that the macroalga *Caulerpa* sp., assimilated leached DOC from its own detritus. Given the high biomass of *E. radiata* at our study sites, DOC released from kelp detritus may be a valuable source of carbon for *P. preissianum*.

Light was also shown to be a correlate of  $\delta^{13}\text{C}$  in both taxa (as an independent variable in *E. radiata*, and within the temporal model in *P. preissianum*). Irradiance is directly linked to macroalgal growth, which in part determines fractionation of  $\delta^{13}\text{C}$  in macroalgae (e.g. *Undaria* sp., Carvalho et al. 2009). Also, for *E. radiata*, the assimilation of  $\text{HCO}_3$  over  $\text{CO}_2$  requires energy through irradiance (Cornelisen et al. 2007). The result of water velocity correlating with  $\delta^{13}\text{C}$  in *E. radiata* (as an independent variable), but not with *P. preissianum*, is likely related to morphology between the taxa. *E. radiata* is a relatively large canopy forming species, growing to around 85 cm in height (Wernberg et al. 2003), and is therefore far more exposed to water motion than *P. preissianum*, which forms part of the understory and grows to around 20 cm in length.

Unlike *E. radiata*,  $\delta^{13}\text{C}$  in *P. preissianum* was highly and positively correlated with water temperature within both the temporal and combined datasets. The temperature range over the study period was high compared to that over the entire study area ( $6.7^\circ\text{C}$  vs.  $0.34^\circ\text{C}$ ), which is likely to at least partly explain the significant relationships using temporal and combined data compared to the spatial data alone. However, temperature itself may not be directly driving  $\delta^{13}\text{C}$  variability in *P. preissianum*, but instead may act as a proxy for a higher uptake of  $\text{CO}_2$  (over  $\text{HCO}_3$ ). For example, phytoplankton can display an increase in  $\delta^{13}\text{C}$  with increasing temperature (Sackett et al. 1965; Fontugne and Duplessy 1981; Rau et al. 1989; MacKenzie et al. 2011), but this is likely to occur through phytoplankton utilising carbon from the available  $\text{CO}_2$  pool, which generally increases with decreasing SST and increasing latitudes (Francois et al. 1993). Because phytoplankton preferentially incorporates the lighter over the heavier isotope (Goericke and Fry 1994), colder waters provide a larger source of the lighter isotope through the larger  $\text{CO}_2$  pool, resulting in lower  $\delta^{13}\text{C}$  values in the phytoplankton. If the same applies to *P. preissianum*, we would expect to see similar latitudinal patterns in  $\delta^{13}\text{C}$  with

temperature. We looked for evidence of this in *P. preissianum*, but as only a single published record exists for this species (Raven et al. 2002), we focused on the congeneric *P. cartilagineum*, for which the greatest amount of published  $\delta^{13}\text{C}$  data was available. We extracted only  $\delta^{13}\text{C}$  data from temperate regions worldwide, and superimposed mean SST data for each sampling time and location (see Appendices Table A1). As with *P. preissianum*,  $\delta^{13}\text{C}$  in *P. cartilagineum* showed a strong significant relationship with temperature ( $p < 0.001$ ,  $R^2 = 0.51$ ), but this was a polynomial rather than linear relationship (Figure 7). The temperate distribution of *P. cartilagineum* is worldwide, and therefore this species experiences a far greater range of temperatures than *P. preissianum*, and appears to have an upper limit to its  $\delta^{13}\text{C}$ . Whether the influence of temperature on the  $\delta^{13}\text{C}$  in *Plocamium* is direct or acting as a proxy for  $\text{CO}_2$ , is beyond the scope the study, but our data show a consistent and significant correlation among two species from this genus.



**Figure 7.** The relationship between temperature and  $\delta^{13}\text{C}$  in *Plocamium cartilagineum*.

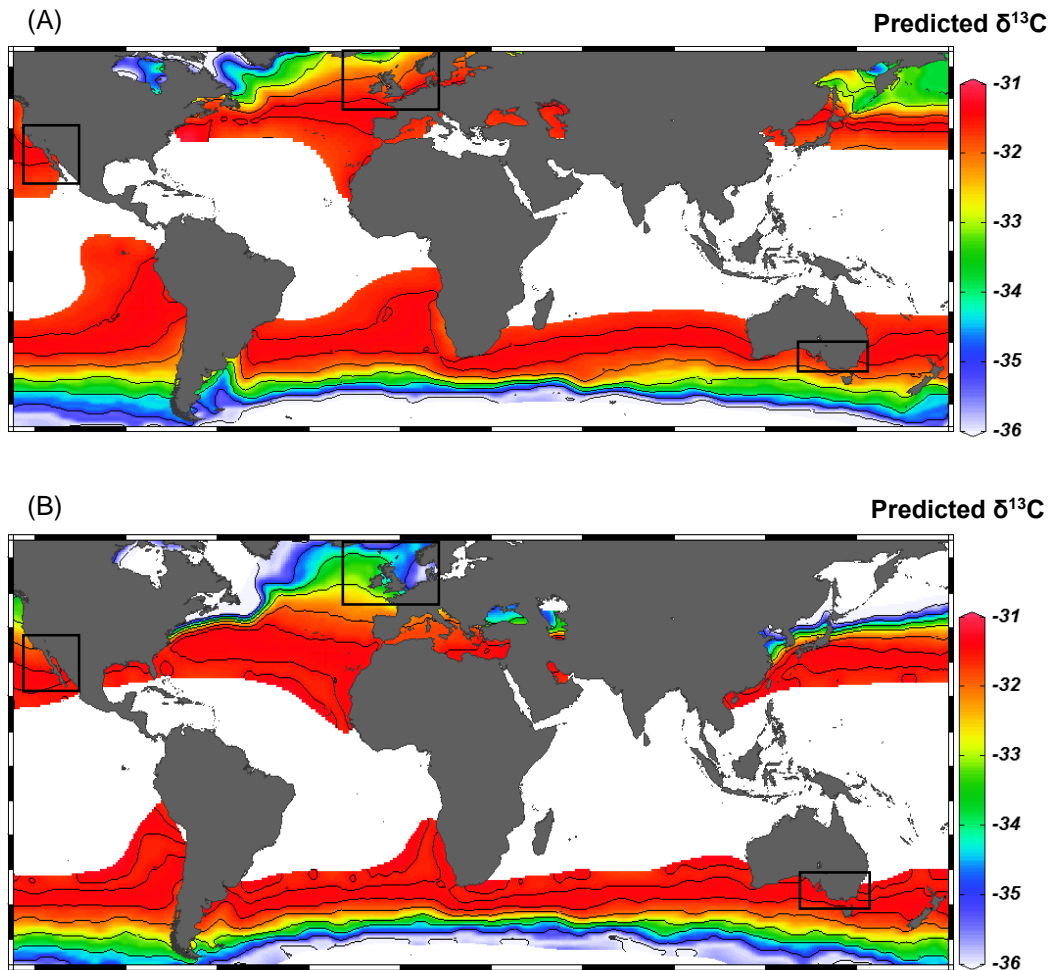
In *E. radiata*, other factors could be more closely related than what we observed. Our environmental data were recorded at the site, not replicate level, where most of the variation in  $\delta^{13}\text{C}$  of *E. radiata* was recorded. At the replicate level, light, water velocity and DIC/DOC availability may not be consistent, as neighbouring kelps could shade light (e.g. Wood 1987) and deflect hydrodynamic forces and uptake of nutrients, as noted by Simenstad et al. (1993). Therefore, possible environmental drivers of  $\delta^{13}\text{C}$  at the individual level may have masked these effects. We were, however, surprised that temperature did not show a relationship in *E. radiata*, given that temperature would be consistent between individual kelps, and temperature,

like irradiance, regulates growth in kelp (e.g. Bearham et al. 2013), and other macroalgae (Bischoff-Basman and Wiencke 1996), subsequently affecting fractionation of  $\delta^{13}\text{C}$  during photosynthesis (Raven et al. 2002; Carvalho 2010), and respiration (Carvalho and Eyre 2011). We therefore took the added step of investigating the effect of temperature on  $\delta^{13}\text{C}$  at a far broader range of values than those of our own sampling. We did this by obtaining  $\delta^{13}\text{C}$  data of *E. radiata* from studies across temperate Australia (from Western Australia to Tasmania), and applied these to mean sea surface temperature data (extracted from: Australian Navy Metrology, <http://www.metoc.gov.au>) in a simple linear regression (Table A2). The result was non-significant ( $p = 0.29$ ), showing that even at the continental level, no such relationship exists.

### **Spatial projections and temporal forecasts of $\delta^{13}\text{C}$ in *P. preissianum***

The significant relationship between water temperature and  $\delta^{13}\text{C}$  in *P. preissianum* has allowed us to produce spatial projections and temporal forecasts of  $\delta^{13}\text{C}$  in *P. preissianum* which could provide a valuable tool for a desktop approach to formulate hypotheses about the variation of  $\delta^{13}\text{C}$  in this species and its trophic interactions where current data do not exist. For example, while the mean projected  $\delta^{13}\text{C}$  for *P. preissianum* in the study region varied by only  $\sim 1\text{‰}$  over a 12-month period, this difference is likely to be more pronounced in regions with greater temperature ranges over the year. Our approach, therefore, would allow for predictions of changes in  $\delta^{13}\text{C}$  of the macroalga and its possible influence on  $\delta^{13}\text{C}$  in food webs of those regions. In comparison, our spatial predictions for  $\delta^{13}\text{C}$  in *P. preissianum* suggest a broad latitudinal shift of  $\sim 3\text{‰}$  over 2000 km, which far exceeds the typical 0-0.5‰ fractionation levels applied to food web studies (Jennings et al. 1997). This is highlighted further through applying our polynomial model between temperature and  $\delta^{13}\text{C}$  for *P. preissianum* to SST data and published  $\delta^{13}\text{C}$  data for *P. cartilagineum* (Figure 7), which has a worldwide distribution in temperate regions. Isoscapes of predicted  $\delta^{13}\text{C}$  for *P. cartilagineum* in summer and winter showed a distinct latitudinal gradient across temperate regions worldwide ( $\sim 2.5\text{‰}$  over 1000 km) (Figure 8). We recognise that obtaining evidence for and against these broad projections requires a greater amount of  $\delta^{13}\text{C}$  data than is currently available, and neglect the complexity of other factors involved. Therefore, we do not suggest that these models predict  $\delta^{13}\text{C}$  *per se*, but rather they predict the effect of temperature, excluding other factors. Further, we do not recommend using our projection maps themselves for predictive purposes, because the maps

alone offer only a very broad perspective of  $\delta^{13}\text{C}$  variation, based on SST data from seasonal means, and spatial means over from  $1^\circ$  grids. Thus, smaller spatial scales and/or temperature anomalies (e.g. El Niño years) are ignored. Instead, we recommend using the models described in Table 3, using more specific SST data from a region(s) and time(s) of interest.



**Figure 8.** Predicted variation in the  $\delta^{13}\text{C}$  of the red alga *Plocamium cartilagineum* across temperate regions of the world for (A) winter; and (B) summer. Boxes indicate the regions from where data was extracted. Contours are extended away from coastlines to clearly illustrate latitudinal patterns. Ratios based on a significant polynomial relationship with temperature (standard error = 0.66‰).

Absence of adequate information on macroalgal  $\delta^{13}\text{C}$  variability is likely to cause miscalculations of their contribution to the diets of consumers, especially when using data from the literature, or single values to represent different regions. In their study of variation in the stable isotopes of marine macrophytes, Dethier et al. (2013) showed that intraspecific differences among sites and dates (including Rhodophyta, e.g. *Neorhodomela*) were greater than is generally assumed in the published literature. They generated mixing models for a hypothetical consumer by using different sets of dietary isotopic values, over different scales of space and time, and showed that failure to account for these variations leads to inaccurate results. Our approach could facilitate predictions in changes of baseline  $\delta^{13}\text{C}$  in food webs over large spatial scales, interpretations of changes in  $\delta^{13}\text{C}$  of consumers over those scales, and generate hypotheses from such predictions.

In recognition of possible issues related to changes in baseline  $\delta^{13}\text{C}$  values over large spatial scales, Barnes et al. (2009) showed high spatial variation in  $\delta^{13}\text{C}$  of filter-feeding scallops in the North Sea, which was strongly correlated with temperature. This was thought to be propagated through their phytoplankton diet, and the relationship with temperature subsequently accounted for up to 79% of the spatial  $\delta^{13}\text{C}$  variation in fishes. Here, we predict that  $\delta^{13}\text{C}$  of *Plocamium* spp. is likely to vary markedly over large spatial scales and this is likely to be propagated through food webs. *Plocamium* is a genus of red algae that is distributed globally and represents some of the most common red algae over subtidal reefs (McClanahan 2008; Shepherd and Edgar 2013), and is also a major constituent in the diet of herbivorous gastropods (e.g. up to 39% in abalone, Barkai and Griffiths (1986), amphipods (Amsler and Amsler 2013) and fish (Jones and Norman 1986). In addition, *Plocamium cartilagineum* is the predominant food source for, and has been shown to enhance growth rates in, juveniles of the Californian sea hare *Aplysia* sp. (Pennings 1990). Therefore, based on our predictions of spatial shifts in  $\delta^{13}\text{C}$  of baseline food sources, we may form the hypothesis that  $\delta^{13}\text{C}$  of *Aplysia*, which is globally distributed, would similarly follow a positive (polynomial) relationship with temperature. If the relationship between temperature and  $\delta^{13}\text{C}$  of *Plocamium* held for red algae, it could benefit interpretations of  $\delta^{13}\text{C}$  in food webs more broadly.



## 2.5 Conclusion

We have shown a high degree of spatial and temporal variability in  $\delta^{13}\text{C}$  for two species of macroalgae in coastal marine waters, which has implications for food web studies using stable isotopes due to the importance of macroalgae as food sources for a wide range of mesograzers (Cruz-Rivera and Hay 2000; Vanderklift and Kendrick 2004). Kelp and red algae are essential sources of production supporting temperate and cold-water ecosystems the world over (Hoek 1984; Steneck et al. 2003). Over temperate Australian reefs, the small kelp *E. radiata* dominates biomass (Wernberg 2003), while species of the genus *Plocamium* are among the most abundant taxa (Shepherd and Edgar 2013). Therefore, understanding  $\delta^{13}\text{C}$  variation in these macroalgae is critically important. While our study confirms the spatial and temporal variation of autotrophs in other studies (e.g. Vanderklift and Wernberg 2010; Hyndes et al. 2013), we show that, in some taxa, spatial and temporal patterns can be broadly predicted.

Our distance based linear models, and marginal tests of independent variables, showed that  $\delta^{13}\text{C}$  variation in both taxa of macroalgae are, in part, driven by the same causes, namely  $\delta^{13}\text{C}$  of dissolved inorganic carbon (DIC) and light. However, there is also disparity between the taxa. The most obvious of these being that  $\delta^{13}\text{C}$  in *P. preissianum* is tightly correlated to temperature, whilst *E. radiata* showed no such relationship. Although this relationship may be indirect (e.g. via  $\text{CO}_2$  absorption), water temperature explained over half of the  $\delta^{13}\text{C}$  variability in *P. preissianum*, and our spatial projections and temporal forecasts illustrate a method by which hypotheses can be generated to predict isotopic variation in this macroalgae. Furthermore, if  $\delta^{13}\text{C}$  variability in other taxa could be linked to factors of their environment, particularly those widely recorded and extensively available, the use of a similar approach we adopted here would facilitate the interpretation of variation in  $\delta^{13}\text{C}$  of consumers in food webs over broad scales, where it can often be difficult to obtain consistent samples of food sources.

## Chapter 3. Physical and biogeochemical correlates of variation in the $\delta^{15}\text{N}$ of marine macroalgae: spatial and temporal predictions to aid food web studies

### 3.1 Abstract

Natural abundance nitrogen stable isotopes ( $\delta^{15}\text{N}$ ) are commonly used in trophic studies to determine the food sources of consumers. Primary producers represent the first trophic step supporting food webs, but their value as baseline  $\delta^{15}\text{N}$  to assign trophic levels to consumers will depend on their intraspecific variability in  $\delta^{15}\text{N}$ . We measured spatial and temporal variation in the  $\delta^{15}\text{N}$  signatures of three taxonomically contrasting macroalgae supporting temperate reefs, along with a suite of physical and biogeochemical variables, at sites along the coast of south-western Australia. Patterns in  $\delta^{15}\text{N}$  variation over different spatial scales differed greatly between taxa. A significant proportion of the spatio-temporal variation in the  $\delta^{15}\text{N}$  of each taxon was explained by models containing several environmental variables (71% in *E. radiata*, 66% in *A. anceps*, and 46% in *P. preissianum*). However, we found that water temperature was the best single explanatory variable of macroalgae  $\delta^{15}\text{N}$  (e.g. 55% in *E. radiata*, 46% in *P. preissianum*, and 66% in *A. anceps*). We suggest that these relationships are largely indirect, with temperature acting as a proxy for other variables associated with macroalgae  $\delta^{15}\text{N}$  and its variation (e.g. concentrations of nitrate and phosphate). Accordingly, we applied these relationships with temperature to project (in space) and forecast (in time) patterns in the  $\delta^{15}\text{N}$  of each taxon. Our  $\delta^{15}\text{N}$  predictions for *E. radiata*, when compared against  $\delta^{15}\text{N}$  data extracted from studies across Australia and New Zealand, through sea surface temperature data from the time and place sampling was conducted, accounted for 44% of the spatio-temporal variation (with a standard error of 1.02‰). While  $\delta^{15}\text{N}$  signatures in primary producers can be highly variable, which has implications for food web studies due to the importance of macroalgae as food sources for a wide range of mesograzers, we show that, in some taxa, spatial and temporal patterns can be broadly predicted.

### 3.2 Introduction

Nitrogen stable isotopes ( $\delta^{15}\text{N}$ ) are becoming a vital tool in determining the trophic structure of marine ecosystems, as  $\delta^{15}\text{N}$  often exhibits a clear enrichment of  $\sim 3\text{‰}$  between trophic levels (DeNiro and Epstein 1981, Peterson and Fry 1987). In most ecological applications, an essential step requires accurate measurements of isotopic signatures in organisms at or near the base of the food web, which act as basal reference points from which the isotopic data in higher trophic animals can be calibrated (Jennings et al. 2008). However, the  $\delta^{15}\text{N}$  in macroalgae that represent the food web base of temperate coastal reefs, is quickly turned-over, and influenced by their surrounding physical and biogeochemical environment (Umezawa et al. 2002; Marconi et al. 2011; Raimonet et al. 2013), which is highly variable over space and time (Fredriksen 2003). Thus, for food web models to accurately reflect trophic structure in coastal temperate regions, and to be comparable with food webs in other regions, basal  $\delta^{15}\text{N}$  variation needs to be measured over relevant spatial and temporal scales. For example, Guest et al. (2010) showed that the  $\delta^{15}\text{N}$  of macroalgae does not necessarily increase with spatial scale, as variation among sites can be greater than that of regions. However,  $\delta^{15}\text{N}$  variability is often overlooked, and data from the literature or single values that represent different regions or seasons are often used instead as proxies (Boecklen and Yarnes 2011).

A major concern is that spatial and temporal variation may mask or distort subsequent interpretations. Alternatively, we can use spatial and temporal  $\delta^{15}\text{N}$  variation to our advantage, by linking it to the physical and biogeochemical factors that are likely to influence it, and so be better equipped to predict where and when  $\delta^{15}\text{N}$  ratios in basal sources are likely to vary. The  $\delta^{15}\text{N}$  of autotrophs can be strongly influenced by these factors. For example,  $\delta^{15}\text{N}$  in autotrophs are strongly dependent on concentrations, composition and  $\delta^{15}\text{N}$  from the available dissolved inorganic nitrogen pool (DIN) (Umezawa et al. 2002; Barr et al. 2013), and fractionation during N assimilation (Fourqurean et al. 2005; Umezawa et al. 2007). The extent of  $\delta^{15}\text{N}$  fractionation varies with cell growth and DIN concentration (Dudley et al. 2010), and the nature of this relationship is further influenced by the degree of light, temperature, water movement and nutrient limitation, particularly nitrate ( $\text{NO}_3^-$ ), ammonium ( $\text{NH}_4^+$ ) and phosphorus ( $\text{PO}_4^{3-}$ ) (Reay et al. 1999; Doi et al. 2009; Dudley et al. 2010). As marine derived  $\text{NO}_3^-$  is  $\delta^{15}\text{N}$  enriched relative to  $\text{NH}_4^+$  (Dudley et al. 2010; Raimonet et al. 2013), higher concentrations of  $\text{NO}_3^-$  results in enriched  $\delta^{15}\text{N}$  values in macroalgae (Reay et al. 1999). Further, high concentrations of  $\text{PO}_4^{3-}$  can promote algal- $\delta^{15}\text{N}$  enrichment (Doi et al. 2009). In

addition, recent studies have identified that water temperature has an inverse relationship with concentrations of  $\text{NO}_3^-$  and  $\text{PO}_4^{3+}$ , both horizontally through the water column, and laterally at the sea surface (Leichter et al. 2007; Sarangi 2012). As sea surface temperature (SST) is both widely recorded and extensive, SST may be a useful proxy of  $\delta^{15}\text{N}$  in macroalgae, as it integrates many of the influencing variables.

Although many studies have focused on the isotopic variation of macroalgae in space and time (Guest et al. 2010; Hyndes et al. 2013; Dethier et al. 2013), and values have been linked to different sources and concentrations of DIN (Matsuo et al. 2010; Barr et al. 2013; Raimonet et al. 2013), few studies have looked to link both physical environmental and biogeochemical variables, and used subsequent relationships as tools to predict  $\delta^{15}\text{N}$  variation. Jennings & Warr (2003) showed that high spatial variation in  $\delta^{15}\text{N}$  of queen scallops in the North Sea was strongly correlated with widely-recorded environmental variables such as temperature, salinity, and depth. This was thought to be propagated through their phytoplankton diet, and models using these environmental correlates subsequently accounted for up to 77% of the spatial  $\delta^{15}\text{N}$  variation in fishes. The case for macroalgae is, however, quite different. Phytoplankton are sampled in bulk, giving a production weighted mean of  $\delta^{15}\text{N}$ , while macroalgae are sampled individually. However, if different species are shown to respond in a similar way to their environment, linking these  $\delta^{15}\text{N}$  responses to physical and biogeochemical environmental changes in space and time, could facilitate predictions in changes of baseline  $\delta^{15}\text{N}$  in food webs over large spatial scales. This could help interpretations of changes in  $\delta^{15}\text{N}$  of consumers over those scales, and generate hypotheses from such predictions.

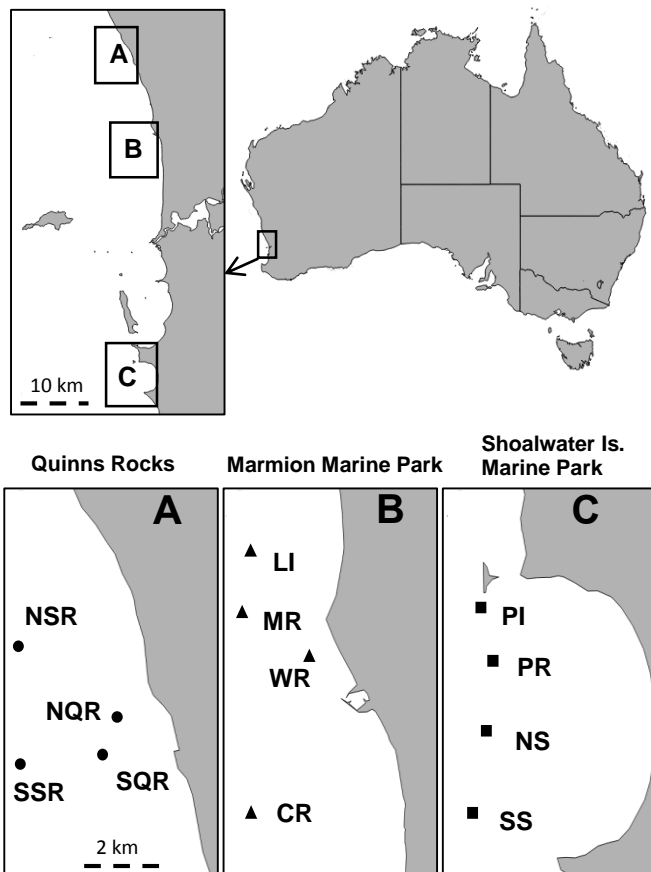
In this study, we describe  $\delta^{15}\text{N}$  variation in three taxonomically contrasting macroalgae supporting temperate subtidal reefs over various spatial (10s m to 100 km), and temporal (weekly to seasonal) scales over a 13-month period. The kelp *Ecklonia radiata* is abundant along the southern coasts of Australia and New Zealand, and supports food webs in that region (Crawley and Hyndes 2007; Guest et al. 2010; Vanderklift and Wernberg 2010). Similarly, the red algae *Plocamium preissianum*, which represents foliose species, and *Amphiroa anceps*, which represents calcareous species, are abundant and support food webs in temperate reef and seagrass systems (McClanahan 2008; Shepherd and Edgar 2013). Secondly, we analysed spatial and temporal variations in  $\delta^{15}\text{N}$  against a suite of physical and biogeochemical variables, including those where relationships could be conveniently applied to extensive oceanographic and remote sensed data. We were particularly interested in water temperature as a potential proxy for  $\delta^{15}\text{N}$  variation in macroalgae. We then used our subsequent models to project (in

space) and forecast (in time) patterns in  $\delta^{15}\text{N}$  to determine if environmental variables could be used for predictions in  $\delta^{15}\text{N}$  and whether these could facilitate interpretation of food web data and generation of hypotheses.

### 3.3 Materials and methods

#### Study area

Our study was conducted over subtidal reefs along the lower west coast of Australia, and encompassed three spatial scales and two temporal scales. For the spatial design, we sampled three regions (Quinn's Rocks (31°41'09'S, 115°40'26'E), Marmion Marine Park (31°49'13'S, 115°43'01'E) and Shoalwater Islands Marine Park (32°19'43'S, 115°41'23'E), four sites within each region, and five replicates within each site (Fig.1) during early autumn 2013. These regions are likely to experience different levels of anthropogenic sources of nitrogen (N). Quinns Rocks is located in close proximity (~ 2 km) to a waste water outflow, whilst Marmion is likely to be influenced by dissolved N via surface and groundwater; being in close proximity to a populated area of Perth. In comparison, Shoalwater is located some distance away from wastewater outflows and is in a less populated region. Each region was separated by 10s of km (regional scale), while sites were spaced >1 km apart (site scale), and replicates taken 10s of m apart (replicate scale). At each site, and from a depth of between 5 and 7 m, we collected by hand using SCUBA, replicate samples of three taxonomically contrasting macroalgae: the small kelp *Ecklonia radiata* (of the order Laminariales), the coralline red alga *Amphiroa anceps* (of the order Corallinales), and the fleshy red alga *Plocamium preissianum* (of the order Plocamiales). Samples were kept on ice before being frozen for later processing. Our temporal sampling followed the same protocol but from just two sites within Marmion Marine Park (Little Island (LI) and Marmion Reef (MR), Fig 1.). At these sites, we sampled every month over 13-months from Sep 2012, with two additional sampling times one to two weeks apart in April and August/September 2013.



**Figure 1.** Locations of reef sites within each region of south-western Australia (from north to south). (A) Quinns Rocks: north Scarpie Reef (NSR), north Quinns Rocks (NQR), south Quinns Rocks (SQR), south Scarpie Reef (SSR). (B) Marmion Marine Park: Little Island (LI), Marmion Reef (MR), Wanneroo Reef (WR), Centaur Reef (CR). (C) Shoalwater Islands Marine Park: Penguin Island (PI), Passage Rocks (PR), north Sisters (NS), and south Sisters (SS)

## Physical and biogeochemical data

Continuous measurements of light (as relative Lux,  $\text{Lx/m}^2$ ), temperature ( $^{\circ}\text{C}$ ) and water velocity (relative.  $\text{m}\cdot\text{sec}^{-2}$ ) were recorded from each site throughout the study using Hobo™ data loggers, Onset computer Corporation, Bourne, Massachusetts, USA. Loggers were mounted on the flat surface of bisected buoys, which were attached to the reef (depth 5-7 m) via a 1 m tether, and oriented towards the surface. All loggers were tested and calibrated before deployment to eliminate discrepancies among them. Loggers deployed spatially (across the 12 sites) and temporally (over 13-months) were fitted and started to record data at least two weeks prior to the first sampling event. Two sets of loggers from our spatial design were lost (site NSR in Quinns Rocks and site NS in Shoalwater Islands Marine Park), and were thus excluded from the analyses. At each site on each sampling occasion, subsurface ( $\sim 4$  m) seawater samples were collected for salinity, and concentrations ( $\mu\text{g/l}$ ) of nitrate + nitrite ( $\text{NO}_x$ ), phosphate ( $\text{PO}_4^{3+}$ ), ammonium ( $\text{NH}_4^+$ ), and the  $\delta^{15}\text{N}$  (‰) of nitrate, using a messenger-activated water bottle. Duplicate subsamples (50 ml) were immediately passed through  $0.2\ \mu\text{m}$  filters into precombusted vials and kept frozen prior to analysis.

## Sample processing and stable isotope analysis

All macroalgae were measured (length mm) and cleaned, using a scalpel if necessary to remove any epibionts or other contaminants. Material was dried at  $60^{\circ}\text{C}$  for 48 hrs and homogenised to a powder using a ball and mill. Samples were then analysed for  $\delta^{15}\text{N}$  by combusting pre-weighed ( $\sim 2\text{-}3\text{mg}$ ) subsamples in a Flash1112 elemental analyser coupled to a Delta V Plus Isotope Ratio Mass Spectrometer (IRMS) via a conflo IV interface (Thermo Scientific). Stable isotope ratios  $^{15}\text{N}/^{14}\text{N}$  are expressed as delta value ( $\delta$ ) relative to the international standard atmospheric  $\text{N}_2$ .

The nitrogen isotopic composition of nitrate + nitrite ( $\delta^{15}\text{N}\text{-NO}_x$ ) was determined using the denitrifier method (Sigman et al. 2001, Casciotti et al. 2002). This method results in the complete conversion of  $\text{NO}_x$  into  $\text{N}_2\text{O}$  by the specific strain of bacteria *Pseudomonas aureofasciens* (ATTC #). The produced  $\text{N}_2\text{O}$  was subsequently concentrated and purified in a custom built purge and trap system and then sent to a delta V Plus IRMS (ThermoScientific) via a modified Gas Bench II interface (ThermoScientific) for analysis of its isotopic composition. Data were subsequently normalised using the certified reference materials USGS

32 and USGS 34 (Boehlke and Coplen 1995) and are reported in per mil (‰) relative to atmospheric N<sub>2</sub>. The analytical precision (1 $\sigma$ ) was 0.3‰ and the detection limit of the method is 0.5 $\mu$ M.

## **Data analysis**

Our initial analysis examined how  $\delta^{15}\text{N}$  for each macroalga species varied across different scales of space and time. The magnitude of variation in  $\delta^{15}\text{N}$  (of each production source) at each spatial and temporal scale (i.e. to determine the variance components) was determined using the permutational analysis of variance (PERMANOVA+) package in PRIMER v6 (Plymouth Routines in Multivariate Research), based on Euclidian distances. A PERMANOVA for each taxon was conducted using three different designs. The patterns in spatial variation were separated into two factors, region and site nested in region. The patterns in temporal variation were separated into: (a) a broad temporal design with month nested in season, and including a spatial factor (site) nested in month; and (b) a contracted temporal design with weeks nested into months. Variation was determined through variance component tests, which are recognised as the best estimates of the contribution of a given factor to variability in the response variable (Graham and Edwards 2001).

## **Environmental variables**

Our physical and biogeochemical variables (here after termed environmental variables) included temperature and water velocity (rel. m.sec<sup>-2</sup>) from data loggers, and the mean values over the previous 30-days (prior to a sampling event) were linked to the  $\delta^{15}\text{N}$  signatures. For light, mean daily lux (Lx/m<sup>2</sup>) from only the first 10 days after deployment or sampling (when sensors were cleaned) were used in the analysis due to fouling. Data on the concentrations of NO<sub>x</sub>, NH<sub>4</sub><sup>+</sup>, PO<sub>4</sub><sup>3+</sup> and  $\delta^{15}\text{NO}_3^-$ , and salinity, were based on the time of sampling. We added to the dataset (temporal only) average precipitation (mm) from the previous 28 d prior to sampling (Greenwood weather station, WA) as a potential correlate of riverine and land runoff sources of N. We applied principle component analysis (PRIMER v6) on normalised environmental data to identify patterns among sites, regions, months and seasons.



Multiple linear regression analyses were used to select predictor variables from all physical and biogeochemical variables, that accounted for spatial, temporal and combined spatial and temporal variability in  $\delta^{15}\text{N}$  in each taxa, using distance-based linear models (DistLM) in PERMANOVA+ (add-on in PRIMER v6). DistLM does not assume normality of data distributions as hypotheses are tested by permutations (Anderson 2005). It also controls for Type I and II errors that may occur through multiple testing using conventional (parametric) data analyses (Ernst 2004). A “step-wise” procedure using the “Bayesian Information Criterion” (BIC) was used to determine models that best fit the data with fewest predictor variables. We included marginal tests to identify individual variables (e.g. independent of other variables) that could be used to predict  $\delta^{15}\text{N}$ . We do not suggest particular variables or models are necessarily causative. These may be variables involved indirectly or simply acting as proxies for other underlying causes. Conventional multiple and simple linear regressions were conducted to derive regression equations for the models identified in our DistLM’s (where assumptions of linear regression were met).

We projected isoscapes (for summer and winter) and produced monthly forecasts for  $\delta^{15}\text{N}$  in each taxa using linear models (for the combined spatial and temporal dataset) with corresponding oceanographic data from the World Ocean Atlas 2009 (NOAA, [www.nodc.noaa.gov/OC5/WOA09](http://www.nodc.noaa.gov/OC5/WOA09)). The World Ocean Atlas 2009 is a set of objectively analysed ( $1^\circ$  grid) fields of *in situ* oceanographic data. Isoscapes were created using Ocean Data View (ODV) version 4 (Schlitzer 2002), and are displayed as colour-shaded maps of contoured data, setup through the Data Interpolating Variational Analysis (DIVA) feature in ODV. We confined our isoscapes to only the ranges of oceanographic data experienced in our own spatial and temporal datasets, and the known distributions for each taxa, limiting our projections to temperate Australasia. Our temporal forecast models of  $\delta^{15}\text{N}$  variation in each taxa represent variation from the spatial margins of our temporal sampling region (e.g. south-western Australia), and are simply plotted as predicted  $\delta^{15}\text{N}$  (‰) for each month.

### **3.4 Results**

#### **Spatial and temporal $\delta^{15}\text{N}$ variation**

For the spatial design, both *E. radiata* and *A. anceps* displayed significant variation in  $\delta^{15}\text{N}$  across sites ( $p = 0.001$  and  $0.004$ , respectively), but not regions ( $p = 0.18$  and  $0.37$ ,

respectively), with the greatest source of variation (in each) at the replicate (Residual: 37 and 70%, respectively) or site scales (49% and 29%, respectively) (Table 1, Figure 2a). *P. preissianum* displayed significant variation across sites ( $p = 0.001$ ) and regions ( $p = 0.01$ ), with the greatest source of variation at the region scale (58%), followed by site (22%), and replicates (20%) (Table 1, Figure 2a). The mean  $\delta^{15}\text{N}$  in *E. radiata* ranged from -0.027 to 4.36‰, and in *A. anceps* from -1.36 to 4.69‰ across all sites and regions. In *P. preissianum*, mean  $\delta^{15}\text{N}$  ranged from 1.88 to 6.98‰ (Figure 2a).

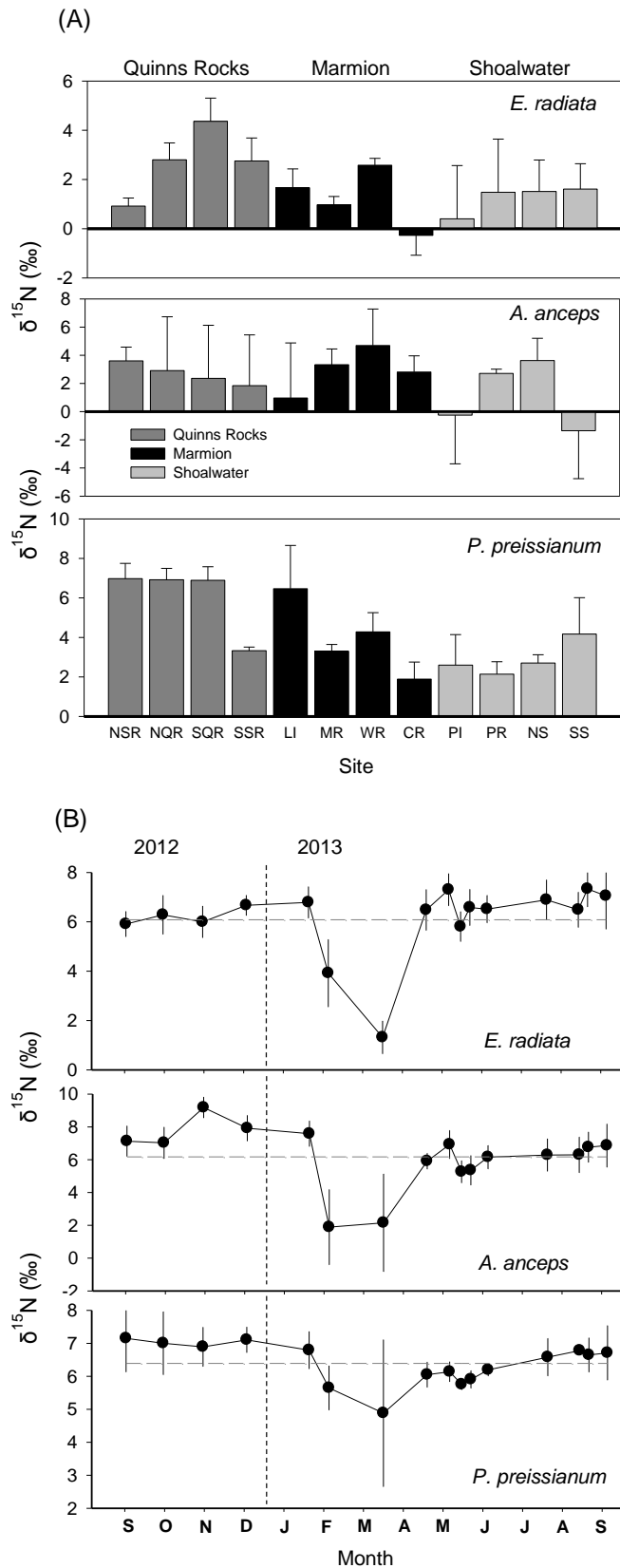
The temporal sources of  $\delta^{15}\text{N}$  variation were also similar for *E. radiata* and *A. anceps*. For the broader temporal dataset, over 13-months of sampling at reefs in Marmion Marine Park, each displayed significant variation across months ( $p < 0.01$ ), but not seasons ( $p = 0.5$  and  $0.45$ , respectively), with the greatest source of variation at the month scale (75 and 57%, respectively) followed by replicates (16 and 28%), with seasons being statistically absent (0%) (Table 1, Figure 2B). At the finer temporal scale in both *E. radiata* and *A. anceps*,  $\delta^{15}\text{N}$  variation was not significant among months or weeks, and the greatest source of variation was at the replicate scale (70 and 68%, respectively) (Table 1, Figure 2B).

For *P. preissianum* in the broad temporal design,  $\delta^{15}\text{N}$  displayed significant variation across seasons ( $p = 0.05$ ), but not months ( $p = 0.7$ ), with the greatest source of variation at the replicate scale (41%) followed by season (23%) (Table 1, Figure 2B). At the finer spatial scale, the greatest source of  $\delta^{15}\text{N}$  variation in *P. preissianum* was among months (54%), which was significant ( $p = 0.019$ ), followed by replicates (46%), and variation among weeks was absent (0%).

The mean  $\delta^{15}\text{N}$  for *E. radiata* ranged from 1.31 to 7.33‰, and in *A. anceps* from 1.88 to 9.18‰, across all months and seasons, whilst in *P. preissianum*, ratios ranged from 4.88 to 7.15‰ (Figure 2B). For both *A. anceps* and *P. preissianum*,  $\delta^{15}\text{N}$  values were relatively enriched from September to January prior to a sharp depletion in February and March, before increasing again to ratios around their annual means (6.19 and 6.42‰). *E. radiata* also showed a distinct depletion in February and March, but ratios before and after closely followed the annual mean (6.04‰).

**Table 1.** Summary results were obtained for Permutational analysis of variance (PERMANOVA), including variance components estimates (and % variance) for the  $\delta^{15}\text{N}$  in macroalgae from: (A) a spatial design, with reefs nested in location; (B) a broad temporal design, with months nested in seasons and sites nested in month; and (C) a constricted temporal design, with weeks nested in month. Analyses were based on Euclidian distance. Negative values for variance components are treated as zero. *P* values in bold indicate significance.

<i>Ecklonia radiata</i>					
Design	Factors	<i>Df</i>	MS	<i>P</i>	Var. (%)
<b>A</b>	Region	2	14.2	0.175	0.40 (14.8)
	<b>Site</b>	<b>9</b>	<b>6.65</b>	<b>0.001</b>	<b>1.31 (48.5)</b>
	Residuals	48	2.87		0.99 (36.7)
<b>B</b>	Season	3	23.3	0.50	-0.2 (0.00)
	<b>Month</b>	<b>8</b>	<b>32.9</b>	<b>0.001</b>	<b>2.66 (74.7)</b>
	<b>Site</b>	<b>12</b>	<b>2.60</b>	<b>0.002</b>	<b>0.34 (9.55)</b>
	Residuals	121	0.56		0.56 (15.7)
<b>C</b>	Month	1	1.68	0.40	-8.9 (0.00)
	Week	4	1.91	0.07	0.39 (30.2)
	Residuals	39	0.81		0.90 (69.7)
<i>Amphiroa anceps</i>					
Design	Factors	<i>Df</i>	MS	<i>P</i>	Var. (%)
<b>A</b>	Region	2	38.8	0.37	0.21 (1.3)
	<b>Site</b>	<b>9</b>	<b>34.7</b>	<b>0.004</b>	<b>4.70 (28.9)</b>
	Residuals	48	11.3		11.3 (69.7)
<b>B</b>	Season	3	44.3	0.45	-6.3 (0.00)
	<b>Month</b>	<b>8</b>	<b>47.9</b>	<b>0.006</b>	<b>3.78 (57.2)</b>
	<b>Site</b>	<b>12</b>	<b>7.45</b>	<b>0.002</b>	<b>0.99 (14.9)</b>
	Residuals	116	1.84		1.84 (27.8)
<b>C</b>	Month	1	6.98	0.13	0.32 (23.2)
	Week	4	1.68	0.15	0.12 (8.70)
	Residuals	35	0.94		0.94 (68.1)
<i>Plocamium preissianum</i>					
Design	Factors	<i>Df</i>	MS	<i>P</i>	Var. (%)
<b>A</b>	<b>Region</b>	<b>2</b>	<b>74.4</b>	<b>0.011</b>	<b>3.41 (57.9)</b>
	<b>Site</b>	<b>9</b>	<b>7.53</b>	<b>0.001</b>	<b>1.27 (21.5)</b>
	Residuals	48	1.20		1.20 (20.4)
<b>B</b>	<b>Season</b>	<b>3</b>	<b>10.9</b>	<b>0.050</b>	<b>0.26 (23.3)</b>
	Month	8	2.65	0.699	-0.1 (0.00)
	<b>Site</b>	<b>12</b>	<b>3.92</b>	<b>0.001</b>	<b>0.61 (42.1)</b>
	Residuals	118			0.59 (40.7)
<b>C</b>	<b>Month</b>	<b>1</b>	<b>6.01</b>	<b>0.019</b>	<b>0.30 (53.5)</b>
	Week	4	0.11	0.43	-2.0 (0.00)
	Residuals	38	0.26		0.26 (46.4)

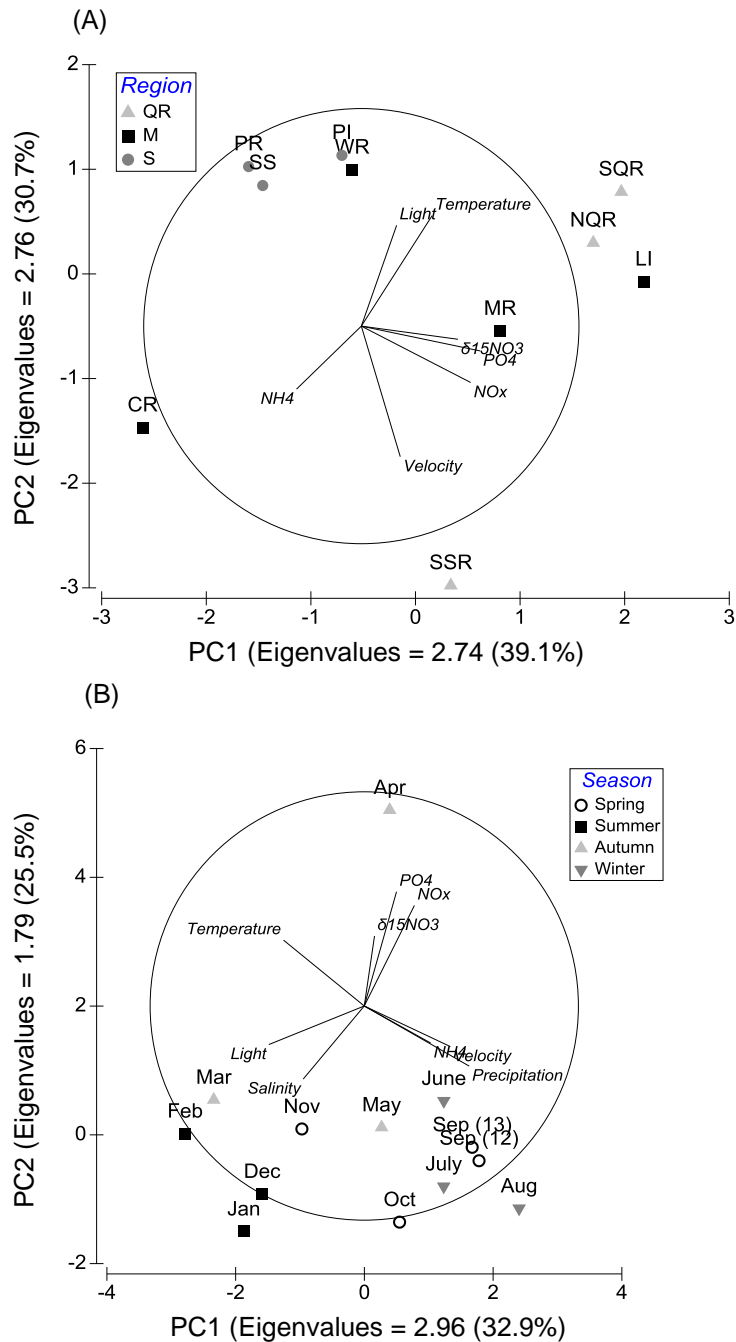


**Figure 2.** Means ( $\pm$  standard deviation) of  $\delta^{15}\text{N}$  in *Ecklonia radiata*, *A. anceps* and *Plocamium preissianum* at (a) sites in each region (See Fig 1 for locations of sites); and (B) over 13-months from Marmion Marine Park, where sampling points are spread by Julian Day, but ticks labelled by month. Dashed lines represent mean  $\delta^{15}\text{N}$  in each source.

## **Principal component analysis of physical and biogeochemical data**

The PCA of environmental data for the spatial design fell into two distinctive groups of sites, with three remaining sites being quite distinct from these groups and each other (Figure 3). The first principal component described 39.1% of the variation, and distinguished NQR, SQR (Quinns Rocks) and LI (Marmion) from the remaining sites by way of higher concentrations of  $\text{NO}_x$ ,  $\text{PO}_4^{3+}$  and  $\delta^{15}\text{N}$  of  $\text{NO}_3^-$ , with the remaining sites interspersed along a gradient in these variables. The second principal component described 30.7% of the variation, and distinguished sites along a gradient of light, temperature and water velocity, with each of the three sites in Shoalwater and WR in Marmion experiencing the least velocities but the highest temperature and light values.

The temporal environmental data were less defined, but the first principal component described 32.9% of the variation, and showed a division between summer months (with higher values of light) and winter months (higher values of  $\text{NH}_4^+$  and precipitation), with autumn and spring having intermediate values. The second principal component described 25.5% of the variation, and distinguished April as being far removed from all other months by way of higher values in  $\text{PO}_4^{3+}$ ,  $\text{NO}_x$  and  $\delta^{15}\text{N}$  of  $\text{NO}_3^-$  (Figure 3).



**Figure 3.** Principle component analysis (PCA) using normalised means of environmental data measured at: (A) 10 sites across three regions in south-western Australia (see Fig 1 for locations of sites); and (B) over 13-months from within Marmion Marine Park from September 2012 to September 2013. Months follow austral seasons, e.g. autumn (March – May), winter (June – August), spring (September – November), and summer (December – February). Eigenvalues for each principle component are given in their respective axis, with the circle overlay scaled to the maximum effect size.

## Environmental models of $\delta^{15}\text{N}$ in macroalgae

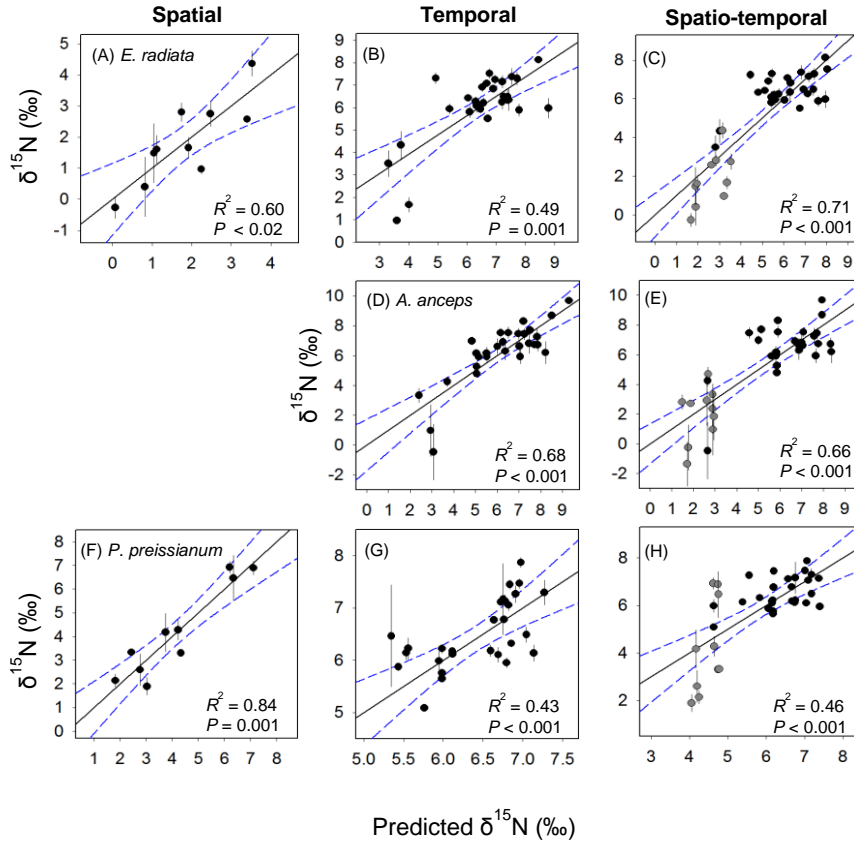
Based on DistLM using spatial data for *E. radiata*,  $\delta^{15}\text{N}$  correlated best with  $\text{PO}_4^{3+}$  and  $\text{NO}_x$  concentrations (Adj.  $R^2 = 0.60$ ). The temporal dataset yielded a significant model for  $\delta^{15}\text{N}$  combining  $\text{PO}_4^{3+}$ , light, temperature and precipitation (Adj.  $R^2 = 0.49$ ), whilst the combined spatial and temporal dataset identified  $\text{PO}_4^{3+}$  concentration, water velocity and temperature in significantly explaining  $\delta^{15}\text{N}$  variation (Adj.  $R^2 = 0.71$ , Table 2). *A. anceps* did not return any significant models explaining spatial variability in  $\delta^{15}\text{N}$ , whereas the model output for the temporal dataset yielded the combination of  $\text{PO}_4^{3+}$ , light and temperature (Adj.  $R^2 = 0.68$ , Table 2). The combined spatio-temporal dataset returned temperature in combination with  $\text{PO}_4^{3+}$  concentration (Adj.  $R^2 = 0.66$ ).

For *P. preissianum*, the spatial variation in  $\delta^{15}\text{N}$  correlated best with light and  $\delta^{15}\text{NO}_3$  (Adj.  $R^2 = 0.84$ ). The temporal dataset yielded a significant model combining light and temperature (Adj.  $R^2 = 0.43$ ), whilst the combined dataset identified  $\text{PO}_4^{3+}$  concentration and temperature in significantly explaining  $\delta^{15}\text{N}$  variation (Adj.  $R^2 = 0.46$ , Table 2). Conventional multiple linear regressions were used to predict  $\delta^{15}\text{N}$  based on the above DistLM's (that yielded identical  $r^2$  values, but a higher significance, e.g.  $p$  values) and are displayed in Figure 4, and equations in Table A3 (Appendices).

**Table 2.** Summary results from Distance based linear models (DistLM), using Bayesian Information Criterion (BIC) (Schwartz 1978), identifying the physical and biogeochemical variables that best explain variation in the  $\delta^{15}\text{N}$  in macroalgae. See Table A3 (in appendices) for regression equations (using conventional multiple regressions) based on these models. Different models were used for spatial, temporal and combined spatial and temporal designs.

(A)	Selected model		
Design		Adj. $r^2$ (%)	BIC
	<i>Ecklonia radiata</i>		
Spatial	$\text{PO}_4^{3+}$ , and $\text{NO}_x$	60.0	-3.39
Temporal	$\text{PO}_4^{3+}$ , light, temperature, precipitation	49.1	19.16
Combined	$\text{PO}_4^{3+}$ , velocity, temperature	71.4	25.36
	<i>Amphiroa anceps</i>		
Temporal	$\text{PO}_4^{3+}$ , light, temperature	68.2	21.79
Combined	$\text{PO}_4^{3+}$ , temperature	66.2	40.70
	<i>Plocamium preissianum</i>		
Spatial	Light and $\delta^{15}\text{NO}_3$	84.4	-6.61
Temporal	Light, temperature	42.7	-16.6
Combined	$\text{PO}_4^{3+}$ , temperature	46.3	16.18





**Figure 4.** Relationship between  $\delta^{15}\text{N}$  in macroalgae (from spatial, temporal and combined spatio-temporal datasets) vs. predicted ratios from multiple regressions. *E. radiata*: (A) spatial vs.  $\text{PO}_4^{3+}$  and  $\text{NO}_x$ ; (B) temporal vs.  $\text{PO}_4^{3+}$  and light; (C) spatio-temporal vs. water velocity and temperature. *A. anceps*: (D) temporal vs. light and temperature; (E) spatio-temporal vs.  $\text{PO}_4^{3+}$  and temperature. *P. preissianum*: (F) spatial vs. light and  $\delta^{15}\text{NNO}_3$ ; (G) temporal vs. light and temperature; (H) spatio-temporal vs.  $\text{PO}_4^{3+}$  and temperature. Dashed lines represent 95% confidence intervals.

## Independent environmental correlates of $\delta^{15}\text{N}$

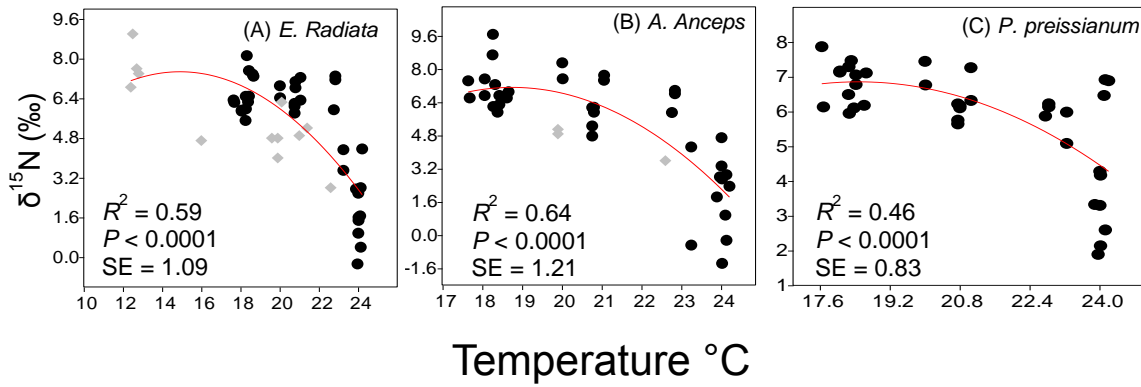
Using marginal tests from DistLM (e.g. independent predictor variables), we looked to identify which individual variables that best explained  $\delta^{15}\text{N}$  variation in each taxon from the combined spatial and temporal dataset. Although many variables showed significant correlations, temperature was the best single predictor, inversely explaining 55, 58 and 42% of the  $\delta^{15}\text{N}$  variation for *E. radiata*, *A. anceps* and *P. preissianum*, respectively (Table 3). However, polynomial relationships yielded greater predictive power for each species, significantly explaining 76, 67, and 46% of the  $\delta^{15}\text{N}$  variation (Table 4a). As our study was conducted along the coastline of metropolitan Perth, where  $\delta^{15}\text{N}$  is likely to be enriched by anthropogenic sources in some regions, we looked to broaden our pool of data to other times and regions using published studies that included  $\delta^{15}\text{N}$  data of our study species (*E. radiata* and *A. anceps* only). This allowed us to add extracted  $\delta^{15}\text{N}$  values to our plots (y-axis), with satellite derived SST data (x-axis). In addition to the data from our study, these consisted of 13 mean  $\delta^{15}\text{N}$  values from across coastal Australia and New Zealand for *E. radiata* (Guest et al. 2010; Vanderklift and Wernberg 2010; Davis and Wing 2012; Hyndes et al. 2013), and three mean  $\delta^{15}\text{N}$  values along coastal mid-western Australia for *A. anceps* (Hyndes et al. 2013) (Table A4, in appendices). For *E. radiata* and *A. anceps*, these additional values reduced the predictive power of the models to 59% and 64%, but extended the temperature range in the regression for the former alga species and were still highly significant for both species ( $P < 0.0001$ ) (Table 4b, Figure 5). Independently, the extracted  $\delta^{15}\text{N}$  values for *E. radiata*, however, showed a significant linear relationship with temperature ( $P < 0.001$ ,  $R^2 = 0.70$ ,  $\delta^{15}\text{N} = -0.3678x + 12.16$ ) (data points shown in Figure 5). A lack of  $\delta^{15}\text{N}$  data for *P. preissianum* prevented us from expanding the dataset for this species.

**Table 3.** Significant correlations between the  $\delta^{15}\text{N}$  in macroalgae and independent environmental variables. Results are from the combined spatial and temporal datasets, and were obtained from Marginal tests within distance based linear models (DistLM) based on Euclidian distance.

Spatio-temporal correlations		
	Adj. $r^2$ (%)	$p$
<i>Ecklonia radiata</i>		
Precipitation (28/d)	40.4	0.001
Velocity (rel. m.sec <sup>-2</sup> )	33.2	0.003
Light (Lx/m <sup>2</sup> )	41.8	0.001
Temperature (°C)	55.2	0.001
Salinity	46.7	0.001
NO <sub>x</sub> (ug/l)	16.1	0.014
PO <sub>4</sub> <sup>3+</sup> (ug/l)	22.7	0.004
<i>Amphiroa anceps</i>		
Precipitation (28/d)	31.9	0.001
Velocity (rel. m.sec <sup>-2</sup> )	12.2	0.031
Light (Lx/m <sup>2</sup> )	24.9	0.003
Temperature (°C)	58.1	0.001
Salinity	28.2	0.002
PO <sub>4</sub> <sup>3+</sup> (ug/l)	16.7	0.009
<i>Plocamium preissianum</i>		
Precipitation (28/d)	25.6	0.003
Velocity (rel. m.sec <sup>-2</sup> )	15.7	0.017
Light (Lx/m <sup>2</sup> )	13.9	0.028
Temperature (°C)	42.3	0.001
Salinity	21.3	0.004
PO <sub>4</sub> <sup>3+</sup> (ug/l)	12.7	0.028

**Table 4.** Significant polynomial relationships between  $\delta^{15}\text{N}$  in macroalgae and water temperature for: (A) combined spatial and temporal data from metropolitan Perth; and (B) added values from the literature.

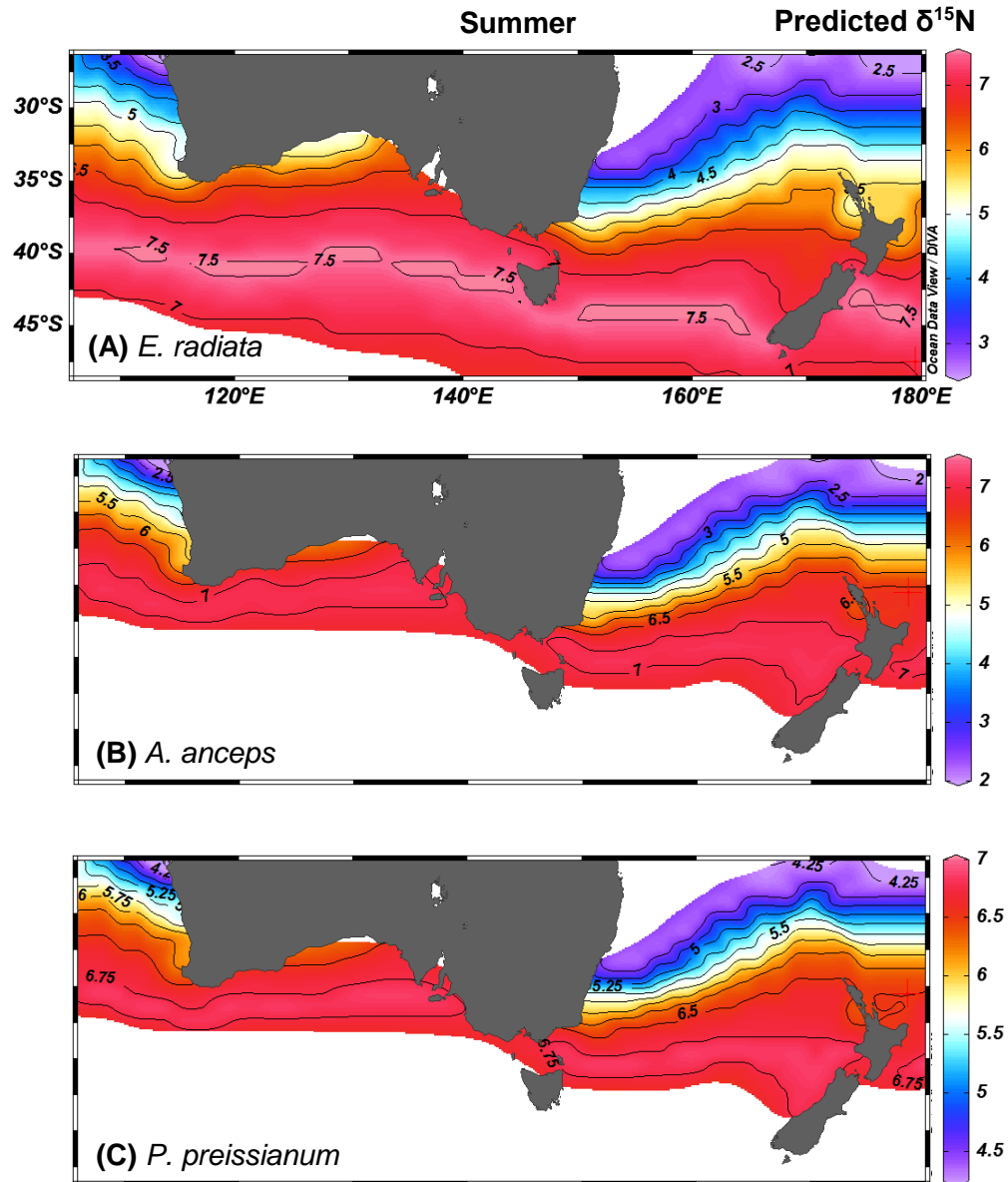
(A) Taxa	Regression equation	$R^2$ (%)	$p$	SE
<i>E. radiata</i>	$\delta^{15}\text{N} = -0.2852 \times 2 + 11.32 \times -105.1$	75.9	<0.001	0.83
<i>A. anceps</i>	$\delta^{15}\text{N} = -0.217 \times 2 + 8.309 \times -72.2$	67.4	<0.001	1.19
<i>P. preissianum</i>	$\delta^{15}\text{N} = -0.07867 \times 2 + 2.909 \times -20.03$	45.9	<0.001	0.83
(B) Taxa	Regression equation	$R^2$ (%)	$p$	SE
<i>E. radiata</i>	$\delta^{15}\text{N} = -0.05734 \times 2 + 1.71 \times -5.276$	59.2	<0.001	1.09
<i>A. anceps</i>	$\delta^{15}\text{N} = -0.1775 \times 2 + 6.656 \times -55.29$	64.5	<0.001	1.21



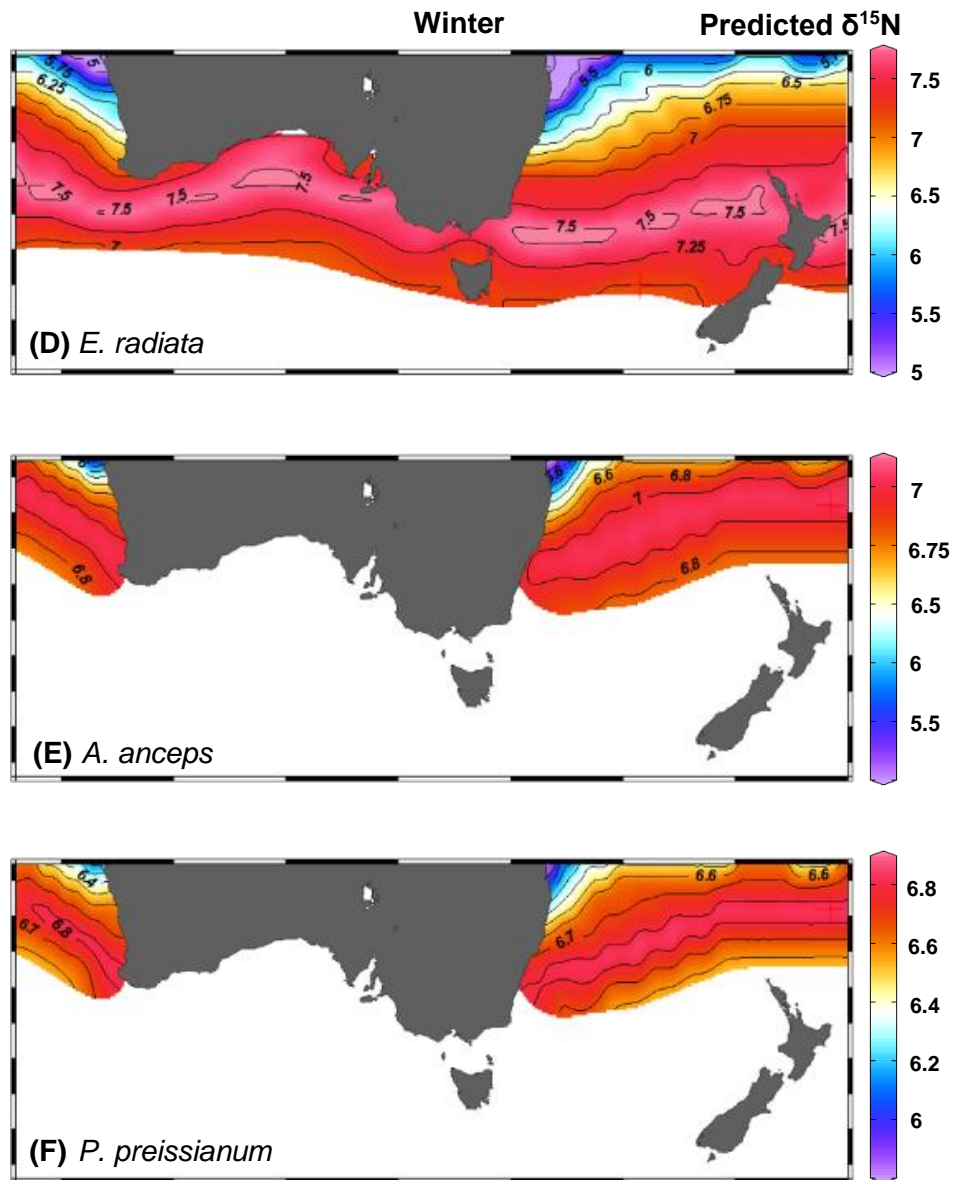
**Figure 5.** Relationships between the  $\delta^{15}\text{N}$  of macroalgae across scales of space and time and water temperature for: (A) *E. radiata*; (B) *A. anceps*; and (C) *P. preissianum*. Added  $\delta^{15}\text{N}$  data (*E. radiata* and *A. anceps* only) extracted from other studies, are linked to sea surface temperature data are shaded in grey.

### Spatial projections and temporal forecasts

Predicted  $\delta^{15}\text{N}$  for each taxon was calculated for temperate Australasia by applying our polynomial models (e.g. Figure 5) to mean seasonal temperature data for summer and winter from the World Ocean Atlas 2013 (NOAA) (Figure 6). For each taxa,  $\delta^{15}\text{N}$  isoscapes for summer show contours following clear latitudinal patterns of relatively enriched and invariable values to the south (e.g. around south-eastern Australia and New Zealand), and progressively depleted values, within tighter latitudinal bands, towards the north. Similar patterns were observed in winter, but with a latitudinal shift northwards (patterns for higher latitudes are not shown due to the lower temperatures in winter extending beyond the data range in the regressions).

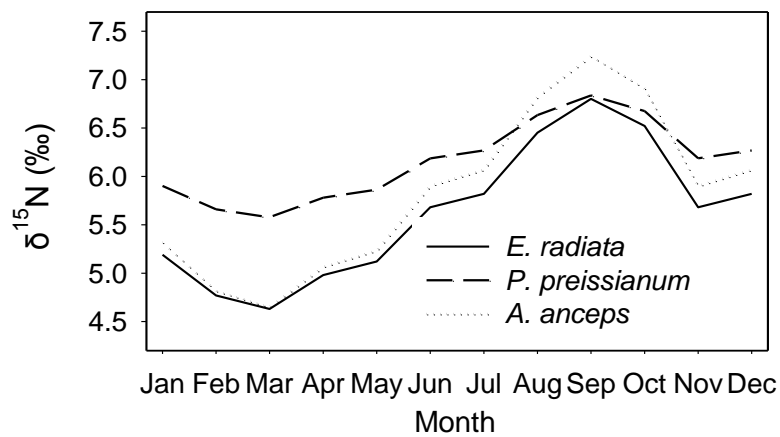


**Figure 6.** Predicted summer and winter variation in the  $\delta^{15}\text{N}$  of macroalgae across temperate Australasia (at a 5 m depth horizon) for *E. radiata* (A and D); *A. anceps* (B and E); and *P. preissianum* (C and F). Ratios based on a significant linear relationships with temperature.



**Figure 6.** (Continued)

A monthly forecast of  $\delta^{15}\text{N}$  for each taxon was calculated by applying our polynomial models for data from our study sites (from our combined spatial and temporal datasets) to mean SST data for south-western Australia, and is therefore restricted to our study region (Figure 7). Our temporal forecast exhibited depleted values in mid-summer and early autumn (e.g. from January to May) and relatively enriched values through winter and spring in each taxon. However, the range of variation in *P. preissianum* ( $\sim 1.3\text{‰}$ ) is far less pronounced than that of *E. radiata* ( $\sim 2.5\text{‰}$ ) and *A. anceps* ( $\sim 3\text{‰}$ ).



**Figure 7.** A predicted forecast of temporal variation in the  $\delta^{15}\text{N}$  of macroalgae for south-western Australia. Ratios based on a significant linear relationships with temperature.

### 3.5 Discussion

#### Spatial and temporal $\delta^{15}\text{N}$ variation in macroalgae

We have shown a high degree of spatial and temporal variability in  $\delta^{15}\text{N}$  for three species of macroalgae in coastal marine waters, which has implications for food web studies using stable isotopes due to the importance of macroalgae as food sources for a wide range of mesograzers (Cruz-Rivera and Hay 2000; Vanderklift and Kendrick 2004). Kelp and red algae are essential sources of production supporting temperate and cold-water ecosystems the world over (Hoek 1984; Steneck et al. 2003). Over temperate Australian reefs, the small kelp *E. radiata* dominates biomass (Wernberg 2003), while species of the genus *Plocamium* and *Amphiroa* are among the most abundant taxa (Shepherd and Edgar 2013). Therefore, understanding  $\delta^{15}\text{N}$  variation in these macroalgae is critically important for interpreting food-web studies using stable isotopes. Our observed spatial patterns for  $\delta^{15}\text{N}$  in *E. radiata* follow those found by Guest et al. (2010), where  $\delta^{15}\text{N}$  variation was significant among sites (e.g. between 100 to 1000 m) but not among regions (100s km apart), and variation among replicates was high. In contrast, Vanderklift et al. (2010) showed that, when sampled over broader spatial scales (e.g. 1000s km, along 6° of latitude),  $\delta^{15}\text{N}$  variation in *E. radiata* can be significant among regions and variation among sites may be relatively minor. This is perhaps not surprising, as the physical and biogeochemical environment is likely to be more varied at greater spatial scales, particularly along latitudinal gradients. The red algae *A. anceps* and *P. preissianum*, also displayed significant variation in  $\delta^{15}\text{N}$  among sites, but *P. preissianum* contrasted at the regional scale, where the greatest source of its variation was found. Significant variation among sites (~1 km apart) have been shown in other Rhodophyta, e.g. *Neorhodomela* (Dethier et al. 2013), *Metagoniolithon* (Hyndes et al. 2013), whilst in other species, e.g. *Rhodymenia sonderi*, significant variation at both regional and site scales has been found (Vanderklift and Wernberg 2010).

In the case of temporal variation, we have shown significant variation in  $\delta^{15}\text{N}$  for both *E. radiata* and *A. anceps* at the monthly scale, but not the seasonal scale, whereas *P. preissianum* was significant at both monthly and seasonal scales. These seasonal results, however, may be deceptive and an effect of how we partition seasons. For example, our principal component analysis of the environmental data showed a high degree of overlap between winter and spring. Nevertheless, our results show temporal variation in the  $\delta^{15}\text{N}$  for three divergent macroalgae



species. While recent studies have noted the need for sampling protocols in trophic studies to encompass, not only the spatial limits of the region of interest, but also at the finer spatial scale (and replication therein) (Guest et al. 2010; Dethier et al. 2013), we clearly demonstrate that temporal variation could also mislead trophic investigations. Although other studies have also identified significant and substantial temporal variation in the  $\delta^{15}\text{N}$  of macroalgae, e.g. among months (Hill et al. 2008), seasons (Vizzini and Mazzola 2003; Dethier et al. 2013), and among years (Hill et al. 2006), static values are still routinely used in mixing-models (see Boecklen et al. 2011). Sampling from a single month, may not represent an entire season, while, sampling from a single week appears to generally represent an entire month, as  $\delta^{15}\text{N}$  variation at the weekly scale was not significant, although this was close for *E. radiata* (e.g.  $P = 0.07$ ).

### **Modelled predictions of $\delta^{15}\text{N}$ variation in macroalgae**

Studies from estuaries or under controlled laboratory conditions have shown that light, temperature, water velocity and nutrient concentrations (and their  $\delta^{15}\text{N}$ ) can each independently affect the  $\delta^{15}\text{N}$  in autotrophs (Lomas and Glibert 1999; Needoba and Waser 2003; Needoba and Harrison 2004; Cornelisen et al. 2007; Campbell and Fourqurean 2009). However, to detect these effects in their natural environment depends on the degree of variation relative to other influences. Our results suggest that spatial variation in  $\delta^{15}\text{N}$  among different macroalgae are driven by different factors. The  $\delta^{15}\text{N}$  in *E. radiata* was best predicted by  $\text{PO}_4^{3+}$  and  $\text{NO}_x$ , whilst light and  $\delta^{15}\text{NO}_3^-$  best explained variation in the  $\delta^{15}\text{N}$  in *P. preissianum*, but the high level of  $\delta^{15}\text{N}$  variation for *A. anceps* at the site scale may have masked any possible spatial associations. However, for the temporal and combined (spatio-temporal) models, light,  $\text{PO}_4^{3+}$ , and particularly temperature, were consistently identified as significant components in predicting  $\delta^{15}\text{N}$  in each taxon.

Although temperature was not a factor in determining the  $\delta^{15}\text{N}$  in macroalgae spatially, the temperature range over the study period was far greater than over the entire study area ( $6.7^\circ\text{C}$  vs  $0.34^\circ\text{C}$ ), and was the single best predictor of  $\delta^{15}\text{N}$  in each taxa for the combined spatio-temporal predictive models. Temperature itself may not be directly driving  $\delta^{15}\text{N}$  variability in macroalgae, but instead may be acting as a proxy for other variables. For example, different sources of N contribute to the DIN pool, with marine derived  $\text{NH}_4^+$  expected to be  $\delta^{15}\text{N}$  depleted relative to  $\text{NO}_3^-$  (Michener and Schell 1994; Raimonet et al. 2013), thus macroalgae-

$\delta^{15}\text{N}$  will be influenced by the concentrations of these different sources. As SST has an inverse relationship with  $\text{NO}_3^-$  (Leichter et al. 2007; Sarangi 2012; Yin et al. 2014), decreasing temperatures with increasing latitude results in a larger  $\text{NO}_3^-$  pool in colder waters, and a greater source of the heavier isotope, i.e. higher  $\delta^{15}\text{N}$  values in macroalgae. The same inverse relationship with temperature has been observed with  $\text{PO}_4^{3+}$  (Leichter et al. 2007), which can be the primary limiting nutrient for growth in macroalgae on oligotrophic reefs (Littler et al. 1991), and high concentrations of  $\text{PO}_4^{3+}$  can enrich algal- $\delta^{15}\text{N}$  (Doi et al. 2009). Therefore, temperature may be acting as an indicator of  $\text{NO}_3^-$  and  $\text{PO}_4^{3+}$  concentration. Macroalgae N in most temperate species tends to increase towards saturation in winter months, as growth rates become limited by temperature and light (DeBoer, 1981), so that increased N-availability may not necessarily be linearly related to macroalgae-N (Barr et al. 2013). Thus, the inverse and polynomial relationships we observed between macroalgae- $\delta^{15}\text{N}$  and temperature may be the result of N saturation. To complicate matters further, human derived DIN (e.g.  $\delta^{15}\text{NO}_3^-$  and  $\text{NH}_4^+$ ) is enriched compared to marine sources (Kendall et al. 2007). Given the above information, it is interesting that our  $\delta^{15}\text{NO}_3^-$  measurements only correlated spatially with the  $\delta^{15}\text{N}$  in *P. preissianum* (in combination with light). Further, there was no apparent relationship between temperature and  $\delta^{15}\text{NO}_3^-$  or  $\text{NO}_x$  (see Figure 3). However, while we recorded the variables of temperature, light and water velocity continuously throughout the study, our biogeochemical measurements were limited to triplicate measurements at single points in space and time. N sources, composition and their concentrations can vary substantially over short timescales (e.g. hours/days, Glibert & Garside 1991), which may only be resolved by high frequency and intensive surveys (Raimonet et al. 2013). Thus, the values we obtained for our N measures may not have been accurate and representative. Many studies have described the relationship between macroalgae- $\delta^{15}\text{N}$  and N pollution, as a function of distance to known sources, e.g. sewage effluent (Gartner et al. 2002), aquaculture (Matsuo et al. 2010), and human population density (Benson et al. 2008), but few studies have looked at macroalgae- $\delta^{15}\text{N}$  at broad regional or latitudinal scales. Marconi et al. (2011) documented that the  $\delta^{15}\text{N}$  of macroalgae did not show any large-scale biogeographic correlations, suggesting that local N enrichment from upwellings or pollution were primarily responsible for any variations. While it seems likely that autotrophic  $\delta^{15}\text{N}$  increases with the amount of urbanisation in coastal water and N enrichment, water temperature was not looked at as a correlate. In contrast, Christiaen et al. (2014) showed that  $\delta^{15}\text{N}$  in seagrass leaves are more influenced by latitudinal differences than the degree of urbanisation or the amount of fertiliser used in local watersheds, suggesting

a latitudinal gradient of increased  $\delta^{15}\text{N}$  assimilated by plants. Further, a review of variation in the isotopes of invertebrate primary consumers by Woodland et al. (2012) reported a winter-to-summer depletion in  $\delta^{15}\text{N}$ , consistent with what we report here in macroalgae. Regardless of the root cause, our results show variation in the  $\delta^{15}\text{N}$  of three divergent macroalgae species shows an inverse and polynomial correlation with water temperature.

### **Spatial and temporal predictions of $\delta^{15}\text{N}$ in macroalgae**

Our relationships have allowed us to produce spatial projections and temporal forecasts of  $\delta^{15}\text{N}$  in macroalgae that give a very broad indication of their natural variability (with a degree of error) across temperate Australasia and over an annual cycle. However, these are clearly very simplistic, using temperature alone, and although temperature may integrate some of the variables that influence algal- $\delta^{15}\text{N}$ , they neglect the complexity of factors involved. Therefore, we do not suggest that these predictions be used as substitutes for real data, particularly over small scales, but rather we illustrate the use of temperature to account for much of the broad latitudinal or seasonal  $\delta^{15}\text{N}$  variation observed in macroalgae and food webs generally. For example, while the mean projected  $\delta^{15}\text{N}$  for *E. radiata* in the study region varied by  $\sim 3\text{‰}$  over a 12-month period, equal to the typical  $3\text{‰}$  fractionation levels identified in controlled studies (Vanderkluft and Ponsard 2003), this difference is likely to be more distinct in regions with greater temperature ranges over the year. For *P. preissianum*, the range in the mean projected  $\delta^{15}\text{N}$  was far lower than that of *A. anceps* and *E. radiata* (at equivalent temperature bands, e.g. Figure 5), suggesting that temporal shifts in  $\delta^{15}\text{N}$  will have a reduced impact on interpretation of trophic levels using some macroalgae species as baselines

Using  $\delta^{15}\text{N}$  in filter-feeding scallops as a proxy for phytoplankton  $\delta^{15}\text{N}$  variation, Jennings and Warr (2002) demonstrated that  $\delta^{15}\text{N}$  over large spatial scales was strongly correlated with temperature and salinity. Accordingly, they generated a predictive model, combining these and other environmental factors, which explained up to 79% of the spatial  $\delta^{15}\text{N}$  variation in fishes. Similarly, but for  $\delta^{13}\text{C}$ , in Chapter 2, I showed that  $\delta^{13}\text{C}$  of *P. preissianum* was positively correlated with water temperature, which was thought to be an indirect influence by way of  $\text{CO}_2$  concentration. We used this relationship to predict  $\delta^{13}\text{C}$  for *P. preissianum* across its distribution in Australasia to illustrate the potential scale in the shift of  $\delta^{13}\text{C}$  in this baseline food source over broad scales, and its implications to food web studies.

Absence of adequate information on variability in macroalgal  $\delta^{15}\text{N}$  is likely to cause miscalculations in the trophic level of consumers, especially when using data from the literature, or single values to represent different regions or seasons. In a study of variation in the stable isotopes of marine macrophytes, Dethier et al. (2013) demonstrated that intraspecific differences among sites and dates were greater than is generally assumed in the published literature. They generated mixing models for a hypothetical consumer by using different sets of dietary isotopic values, over different scales of space and time, and showed that failure to account for these variations leads to inaccurate results. Our approach could facilitate predictions in changes of baseline  $\delta^{15}\text{N}$  in food webs over large spatial and seasonal scales, interpretations of changes in  $\delta^{15}\text{N}$  of consumers over those scales, and generate hypotheses from such predictions. However, it must also be noted that, although the  $\delta^{15}\text{N}$  of macroalgae may follow a particular trajectory over a period of time, consumers (especially those long-lived and slow-growing species) will neutralise much of this variability in their diet. Therefore, the potential to miss-quantify the role of different autotrophic sources to the diet of consumers (and their trophic level) is considerable.

### 3.6 Conclusion

We have shown a high degree of spatial and temporal variability in  $\delta^{15}\text{N}$  for three species of macroalgae in coastal marine waters, which has implications for food web studies using stable isotopes due to the importance of macroalgae as food sources for a wide range of mesograzers (Cruz-Rivera and Hay 2000; Vanderklift and Kendrick 2004). Kelp and red algae are essential sources of production supporting temperate and cold-water ecosystems the world over (Hoek 1984; Steneck et al. 2003). Therefore, understanding  $\delta^{15}\text{N}$  variation in these macroalgae is vital.

Our distance based linear models, and marginal tests of independent variables, showed that although  $\delta^{15}\text{N}$  variation among species appears to be influenced by different factors,  $\delta^{15}\text{N}$  of each taxon of macroalgae is tightly linked to temperature. Our spatial projections and temporal forecasts of  $\delta^{15}\text{N}$  in macroalgae illustrate a method by which hypotheses can be generated to predict isotopic variation in other macroalgae, and indeed, other primary sources and consumers. Furthermore, the use of a similar approach we adopted to explain broad-scale variation in  $\delta^{15}\text{N}$ , would facilitate the interpretation of variation in  $\delta^{15}\text{N}$  of consumers in food webs over broad scales, where it can often be difficult to obtain consistent samples of potential food sources.

## **Chapter 4. Temporal and spatial variation in $\delta^{15}\text{N}$ of primary consumers: inferences and implications for baseline $\delta^{15}\text{N}$ and trophic level estimates in marine food webs**

### **4.1 Abstract**

As muscle-tissue of long-lived suspension-feeders and grazers are thought to time-integrate the seasonal and episodic  $\delta^{15}\text{N}$  fluctuations of their diet, they are assumed to accurately represent  $\delta^{15}\text{N}$  baselines of primary sources. However, these inferences will be interpreted incorrectly if  $\delta^{15}\text{N}$  variations are not accounted for over time and space. We examined the variation in  $\delta^{15}\text{N}$  for a range of species representing long-lived suspension-feeders and grazers from temperate coastal reefs over months, seasons, sites and regions. In general,  $\delta^{15}\text{N}$  was more variable over sites (1s km) and regions (10s km) than months and seasons. However, we detected a significant level of temporal  $\delta^{15}\text{N}$  variation from both of these functional groups. In using our consumers as  $\delta^{15}\text{N}$  baselines, trophic level (TL) estimates of second order consumers varied by 0.9 TLs among baseline sources, and up to ~1 TL among months within baselines. Thus, sampling at discrete points in space and time may be insufficient to accurately capture consumer  $\delta^{15}\text{N}$  in temperate coastal systems. Furthermore, in this case, it would be inappropriate to assume that primary consumers, even from the same functional groups, exhibit similar  $\delta^{15}\text{N}$  values, or patterns of temporal and spatial variation. However, while we show a high level of temporal variability in the  $\delta^{15}\text{N}$  in some species, we also show a reasonable level of consistency in others. Due to their wide distribution, the ledge mussel *S. bilocularis*, and the gastropod *T. torquatus*, may provide reliable proxies of baseline  $\delta^{15}\text{N}$  across coastal regions at continental scales.

## 4.2 Introduction

Nitrogen stable isotopes can provide a powerful and flexible tool for ecologists to describe the trophic structure within food webs (Vander Zanden et al. 1997), absolute differences between food webs (Jennings and Warr 2003), and the movement of migratory or wide-ranging animals (Hobson 1999). In each of these applications, an essential step requires accurate measurements of  $\delta^{15}\text{N}$  in organisms at or near the base of the food web. Because the  $\delta^{15}\text{N}$  of consumers is generally assumed to be enriched by  $\sim 3\text{‰}$  following each trophic step (Post 2002; Boecklen et al. 2011), these “baseline” signatures provide trophic reference points. However, without appropriate estimates in baseline  $\delta^{15}\text{N}$ , it cannot be determined whether isotopic variation in consumers reflect changes in their trophic level or simply variation in the same baseline sources (Post 2002).

Although autotrophs represent the true base of food webs, the relatively high turnover times, in tandem with the temporal and spatial variation in biochemical and physical conditions, can significantly alter their  $\delta^{15}\text{N}$  (see Chapter 3, e.g. sources and concentrations of dissolved inorganic nitrogen and phosphate, temperature and light), resulting in highly variable  $\delta^{15}\text{N}$  signatures (Cornelisen et al. 2007; Chapter 2). Another approach is to use primary consumers with variable and non-specialist diets, where their isotopic values represent an assimilated and time-integrated weighted mean of dietary sources (Post 2002; Jennings and Warr 2003; Sweeting et al. 2005). The level of time-integration relates to biomass and metabolic rate (Sweeting et al. 2005; Hill and McQuaid 2009). Since highly metabolic tissue rapidly turns over the assimilated isotopic composition of its diet, small short-lived species (e.g. zooplankton), or highly metabolic tissue in larger longer-lived species (e.g. blood plasma and liver) often reflect the short-term variations in their diets (Sweeting et al. 2005; Perga and Gerdeaux 2005). Whereas, less-active muscle tissue from larger longer-lived consumers reflect a longer-term mean (Sweeting et al. 2005; Hill and McQuaid 2009). Accordingly, ecologists have used long-lived, slow-growing primary consumers, such as bivalves and gastropods, with recognised trophic roles (e.g. suspension feeders vs benthic grazers) as time-integrated references of  $\delta^{15}\text{N}$  to identify the trophic levels of second and higher order consumers within and among food webs (Cabana and Rasmussen 1996; Post 2002; Jennings and Warr 2003).

The muscle-tissue in a number of primary-consumers has been shown to slowly equilibrate with the isotopic values of their diet (e.g. weeks to months, Gustafson et al. 2007; Fukumori et

al. 2008; Boecklen et al. 2011), but the assumption that consumers simply reflect dietary  $\delta^{15}\text{N}$  could be misleading. For example, growth rate, linked to temperature and food availability, can induce a switch from anabolism to catabolism, where isotopically lighter compounds are preferentially metabolised over heavier compounds (see Gannes et al. 1997), whereas physical stress and subsequent condition, can also induce  $\delta^{15}\text{N}$  variation (Fuller et al. 2004). A recent meta-analysis by Woodland et al (2012), that covered a wide range of taxa and aquatic systems, found that primary consumers incurred a winter-to-summer depletion in  $\delta^{15}\text{N}$ . Further, other studies have demonstrated that autotrophic  $\delta^{15}\text{N}$  can vary with N pollution and biogeochemistry, as a function of distance to known sources, e.g. sewage effluent (Gartner et al. 2002), aquaculture (Matsuo et al. 2010), and human population density (Benson et al. 2008), which may enrich the  $\delta^{15}\text{N}$  of reference taxa over small spatial scales. Thus, seasonally and regionally shifting environmental conditions and productivity in temperate coastal regions could lead to sources of variation in  $\delta^{15}\text{N}$ , and if unaccounted for, may end in a miscalculation of trophic structure. Despite these concerns,  $\delta^{15}\text{N}$  variation of primary consumers are often assumed to be minor relative to trophic fractionation, and are thus considered to reflect a consistent time-integrated  $\delta^{15}\text{N}$  value of their diet (see Boecklen et al. 2011; Woodland et al. 2012a).

Based on the above, it is critically important to quantify and compare temporal and spatial  $\delta^{15}\text{N}$  variation in potential ‘baseline’ primary consumers, and to determine how well they reflect (and possibly time-integrate) primary sources. Furthermore, it is important to generate estimates of enrichment between consumers and sources, and whether these patterns are consistent across different taxa to examine the consequence of using different primary consumers to calculate trophic levels. Such studies should give ecologists greater confidence that  $\delta^{15}\text{N}$  values of baseline consumers are representative of baseline sources, and so can be used to estimate trophic levels in higher consumers across spatial scales.

We, therefore, evaluate the suitability of different taxa of primary consumers, representing suspension and grazer functional groups as baseline proxies of  $\delta^{15}\text{N}$ . Due to their wide distribution and high abundances across Australia and New Zealand, we chose the ascidian *Herdmania momus* and the bivalve *Septifer bilocularis* to represent suspension feeders, and the echinoderm *Heliocidaris erythrogramma*, the gastropod *Turbo torquatus*, and two fish species, *Kyphosus cornelii* and *Kyphosus gladius*, to represent benthic grazers (Table 1). We examined the intraspecific variations of  $\delta^{15}\text{N}$  in these consumers over different scales of space, among regions with different biogeochemical profiles, and time, over 13 continuous months of

sampling. In addition, we recorded their isotopic response to  $\delta^{15}\text{N}$  variation in benthic (macroalgae) and suspended (particulate organic matter) primary sources, to determine whether the trophic enrichment for consumers is consistent across time and space relative to sources. Accordingly, we highlight the effect of using each species on estimations and interpretations of trophic level in higher level consumers in the region. In identifying consumers that appeared to effectively time-integrate  $\delta^{15}\text{N}$  variation, we used  $\delta^{15}\text{N}$  data from one of these species, extracted from studies conducted across southern Australia, to compare  $\delta^{15}\text{N}$  baselines at the sub-continental scale. This “baseline” may provide an accurate  $\delta^{15}\text{N}$  reference point from which to estimate trophic levels in higher consumers across coastal Australasia.

### 4.3 Materials and methods

#### Sampling and general information

We sampled muscle-tissue from ascidians, bivalves, gastropods, urchins and fish primary consumers. With the exception of relatively short-lived ascidians, these taxa were considered potentially suitable references of isotopic baselines, as each species is considered long-lived and slow-growing (see Table 1), and thought likely to negate or “dampen” any dietary  $\delta^{15}\text{N}$  variations (Post 2002). In contrast, ascidians are shorter-lived, and allowed for comparisons in  $\delta^{15}\text{N}$  variations among short and long-lived taxa.

The diet of the suspension-feeders *H. momus* and *S. bilocularis* likely consists of macroalgal detritus and phytoplankton (Kang et al. 2009; Leclerc et al. 2013), thus potentially coupling both benthic and suspended primary sources. Of our grazers, the diet of *H. erythrogramma* and *K. gladius* likely consists primarily of the small kelp, *Ecklonia radiata* (e.g. Vanderklift and Wernberg 2010, A Turco, unpublished data), which is the dominant source of production over temperate Australian reefs (Kendrick et al. 1999). Whereas *T. torquatus* and *K. cornelii* consume a range of red, green and brown macroalgae (Wernberg et al. 2008, A. Turco, unpublished data). Thus,  $\delta^{15}\text{N}$  values from our grazers may reflect either a general  $\delta^{15}\text{N}$  baseline of macroalgae or kelp.



**Table 1.** Summary of consumer taxa analysed in this study with ecological information relevant to their suitability as  $\delta^{15}\text{N}$  references for trophic baselines.

	Suspension feeders			Grazers		
	<i>H. momus</i>	<i>S. bilocularis</i>	<i>T. torquatus</i>	<i>H. erythrogramma</i>	<i>K. cornelii</i>	<i>K. gladius</i>
	<b>Ascidian</b>	<b>Bivalve</b>	<b>Gastropod</b>	<b>Echinoderm</b>	<b>Teleost</b>	<b>Teleost</b>
Lifespan	~4 <sup>1</sup>	~11 <sup>3</sup>	~10 <sup>4</sup>	~30 <sup>5</sup>	~15 <sup>7</sup>	~18 <sup>7</sup>
Diet	Seston	Seston	Macroalgae <sup>4</sup>	Macroalgae <sup>6</sup>	Macroalgae	Macroalgae
Distribution	Worldwide <sup>2</sup>	Worldwide <sup>2</sup>	Australia <sup>2</sup>	Australia <sup>2</sup>	Australia	Australia
Size (mm)	~30	~25 <sup>2</sup>	~40	~29	~49 (tl) <sup>7</sup>	~67 (tl)
Home range	Sessile, 0	Sessile, 0	10s m	10s m	10s km <sup>7</sup>	10s km <sup>7</sup>
Predators	Echinoderms	Gastropod	Teleosts	Lobster/ teleosts	teleosts/elasmobranchs/ mammals	
	/gastropods	-molluscs				

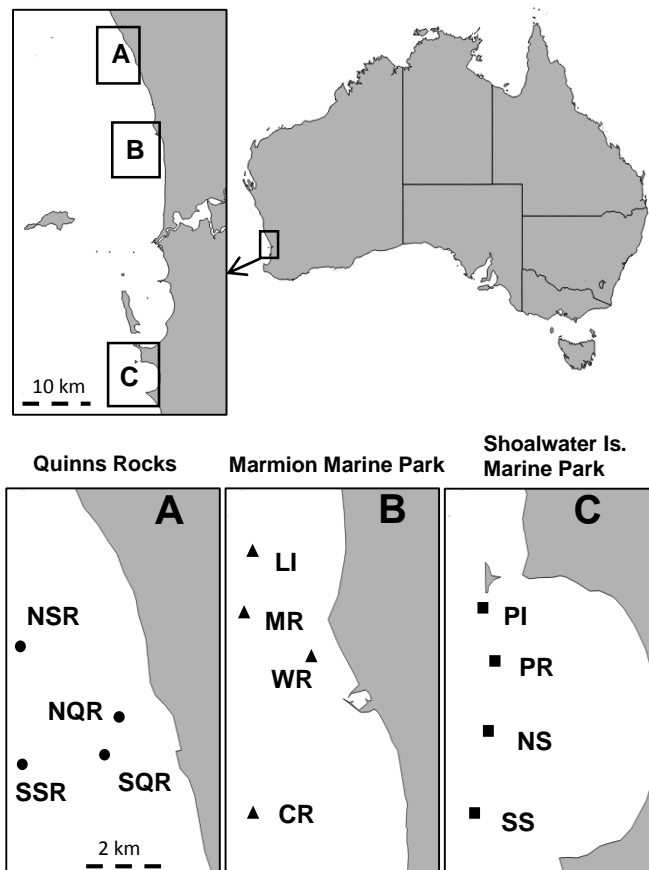
<sup>1</sup>Panadian (2012), <sup>2</sup>Encyclopedia of Life, <sup>3</sup>Selin and Latypov (2006), <sup>4</sup>Wernberg et al. (2008), <sup>5</sup>Pederson and Johnson (2007), <sup>6</sup>Vanderklift and Wernberg (2010), <sup>7</sup>Turco (Unpublished)

To measure spatial variability among individuals of invertebrate species, sites and regions, five replicate samples of each species were collected at depths between 5-7 m from within each of four sites (spaced > 1 km apart) within each of three regions (spaced > 10 km apart): Marmion Marine Park, Quinn's Rocks and Shoalwater Islands Marine Park (Figure 1). However, due to the limited number of replicate samples of *H. erythrogramma* across regions and sites, this species was excluded from our spatial analysis. We preferentially selected individuals of each species within a narrow size-range to reduce any size-related variability in  $\delta^{15}\text{N}$ . To measure temporal variability among months and seasons for invertebrate species, sampling was conducted at monthly intervals from September 2012 to September 2013 from within Marmion Marine Park, following an identical protocol as spatial sampling.

Each region experiences different levels of anthropogenic sources of nitrogen. Quinns Rocks is located in close proximity (~ 2 km) to a waste water outflow, whilst Marmion is likely to be influenced by dissolved nitrogen via surface and groundwater, being in close proximity to a populated area of Perth (Gartner et al. 2002). In comparison, Shoalwater Bay is located some distance away from waste-water outflows and is in a less populated region. Chapter 2 showed

that Quinns Rocks is marginally enriched in dissolved  $\delta^{15}\text{NO}_3$  to Marmion, but both regions were considerably enriched compared to Shoalwater Bay.

We also obtained from fishers, five replicate samples of two grazing fish species, *Kyphosus cornelii* and *K. gladius*, in April/May and September 2013. Although, these represent a limited analysis relative to invertebrates, these grazers are relatively large (adult total length ~50 cm), and muscle- $\delta^{13}\text{C}$  is expected to represent their diet over extended periods (several months).



**Figure 1.** Locations of reef sites within each region of south-western Australia (from north to south). (A) Quinns Rocks: north Scarpie Reef (NSR), north Quinns Rocks (NQR), south Quinns Rocks (SQR), south Scarpie Reef (SSR). (B) Marmion Marine Park: Little Island (LI), Marmion Reef (MR), Wanneroo Reef (WR), Centaur Reef (CR). (C) Shoalwater Islands Marine Park: Penguin Island (PI), Passage Rocks (PR), north Sisters (NS) and south Sisters (SS).

Additionally, we incorporated  $\delta^{15}\text{N}$  data of macroalgae (extracted from Chapter 3) and suspended particulate organic matter (POM, see appendix), incorporating only data from corresponding times and places as our consumers. These included three species, the small kelp *Ecklonia radiata* (of the order Laminariales), the foliose red alga *Plocamium preissianum* (of the order Plocamiales), and the smaller coralline red alga *Amphiroa anceps* (of the order Corallinales). These were chosen as each source (macroalgae and POM) are thought to represent dietary components in ascidians (e.g. POM, Kang et al. 2009), bivalves (e.g. macroalgae and phytoplankton, Hill et al. 2008; Kang et al. 2009), gastropods (e.g. brown, green and red macroalgae, Wernberg et al. 2008), and urchins (e.g. kelp, Vanderklift and Wernberg 2010).

### **Sample processing and stable isotope analysis**

For the consumers, muscle tissue was taken from the body wall of ascidians, the foot of gastropods, and the abductor muscle of bivalves, the Aristotle's Lantern of echinoderms, and from between the dorsal fin and lateral line of fish. For the macroalgae, a 4 cm section of the stipe was removed from the thallus of *E. radiata*, whereas an entire section of thallus was used for *A. anceps* and *P. preissianum*. Each seawater sample (3.5 L, containing particles <100  $\mu\text{m}$ ) was filtered through precombusted (450°C 4 hrs.) Whatman GF/F filters (nominal pore size = 0.7  $\mu\text{m}$ ) to obtain POM. Tissue from both consumer and macroalgae was dried at 60°C for 48 hours and homogenised to a powder using a ball and mill. All samples were then weighed into tin capsules ready for stable isotope analysis.

Samples were then analysed for  $\delta^{15}\text{N}$  using a Thermo Finnigan Flash EA 112 interfaced via a Thermo Conflo III with a Thermo Delta V Plus IRMS. Reference materials of known elemental composition of  $\delta^{15}\text{N}$  were interspersed with the samples for calibration. Ratios  $^{15}\text{N}/^{14}\text{N}$  are expressed as delta ( $\delta$ ) relative to the international carbon standard atmospheric  $\text{N}_2$  as:

$$\delta X (\text{‰}) = ((R_{\text{sample}}/R_{\text{standard}}) - 1) \times 1000, \text{ where } X = ^{15}\text{N}, \text{ and } R = ^{15}\text{N}:^{14}\text{N}.$$

## Data analyses

Our initial analysis examined how  $\delta^{15}\text{N}$  for each invertebrate species varied across different scales of space and time. The magnitude of variation in  $\delta^{15}\text{N}$  (of each species) at each temporal and spatial scale (i.e. to determine the variance components) was determined using the permutational analysis of variance (PERMANOVA+) package in PRIMER v6 (Plymouth Routines in Multivariate Research), based on Euclidian distances. The patterns in temporal and spatial variation were separated into two factors for each, month nested in season (temporal), and site nested in region (spatial). Variance component tests are recognised as the best estimates of the contribution of a given factor to variability in the response variable (Graham and Edwards 2001).

Relative enrichment between each consumer and source was calculated by subtracting annual mean  $\delta^{15}\text{N}$  values of each primary source from that of each consumer. We used linear correlations between corresponding mean values of consumers with those of sources (months and sites) to determine if source values were tracked by consumers. Additionally, we also linked values of sources with values of consumers from the following month (e.g. source values in January linked to consumer value in February), to factor in a time lag.

We estimated the trophic positions of secondary consumer teleosts by calibrating published muscle- $\delta^{15}\text{N}$  data from the long-ray weed whiting *Siphinognathus radiatus* (11.2‰), the sea trumpeter *Pelsartia humeralis* (11.5‰), and the long-head flathead *Leviprora inops* (11.4‰) previously collected from within the study area (Smit et al. 2005), against annual mean  $\delta^{15}\text{N}$  from our primary sources and consumers, and highlight differences among baselines. *S. radiatus*, *P. humeralis* and *L. inops* feed on first order consumers of benthic production (Howard 1988; Crawley et al. 2006; Coulson et al. 2013). We also highlight the effect of temporal  $\delta^{15}\text{N}$  variation on subsequent trophic calculations, by comparing monthly mean trophic level estimates in *L. inops* against each of our consumers (and *E. radiata*). We could only use a static mean  $\delta^{15}\text{N}$  value for *L. inops*, as there are no time-series data available, however, we feel this is reasonable given *L. inops* is relatively large (adult total length >50 cm), and muscle- $\delta^{15}\text{N}$  is likely to represent its diet over extended periods. The  $\delta^{15}\text{N}$  in our different primary consumers were set at trophic level 2 (and TL1 for *E. radiata*), and we assumed a consistent  $\delta^{15}\text{N}$  trophic enrichment of 3.0‰ using the following equation:

$$\text{TL} = [(\delta^{15}\text{N}_{\text{teleost}} - \text{mean } \delta^{15}\text{N}_{\text{consumer/producer}})/3] + 1_{\text{consumer}/2_{\text{producer}}}$$

## 4.4 Results

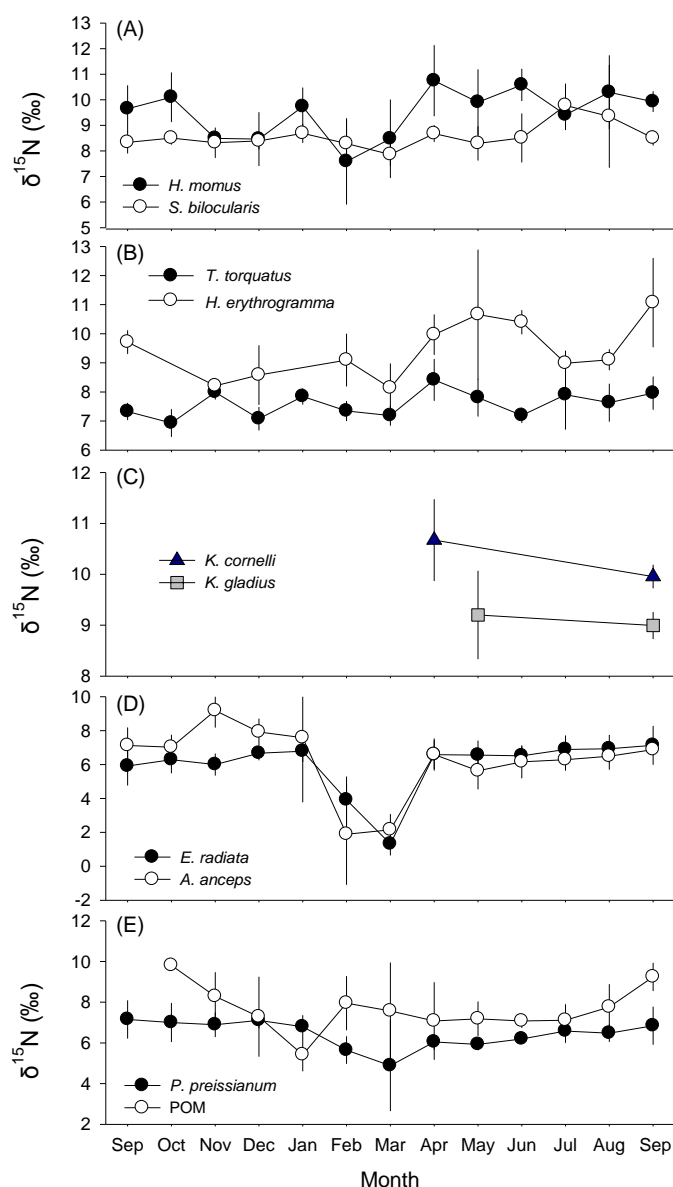
### Consumer $\delta^{15}\text{N}$ variation

Annual mean (12-months)  $\delta^{15}\text{N}$  was 9.49‰ ( $\pm 0.26$ ) for *H. momus*, 8.57‰ ( $\pm 0.13$ ) for *S. bilocularis*, 7.59‰ ( $\pm 0.12$ ) for *T. torquatus* and 9.45‰ ( $\pm 0.30$ ) for *H. erythrogramma*. *H. momus*, *T. torquatus* and *H. erythrogramma* each displayed significant variation in  $\delta^{15}\text{N}$  across months within seasons ( $P = 0.01$ ,  $0.001$  and  $0.006$ , respectively), but not among seasons ( $P = 0.32$ ,  $0.82$ , and  $0.83$ , respectively) (Table 2, Figure 2). For each of these species, the greatest source of variation was at the replicate (residual) scale (63, 68 and 58%, respectively), followed by months (30, 32 and 42%, respectively), and then seasons, which was either negligible or statistically absent (Table 2, Figure 3). The  $\delta^{15}\text{N}$  for *S. bilocularis* was not significant across months or seasons ( $P = 0.21$  and  $0.08$ , respectively), with the greatest source of variation at the replicate scale (78%).

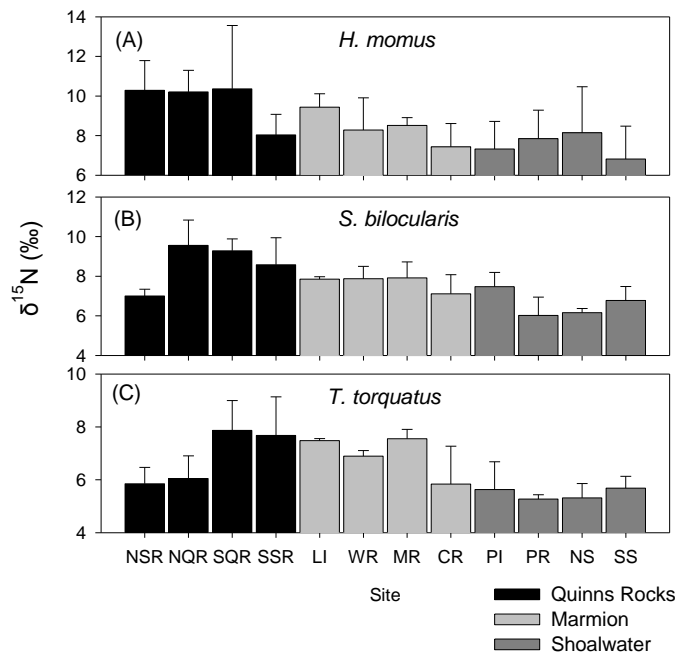
Across sites and regions, mean  $\delta^{15}\text{N}$  in suspension-feeders ranged from 6.81 to 10.35‰ in *H. momus*, and 6.02 to 9.56‰ in *S. bilocularis*, each displaying significant variation across regions ( $P = 0.006$  and  $0.025$ , respectively), but not sites within regions ( $P = 0.85$  and  $0.47$ , respectively) (Table 2, Figure 3). The greatest source of variation in *H. momus* was at the replicate scale (69%), while that of *S. bilocularis* was at the regional scale (42%) followed by replicate scale (34%). The mean  $\delta^{15}\text{N}$  in the grazer *T. torquatus* ranged from 5.27 to 7.87‰, and displayed significant variation across sites ( $P = 0.003$ ) and regions ( $P = 0.046$ ), with the greatest source of variation at the replicate (41%) and region (32%) scales (Table 2).

**Table 2.** Permutational analysis of variance (PERMANOVA) results, including variance components estimates (and % variance), for the  $\delta^{15}\text{N}$  in primary consumers, with months nested in seasons. Results were obtained from PERMANOVA based on Euclidian distance. Negative variance components estimates were treated as zero. *P* values in bold indicate significance.

Taxa		Temporal variation			
Var. (%)	Factors	<i>df</i>	MS	<i>P</i>	Var. (%)
<b>Suspension feeders</b>					
<i>Herdmania</i>	Season	3	5.22	0.296	0.11 (5.70)
<i>momus</i>	<b>Month (S)</b>	<b>8</b>	<b>3.88</b>	<b>0.010</b>	<b>0.61 (31.8)</b>
	Residuals	43	1.20		1.20 (62.5)
<i>Septifer</i>	Season	3	2.72	0.079	0.13 (16.9)
<i>bilocularis</i>	Month (S)	8	0.85	0.212	0.04 (5.19)
	Residuals	52	0.60		0.60 (77.9)
<b>Grazers</b>					
	Season	3	0.42	0.820	0
<i>Turbo torquatus</i>	<b>Month (S)</b>	<b>8</b>	<b>1.26</b>	<b>0.001</b>	<b>0.18 (31.6)</b>
	Residuals	55	0.30		0.39 (68.4)
<i>Heliocidaris</i>	Season	3	1.11	0.825	0
<i>erythrogramma</i>	<b>Month (S)</b>	<b>6</b>	<b>4.53</b>	<b>0.006</b>	<b>0.87 (42.2)</b>
	Residuals	38	1.20		1.20 (57.9)
Taxa		Spatial variation			
	Factors	<i>df</i>	MS	<i>P</i>	Var. (%)
<b>Suspension feeders</b>					
<i>Herdmania</i>	<b>Region</b>	<b>2</b>	<b>20.9</b>	<b>0.006</b>	<b>1.41 (30.7)</b>
<i>momus</i>	Site (R)	7	1.48	0.851	0
	Residuals	34	3.19		3.19 (69.3)
<i>Septifer</i>	<b>Region</b>	<b>2</b>	<b>18.6</b>	<b>0.025</b>	<b>0.84 (42.5)</b>
<i>bilocularis</i>	Site (R)	9	2.95	0.47	0.47 (23.7)
	Residuals	45	0.67		0.67 (33.8)
<b>Grazers</b>					
	<b>Region</b>	<b>2</b>	<b>13.5</b>	<b>0.046</b>	<b>0.54 (32.5)</b>
<i>Turbo torquatus</i>	<b>Site (R)</b>	<b>9</b>	<b>2.88</b>	<b>0.003</b>	<b>0.44 (26.5)</b>
	Residuals	47	0.68		0.68 (40.9)



**Figure 2.** Monthly means ( $\pm$ SD) of muscle- $\delta^{15}\text{N}$  in: (A) suspension-feeders, the ascidian *Herdmania momus* and the bivalve *Septifer bilocularis*; (B) invertebrate grazers, the gastropod *Turbo torquatus*, and the urchin *Heliocidaris erythrogramma*; (C) fish grazers *Kyphosis cornelli* and *Kyphosis gladius*; and (D) the brown alga *Ecklonia radiata* and red algae *Amphiroa anceps*; and (E) the red algae *Plocamium preissianum* and particulate organic matter (POM); sampled over 13-months from Marmion Marine Park.



**Figure 3.** Means ( $\pm$  standard deviation) of  $\delta^{15}\text{N}$  in the ascidian *Herdmania momus*, the bivalve *Septifer bilocularis* and the gastropod *Turbo torquatus*, at sites in each region. See Figure 1 for site locations.

### Relative $\delta^{15}\text{N}$ enrichment between primary consumers and sources

Mean annual  $\delta^{15}\text{N}$  was elevated for primary consumers relative to sources, except for *T. torquatus* relative to POM (Table 3). However, patterns of enrichment in consumers did not fall into functional suspension feeding or grazing groups. The suspension feeder *H. momus* and grazer *H. erythrogramma* were most enriched (3.0-3.5‰ relative to macroalgae and ~1.8‰ relative to POM), whilst the suspension feeder *S. bilocularis* and grazer *T. torquatus* were least enriched (2.1-2.5‰ and 1.1-1.6‰ relative to macroalgae, respectively, and 0.99‰ and 0.03‰ relative to POM, respectively). The mean  $\delta^{15}\text{N}$  in our two grazing teleosts *K. cornelii* and *K. gladius* only represent two sampling points (Mar/Apr and Sep), but were 10.31‰ ( $\pm$  0.21) and 9.10‰ ( $\pm$  0.06), respectively (Figure 3c), and were enriched relative to macroalgae by 3.8-4.3‰ and 2.6-3.1‰, respectively (Table 3).



**Table 3.** Mean annual consumer  $\delta^{15}\text{N}$  enrichment relative to primary sources.

Consumer species	Primary source			
	<i>A. anceps</i>	<i>E. radiata</i>	<i>P. preissianum</i>	POM
<u>Suspension-feeders</u>				
<i>H. momus</i>	3.35	3.50	3.12	1.99
<i>S. bilocularis</i>	2.35	2.51	2.13	0.99
<u>Grazers</u>				
<i>T. torquatus</i>	1.39	1.54	1.16	0.03
<i>H. erythrogramma</i>	3.22	3.48	3.02	1.79
<i>K. cornelii</i>	4.12	4.27	3.89	2.76
<i>K. gladius</i>	2.90	3.06	2.68	1.54

### Correlations of spatial and temporal $\delta^{15}\text{N}$ patterns in primary consumers and sources

Using correlation coefficients ( $r$ ), the  $\delta^{15}\text{N}$  in *H. momus* and *H. erythrogramma* each correlated significantly with corresponding monthly values of *E. radiata* ( $P = 0.03$ ,  $r = 0.36$  and  $P = 0.04$ ,  $r = 0.39$ , respectively), but not other sources, even when factoring in a one-month time-lag (Table A5, appendices). The  $\delta^{15}\text{N}$  in the suspension feeder *S. bilocularis* and grazer *T. torquatus* did not correlate with corresponding monthly values in any primary source (Table 4, Figure 3), or over one-month time-lag (Table A5).

Across sites, mean  $\delta^{15}\text{N}$  in the suspension feeders *H. momus*, and *S. bilocularis* each correlated significantly with corresponding values of *E. radiata* ( $P = 0.02$ ,  $r = 0.41$  and  $P = 0.02$ ,  $r = 0.43$ , respectively) and *P. preissianum* ( $P = 0.01$ ,  $r = 0.58$  and  $P = 0.04$ ,  $r = 0.35$ , respectively), but not other sources (Table 4). *T. torquatus*  $\delta^{15}\text{N}$  correlated with corresponding values of *E. radiata* ( $P = 0.03$ ,  $r = 0.40$ ), but not other sources.

**Table 4.** Linear correlations ( $r$ ) between mean  $\delta^{15}\text{N}$  in primary consumers and primary sources over sites (upper panel) and months (lower panel). Significant correlations ( $p = <0.05$ ) are highlighted in bold type.

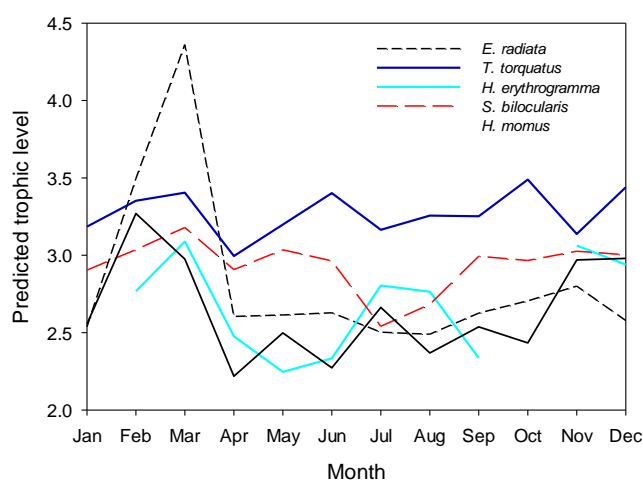
Primary source	Suspension-feeders				Grazers			
Temporal	<i>H. momus</i>	<i>S. bilocularis</i>	<i>T. torquatus</i>	<i>H. erythrogramma</i>				
	<i>P</i>	<i>r</i>	<i>P</i>	<i>r</i>	<i>P</i>	<i>r</i>	<i>P</i>	<i>r</i>
<i>A. anceps</i>	0.178	0.15	0.372	0.08	0.334	0.01	0.852	0.01
<i>E. radiata</i>	<b>0.027</b>	<b>0.36</b>	0.063	0.33	0.212	0.14	<b>0.043</b>	<b>0.39</b>
<i>P. preissianum</i>	0.454	0.06	0.281	0.01	0.963	0.01	0.873	0.01
POM	0.629	0.01	0.571	0.03				
Spatial	<i>H. momus</i>	<i>S. bilocularis</i>	<i>T. torquatus</i>					
	<i>P</i>	<i>r</i>	<i>P</i>	<i>r</i>	<i>P</i>	<i>r</i>		
<i>A. anceps</i>	0.256	0.14	0.849	0.03	0.638	0.02		
<i>E. radiata</i>	<b>0.017</b>	<b>0.41</b>	<b>0.019</b>	<b>0.43</b>	<b>0.026</b>	<b>0.40</b>		
<i>P. preissianum</i>	<b>0.003</b>	<b>0.58</b>	<b>0.039</b>	<b>0.35</b>	0.193	0.35		
POM	0.183	0.26	0.121	0.29				

### Trophic level estimation

Estimated trophic level of benthic secondary consumers, *Pelsartia humeralis*, *Siphinognathus radiatus*, and *Leviprora inops*, using different annual means of  $\delta^{15}\text{N}$  in primary producers and consumers as baselines, elicited similar results among the macroalgae, but dissimilar results among the consumers (Table 5). The two most disparate consumer baselines were *T. torquatus* and *K. cornelii*, which elicited estimates of almost one TL apart for each fish species. Estimated trophic level of *Leviprora inops*, using monthly means of  $\delta^{15}\text{N}$  in *E. radiata*, showed a large episodic fluctuation ( $\pm 1.0$  TL), due to a sharp depletion-enrichment event in February and March, but estimates were relatively consistent throughout the rest of the year (Figure 5). In contrast, against primary-consumers, estimates were consistently variable among months, but limited in the degree to which they varied. Estimates varied by about half a trophic level for *H. momus* ( $\pm 0.55$ ) and *H. erythrogramma* ( $\pm 0.42$ ), and approximately a quarter a trophic level for *T. torquatus* ( $\pm 0.25$ ) and *S. bilocularis* ( $\pm 0.32$ ) (Figure 5).

**Table 5.** Differences in trophic level estimates of fish using different organisms as  $\delta^{15}\text{N}$  baselines.

Baseline taxa	Trophic level			
	<i>Pelsartia humeralis</i>	<i>Siphinognathus radiatus</i>	<i>Leviprora inops</i>	
<u>Primary sources</u>				
<i>A. anceps</i>	1	2.8	2.7	2.7
<i>E. radiata</i>	1	2.8	2.7	2.8
<i>P. preissianum</i>	1	2.7	2.6	2.7
POM	1	2.3	2.2	2.3
<u>Suspension-feeders</u>				
<i>H. momus</i>	2	2.7	2.6	2.6
<i>S. bilocularis</i>	2	3.0	2.9	3.0
<u>Grazers</u>				
<i>T. torquatus</i>	2	3.3	3.2	3.3
<i>H. erythrogramma</i>	2	2.6	2.5	2.6
<i>K. cornelii</i>	2	2.4	2.3	2.4
<i>K. gladius</i>	2	2.8	2.7	2.8



**Figure 5.** Estimated trophic level of *Leviprora inops*, using monthly means of  $\delta^{15}\text{N}$  in the macroalgae *Ecklonia radiata* and invertebrate primary consumers, *Herdmania momus*, *Septifer bilocularis*; *Turbo torquatus* and *H. erythrogramma* as  $\delta^{15}\text{N}$  baselines.

## 4.5 Discussion

It is becoming increasingly common to use the  $\delta^{15}\text{N}$  of suspension-feeders and grazers as general baselines for quantifying the trophic level of consumers in food webs (Post 2002; Guzzo et al. 2011; Xu et al. 2011; Woodland et al. 2012a). The scales at which variation in  $\delta^{15}\text{N}$  occurs in primary consumers, must be considered for this approach to be accurate (Woodland et al. 2012a). The  $\delta^{15}\text{N}$  in each of our consumers was more variable over sites (1s km) and regions (10s km) than months and seasons. However, we have shown a significant level of temporal  $\delta^{15}\text{N}$  variation in species from both of these functional groups. Our simple predictions of TL in the teleost secondary consumer, *L. inops*, clearly highlights the potential for trophic miscalculations. However, while we show a high level of temporal variability in the  $\delta^{15}\text{N}$  (and subsequent TL estimates) in some species, we also show a reasonable level of consistency in others. Our quantification of annual mean  $\delta^{15}\text{N}$  in primary consumers reveals  $\delta^{15}\text{N}$  from taxa within and among functional groups can be quite disparate, and highlights the potential for TL discrepancies in using different species as  $\delta^{15}\text{N}$ /trophic baselines.

### Temporal variation

With the exception of bivalves, the  $\delta^{15}\text{N}$  in our consumer species, regardless of functional role, displayed significant variability among months, and all consumers lacked clear temporal trends with inconsistent enrichment/depletions in following months. Ascidiarians and urchins exhibited a wider range of mean values than bivalves and gastropods, and demonstrate how trophic estimates could be misinterpreted if temporal variations in the  $\delta^{15}\text{N}$  of primary consumers are not properly considered (Woodland et al., 2012a). For example, as the urchin *H. erythrogramma* is extremely long-lived (e.g. ~30 yrs., Pederson and Johnson, 2007), it could be assumed that  $\delta^{15}\text{N}$  values would be sufficiently alike across months to reflect a long-term average. This was not the case, as values were in constant flux, with mean values varying by up to ~3‰ among months (similar to short-lived ascidians), equating to discrepancies of an entire trophic level in the estimate of a secondary consumer. Clearly, sampling urchins across the year is required to accurately describe a representative value. In contrast, monthly mean  $\delta^{15}\text{N}$  in bivalves and gastropods remained relatively consistent, equating to only a minor bias in trophic estimates among months (e.g. 0.5 TLs). Interestingly,  $\delta^{15}\text{N}$  did not differ among seasons for any of our consumers, which contrasts with a meta-analysis by Woodland et al.

(2012a) that covered a wide range of taxa and aquatic systems, and found that primary consumers incur a winter-to-summer depletion in  $\delta^{15}\text{N}$ . The lack of any seasonal effect likely reflects the high variability among months in any one season, but the potential causes of this variability are difficult to resolve.

Although we sampled highly abundant and likely dietary sources, we cannot discount other primary sources contributing to the consumer diets. Further, consumer  $\delta^{15}\text{N}$  does not simply reflect dietary  $\delta^{15}\text{N}$ , but is complicated by temporal changes in metabolic rate and food availability, and subsequent growth and repair, which can result in changes in turnover and  $\delta^{15}\text{N}$  fractionation, two important sources of variation (Tieszen et al. 1983; Gannes et al. 1997; Overman and Parrish 2001; Sweeting et al. 2007; Woodland et al. 2012a).

Despite the potential for the above causes of variation, the  $\delta^{15}\text{N}$  of ascidians and urchins correlated with corresponding values of the dominant source of production, kelp, in the region. Although the correlations between ascidian, urchin and kelp  $\delta^{15}\text{N}$  were significant, monthly trajectories were not tightly linked, and each of our consumers displayed a far narrower range of values than primary producers, particularly the macroalgae, which underwent sharp depletion-enrichment events in the early part of the year. This is perhaps not surprising, given that the  $\delta^{15}\text{N}$  of autotrophs are subject to more variation than their consumers, as they respond rapidly to changes in nutrient  $\delta^{15}\text{N}$  and coastal hydrography (O'Reilly and Hecky, 2002), while consumers time-integrate (weeks to months) dietary source  $\delta^{15}\text{N}$ , which should act to dampen any variability. These data further highlight the problems of using autotrophs for base  $\delta^{15}\text{N}$  (e.g. Cabana and Rasmussen, 1996; Gustafson et al., 2007; Post, 2002, Chapter 2), as variation in TL estimates among months were particularly large (e.g. kelp,  $\sim 2$  TL). In contrast to ascidians and urchins, the muscle-tissue  $\delta^{15}\text{N}$  of bivalves and gastropods did not correlate with corresponding values any of the sources sampled (or when factoring a one month time-lag). These data highlight the need to consider temporal integration when comparing values which average  $\delta^{15}\text{N}$  over different timescales. Our results concur with other studies that have shown bivalves and gastropods to be good temporal integrators of highly variable  $\delta^{15}\text{N}$  among primary producers (Post 2002; Gustafson et al. 2007; Fukumori et al. 2008). Measurements of  $\delta^{15}\text{N}$  turnover in mussels and gastropods can be slow, equilibrating to that of their diet over extended periods, e.g. months to years (Hawkins 1985; Hill and McQuaid 2009, E. Gates, unpub. data), so it is no surprise that  $\delta^{15}\text{N}$  in these taxa did not reflect the monthly measurements of primary sources. Therefore, it is surprising that both ascidians and urchins significantly tracked values

of kelp, but this may be an effect of other co-varying environmental variables. Notwithstanding this, our data suggest that dietary  $\delta^{15}\text{N}$  is rapidly equilibrated by ascidians and urchins.

Although the stable isotopes in different consumers at single points in time represent an integration of dietary sources over different timescales, annual mean values over an entire year should neutralise this effect. Thus, by consistently sampling throughout the year from the same location, we have used the best feasible approach to capture annual mean  $\delta^{15}\text{N}$ , and quantify relative enrichment between primary consumers and sources. The macroalgae had similar values (e.g.  $\sim 6.2\text{‰}$ ), whereas bulk POM was relatively enriched (e.g.  $\sim 7.5\text{‰}$ ). Using  $3\text{‰}$  as a benchmark for herbivore specific trophic fractionation (e.g. Vanderklift and Ponsard 2003), as expected for grazers, the level of enrichment in urchins was consistent with a diet a macroalgae (e.g.  $\sim 3.2\text{‰}$ ), but this was not the case for gastropods (e.g.  $\sim 1.4\text{‰}$ ). However, this species appears to be an exception, as our results were consistent with fractionation estimates from controlled feeding studies specific to this species, e.g.  $\sim 1.5\text{‰}$  (E. Gates, unpub. data). Interestingly, our suspension-feeders were also more aligned to macroalgae than bulk POM, which suggests that both ascidians and bivalves are exhibiting size-selective feeding of macroalgal detritus from bulk POM (discussed further below).

The trophic level estimates for the long-ray weed whiting *S. radiatus*, the sea trumpeter *P. humeralis*, and the long-head flathead *L. inops*, combining mean values of macroalgae, equated to  $\sim 2.8$ , which is approximately what we would expect, given that these species are considered secondary consumers from benthic food chains (Howard 1988; Crawley et al. 2006; Coulson et al. 2013). However, annual mean differences in  $\delta^{15}\text{N}$  among suspension-feeding ascidians and bivalves, and among grazing gastropods, urchins and fish, equated to large discrepancies in the trophic level estimates of secondary consumers (differences of 0.9 TLs). While we recognise that these fish species are unlikely to consume the primary consumers we examined, they are representative of that trophic level, and therefore the potential effects any variability among primary consumers is likely to have on higher trophic levels. This differences we observed is clearly enough to mislead trophic studies, and far greater than discrepancies among macroalgae (e.g.  $\sim 0.1$  TL's). While these results further highlight the importance of evaluating the effects of selecting different baselines on trophic level estimates (e.g. Guzzo et al. 2011; Xu et al. 2011), they also provide a reference to correct for different levels of  $\delta^{15}\text{N}$  enrichment in these consumers, relative to macroalgae. For example, the trophic levels of higher consumers are often estimated relative to a primary consumer (set a TL 2), and often the level of enrichment between the primary consumer and the source(s) of production they are assumed

to proxy are unknown. Therefore, calculations of trophic level in higher consumers using this method are relative not definitive (see Jennings et al. 2002). By correcting for enrichment (e.g. gastropods relative to macroalgae, ~1.5‰), this value could be subtracted to infer an absolute value of base  $\delta^{15}\text{N}$  (e.g. trophic level 1, macroalgae).

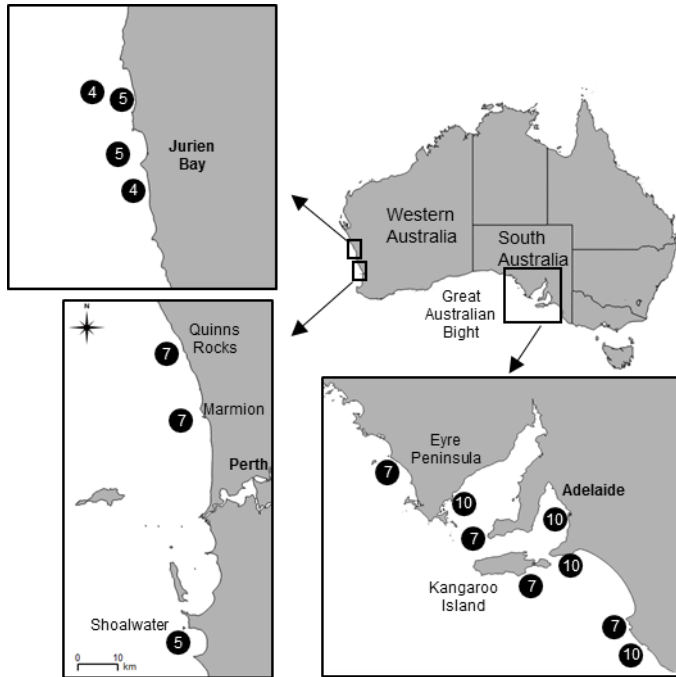
## Spatial variation

Each primary consumer showed significant variability among regions separated by 10s of km, while differences were also significant for gastropods among sites separated by 1s of km. Broadly, differences in primary consumers followed a south to north pattern of enrichment, with Shoalwater being depleted relative to Marmion (~1‰) and Quinns Rocks (~2‰). This trend appears to reflect differences in human derived dissolved inorganic N documented in Chapter 3, likely enriching primary sources, and propagated through to consumers. Numerous studies have shown anthropogenic derived sources of nutrients enriching the  $\delta^{15}\text{N}$  of local foodwebs (Cabana and Rasmussen 1996; Gorman 2009; Vanderklift and Wernberg 2010), and these results further highlight the need to correct for such baseline variations in  $\delta^{15}\text{N}$  when apportioning trophic levels, particularly across urbanised regions where nutrient enrichment can influence autotrophic  $\delta^{15}\text{N}$ .

Patterns of variability among sites differed among taxa and trophic groups, but by using baseline  $\delta^{15}\text{N}$  variation to facilitate the exploration of trophic links, by correlating differences among primary sources and consumers, we showed that  $\delta^{15}\text{N}$  of consumers significantly tracked those of kelp for all three species (*H. momus*, *S. bilocularis*, and *T. torquatus*) and those of the red alga *P. preissianum* for the former two species. Whether both kelp and *P. preissianum* are contributing to the diets of ascidians and gastropods is difficult to determine, but as the biomass of kelp far exceeds that of other macroalgae (Wernberg and Vanderklift 2010), it seems possible that kelp is responsible. Regardless, this is further evidence that  $\delta^{15}\text{N}$  in suspension-feeders and grazers over macroalgal dominated reefs, represent values of macroalgae, particularly kelp. Kelp can be a significant component in suspension-feeder diets on kelp dominated reefs (Fredriksen 2003; Leclerc et al. 2013), and although debated in the literature (Miller and Page 2012; Miller et al. 2013), our results point to a trophic link between kelp and suspension feeders.

Bivalves and gastropods have been used to represent spatial differences in  $\delta^{15}\text{N}$  baselines in aquatic systems elsewhere (Post 2002; Gustafson et al. 2007), but often without prior knowledge of temporal variation (Xu et al. 2011). We have shown the suspension feeding bivalve *S. bilocularis* and the grazing gastropod *T. torquatus* display limited to no temporal variation in  $\delta^{15}\text{N}$ , and therefore appear to effectively time-integrate  $\delta^{15}\text{N}$  variation, but display high regional variation. Several lines of evidence show that the  $\delta^{15}\text{N}$  in both these species represent values of macroalgae, but are enriched to different degrees. Notwithstanding variation at the individual level, we feel it is reasonable to assume that spatial differences in these species reflect general and time-integrated estimates of spatial variation in macroalgae  $\delta^{15}\text{N}$  (with a degree of error). Since *T. torquatus* displayed limited temporal variation and its  $\delta^{15}\text{N}$  has been reported for other regions in southern Australia (e.g. Gorman 2009; Hyndes et al. 2013), we have combined our data with these studies to estimate and compare  $\delta^{15}\text{N}$  baselines across a sub-continental scale (Figure 6). This extended spatial analysis, unsurprisingly, shows  $\delta^{15}\text{N}$  values within each coastline to be elevated in the major metropolitan areas (e.g. Perth and Adelaide), but more interesting, the South Australian coastline is enriched relative to the mid and lower-western Australian coastline ( $\sim 2\text{‰}$ ). These cross continental trends match those of  $\delta^{15}\text{N}$  in macroalgae reported in Chapter 2, where latitudinal differences (also  $\sim 2\text{‰}$ ), were linked indirectly to water temperature. Although, small scale differences in human derived N are likely to overlay some of these latitudinal differences, broadly speaking, this species may provide an accurate  $\delta^{15}\text{N}$  reference from which to estimate trophic levels in higher consumers across coastal Australasia.





**Figure 6.** Spatial differences in the muscle- $\delta^{15}\text{N}$  of *Turbo torquatus* in coastal Western and South Australia.  $\delta^{15}\text{N}$  values (within black circles) are rounded to whole numbers.

## 4.6 Conclusion

Inferences regarding the trophic levels of consumers from  $\delta^{15}\text{N}$  rely on predictable values of baseline food sources (Post 2002; Hyndes et al. 2013). While primary consumers are often thought to provide a general baseline for quantifying estimates of trophic levels, we have demonstrated that they can be susceptible to substantial temporal and spatial variation that could undermine trophic investigations. Our trophic level estimates, calibrated against different months and species, highlight this point. Further, in this case, it would be inappropriate to assume that primary consumers, even from the same functional groups, exhibit similar  $\delta^{15}\text{N}$  values. However, while we show a high level of temporal variability in the  $\delta^{15}\text{N}$  in some species, we also show a reasonable level of consistency in others. Due to their wide distribution, the ledge mussel *S. bilocularis*, and the gastropod *T. torquatus*, may provide reliable proxies of baseline  $\delta^{15}\text{N}$  across coastal regions at continental scales.

## **Chapter 5. Spatial and temporal variation of $\delta^{13}\text{C}$ in suspension-feeding and grazing marine consumers**

### **5.1 Abstract**

Because of high temporal variation in the  $\delta^{13}\text{C}$  of autotrophs, long-lived primary-consumers are often used instead as proxies (often bivalves and gastropods) to interpret food webs, as they are assumed to time-integrate some of the episodic or seasonal fluctuations of their diet. However, these inferences will be interpreted incorrectly if values of consumer- $\delta^{13}\text{C}$  are not linked to the primary sources they are assumed to proxy, and variations are not accounted for. We measured spatial and temporal variation in the  $\delta^{13}\text{C}$  of primary consumers from suspension-feeding and grazing functional groups, and compared temporal variability of these consumers over 13 continuous months, against corresponding values of likely dietary sources (i.e. particulate organic matter and macroalgae). We showed significant temporal and spatial variation in the  $\delta^{13}\text{C}$  of each taxon, but patterns differed among species. We used relative enrichment of primary consumers against primary sources, from annual mean values, to infer a simple representation of likely dietary source(s). The  $\delta^{13}\text{C}$  of the suspension-feeders were clearly separated from bulk POM ( $\sim 5\text{‰}$ ), far greater than typical values of  $\delta^{13}\text{C}$  fractionation (e.g.  $\sim 0\text{--}2\text{‰}$ ), and more aligned with kelp ( $\sim 1.5\text{‰}$ ), as were urchins (e.g.  $1.6\text{‰}$ ). Gastropods had intermediate values between kelp (depleted,  $1.8\text{‰}$ ) and red algae (enriched,  $\sim 10\text{‰}$ ). Surprisingly, both absolute values of variance components, and ranges of mean  $\delta^{13}\text{C}$  among months were generally greater for primary consumers (e.g. ascidians  $2.97\text{‰}$ , bivalves  $2.61\text{‰}$ , gastropods  $2.25\text{‰}$ , and urchins  $2.92\text{‰}$ ) than primary sources (e.g. kelp  $1.70\text{‰}$ , red algae  $2.25\text{‰}$ , and POM  $2.50\text{‰}$ ). While suspension-feeding and grazing primary consumers have been used as reference taxa for sestonic and benthic  $\delta^{13}\text{C}$  baselines, we show that taxa from these functional groups may not necessarily represent these sources or be any less variable. In this case, there are no advantages in using primary consumers over actual primary sources to describe baseline  $\delta^{13}\text{C}$ .

## 5.2 Introduction

Carbon stable isotopes ( $\delta^{13}\text{C}$ ) are often used to describe energy pathways through food webs and the primary sources that support them (DeNiro and Epstein 1977; Jennings and Reñones 1997; Smit et al. 2005; Barnes et al. 2009). As  $\delta^{13}\text{C}$  undergoes small and predictable changes following each trophic step (Vander Zanden and Rasmussen 2001; Post 2002), and different functional groups of marine autotrophs have fairly distinct  $\delta^{13}\text{C}$  values, e.g. seagrasses, macroalgae and phytoplankton (Farquhar 1989; Raven et al. 2002),  $\delta^{13}\text{C}$  has proved useful in proportioning their relative trophic role in food webs (Simenstad and Wissmar 1985; Lepoint et al. 2000; Melville and Connolly 2003; Hill et al. 2008). However, these inferences are only valid if measurements of  $\delta^{13}\text{C}$  of autotrophs and consumers are accurate. The relatively high turnover times of autotrophs, together with temporally and spatially variable biogeochemical and physical conditions, can result in highly variable  $\delta^{13}\text{C}$  signatures (Boon and Bunn 1994; Raven et al. 2002; Dethier et al. 2013), which can lead to ambiguous results. Consequently, long-lived primary consumers (e.g. sestonic filter feeders or benthic grazers), are often used instead to broadly represent the  $\delta^{13}\text{C}$  of sestonic or benthic sources, as they are assumed to integrate much of the episodic or seasonal fluctuations of their diet, representing a more temporally stable baseline from which to assess the flow of material through food webs (Post 2002; Gustafson et al. 2007). Further, such primary consumers may act as reliable references for spatial differences in baseline  $\delta^{13}\text{C}$ , from which to compare geographically separated food webs (Post 2002; Barnes et al. 2009). However, while several studies have shown primary consumers such as filter-feeding bivalves and grazing gastropods to represent consistent  $\delta^{13}\text{C}$  values relative to their likely dietary source(s), in the main these have been conducted in freshwater environments (e.g. Post 2002; Gustafson et al. 2007), which may not hold for open marine systems.

Although studies have shown that the muscle-tissue in a number of primary consumers slowly equilibrates with the isotopic values of their diet (e.g. weeks to months) (see Boecklen et al. 2011), this assumption could be easily violated. Studies are generally conducted in controlled environments (e.g. aquaria/mesocosm), but consumer  $\delta^{13}\text{C}$  can vary for reasons other than their diet. Fractionation, reflecting differences in food quality (Oelbermann and Scheu 2002) or physiological differences between genders and among life-history stages (Vizzini and Mazzola 2004; Reich et al. 2008), can each influence the stable isotopes of consumers. Even when presented with a consistent and controlled food source, consumers can exhibit variability in

$\delta^{13}\text{C}$  among individuals (Barnes et al. 2008). Further, growth rate, linked to temperature and food availability, can induce a switch from anabolism to catabolism, where isotopically lighter compounds are preferentially metabolised over heavier compounds (see Gannes et al. 1997). Thus, since environmental conditions and productivity can vary both seasonally and spatially in temperate coastal regions, any variation in consumer- $\delta^{13}\text{C}$  could be unrelated to diet. Despite these concerns,  $\delta^{13}\text{C}$  variation of marine primary consumers are assumed to be minor relative to autotrophic variation, thus representing stable and time-integrated baseline (Post 2002; Gustafson et al. 2007). These inferences are rarely tested, particularly in temperate coastal systems, and there has been limited focus on whether shifts in  $\delta^{13}\text{C}$  occur consistently across functional groups.

Based on the above, it is therefore critically important to quantify and compare temporal and spatial  $\delta^{13}\text{C}$  variation in potential “baseline” primary consumers, and to equate their temporal stability against the primary sources they are assumed to proxy. We have therefore quantified, compared and contrasted  $\delta^{13}\text{C}$  values and variability in the muscle-tissue of a range of primary consumers (ascidians, bivalves, gastropods, and urchins) on temperate coastal reefs over different scales of space and time. Furthermore, we equate temporal variability in primary consumers to corresponding data of sestonic (suspended particulate organic matter, POM) and benthic primary sources (brown and red macroalgae) based on data from Chapter 2. Specifically, we have determined the: (1) spatial and temporal scales of variation in  $\delta^{13}\text{C}$  of primary consumers; (2) consistency in the patterns of  $\delta^{13}\text{C}$  variation across taxa within functional groups; and (3) variability in primary consumer- $\delta^{13}\text{C}$  relative to their likely dietary sources. Overall, I assess the implications of using primary consumer  $\delta^{13}\text{C}$  as  $\delta^{13}\text{C}$  baselines for suspended or benthic sources.

## 5.3 Materials and methods

### Chosen consumers

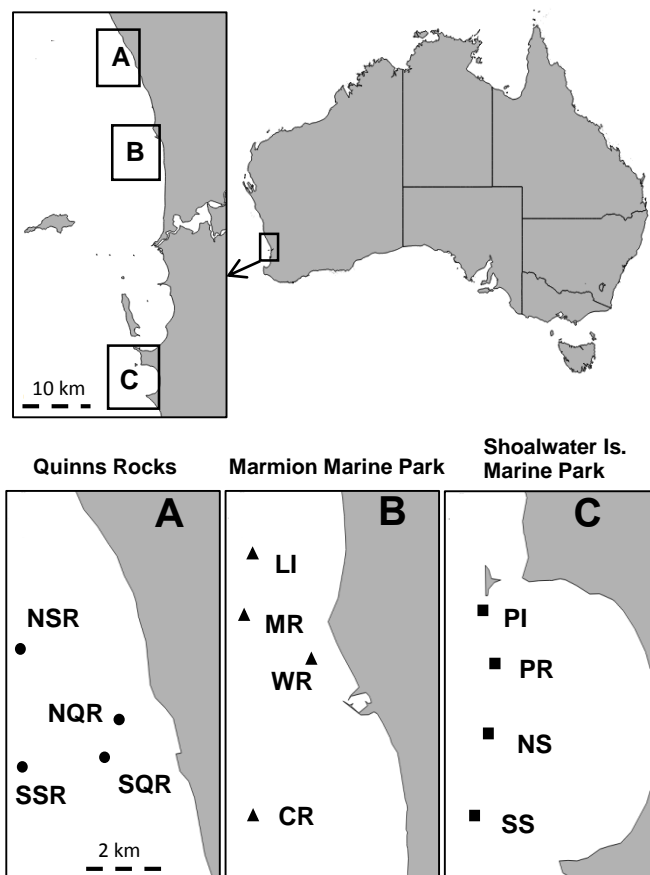
We sampled muscle tissue from ascidians, bivalves, gastropods and urchin primary consumers. With the exception of relatively short-lived ascidians, these taxa were considered potentially suitable references of isotopic baselines, as each species is considered long-lived and slow-growing (see Table 1), and thought likely to negate or “dampen” any dietary  $\delta^{13}\text{C}$  variations.

The diets of the suspension feeders, the ascidian *Herdmania momus* and bivalve *Septifer bilocularis* are sestonic, and have been shown to reflect  $\delta^{13}\text{C}$  values of particulate organic matter (Kang et al. 2009; Miller et al. 2013). Of the grazers, the diet of the urchin *Heliocidaris erythrogramma* consists primarily of the small kelp, *Ecklonia radiata* (e.g. Vanderklift and Wernberg 2010), which is the dominant source of production over temperate Australian reefs (Kendrick et al. 1999). In comparison, the gastropod *Turbo torquatus* consumes a range of red, green and brown macroalgae (Wernberg et al. 2008). Thus,  $\delta^{13}\text{C}$  values from our consumers were assumed to reflect  $\delta^{13}\text{C}$  baselines of particulate organic matter (i.e. ascidians and bivalves), kelp (e.g. urchins) or more broadly macroalgae in general (gastropods).

To measure spatial variability among individuals of invertebrate species, sites and regions, five replicate samples of each species were collected at depths between 5-7 m from within each of four sites (spaced > 1 km apart) within each of three regions (spaced > 10 km apart), Marmion Marine Park, Quinn’s Rocks and Shoalwater Islands Marine Park (Figure 1). However, due to the limited number of replicate samples for *H. erythrogramma* across regions and sites, this species was excluded from our spatial analysis. We preferentially selected individuals of each species within a narrow size-range to reduce any size-related variability in  $\delta^{13}\text{C}$ . To measure temporal variability among months and seasons among individuals of invertebrate species, sampling was conducted at monthly intervals from September 2012 to September 2013 from Marmion Marine Park, following an identical protocol as spatial sampling.

Additionally, we included  $\delta^{13}\text{C}$  data of macroalgae (extracted from Chapter 2) and suspended particulate organic matter (POM, see appendix), incorporating only data from corresponding times and places as our consumers. These included the small kelp *Ecklonia radiata* (of the order Laminariales), and the foliose red alga *Plocamium preissianum* (of the order Plocamiales), and were chosen as each source (macroalgae and POM) are thought to represent

dietary components in ascidians (e.g. POM, Kang et al. 2009), bivalves (e.g macroalgae and phytoplankton, Hill et al. 2008; Kang et al. 2009), gastropods (e.g. brown, green and red macroalgae, Wernberg et al. 2008b), and urchins (e.g. kelp, Vanderklift and Wernberg 2010).



**Figure 1.** Locations of reef sites within each region of south-western Australia (from north to south). (A) Quinns Rocks: north Scarpie Reef (NSR), north Quinns Rocks (NQR), south Quinns Rocks (SQR), south Scarpie Reef (SSR). (B) Marmion Marine Park: Little Island (LI), Marmion Reef (MR), Wanneroo Reef (WR), Centaur Reef (CR). (C) Shoalwater Islands Marine Park: Penguin Island (PI), Passage Rocks (PR), north Sisters (NS), and south Sisters (SS).

## Sample processing and stable isotope analysis

Muscle tissue was taken from the body wall of ascidians, the foot of gastropods, the abductor muscle of bivalves, and the Aristotle's Lantern of echinoderms. All samples were dried at 60°C for 48 hours, and homogenised to a powder using a ball and mill. All samples were then weighed into tin capsules ready for stable isotope analysis. Samples were then analysed for  $\delta^{15}\text{N}$  using a Thermo Finnigan Flash EA 112 interfaced via a Thermo ConFlo III with a Thermo Delta V Plus IRMS. Reference materials of known elemental composition of  $\delta^{15}\text{N}$  ratios were interspersed with the samples for calibration. Ratios  $^{13}\text{C}/^{12}\text{C}$  are expressed as delta ( $\delta$ ) relative to the international carbon standard *Pee Dee Belemnite* as:

$$\delta X (\text{‰}) = ((R_{\text{sample}}/R_{\text{standard}}) - 1) \times 1000, \text{ where } X = ^{13}\text{C}, \text{ and } R = ^{13}\text{C}:^{12}\text{C}.$$

## Data analyses

Spatial and temporal  $\delta^{13}\text{C}$  variability in our primary consumers and sources is demonstrated with graphs and descriptive statistics. Our initial analysis examined how  $\delta^{13}\text{C}$  for each invertebrate species varied across different scales of space and time. The magnitude of variation in  $\delta^{13}\text{C}$  (of each species) at each spatial and temporal scale (i.e. to determine the variance components) was determined using the permutational analysis of variance (PERMANOVA+) package in PRIMER v6 (Plymouth Routines in Multivariate Research), based on Euclidian distances. The patterns in spatial and temporal variation were separated into two factors for each, site nested in region (spatial), and month nested in season (temporal). Variance component tests are recognised as the best estimates of the contribution of a given factor to variability in the response variable (Graham and Edwards 2001).

We calculated relative enrichment between consumers and sources by subtracting annual mean  $\delta^{13}\text{C}$  values of each primary source from that of each consumer. By referring to typical values of trophic  $\delta^{13}\text{C}$  fractionation, e.g. ~0-2‰ (Post 2002; McCutchan et al. 2003; Barnes et al. 2008), we used the above enrichment values to infer a simple representation of likely dietary source(s) for each consumer. This allowed us to compare absolute values of variance components and ranges of monthly mean  $\delta^{13}\text{C}$  among consumers and their likely dietary sources.

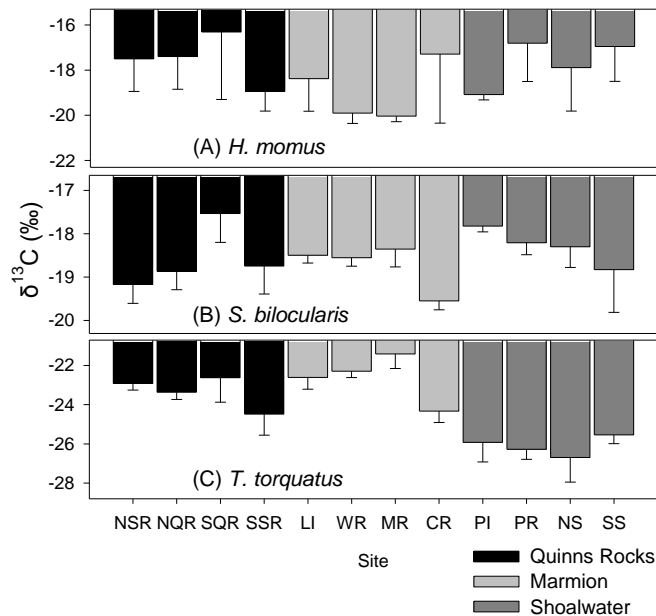
## 5.4 Results

Of the filter-feeders,  $\delta^{13}\text{C}$  in the ascidian *H. momus* and the bivalve *S. bilocularis* across sites and regions ranged from -20.03 to -16.46‰, and -19.54 to -17.53‰, respectively, and each displayed significant variation across sites within regions ( $P = 0.001$ ), but not among regions ( $P = 0.92, 0.56$ , respectively), where there was no discernible pattern (Table 1, Figure 2). The greatest source of variation for each species was at the site scale (61%, 56%, respectively), followed by replicate scale (39.2%, 44.2%). The  $\delta^{13}\text{C}$  in the gastropod *T. torquatus* ranged from -26.9 to -21.4‰, and displayed significant variation across sites ( $P = 0.001$ ) and regions ( $P = 0.002$ ), with the greatest source of variation at the regional scale (71%), followed by the site scale (15%) (Table 1).

**Table 1.** Variance components estimates (and % variance) for the  $\delta^{13}\text{C}$  in primary consumers from: (A) spatial design, with reefs nested in location; and (B) a temporal design, with months nested in seasons. Results were obtained from Permutational analysis of variance (PERMANOVA) based on Euclidian distance. Negative values are treated as zero.  $P$  values in bold indicate significance.

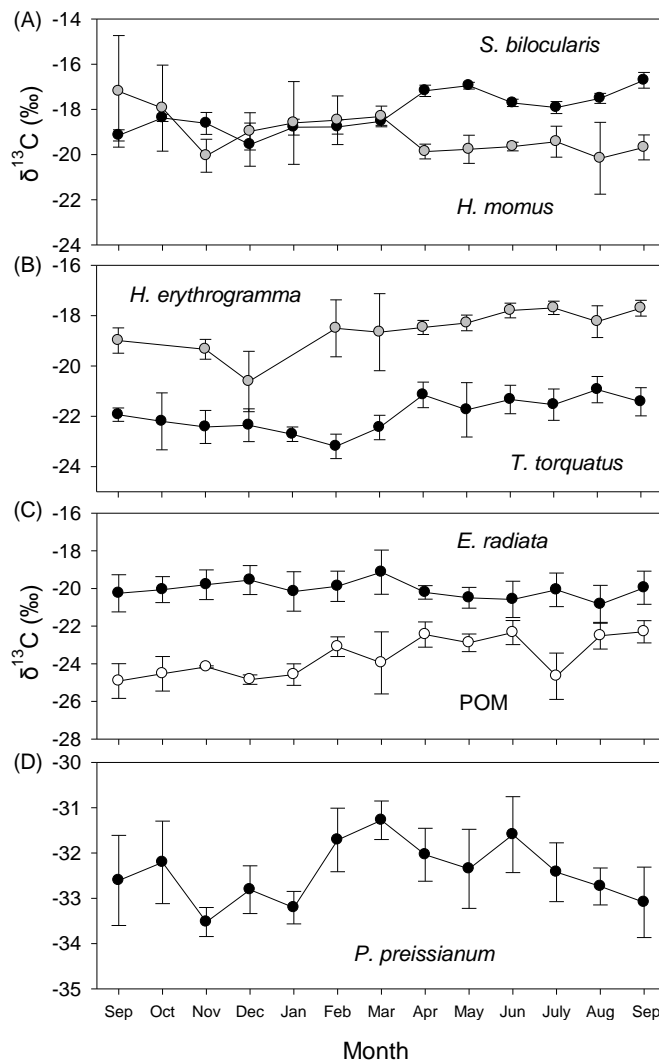
(A) Taxa		Spatial variation			
	Factors	<i>df</i>	MS	<i>P</i>	Var. (%)
<i>Herdamania momus</i>	Region	2	2.01	0.921	0
	<b>Site</b>	<b>7</b>	<b>25.1</b>	<b>0.001</b>	<b>5.08 (60.8)</b>
	Residuals	34	3.28		3.28 (39.2)
<i>Septifer bilocularis</i>	Region	2	0.97	0.56	0
	<b>Site</b>	<b>9</b>	<b>1.61</b>	<b>0.001</b>	<b>0.29 (55.8)</b>
	Residuals	45	0.23		0.23 (44.2)
<i>Turbo torquatus</i>	<b>Region</b>	<b>2</b>	<b>66.3</b>	<b>0.002</b>	<b>3.18 (71.5)</b>
	<b>Site</b>	<b>9</b>	<b>3.94</b>	<b>0.001</b>	<b>0.68 (15.3)</b>
	Residuals	47	0.59		0.59 (13.2)
(B) Taxa		Temporal variation			
Var. (%)	Factors	<i>df</i>	MS	<i>P</i>	Var. (%)
<i>Herdamania momus</i>	Season	3	4.39	0.09	0.25 (14.6)
	Month	8	2.88	0.18	0.18 (10.5)
	Residuals	43	5.12		74.9 (74.9)
<i>Septifer bilocularis</i>	Season	3	6.23	0.081	0.30 (30.6)
	<b>Month</b>	<b>8</b>	<b>2.87</b>	<b>0.001</b>	<b>0.29 (29.6)</b>
	Residuals	52	0.39		0.39 (39.8)
<i>Heliocidaris erythrogramma</i>	Season	3	5.25	0.086	0.31 (24.8)
	<b>Month</b>	<b>6</b>	<b>2.15</b>	<b>0.008</b>	<b>0.36 (28.8)</b>
	Residuals	38	0.58		0.58 (46.8)
<i>Turbo torquatus</i>	<b>Season</b>	<b>3</b>	<b>5.80</b>	<b>0.044</b>	<b>0.29 (34.1)</b>
	<b>Month</b>	<b>8</b>	<b>1.33</b>	<b>0.002</b>	<b>0.18 (21.2)</b>
	Residuals	55	0.38		0.38 (44.7)





**Figure 2.** Means ( $\pm$ SD) of  $\delta^{13}\text{C}$  at sites within each region over temperate reefs of the lower west coast of Australia, for: (A) the ascidian, *Herdmania momus*; (B) the bivalve, *Septifer bilocularis*; and (C) the gastropod, *Turbo torquatus*.

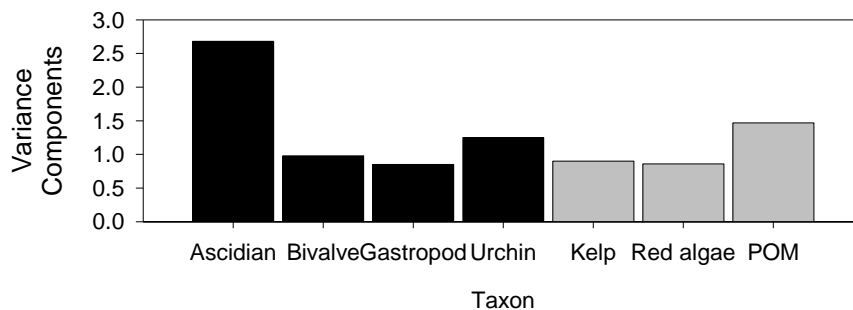
Annual (e.g. 12-months) mean  $\delta^{13}\text{C}$  for bivalves ( $-18.12\text{‰}$ ) and urchins ( $-18.51\text{‰}$ ) were similar, and enriched relative to ascidians ( $-19.21\text{‰}$ ) and gastropods ( $-21.99\text{‰}$ ) (Figure 3a and b). Temporal patterns contrasted among taxa. Bivalves and urchins (*H. erythrogramma*) displayed significant  $\delta^{13}\text{C}$  variation across months within seasons ( $P < 0.01$ ), but not among seasons ( $P > 0.08$ ), with the greatest source of variation at the replicate (residual) scale (40 and 47%), followed by month and season (25-30%) (Table 1, Figure 3a and b). The  $\delta^{13}\text{C}$  for ascidians was not significant at either month or seasonal scales ( $P = 0.17$  and  $0.09$ , respectively), with the greatest source of variation at the replicate scale (75%). In comparison,  $\delta^{13}\text{C}$  for gastropods was significant at both month and seasonal scales ( $P = 0.002$  and  $0.044$ , respectively), with the greatest source of variation at the replicate scale (45%) (Figure 3c and d). Filter-feeders (*H. momus* and *S. bilocularis*) had similar and overlapping  $\delta^{13}\text{C}$  values from September 2012 to March 2013, before an enrichment in bivalves and a depletion in ascidians in April 2013, after which a consistent separation of  $\sim 2\text{‰}$  continued until September 2013. Grazers (*T. torquatus* and *H. erythrogramma*) maintained a consistent separation between taxa of  $\sim 3\text{‰}$  throughout the year, and were marginally enriched in the latter eight months relative to earlier samples.



**Figure 3.** Monthly mean  $\delta^{13}\text{C}$  ( $\pm$ SD) for: (A) suspension-feeders, the ascidian *Herdmania momus*, and the bivalve *Septifer bilocularis*; (B) grazers, the gastropod *Turbo torquatus*; and the urchin *Heliocidaris erythrogramma*; (C) autotrophs, the kelp *Ecklonia radiata* and suspended particulate organic matter (POM); and (D) the red algae *Plocamium preissianum*. Values are from 13-months over temperate reefs of Marmion Marine Park, the lower west coast of Australia.

The level of enrichment in suspension-feeders were clearly separated from bulk POM (e.g. 4.6‰ ascidians, 5.5‰ bivalves), and more aligned with values of kelp (e.g. 1.2‰ ascidians, 2‰ bivalves). Gastropods had intermediate values between kelp (depleted, 1.8‰) and red algae (enriched, ~10‰), while urchins were aligned kelp (e.g. 1.6‰), and clearly separated from red algae (e.g. ~14‰). Therefore, ascidian, bivalve and urchin  $\delta^{13}\text{C}$  appear to represent kelp- $\delta^{13}\text{C}$ , and gastropod- $\delta^{13}\text{C}$  appears to represent an intermediate value between kelp and red alga (Figure 3a-d).

Based on the above trophic associations, absolute values of variance components (VC) and ranges of monthly mean  $\delta^{13}\text{C}$  were generally higher for consumers than autotrophs. Ranges and VC were lower for kelp (e.g. kelp  $\delta^{13}\text{C}$  = 1.70‰, VC = 0.90), than likely consumers, e.g. ascidians ( $\delta^{13}\text{C}$  = 2.96‰, VC = 2.68), bivalves (2.61‰, 0.98), gastropods (2.25‰, 0.85), and urchins (2.92‰, 1.25). Whereas ranges and VC of gastropods and red algae (e.g. 2.25‰, 0.86), were almost identical. Ranges and VC of bulk POM were 2.50‰ and 1.47, respectively (Figures 3 and 4).



**Figure 4.** Absolute values of variance components (VC) for: the ascidian *Herdmania momus*; the bivalve *Septifer bilocularis*; the gastropod *Turbo torquatus*; the urchin *Heliocidaris erythrogramma*; the kelp *Ecklonia radiata*; the red algae *Plocamium preissianum*; and particulate organic matter (POM). Values represent total VC for primary consumers (from Table 1b, this Chapter), and autotrophs, from Table 1 (temporal), Chapter 2.

## 5.5 Discussion

Our study demonstrates that  $\delta^{13}\text{C}$  in the muscle-tissue of ascidians, bivalves, gastropods and urchins can vary substantially across time (e.g. months to seasons) and space (1s to 10s km), and patterns in variability can differ across taxa within functional groups. This has implications for the use of long-lived primary-consumers as proxies for baseline  $\delta^{13}\text{C}$ , as they are often assumed to reduce variation in the  $\delta^{13}\text{C}$  of autotrophs through time-integrating some of the episodic or seasonal fluctuations of their diets (Barnes et al. 2009). However, we show that primary consumer- $\delta^{13}\text{C}$  can be more variable than the primary sources they are assumed to proxy.

Without knowledge of variations in primary-consumer  $\delta^{13}\text{C}$ , trophic inferences could be miscalculated. For example, gastropods have been used to proxy baseline isotopic sources (Post 2002; Pruell et al. 2006; Xu et al. 2011), yet  $\delta^{13}\text{C}$  in the gastropod *T. torquatus* was significantly different at every scale of time and space. The assumption that the  $\delta^{13}\text{C}$  in gastropods from one site or time is representative of benthic production at neighbouring sites, seasons or even a full year, therefore could be highly deceptive. Although  $\delta^{13}\text{C}$  variation was not significant at the season or region levels for other taxa, variation at the site level was significant for ascidians and bivalves, and at the month level for bivalves and urchins. Indeed, the urchin *H. erythrogramma*, which has been shown to track large-scale spatial patterns of  $\delta^{13}\text{C}$  in kelp (Vanderklift and Wernberg 2010), was more variable and covered a wider range of values. Further, gastropods covered a wider range of values than kelp and red algae, while bivalves and ascidians tracked a wider range than kelp, red algae and POM. Absolute values of variance components were more comparable among primary sources and consumers, likely reflecting the higher replicate variation in primary sources.

It is hard to reconcile the level of variation we observed, particularly the monthly variation, relative to primary sources.  $\delta^{13}\text{C}$  in the muscle-tissue of consumers is considered to reflect a time-integrated assimilation of their diet (weeks to months), which should act to dampen any variability (Sweeting et al. 2007). Certainly, several field-based studies have shown  $\delta^{13}\text{C}$  values of primary consumers to be generally more stable over time than values of their likely diets. For example, Post et al. (2002) showed that muscle tissue from freshwater bivalves and gastropods accurately reflected spatial and temporal  $\delta^{13}\text{C}$  variation from their corresponding food chains (e.g. pelagic and littoral). Further, Gustafson (2007) found that the  $\delta^{13}\text{C}$  of freshwater bivalves reflected a stable baseline relative to POM. However, consumer  $\delta^{13}\text{C}$  does

not simply reflect dietary  $\delta^{13}\text{C}$ , but is complicated by temporal changes in metabolic rate and food availability, and subsequent growth and repair, which can result in changes in turnover and  $\delta^{13}\text{C}$  fractionation (Woodland et al. 2012a; Woodland et al. 2012b), two important sources of variation. While the above studies were conducted in freshwater environments, ours was conducted in a coastal marine system, where consumers may be subject to different physiological pressures. Although we standardised our collections to individuals of similar sizes, to limit some of these factors, we could not control for the physical and biotic environment, and it may be a combination of these factors that are driving the variability we observed.

Interestingly, our results for  $\delta^{13}\text{C}$  contrast with those of corresponding data for  $\delta^{15}\text{N}$  (e.g. Chapter 4). Although ascidians and urchins also displayed substantial  $\delta^{15}\text{N}$  variation across the year, the  $\delta^{15}\text{N}$  in bivalves and gastropods were relatively stable and far less variable than corresponding primary sources (e.g. among replicates and months). This suggests that intraspecific consistency in  $\delta^{15}\text{N}$  does not necessarily guarantee consistency in  $\delta^{13}\text{C}$ . Further evidence of this is reported by Hill et al. (2009), who found through controlled feeding experiments that muscle-tissue in the bivalve *Perna perna* incorporated dietary- $\delta^{13}\text{C}$  substantially faster than that of  $\delta^{15}\text{N}$ . However, other studies have not found this trend (e.g. Hawkins 1985; Dattagupta et al. 2004), and  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  turnover rates are typically assumed to be roughly equal (Boecklen et al. 2011). It is clear that there is a need to better understand the causes of  $\delta^{13}\text{C}$  variation in primary consumers, and to account for variation both related and unrelated to their diet.

While the  $\delta^{13}\text{C}$  in different consumers at single points in time represent an integration of dietary sources over different timescales, annual mean values over an entire year should neutralise this effect. Thus, by consistently sampling throughout an entire year from the same location, we have used the best feasible approach to capture annual mean  $\delta^{13}\text{C}$ , and quantify relative enrichment between primary consumers and sources. The  $\delta^{13}\text{C}$  of the suspension-feeders across the year were clearly separated from bulk POM ( $\sim 5\text{‰}$ ), far greater than typical values of  $\delta^{13}\text{C}$  fractionation, e.g.  $\sim 0\text{--}2\text{‰}$  (Post 2002; McCutchan et al. 2003; Barnes et al. 2008), and more aligned with kelp ( $\sim 1.5\text{‰}$ ). These results are in close agreement with those outlined in Chapter 4, but using  $\delta^{15}\text{N}$ , and the data presented here adds to the evidence that both ascidians and bivalves exhibit selective feeding of macroalgal detritus from the available bulk POM pool, which is likely to reflect selection of particular particle sizes. This has been demonstrated for a range of filter feeders, e.g. ascidians and oysters (Kang et al. 2009), mussels (Defosse and

Hawkins 1997), oligochaetes (Tevesz 1980) and zooplankton (DeMott 1986). Macroalgae, particularly kelp, can be a substantial component in the diets of suspension-feeders over temperate reefs (Fredriksen 2003; Hill et al. 2006; Leclerc et al. 2013), and although debated in the literature (Miller and Page 2012; Miller et al. 2013), our results also suggest a strong trophic link between kelp and these suspension-feeding consumers. This was beyond the scope of this study, but deserves a closer examination of data in the future.

Urchins also, were more aligned to kelp ( $\sim 1.5\%$ ), while gastropods were relatively depleted to kelp. Turban gastropods can consume a range of red, green and brown macroalgae (Wernberg et al. 2008). The  $\delta^{13}\text{C}$  values observed here suggest that a significant component of this gastropod's diet consists of highly negative red algae (e.g.  $< -30\%$ ), as shown here with *P. preissianum*, and common in the Floridiophyceae class generally (Raven et al. 2002; Marconi et al. 2011). Surprisingly, bivalves and urchins in different functional feeding groups had similar and overlapping monthly  $\delta^{13}\text{C}$  values, compared to urchins and gastropods within the same functional group. Although, bivalves and ascidians had similar values for the first half of the year, they diverged by  $\sim 2\%$  over the latter half of the year. Therefore, even if these consumers mitigated some of the variability of kelp, which they clearly do not, it would neither be valid to assume that filter-feeders represent a general kelp baseline, nor that grazers represent a general benthic baseline, as some level of selection bias will exist.

In Chapter 3, we showed spatial  $\delta^{15}\text{N}$  variation of corresponding primary consumers had marked differences among regions, but no such distinction was found for  $\delta^{13}\text{C}$ . The exception being gastropods, with  $\delta^{13}\text{C}$  values significantly depleted in Shoalwater relative to other regions. As some red algae are substantially depleted in  $\delta^{13}\text{C}$  relative to brown and green algae (see Chapter 2; Raven et al. 2002), and abundant in patches within our regions (e.g. *Plocamium* sp., Smale et al. 2011), it seems likely that these regional differences are driven by a preference for, or greater availability of, red algae over other sources where gastropod  $\delta^{13}\text{C}$  values are particularly low. It is therefore critically important to predetermine the dietary preferences of consumers, if they are to be used to represent the  $\delta^{13}\text{C}$  of particular or general primary sources. The lack of any regional effect for ascidians and bivalves likely reflects the high variability among sites, but the potential causes of this variability is unknown. Regardless, the level of temporal variability observed in each primary consumer creates uncertainty as to whether spatial differences (or lack of) are truly representative, or simply represent a short term fluctuation.

## 5.6 Conclusion

The spatial and temporal variation observed here in the  $\delta^{13}\text{C}$  of primary consumers likely reflects complex interactions between their dietary sources and the physical environment. It is clear that there is a need to better understand the causes of isotopic variation in primary consumers. The  $\delta^{13}\text{C}$  values of primary consumers were no less variable than those of primary sources, and their dietary source(s) were somewhat ambiguous. In this case, there would be little advantage in using primary consumers over actual primary sources to describe baseline  $\delta^{13}\text{C}$ .

## **Chapter 6. Isotopic turnover in the marine ledge mussel, *Septifer bilocularis*, and simulations in the $\delta^{15}\text{N}$ response of mussels (primary consumers) and fish (secondary consumers) to a dynamic kelp baseline**

### **6.1 Abstract**

Nitrogen isotopes are widely used to determine the trophic positions of aquatic consumers in foodwebs, but require accurate measurements in organisms at or near the base of the food web. As the stable isotopes of autotrophs can undergo significant seasonal or episodic variations, long-lived, slow-growing primary consumers are used as baselines instead, as they gradually integrate and average out short-term variation in their diet. There is, however, a lack of information on how temporally dynamic baselines propagate through food webs. Through controlled feeding experiments, we determined the turnover rate of  $\delta^{15}\text{N}$  in the suspension-feeding ledge mussel *Septifer bilocularis*. We then used data from the literature to simulate the  $\delta^{15}\text{N}$  response of the mussel and a secondary consumer to a time-series of highly variable field-observed isotopic values of a representative autotroph (kelp), to assess the value of using mussels as a baseline compared to the autotroph, and simulate temporal trajectories of  $\delta^{15}\text{N}$  at multiple trophic levels. The mussel displayed slow turnover estimates (e.g. 56 days), with a mean daily depletion rate of 0.014%. Large episodic fluctuations in kelp- $\delta^{15}\text{N}$  caused large disparities in the trajectories between kelp and consumers (~5‰), but only a minor disparity between mussels and fish (up to ~1.5‰). These results provide evidence of the suitability of ledge mussels to represent general estimates of  $\delta^{15}\text{N}$  baselines. Since the ledge mussel has a worldwide distribution, it may provide a suitable and convenient reference for baseline  $\delta^{15}\text{N}$  in other regions.



## 6.2 Introduction

The stable isotopes  $^{15}\text{N}/^{14}\text{N}$  ( $=\delta^{15}\text{N}$ ) are widely used to determine the trophic position of consumers, due to the broadly predictable trophic enrichment (TE) in the heavier isotope from diet to consumer (DeNiro and Epstein 1977; Post 2002). This requires accurate measurements of stable isotopes in organisms at or near the base of the food web (Post 2002; Woodland et al. 2012a). As the stable isotopes of autotrophs can undergo significant seasonal or episodic variations (see Chapter 3), primary consumers are often used instead (Cabana and Rasmussen 1996; Jennings and Warr 2003; Gustafson et al. 2007; Xu et al. 2011). Variations in these isotopic baselines are not immediately evident in the tissues of consumers, but are gradually integrated over time, as old tissue is either diluted or replaced, thus representing a relatively invariable proxy for trophic baselines (e.g. Sweeting et al. 2007; Woodland et al. 2012a).

The level of time-integration of stable isotopes in consumers relates to biomass and metabolic rate (Peters 1986). Since highly metabolic tissue has rapid tissue/isotopic-turnover, the stable isotope signatures of small short-lived species (e.g. zooplankton), or highly metabolic tissue in larger, longer-lived species (e.g. blood plasma and liver) often reflect the short term variations in their diet (Gu and Schelske 1996). In comparison, less-active muscle tissue from larger, longer-lived consumers reflect a longer-term mean (Cabana and Rasmussen 1996; Post 2002; Hill and McQuaid 2009). Effectively, the longer the timeframe over which dietary isotopes are incorporated into consumers, the more broadly they represent the broad isotopic values of their diet. However, there is a lack of information on how these turnover rates propagate stable isotopes of temporally dynamic food sources through food webs.

There is growing acknowledgement that seasonal enrichment-depletion cycles occur in primary consumers, even in those used as baseline references (e.g. Woodland et al. 2012a). For example, when the diet of a consumer undergoes a distinct change in its isotopic signature (either through a change in diet or a change in the isotopic signature of the same diet), consumer signatures will be in disequilibrium, until both diet and consumer values correspond (correcting for trophic enrichment) (Sweeting et al. 2005). The time lapse at which consumer isotopic values reflect equally old and new dietary values is termed the ‘half-life’ (Boecklen et al. 2011). Consequently, distinct baseline changes in isotopic values propagate through food chains, but are lagged over different temporal scales, causing a time disparity in the isotopic values at different trophic levels. Longer half lives in consumers will have a “dampening” effect on the fluctuating  $\delta^{15}\text{N}$  values of food sources.

Filter-feeding bivalves have been shown to be good indicators of baseline stable isotopes of combined suspended and benthic sources, in both freshwater and marine systems, due to their longevity, and relatively slow growth and metabolism (e.g. Jennings and Warr 2003; Gustafson et al. 2007; Fukumori et al. 2008; Barnes et al. 2009; Lefebvre et al. 2009). However, the extent to which these species can be used are limited to the regions of their distribution. Different species cannot be substituted to extend comparisons, as isotopic values vary among consumer species (e.g. Chapter 4). Therefore, it is important to identify suitable species with wide distributions. In Chapter 4, we showed that abductor-muscle in the globally distributed suspension feeding ledge mussel *S. bilocularis* was relatively invariable in its mean  $\delta^{15}\text{N}$  over monthly and seasonal scales, and relative to the mean  $\delta^{15}\text{N}$  of primary sources (e.g. seston and macroalgae). These field observations gave us reason to hypothesise that *S. bilocularis* integrates  $\delta^{15}\text{N}$  variation at a timescale useful for describing baselines in food webs. Nonetheless, it is important to experimentally quantify the rate and time-period over which isotopic signatures are incorporated into the tissue of consumers. Ideally baseline proxies should integrate primary source isotopes at a timescale similar to that of higher consumers (which are generally larger and longer lived), so as to nullify any temporal disparity between them (Post 2002).

In this chapter, we aim to determine the  $\delta^{15}\text{N}$  half-life of the ledge mussel *S. bilocularis* by examining changes in its mean  $\delta^{15}\text{N}$  values as it shifts from isotopically enriched to depleted kelp diets. This estimate of integration in mussels, and that of a secondary consumer (using values from the literature), was applied to a time-series of highly variable field-observed isotopic values of a representative autotroph (kelp), to assess the value of using mussels as a baseline compared to the autotroph, and simulate temporal trajectories of isotopes at multiple trophic levels. Accordingly, we highlight the potential effect of temporal disparity on defining trophic relationships in food webs.

## 6.3 Materials and methods

### Controlled feeding study: isotopic labelling of kelp diet

Experimental diets were manipulated to produce large differences in dietary isotopic signatures. Fresh *Ecklonia radiata* (kelp) were collected in the field, and placed in containers with an enriched medium of  $^{15}\text{N}$ , consisting of 10L of filtered seawater mixed with 30 mg of  $\text{NH}_4\text{Cl}$  ( $^{15}\text{N}$ , 98%, Novachem Ltd). The thalli were kept in the medium under natural light conditions in a mesocosm for three days, before being transferred to plastic containers with flowing filtered seawater for 24 hours, to allow the labels to diffuse through the tissue. After enrichment, the thalli were washed in distilled water, and dried at  $60^\circ\text{C}$  to a constant weight. An identical procedure was conducted to prepare the control diet, but without the added  $\text{NH}_4\text{Cl}$ . This labelling procedure resulted in an elevated  $\delta^{15}\text{N}$  signature in the enriched diet ( $45.78\text{‰}$ ,  $\pm 0.13$ ), relative to the control diet ( $7.10\text{‰}$ ,  $\pm 0.9$ ).

### Controlled feeding study: isotopic turnover in the ledge mussel *S. bilocularis*

The isotopic turnover in the ledge mussel *S. bilocularis* was estimated under controlled laboratory conditions, by running concurrent treatment and control feeding experiments. Mussels were collected from reefs within Marmion Marine Park in the lower west coast of Australia. Prior to controlled feeding, a subsample of mussels was separated, placed in holding tanks for 24 h (for gut clearance), and prepared for stable isotope analysis, to determine  $\delta^{15}\text{N}$  value prior to food source manipulation. In each treatment, mussels of a similar size (20-25 mm) were collected from the field and separated into one of three replicate aquaria (50 mussels in each). Each aquarium was filled with 20 l of filtered seawater and equipped with a submersible corner pump (and filter) to maintain suspension of food particles. In the control, a diet of powdered *E. radiata* was fed at a consistent rate to mussels over a period of 10 weeks. In the treatment, powdered *E. radiata* labelled with elevated  $\delta^{15}\text{N}$  was initially fed to mussels for two weeks, to raise their isotopic signatures, before being fed natural powdered *E. radiata*. Due to complications with the aquaria facility and periods of large mortality in mussels, several attempts to run the experiment were started but aborted. After fine-tuning to the aquarium set-up described above, we were able to run the experiment for 70 days, with mussels from control and treatment tanks removed after 0, 7, 14, 28, and 49 and 70 days, before which a high level of mortality in mussels prevented further analyses. Mussels that were removed at each time

point were placed in holding tanks for 24 h (for gut clearance), and prepared for stable isotope analysis, as described in Chapter 4.

## Data analysis

To estimate isotopic half-life in mussels, we simply recorded the time lapsed from which treatment mussels changed to half way to the  $\delta^{15}\text{N}$  in control group mussels (Hill and McQuaid 2009; Boecklen et al. 2011). We used this half-life value (in time/days) to calculate a daily rate of exponential decay, using the equation:

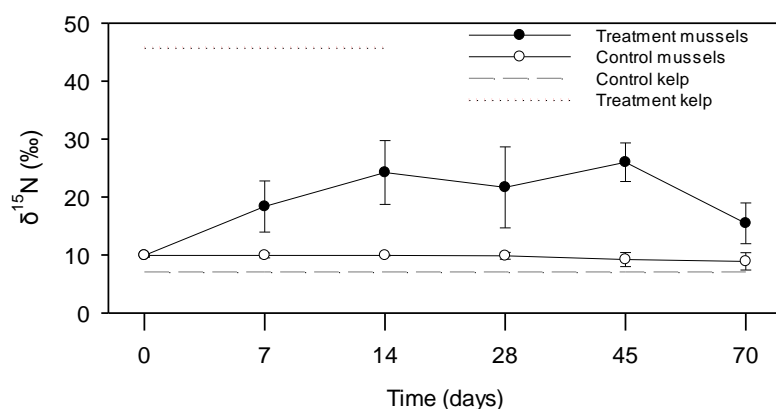
percentage decay = Exponential  $-\ln(2) \times t/T_{1/2}$ , where  $t$  is time passed in days, and  $T_{1/2}$  is half-life.

Using this half-life, and monthly mean  $\delta^{15}\text{N}$  values collected over a full year (September 2012 to August 2013) for *Ecklonia radiata* from Chapter 2, we predicted temporal trajectories of stable isotopes at higher trophic levels. Initially, daily values of  $\delta^{15}\text{N}$  in kelp were extrapolated from the monthly kelp data over a year, forming an annual trajectory of  $\delta^{15}\text{N}$  for kelp. We then applied our daily rate of exponential decay in mussels, based on the half-life of  $\delta^{15}\text{N}$ , to our annual trajectory in kelp, and so modelled the isotopic response of mussels. Trophic enrichment between kelp and mussels was incorporated into the model at a level of 2.51‰, the mean relative enrichment observed in the field (see Chapter 4). To predict the isotopic trajectory of a secondary consumer, we used published  $\delta^{15}\text{N}$  values (sampled from our region) of the fish *Leviprora inops* (Smit et al. 2005), and an exponential decay equation based on the isotopic half-life of 49 days in a fish of similar size and trophic role, the flounder *Paralichthys dentatus* (Buchheister and Latour 2010). Both *L. inops* and *P. dentatus* feed on first order consumers of benthic production (Buchheister and Latour 2010; Coulson et al. 2013). Trophic enrichment between mussels and fish was incorporated into the model at a level of 2.8‰, the difference in  $\delta^{15}\text{N}$  between annual mean values of mussels and published accounts in fish (e.g. Smit et al. 2005). We do not imply that this is a realistic food chain (e.g. kelp, bivalve, fish), but instead use these species to simulate the effect of turnover on isotopic trajectories at multiple trophic levels.

## 6.4 Results

### Controlled feeding experiment

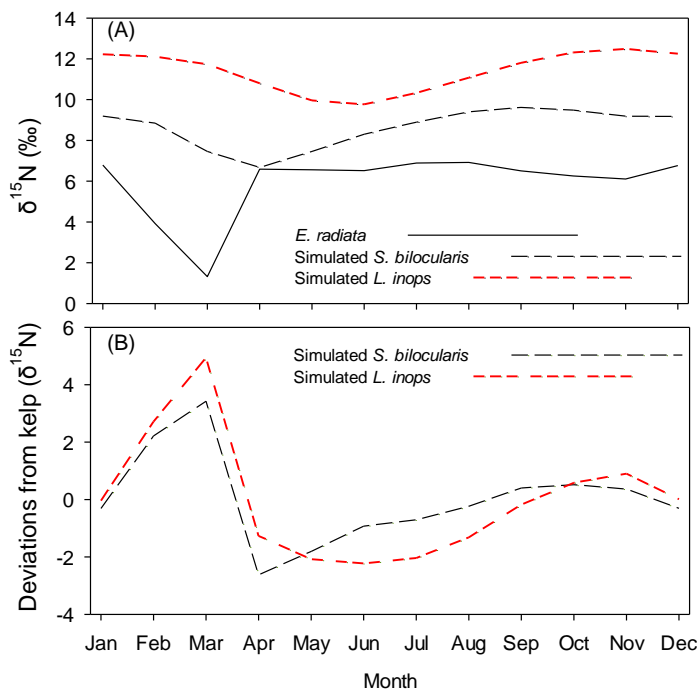
The control mussels, which were fed on unaltered kelp, maintained consistent  $\delta^{15}\text{N}$  prior to and throughout the trial ( $\sim 9.6\text{‰}$ ). Mussels fed on  $\delta^{15}\text{N}$  labelled kelp for 14 days ( $\delta^{15}\text{N} = 45.78\text{‰}$ ,  $\pm 0.23\text{‰}$ ), attained an elevated mean value of  $\sim 24\text{‰}$  and maintained highly elevated values ( $\sim 25.5\text{‰}$ ) through to Day 45, before declining to  $\sim 15\text{‰}$  on Day 70 (Figure 1). However, due to high mortality of mussels after Day 70 (in both control and experimental treatments), the experiment could not be continued. Nevertheless, the value at day 70, 56 days after a switch from enriched to unaltered kelp, reflects the half-life if the trajectory continued to the control mussel level. Thus, 56 days was used as an approximate half-life estimate, resulting in a mean daily rate of decay equating to 0.014%.



**Figure 1.** Mean ( $\pm$ S.E.,  $n = 3$ ) of muscle- $\delta^{15}\text{N}$  for the ledge mussel *S. bilocularis* fed  $\delta^{15}\text{N}$ -enriched kelp for 14 days then switched to a diet of natural kelp for a following 45 days.

## Isotopic trajectories

Our model on isotope trajectories was based on a simple, hypothetical food chain, using ‘real’  $\delta^{15}\text{N}$  values from kelp, with simulated responses to this baseline in a primary and secondary consumer. The sharp depletion in kelp values in February and March had a delayed knock-on effect through the food chain. The  $\delta^{15}\text{N}$  trajectories of kelp and mussels varied from  $\sim 6\text{‰}$  in March to  $0.08\text{‰}$  in April, whereas kelp and fish varied from  $\sim 11.7\text{‰}$  in March to  $3.2\text{‰}$  in June, and mussels and fish varied from  $\sim 4.3\text{‰}$  in March/April to  $\sim 1.5\text{‰}$  in June/July (Figure 2A). The  $\delta^{15}\text{N}$  values ranged by  $5.61\text{‰}$  for kelp,  $3.03\text{‰}$  for mussels and  $2.72\text{‰}$  for fish. After correcting for fractionation, and setting kelp values at zero (y-axis), mussels deviated from kelp by up to  $\sim 3.4\text{‰}$  and fish  $\sim 4.9\text{‰}$ , whereas mussels deviated from fish by up to  $\sim 1.5\text{‰}$  (Figure 2B). Although results from our feeding study are somewhat ambiguous, the ledge mussel *S. bilocularis* appears to respond slowly to the  $\delta^{15}\text{N}$  of its diet, taking 56 days to reach a half-life after a diet switch, and mitigated much of the kelp variability in our simulation ( $\sim 55\%$ ).



**Figure 2.** Simulated  $\delta^{15}\text{N}$  response trajectories of mussels (*S. bilocularis*) to a diet of kelp (*E. radiata*), and fish (*L. inops*) to a diet of mussels. Kelp represent observed values over temperate reefs on the lower west coast of Australia. Simulated responses of consumers are based on exponential decay equations, with: (A) incorporating trophic enrichment relative to kelp; and (B) variations from kelp values.

## 6.5 Discussion

The muscle tissue of ledge mussel (*S. bilocularis*) exhibited a slow turnover rate, with a half-life of 56 days and a mean daily rate of decay 0.014%. This slow turnover rate had implications to the projected response of tissue- $\delta^{15}\text{N}$  to the large episodic fluctuation in  $\delta^{15}\text{N}$  of kelp. The large fluctuations in kelp caused large disparities in the trajectories of kelp with consumers, but only a minor disparity between mussels and fish (e.g. up to  $\sim 1.5\text{‰}$ ). These results are further evidence of the suitability of these mussels to represent general estimates of  $\delta^{15}\text{N}$  baselines, e.g. Chapter 4, where mean  $\delta^{15}\text{N}$  in mussels from reefs remained relatively consistent throughout the year.

The turnover rate in the isotopic values of abductor tissue in ledge mussels of  $\sim 56$  days in this study is similar to estimates in whole-body tissue in the oyster *Crassostrea gigas* (e.g. 30-60 days) using similar controlled feeding methods (Riera and Richard 1997). The slow turnover rate for *C. gigas* has allowed it to be used to represent isotopic baselines in north-west coastal France (Lefebvre et al. 2009). Other estimates of isotopic turnover in bivalves have, in the main, come from field observations rather than experimentally (e.g. Gustafson et al. 2007; Fukumori et al. 2008). They are generally assumed to be good indicators of baseline stable isotopes, in both freshwater and marine systems, due to their longevity, relatively slow growth and metabolism (Jennings and Warr 2003; Gustafson et al. 2007; Fukumori et al. 2008; Barnes et al. 2009; Lefebvre et al. 2009; Hill and McQuaid 2009). The slow turnover rate for *S. bilocularis* in our study supports this assumption. Furthermore, it is likely to be a major factor in reducing any monthly or seasonal effects in its  $\delta^{15}\text{N}$  over the year in the study region (Chapter 4), making it a good candidate as a proxy baseline of  $\delta^{15}\text{N}$  for higher trophic levels.

The ledge mussel appears to integrate  $\delta^{15}\text{N}$  at a similar rate (as per our simulation) to fish, based on a meta-analysis by Boecklen et al. (2011), who determined that the half-life in muscle-tissue of fish ranged from  $\sim 20$  to 60 days. As reference taxa should integrate primary source isotopes at a timescale similar to that of higher consumers, to nullify any temporal disparity between them (Post 2002; Woodland et al. 2012a), ledge mussels appear to be ideal for this purpose. This was supported through our simulations of  $\delta^{15}\text{N}$  trajectories of mussels and fish, based on large episodic fluctuations in primary sources. While there were large disparities between kelp and consumers (mussels and fish), these disparities were limited between mussels and fish (e.g. up to  $\sim 1.5\text{‰}$ ), as mussels were slow to respond to shifts in  $\delta^{15}\text{N}$  of kelp. These results support the use of consumers with slow tissue turnover as baselines in

food web studies, but primary-consumers with unknown turnover rates, or even consumers known to have high turnover rates (e.g. amphipods) are often used as baseline references (see Woodland et al. 2012a). Therefore, it is important to consider the timescale over which consumer isotopic values represent, to avoid misinterpreting isotopic data. Conversely, our simulations raise the issue of temporal disparity between autotrophic sources and their consumers. This is important because, although primary-consumers can represent isotopic baselines, studies often sample autotrophs and consumers at single points-in-time, and infer relationships between them (Gillies et al., 2012; Jennings et al., 1997; Smit et al., 2005). Although we deal with the subject of autotrophic variation in detail in Chapters 2 and 3, our simulations clearly highlight the effect of temporal disparity, and how important it is to account for temporal variation when linking primary sources to their consumers.

We do not suggest that our simulation is a realistic relationship. While kelp detritus can be a major dietary component of suspension-feeders on kelp dominated reefs, in reality they consume a mix of detritus, phytoplankton, bacteria and even small zooplankton, whose stable isotopes in tissue are likely to vary with season (Fredriksen 2003; Leclerc et al. 2013). Moreover, mussels are unlikely to be a major component in the diet of *L. inops*. Our simulation also assumes that  $\delta^{15}\text{N}$  in our consumers are driven by diet alone. In fact, consumer  $\delta^{15}\text{N}$  can vary for reasons other than the  $\delta^{15}\text{N}$  in their diet. For example, growth rate, which is often linked to temperature and food availability, can induce a switch from anabolism to catabolism, where isotopically lighter compounds are preferentially metabolised over heavier compounds (see Gannes et al. 1997). Instead, we use these species to simulate a hypothetical food chain, based on natural temporal variability in baseline  $\delta^{15}\text{N}$ , and with experimentally determined rates of consumer integration.

In conclusion, the ledge mussel is found in high abundances worldwide, particularly in coastal regions of east Africa, Asia and Australasia (Albayrak and Calgar, 2006; Mavuti et al., 2008; Selin and Latypov, 2006; Sheppard, 1984). This, combined with the species' slow turnover rate of  $\delta^{15}\text{N}$ , suggests it may provide a suitable and convenient reference for baseline  $\delta^{15}\text{N}$  across these regions. Our simulation of a simple three tier food chain, using 'real' baseline (kelp)  $\delta^{15}\text{N}$  values and simulated responses in primary and secondary consumers, highlights how fluctuations in baseline  $\delta^{15}\text{N}$  can propagate through the food chain and cause disparities between autotrophs and their consumers. However, these disparities are reduced considerably when comparing  $\delta^{15}\text{N}$  values of higher level consumers with those of the mussel.



## Chapter 7. General Discussion

### 7.1 Introduction

This study set out to evaluate the “basal” (e.g. primary producers/consumers) isotopic variations in a temperate coastal food web, and their relationships with surrounding environmental factors. This has allowed me to determine the suitability and issues for using such baselines in interpreting trophic links and positions in food webs, and predicting shifts in isotopic signatures across broad spatial and temporal scales. Understanding variations in these isotopic baselines, and the mechanisms leading to that variability is crucial for food web ecology, since isotopic baselines form a major component of resolving fundamental questions in trophic ecology (Gannes et al. 1997; Post 2002). Firstly, the stable isotopes of basal resources (autotrophs and their consumers), from species to production pathways (e.g. seagrass or macroalgae, benthic or pelagic sources), can be used to estimate their contribution to consumers (Barkai and Griffiths 1986; Jennings et al. 1997; Melville and Connolly 2003; Xu et al. 2011; Parnell et al. 2013). Secondly, isotopic differences among food webs can be standardised to a common reference, thus the  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  of geographically distinct food webs may be compared (Cabana and Rasmussen 1996; Post 2002; Barnes et al. 2009), or the movement of migratory or wide-ranging consumers traced (Post 2002). And thirdly, they can be applied to assist with the monitoring of human derived pollutants (Gartner et al. 2002; Gustafson et al. 2007; Gorman et al. 2009).

Each of these applications requires reference taxa that are wide-ranging, abundant and form an important basal component in the food web. However, even in meeting these fundamental criteria, large spatial and temporal variations in the stable isotopes of basal sources and their proxies (e.g. primary-consumers) can potentially mislead baseline estimates (Boon and Bunn 1994; Dethier et al. 2013). Thus, it is critically important to quantify the scales over which stable isotopes in reference taxa vary before conclusions can be drawn (Guest et al. 2010; Hyndes et al. 2013). However, scale itself is only relevant if environmental factors that cause isotopic variation change in space and time. Therefore, using baseline isotopic variation to our advantage, by linking variation to factors of their environment, may account for much of the spatial and temporal isotopic variation. Predictions of baseline  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  could simplify broad-scale comparisons of trophic structure (e.g. energy pathways and trophic level) (see Jennings and Warr 2003; Barnes et al. 2009). Here, I have shown that the stable isotopes of

autotrophs and primary consumers can vary substantially in space and time, but that values of important taxa of macroalgae can be broadly predicted by factors of their physical and biogeochemical environment (Chapters 2 and 3). Furthermore, I have identified wide ranging and abundant primary consumers that effectively time-integrate dietary  $\delta^{15}\text{N}$ , thus represent convenient proxies of baseline  $\delta^{15}\text{N}$  across latitudinal scales (Chapters 3 and 5). Overall, I demonstrate the difficulty in capturing accurate and representative values of baseline sources, as variability differed between autotrophs and consumers, among species within these groups, and between isotopes (i.e.  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$ ) (Chapters 2, 3, 4 and 5).

The reference taxa chosen for this study were based on their wide-ranging distribution, along with their abundance and importance in the reef ecosystem of the study region. *Ecklonia radiata* was chosen on the basis that it represents a kelp, which has amongst the highest known biomass per unit area in marine systems (Mann 1982; Abdullah and Fredriksen 2004), and is important as a food resource (Rodriguez 2003; Vanderklift et al. 2009; Leclerc et al. 2013). Kelp has been shown to contribute ~ 60% of the carbon found in coastal invertebrates (Duggins et al. 1989), and indirectly, up to 37% of the carbon in apex predators (e.g. Cormorants *Phalacrocorax carbo*, Fredriksen 2003). Moreover, kelp is an important component of particulate organic matter, capable of out producing phytoplankton by an order of magnitude (Mann 2000). In the case of *Plocamium preissianum*, the species belongs to a genus of red algae that is distributed globally and represents some of the most common red algae over subtidal reefs (McClanahan 2008; Shepherd and Edgar 2013). It also forms a major dietary component of herbivorous gastropods (e.g. up to 39% in abalone, Barkai and Griffiths (1986), amphipods (Amsler and Amsler 2013) and fish (Jones and Norman 1986). Over temperate reefs of Australia, *E. radiata* is the dominant source of production, while *P. preissianum* is one of the most abundant macroalgae (Andrew 1999; Shepherd and Edgar 2013). In comparison, suspended particulate organic matter (POM, < 100  $\mu\text{m}$ ) forms an important component of the water (Newell et al. 2001) and a major part of the diet of consumers (Fegley et al. 1992). However, bulk POM represents a dynamic mix of macroalgal detritus, phytoplankton and microorganisms (Hill et al. 2008) derived from both benthic and pelagic production (Fredriksen 2003).

As potential proxies of isotopic baselines, long-lived primary consumers are often assumed to time-integrate the often episodic or seasonal isotopic values of their diet (Jennings and Warr 2003; Gustafson et al. 2007). The urchin *Heliocidaris erythrogramma* is extremely long-lived, e.g. > 30 years (Pederson and Johnson 2007), and has a diet thought to primarily consist of *E.*

*radiata* (e.g. Vanderklift and Wernberg 2010). Therefore, its isotopic signature was thought to potentially constitute a relatively invariable measure of kelp. In comparison, temperate coastal suspension-feeders (e.g. the ascidian *Herdmania momus*, and the bivalve *Septifer bilocularis*) assimilate POM, thus possibly coupling benthic and pelagic production suspended in the water column (see Newell 2004; Hill et al. 2006), while the grazing gastropod, *Turbo torquatus*, consumes a mix of macroalgae and benthic detritus (Wernberg et al. 2008). Therefore, it is a valuable endeavour to describe (in detail) the spatial and temporal dynamics in the stable isotopes of these *baselines*, and their proxies.

## 7.2 Empirical findings and practical applications

Using those taxa, I have highlighted isotopic variability among sites, regions, months and seasons, and among individuals separated by metres within a dynamic temperate marine system. The shallow temperate reefs along the lower west coast of Australia vary in their exposure to different levels of oceanic swells and therefore water velocity (Sanderson 2000) and their proximity to terrestrial outputs and human population density (Gartner et al. 2002; Vanderklift and Wernberg 2010). Overall, I demonstrate the difficulty in capturing accurate and representative values of baselines sources, as variability differed between autotrophs and consumers, among species within these groups, and between isotopes (i.e.  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$ ) (Chapters 2, 3, 4 and 5). These results call in to question the assumption of spatial and temporal consistency in isotopic baselines in coastal temperate systems (particularly consumers), but also provide a reference from which to determine the appropriate sampling design to capture variation. For example, patterns of variations for the  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  in *E. radiata*, and the  $\delta^{13}\text{C}$  in *H. momus* and *S. bilocularis*, were strongly influenced by small spatial scales (1s km and 10s of m). Broadly, this suggests that obtaining regionally representative isotopic values across metropolitan Perth would be better served by high sampling intensity over small spatial scales. However, the reverse was true for  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  in *P. preissianum*,  $\delta^{15}\text{N}$  in *S. bilocularis*, and  $\delta^{13}\text{C}$  in *T. torquatus*, with regional differences being the greatest influence. Thus, obtaining regionally representative stable isotope data for these species requires a higher sampling intensity at larger spatial scales. The fact that different autotrophs and consumers elicit different spatial patterns of variation, show that sampling designs need to be compatible to the research questions of interest. Otherwise, improper allocation of sampling effort may decrease the probability of detecting ecologically important differences.

The absence of adequate information on macroalgal  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  variability is likely to cause inaccurate estimates of their contribution to the diets of consumers, especially when using limited data, or worse, static values from the literature (Boecklen et al. 2011; Dethier et al. 2013). In their study of variation in the stable isotopes of marine macrophytes, Dethier et al. (2013) showed that intraspecific differences among sites and dates (including Rhodophyta, e.g. *Neorhodomela*) were greater than is generally assumed in the published literature. They generated mixing models for a hypothetical consumer by using different sets of dietary isotopic values, over different scales of space and time, and showed that failure to account for these variations leads to inaccurate results. While examining the effect of such variability in  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  of macroalgae was beyond the scope of my study, the large range (e.g.  $\sim 2\text{‰}$  and  $\sim 5\text{‰}$  in the  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  of *E. radiata*) that exceeds the typical trophic fractionation levels applied to food web studies suggests that this would also occur using my data. My approach could facilitate predictions in changes of baseline  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  in food webs over large spatial scales, interpretations of changes in  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  of consumers over those scales, and generate hypotheses from such predictions.

Temporally, the patterns of  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  variation in each taxa and trophic group were more comparable, with seasonal variation eliciting a weak or negligible effect, but monthly variation often resulting in a strong effect. However, there was a stark contrast between isotopes in the level of temporal variation in autotrophs versus primary consumers. Variation was particularly high for  $\delta^{15}\text{N}$  in autotrophs, even between neighbouring months (e.g.  $\sim 5\text{‰}$  in *E. radiata*, March – April). While  $\delta^{15}\text{N}$  values of primary consumers were less variable, these were still substantial for ascidians and urchins, roughly equalling  $\delta^{15}\text{N}$  fractionation (e.g.  $\sim 3\text{‰}$ ), and therefore, enough to mislead trophic studies. Conversely, the  $\delta^{15}\text{N}$  in the bivalve *S. bilocularis* and the gastropod *T. torquatus* were relatively stable over time, and appeared to time-integrate the  $\delta^{15}\text{N}$  of their diet. Further, by conducting a controlled feeding study, I showed that *S. bilocularis* exhibited slow  $\delta^{15}\text{N}$  turnover estimates (e.g. half-life of 56 days), which from simulations was shown to negate and “dampen” the effect of fluctuating  $\delta^{15}\text{N}$  values of food sources (e.g. Chapter 6). These data suggest that, owing to their abundance and wide distributions, these species can be used to compare  $\delta^{15}\text{N}$  baselines over large spatial scales. The current theory that  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  variations in consumers mitigate at least some of the temporal variation of their diet appears to be true for  $\delta^{15}\text{N}$ . My results support this, especially when considering that kelp is the major contributor to the diets of ascidians, bivalves and urchins (but less so gastropods), as each consumer was less variable over months and seasons than *E.*

*radiata*. However, this was not the case for  $\delta^{13}\text{C}$ , where consumers were generally more variable than the primary sources they are assumed to proxy. Therefore, consistency in consumer  $\delta^{15}\text{N}$  does not necessarily guarantee consistency in  $\delta^{13}\text{C}$ . Clearly, sampling the  $\delta^{13}\text{C}$  in autotrophs and consumers, and  $\delta^{15}\text{N}$  in autotrophs, ascidians and urchins, throughout the year is required to fully characterise their representative values.

By comparing spatial and temporal trends in the stable isotopes of autotrophs and primary consumers, I was able to ascertain relationships between these two groups. The close tracking of suspension-feeders with kelp suggests that kelp components of POM are likely forming the main source of production for these consumers. This is further supported by the consistent levels of enrichment in suspension-feeders relative to kelp, which were within the typical range expected for trophic fractionation. The subject of the sources of production for suspension feeders over kelp dominated reefs has been heavily debated in recent years (see Hill et al. 2008; Page et al. 2008; Miller and Page 2012; Miller et al. 2013; Leclerc et al. 2013), but my data suggest that ascidians and bivalves within this food web, exhibit size-selective feeding of kelp detritus from the available seston pool. The  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  of urchins were also closely aligned to kelp, which agree with results from other studies (Vanderklift and Wernberg 2010), while the  $\delta^{13}\text{C}$  in gastropods were depleted relative to kelp, suggesting a significant proportion of their diet consisting of highly negative algae, as shown here with *P. preissianum* (e.g.  $< -30\text{‰}$ ), and common in the Floridiophyceae class generally. Therefore, each of these consumers, including suspension-feeders, appear to represent isotopic values from benthic production.

While the initial analysis identified the critical scales for accurately capturing isotopic variation in macroalgae, scale itself is only relevant if environmental factors that cause isotopic variation change in space and time. Thus, a critical scale in one region may not be important in another region. Previous work from laboratory experiments and from estuaries, has shown that stable isotopes in macroalgae are influenced by light, water motion, temperature, and sources and composition of dissolved inorganic carbon, nitrogen and phosphorous (Littler et al. 1991; Umezawa et al. 2002; Cornelisen et al. 2007; Carvalho and Eyre 2011; Barr et al. 2013). In Chapters 2 and 3, I showed that some of these factors correlated to the spatial and temporal variation. For example, the  $\delta^{13}\text{C}$  variation in both *E. radiata* and *P. preissianum* was, in part, related to the same environmental factors, namely concentrations and  $\delta^{13}\text{C}$  of DOC and light. However, there was also disparity among taxa. The most obvious being that temporal (and combined spatio-temporal)  $\delta^{13}\text{C}$  variation in *P. preissianum* was tightly linked to temperature, whilst *E. radiata* showed no such relationship, even once extending the analyses to other

studies at different latitudes. In comparison,  $\delta^{15}\text{N}$  variation was related to similar factors among species, e.g. water temperature and  $\text{PO}_4$  for *E. radiata*, *A. anceps* and *P. preissianum*. Interestingly, as with  $\delta^{13}\text{C}$  for *P. preissianum*, a substantial amount of macroalgal- $\delta^{15}\text{N}$  was explained by water temperature alone (e.g. 55% *E. radiata*, 46% *P. preissianum*, 66% *A. anceps*). However, it is unlikely that these temperature/isotopic relationships are causal, but rather indirect, with temperature acting as a proxy for other variables.

In the case of  $\delta^{13}\text{C}$ , I suggested that a higher uptake of  $\text{CO}_2$  in *P. preissianum* would explain the depletion of  $\delta^{13}\text{C}$  I observed over cooler months. Unlike *E. radiata*, *P. preissianum* preferentially incorporates available  $\text{CO}_2$  ( $\delta^{13}\text{C}$ -depleted) over  $\text{HCO}_3^-$  ( $\delta^{13}\text{C}$ -enriched) (Raven et al. 2002), which generally increases with decreasing temperature (Rau et al. 1989). Therefore, as *P. preissianum* preferentially incorporates the lighter over the heavier isotope, a larger  $\text{CO}_2$  pool in colder waters would provide a larger source of the lighter isotope, resulting in lower enrichment, i.e. lower  $\delta^{13}\text{C}$  values. In the case for  $\delta^{15}\text{N}$ , temperature has an inverse relationship with concentrations of  $\text{NO}_3^-$  (Silió-Calzada et al. 2008), which is generally enriched relative to other DIN sources (Raimonet et al. 2013). Therefore, decreasing temperatures with increasing latitude results in a larger  $\text{NO}_3^-$  pool for macroalgae in colder waters (Sarangi 2012; Yin et al. 2014), and a greater source of the heavier isotope, i.e. higher  $\delta^{15}\text{N}$  values in macroalgae. These hypotheses explain both the temperature/isotopic relationships I observed, but also the lack of a relationship for  $\delta^{13}\text{C}$  in *E. radiata*.

By linking isotopic values to environmental parameters, any variations in those significant factors could be used to further improve upon estimating appropriate sampling designs. For example, a region known to vary substantially in environmental factors that influences isotopic values in a target species, would require a greater sampling scale to capture a representative value than a region that is more spatially homogenous. Thus, by identifying significant relationship between  $\delta^{15}\text{N}$  in *E. radiata* and water temperature, knowledge of any variations in temperature could be used to estimate the appropriate sampling scale to capture  $\delta^{13}\text{C}$  variation in *E. radiata*. As noted above,  $\delta^{15}\text{N}$  in *E. radiata* is more variable among sites 100s to 1000s of m apart, than those 10s of km apart. However, temperature over these distances is relatively stable, while over degrees of latitude, they can vary substantially. Based on this, it could be hypothesised that  $\delta^{15}\text{N}$  would have a greater level of variation across this broader spatial scale. Indeed, the same could be hypothesised for  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  in other macroalgae, where significant relationships were found with temperature and other environmental factors. My predictive models take this one step further, and provide a hypothesis for the potential effects

of water temperature that is easily testable, since data of water temperature is extensive and freely available (e.g. Chapter 2 and 3; Ryu et al. 2012). Such a method to predict isotopic baselines in key sources and the identification of widely distributed taxa that integrate the variation of resource pools (benthic or pelagic production pathways), would be useful as common references to standardise the trophic structure (i.e.  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  bi-plots) of geographically distinct food webs, aid in the tracking of migratory or wide-ranging consumers, and the monitoring of human derived pollutants.

While their use has been limited, models to predict, and correct for, baseline isotopic variation have been used before to great effect. Jennings and Warr (2003) and Barnes et al. (2009) showed that spatial variation in  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  of filter-feeding scallops was strongly correlated with their environment, a relationship that was thought to propagate through their phytoplankton diet. As a result, they were able to predict nearly 80% of the spatial variation of both  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  in species of fish, using either a single variable model of bottom temperature ( $\delta^{13}\text{C}$ ) or multiple variable model of temperature, salinity and depth ( $\delta^{15}\text{N}$ ). Furthermore, the identification of regions with isotopically distinct baselines has been used to trace the movement of animals. For example, MacKenzie et al. (2011), showed that the effects of sea surface temperature (SST) was indirectly linked to the  $\delta^{13}\text{C}$  in Atlantic salmon, through phytoplankton, and used this link to determine the geographic locations of feeding grounds. Another study (Fry 1983) tracked the migration of shrimp in the Gulf of Mexico by linking their tissue- $\delta^{13}\text{C}$  to on-and-offshore regions. Furthermore, the dual use of stable isotopes of seabird feathers, combined with tracking-loggers, has been applied to describe isoscapes of foraging regions in the south Atlantic, and determine differences among species and sexes within species, during the little known non-breeding period (Phillips et al. 2009). Similar approaches have been used to describe the migration routes of terrestrial fauna, where vegetation, and subsequent isotopic values differ (Hobson 1999; Powell et al. 2012).

Despite the ever increasing use of stable isotopes in ecology (Boecklen et al. 2011), and evidence of spatial differences in the  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  at the base of food webs (Jennings and Warr 2003; Barnes et al. 2009; Phillips et al. 2009), there are few available spatial references (or “isoscapes”) of isotopic baselines. The models developed here, for important autotrophic sources ( $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$ ), and identification of reference taxa of baseline  $\delta^{15}\text{N}$ , could be an important step in providing a standard to facilitate spatial (and temporal) comparisons, and track the movement of animals in coastal regions. For example, as shown here and in other

studies, kelp represents the primary source of production supporting consumers over temperate Australasian reefs. Therefore, my  $\delta^{15}\text{N}$  isoscapes of kelp provide a reference of isotopically distinct regions in the primary source of production supporting consumers over these regions.

Water temperature explained a substantial degree of macroalgal isotopic variation, particularly in  $\delta^{15}\text{N}$  (albeit indirectly), and it would be useful to determine if these relationships hold for other taxa, or indeed food webs generally. This is certainly possible, given the extensive amount of published isotopic data from coastal systems, which could be easily linked to freely available water temperature data (as sea surface temperature). I recognise that obtaining evidence for and against these broad predictions requires a greater amount of isotopic data than is currently available, and a greater understanding of the interactions of other environmental factors. However, the link between variability in  $\delta^{15}\text{N}$  of macroalgae and temperature, whether direct or indirect, suggests that temperature could be used to correct  $\delta^{15}\text{N}$  against environmental variables, for example, when using macroalgae- $\delta^{15}\text{N}$  as a measure of pollution. The use of  $\delta^{15}\text{N}$  is recognised as an effective means to identify the level of human derived pollution, e.g. sewage effluent, (Gartner et al. 2002; Gorman 2009), agricultural run-off, (Costanzo et al. 2003; Diebel and Zanden 2009), and from aquaculture (Vizzini and Mazzola 2012). The simple predictive models described within this thesis may provide a standard from which to correct for temperature when using  $\delta^{15}\text{N}$  in this way, particularly when using data collected from over large spatial scales. Furthermore, such relationships could be used to predict the response of  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  in marine autotrophs to continued increases in seawater temperatures associated with climate change (e.g. Wernberg et al. 2011), and so adjust for such differences when comparing past, present and future  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  data from temperate systems.

### **7.3 Conclusion**

It is difficult to make accurate conclusions regarding food-web structure, fate of baseline food sources, and shifts in trophic levels and dietary specialisation without an understanding of the level of variation, and what drives variation, in isotopic signatures among baseline food sources and their consumers in marine systems. The models developed here for important autotrophic sources ( $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$ ), and the identification of reference taxa of baseline  $\delta^{15}\text{N}$ , could be an important step in providing common references to standardise the trophic structure (i.e.  $\delta^{13}\text{C}$



and  $\delta^{15}\text{N}$  bi-plots) of geographically distinct food webs, aid in the tracking of migratory or wide-ranging consumers, and the monitoring of human derived pollutants in coastal regions.

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## APPENDICES

### **Sampling, sample processing and stable isotope analysis of suspended particulate organic matter**

Spatial sampling was conducted at three of the four sites in each region, with triplicate subsurface (~4 m) seawater samples (3.5 L acid-washed clear plastic bottles) collected using a messenger activated water bottle and immediately passed through a 100  $\mu\text{m}$  mesh to remove zooplankton and large particles. Filtered seawater were kept on ice before being frozen for later processing. Temporal sampling followed the same protocol but from just two sites within Marmion Marine Park (Little Island (LI) and Marmion Reef (MR) (Figure 1, Chapters 2 and 3). At these sites, we sampled every month over 13-months, from September 2012 to September 2013.

Each triplicate seawater sample was filtered through precombusted (450°C 4 hrs.) Whatman GF/F filters (nominal pore size = 0.7  $\mu\text{m}$ ) to obtain POM. Filters were dissected and designated for either  $\delta^{13}\text{C}$  or  $\delta^{15}\text{N}$  analysis. Filters for  $\delta^{13}\text{C}$  were exposed to HCL (35%) fumes for 12 hrs in a desiccator to remove carbonates (as described in Carabel and Godínez-Domínguez (2006)). Sample filters for both  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  analysis were dried and packed into 12 x 10 mm tin capsules ready for stable isotope analysis.

Stable isotope analysis was performed using a continuous flow isotope ratio mass spectrometry and ratios  $^{13}\text{C}/^{12}\text{C}$  and  $^{15}\text{N}/^{14}\text{N}$  are expressed as delta ( $\delta$ ) relative to the international carbon and nitrogen standards (e.g. Pee Dee Belemnite and atmospheric  $\text{N}_2$ , respectively).

**Table A1.** Summary  $\delta^{13}\text{C}$  data of the red alga *Plocamium cartilagineum* extracted and analysed to produce Figures 7 and 8. Where only mean and variation was given (i.e. individual values were not), we plotted randomly generated values with the same mean, and variation from the  $n$  of samples stated in the reference source. Where  $n$  was not given, but variation was, we assumed  $n$  was three. References relate to the specific species *P. cartilagineum* and only to regions considered temperate. \* indicates samples that were collected over two months (i.e. April/May), in which case we plotted the same mean values against temperature data from each of those stated months. Data are ordered chronologically by publication date followed by sampling date.

Location (coordinates)	$n$	Date	Temp ( $^{\circ}\text{C}$ )	$\delta^{13}\text{C}$ ( $\pm$ )	Source
Sorrento, Australia (145.53 $^{\circ}\text{E}$ /39.49 $^{\circ}\text{S}$ )	1	Mar, 1995	17.44	-31.93	1
East Haven, UK (1.51 $^{\circ}\text{W}$ /56.55 $^{\circ}\text{N}$ )	3	July, 1995	12.64	-32.0 (0.37)	1
Mission Bay, USA (118.3 $^{\circ}\text{W}$ /32.74 $^{\circ}\text{N}$ )	8	Aug, 1995	18.54	-31.04 (0.22)	1
Barnhill, UK (1.49 $^{\circ}\text{W}$ /57.5 $^{\circ}\text{N}$ )	2	Jan, 1996	7.92	29.14; -31.48	1
Helmsdale, UK (2.43 $^{\circ}\text{W}$ /58.5 $^{\circ}\text{N}$ )	1	July, 1998	12.26	-32.86	1
Kingsbarns, UK (1.42 $^{\circ}\text{W}$ /56.46 $^{\circ}\text{N}$ )	2	May, 1999	7.63	-34.27; -31.99	1
Santa Catalina, USA (119.1 $^{\circ}\text{W}$ /33.7 $^{\circ}\text{N}$ )	6	May, 2000	15.68	-31.94 (0.43)	2
Perth, W. Australia (114.53 $^{\circ}\text{E}$ /32.37 $^{\circ}\text{S}$ )	3*	Apr, 1998	22.35	-31.7 (1.3)	3
Perth, W. Australia (114.53 $^{\circ}\text{E}$ /32.37 $^{\circ}\text{S}$ )	3*	May, 1998	21.99	-31.7 (1.3)	3
Helgoland, Germ. (7.52 $^{\circ}\text{E}$ /54.43 $^{\circ}\text{N}$ )	2	Mar, 2008	3.92	-38.1 (0.1)	4
Helgoland, Germ. (7.52 $^{\circ}\text{E}$ /54.43 $^{\circ}\text{N}$ )	2	May, 2008	9.67	-34.2 (0.2)	4
Galway, Ireland (10.2 $^{\circ}\text{W}$ /53.51 $^{\circ}\text{N}$ )	2	June, 2008	12.97	-34.2 (0.1)	4
Galway, Ireland (10.2 $^{\circ}\text{W}$ /53.51 $^{\circ}\text{N}$ )	2	Oct, 2008	13.05	-33.1 (0.1)	4

*Note:* Reference sources are: (1) Raven et al. (2002); (2) Korb (2003); (3) Smit et al. (2005); and (4)

Rosenfelder and Vetter (2012).



**Table A2.** Summary  $\delta^{13}\text{C}$  data of the brown alga *Ecklonia radiata* from studies across temperate Australasia. Estimates of sea surface temperature are of monthly means extracted from the from the Generalized Digital Environment Model database from the Australian Navy Metrology (<http://www.metoc.gov.au>). Data are ordered chronologically by publication date followed by sampling date.

Location (coordinates)	n	Date	Temp ( $^{\circ}\text{C}$ )	$\delta^{13}\text{C}$ ( $\pm$ )	Source
Hamelin Bay, Western Australia	8	Dec, 1999	19.6	-18.1	1
Carnac Island, Western Australia	6	Jan, 2000	20.9	-19.3	1
Hamelin Bay, Western Australia	3	Dec, 1999	19.6	-21.8*	2
Jurien Bay, Western Australia	3	Dec, 1999	21.0	-21.2*	2
Perth, Western Australia	3	Dec, 1999	20.1	-21.9*	2
Kalbarri, Western Australia	3	Dec, 1999	21.4	-23.1*	2
Maria Island, Tasmania	3	July, 2009	12.4	-18.5	3
Jurien Bay, Western Australia	3	Oct, 2005	19.9	-21.0*	4
Jurien Bay, Western Australia	3	April, 2005	22.6	-19.2*	5
Jurien Bay, Western Australia	3	Oct, 2005	19.9	-20.6*	5

*Note:* Reference sources are: (1) Raven et al. (2002); (2) Vanderklift and Wernberg (2010); (3) Guest et al.

2010; (4) Hanson et al. (2010); and (5) Hyndes et al. (2013)

**Table A3.** Regression equations based on the identified models in Table 2 and Figure 4 (Chapter 3). Different models were used for spatial, temporal and combined spatial and temporal designs.

(B)	Regression equation			
Design	<i>Ecklonia radiata</i>	Adj. $r^2$ (%)	$p$	SE
Spatial	$\delta^{15}\text{N} = 0.84 + 0.89 \times \text{PO}_4^{3+} \text{ concentration} + -0.26 \times \text{NO}_x$	60.0	0.016	0.84
Temporal	$\delta^{15}\text{N} = 7.59 + 0.34 \times \text{PO}_4^{3+} + + 0.01 \times \text{light} +$ $-0.28 \times \text{temperature} + 0.78 \times \text{precipitation}$	49.1	0.001	1.14
Combined	$\delta^{15}\text{N} = 14.0 + 0.35 \times \text{PO}_4^{3+} + 1.0 \times \text{velocity} + -0.5 \times \text{temperature}$	71.4	<0.001	1.22
	<i>Amphiroa anceps</i>			
Temporal	$\delta^{15}\text{N} = 19.9 + 0.44 \times \text{PO}_4^{3+} + 0.000656 \times \text{light} +$ $-0.85 \times \text{temperature}$	68.2	<0.001	1.24
Combined	$\delta^{15}\text{N} = 20.54 + 0.34 \times \text{PO}_4^{3+} + -0.79 \times \text{temperature}$	66.2	<0.001	1.56
	<i>Plocamium preissianum</i>			
Spatial	$\delta^{15}\text{N} = -7.48 + 0.01 \times \text{light} + 0.93 \times \delta^{15}\text{N}\text{-NO}_3$	84.4	0.001	0.76
Temporal	$\delta^{15}\text{N} = 11.8 + 0.0002 \times \text{light} + -0.29 \times \text{temperature}$	42.7	<0.001	0.66
Combined	$\delta^{15}\text{N} = 13.2 + 0.17 \times \text{PO}_4^{3+} + -0.38 \times \text{temperature}$	46.3	<0.001	1.12

**Table A4.** Summary  $\delta^{15}\text{N}$  data of *Ecklonia radiata* and *Amphiroa anceps* from studies across temperate Australasia. Estimates of sea surface temperature are of monthly means extracted from the from the Generalized Digital Environment Model database from the Australian Navy Metrology (<http://www.metoc.gov.au>). Data are ordered chronologically by publication date followed by sampling date.

Location	<i>n</i>	Date	Temp ( $^{\circ}\text{C}$ )	Mean $\delta^{15}\text{N}$	Reference
<b><i>Ecklonia radiata</i></b>					
Hamelin Bay, Western Australia	3	Dec, 1999	19.6	4.8	Vanderklift and Wernberg (2010)
Perth, Western Australia	3	Dec, 1999	20.1	6.25	Vanderklift and Wernberg (2010)
Jurien Bay, Western Australia	3	Dec, 1999	21.0	4.9	Vanderklift and Wernberg (2010)
Kalbarri, Western Australia	3	Dec, 1999	21.4	5.21	Vanderklift and Wernberg (2010)
Maria Island, Tasmania	3	July, 2009	12.5	9.0	Guest et al. (2010)
St. Helens, Tasmania	3	June to August	12.7	7.6	Guest et al. (2010)
Recherche Bay, Tasmania	3	June to August	12.4	6.85	Guest et al. (2010)
West Coast, Tasmania	3	June to August	12.8	7.4	Guest et al. (2010)
Jurien Bay, Western Australia	3	Oct, 2005	19.9	4.8	Hyndes et al. (2013)
Jurien Bay, Western Australia	3	April, 2005	22.6	2.8	Hyndes et al. (2013)
Jurien Bay, Western Australia	3	Oct, 2005	19.9	4	Hyndes et al. (2013)
Southwest coast of South Island, New Zealand	3	April, 2004	16.0	4.7	Davis and Wing (2012)
<b><i>Amphiroa anceps</i></b>					
Jurien Bay, Western Australia	4	October, 2005	19.9	5.1	Hyndes et al. (2013)
Jurien Bay, Western Australia	5	April, 2005	22.6	3.59	Hyndes et al. (2013)
Jurien Bay, Western Australia	7	October, 2005	19.9	4.88	Hyndes et al. (2013)

**Table A5.** Linear correlations (r) between mean  $\delta^{15}\text{N}$  in primary consumers and primary sources among months factoring in a one month time-lag.

Primary source	Suspension-feeders				Grazers			
	<i>H. momus</i>		<i>S. bilocularis</i>		<i>T. torquatus</i>		<i>H. erythrogramma</i>	
	<i>P</i>	<i>r</i>	<i>P</i>	<i>r</i>	<i>P</i>	<i>r</i>	<i>P</i>	<i>r</i>
<i>A. anceps</i>	0.38	0.29	0.69	0.13	0.39	0.28	0.63	0.16
<i>E. radiata</i>	0.53	0.21	0.53	0.21	0.22	0.40	0.79	0.09
<i>P. preissianum</i>	0.51	0.21	0.17	0.44	0.93	0.03	0.53	0.21
POM	0.69	0.13	0.62	-0.16				

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