

# Variation in $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ of kelp is explained by light and productivity

Mathew A. Vanderklift\*, Douglas Bearham

CSIRO Wealth from Oceans Flagship, Private Bag 5, Wembley, Western Australia 6913, Australia

**ABSTRACT:** Stable isotope ratios of autotrophs commonly encompass a wide range and exhibit high variation. It is important to understand how the environments in which autotrophs grow influence stable isotope ratios because variation can limit our ability to make inferences. We sought to understand whether light intensity, water temperature and productivity influenced  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  of the kelp *Ecklonia radiata*.  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  exhibited a wide range: 10.9‰ for  $\delta^{13}\text{C}$  and 5.3‰ for  $\delta^{15}\text{N}$ . Spatial variability was high but could largely be accounted for by patterns in the intensity of light measured at the sea floor. Temperature typically had poorer explanatory power. Partial regression analyses indicated that light intensity was still a good predictor of patterns in  $\delta^{13}\text{C}$  after accounting for depth, providing confidence in inferences about the effects of light. In contrast, patterns in  $\delta^{15}\text{N}$  could be explained by depth alone after accounting for light intensity, indicating that other influences that change with depth are also plausible explanations for patterns in  $\delta^{15}\text{N}$ . Individual productivity was also positively correlated with  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$ , but the amount of variation accounted for was always low. Sections of tissue taken from different parts of the thalli were positively correlated when all data were pooled, but there was significant spatial and temporal variation within this overall trend. Our results suggest that light intensity is an important influence on variation in  $\delta^{13}\text{C}$ . The results support a mechanism in which increased rates of photosynthesis (because of higher light availability) increase the demand for carbon so that a greater proportion of the available carbon is used, which in turn decreases fractionation.

**KEY WORDS:** Carbon · *Ecklonia radiata* · Growth · Nitrogen · Stable isotope analysis

Resale or republication not permitted without written consent of the publisher

## INTRODUCTION

The physiological processes by which autotrophs obtain carbon and nitrogen, and incorporate these elements into tissues, lead to relatively predictable patterns in the relative abundance of  $^{13}\text{C}:^{12}\text{C}$  and  $^{15}\text{N}:^{14}\text{N}$  (Peterson & Fry 1987, West et al. 2006). The ability to rely on these patterns has enabled ecologists to make inferences about trophic interactions that were previously difficult or impossible. However, within this generally predictable framework, there is considerable variation. Stable isotope ratios within a single species can vary from place to place, fluctuate over time, differ between individuals and even vary within an individual (e.g. Fourqurean et al. 2005, Newsome et al. 2009).

Variation in stable isotope ratios can tend to obscure differences between otherwise isotopically distinct groups. Mixing models have been developed to incorporate this variation (e.g. Phillips 2001, Moore & Semmens 2008, Parnell et al. 2010), but too much variation leads to uncertainty in calculations of the contributions of different sources. Variation in stable isotope ratios can also indicate the presence of patterns that offer ecologists the opportunity to enhance their understanding of ecological processes and of trophic pathways. For example, understanding geographic patterns in stable isotope ratios has enabled improved predictions of animal movements (Rubenstein & Hobson 2004), and disentangling discordant spatial patterns in stable isotope ratios of autotrophs has allowed identification of otherwise obscured

\*Corresponding author: mat.vanderklift@csiro.au

trophic pathways (Melville & Connolly 2003, Vanderklift & Wernberg 2010).

Developing an ability to accurately predict (not simply explain) stable isotope ratios ideally requires an understanding of the mechanisms underlying those patterns. Nevertheless, in the absence of mechanistic understanding, the presence of correlations with important environmental variables can yield useful insights. For autotrophs, the most important environmental variables influencing  $\delta^{13}\text{C}$  are light and temperature because these exert a strong influence on rates of photosynthesis, which in turn influence rates of carbon uptake and therefore the magnitude of fractionation between  $^{13}\text{C}$  and  $^{12}\text{C}$  (Farquhar et al. 1989, Raven et al. 1995). Higher light intensity and higher temperatures typically tend to increase rates of photosynthesis (although this increase is asymptotic) and therefore increase the demand for carbon so that a greater proportion of the available carbon is used; this should tend to decrease fractionation, and we should expect a positive relationship between  $\delta^{13}\text{C}$  and light and temperature. Indeed, this has been shown in laboratory and field experiments (e.g. Grice et al. 1996, Cornelisen et al. 2007). These simple predictions ignore the potential influence of carbon-concentrating mechanisms, which are present in most marine algae (Hurd et al. 2009); the relative influence of these mechanisms on  $\delta^{13}\text{C}$  might also be influenced by light and temperature (Raven et al. 2011), but the nature of this influence is not yet well understood. In the absence of this understanding, simple models can still yield useful insights. These simple models predict that there should be correlations between  $\delta^{13}\text{C}$  and light or temperature; thus, tests of the presence of such correlations provide tests of the strength of evidence supporting the models.

In addition, aquatic plants are often able to use both dissolved  $\text{CO}_2$  and bicarbonate ( $\text{HCO}_3^-$ ), which can have  $\delta^{13}\text{C}$  values that differ by ~9 to 10‰ (Mook 2001, Raven et al. 2002). Potentially, variation in the relative availability of each of these (for example, the relative proportion of  $\text{CO}_2$  and  $\text{HCO}_3^-$  in seawater is influenced by pH and to a lesser extent by temperature and salinity; Raven et al. 2005), or in the physiological ability to use one or the other (Raven et al. 2002), could introduce additional variation.

In the ocean,  $\delta^{13}\text{C}$  in autotrophs might also be influenced by the partial pressure of dissolved  $\text{CO}_2$ . The  $\delta^{13}\text{C}$  of inorganic carbon, and subsequently of autotrophs, can be influenced by partial pressure (Fiorini et al. 2010). Because partial pressure is influenced by depth, this could contribute to variation with depth beyond that caused by light attenuation.

The causes of variation in  $\delta^{15}\text{N}$  are likely to be more complex because there are more potential sources of nitrogen for autotrophs (e.g.  $\text{NH}_4^+$ ,  $\text{NO}_3^-$ ,  $\text{NO}_2^-$ ), and the  $\delta^{15}\text{N}$  of these sources are themselves variable (Robinson 2001); in the ocean, these sources can differ by 20‰ or more (Sigman et al. 2009). Nevertheless, any increase in photosynthesis that leads to increases in growth should in turn cause increased demand for nitrogen. This should result in reduced fractionation as a higher proportion of the available nitrogen is used; because fractionation tends to discriminate against  $^{15}\text{N}$ , in simple situations (i.e. one main source of nitrogen), we should again see a positive correlation between  $\delta^{15}\text{N}$  and light and temperature.

Spatial and temporal patterns in environmental variables such as light and temperature might help explain the spatial and temporal variation in stable isotope ratios of autotrophs that has been observed repeatedly (e.g. Campbell & Fourqurean 2009, Vanderklift & Wernberg 2010, Hu et al. 2012). Importantly, understanding the nature (e.g. the direction, slope and strength) of the correlations between environmental variables and stable isotope ratios, alone and when combined with other variables, is likely to help ecologists to enhance their understanding of food webs.

In addition, measurable variation among individuals frequently persists within these broader patterns (e.g. Loader et al. 2007, Guest et al. 2010). This among-individual variation might be due to localised patterns in the surrounding environment (for example, localised changes in light intensity because of shading) but also might be due to among-individual differences in physiology and growth rate. Understanding among-individual variation in stable isotope ratios of autotrophs might therefore yield important insights, in the same way that understanding among-individual variation in heterotrophs has led to insights about niche width (Bearhop et al. 2004, Newsome et al. 2007).

To uncover differences among individuals, patterns within an individual must also be characterised, especially patterns within a single tissue. In autotrophs, intra-individual differences are often encountered (Evans 2001). In part, this might be related to patterns of growth because tissues grown at different times will experience different conditions. One simple test of this explanation is therefore to determine whether the sign and magnitude of intra-individual differences is consistent among individuals within a population.

In our study, we examined some of these patterns in the kelp *Ecklonia radiata*, a dominant canopy-forming alga in southern Australia, in which stable

isotope ratios have previously been shown to vary among places and times (Guest et al. 2010, Vanderklift & Wernberg 2010, Hyndes et al. 2013) and which has distinct spatial and temporal patterns in growth and productivity (Kirkman 1984, Fairhead & Cheshire 2004, Bearham et al. 2013). We set out to test whether spatial and temporal patterns in  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  could be explained by light, temperature and growth history. We asked first whether  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  of different parts of the thallus varied in a way that was spatially and temporally consistent. We then sought to determine whether spatial patterns in  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  could be explained by light and temperature and whether these relationships would be consistent at different times of the year. Finally, we tested whether growth and productivity of individuals could also account for patterns in  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$ .

## MATERIALS AND METHODS

### Study area

The study was focussed on Marmion Lagoon, southwestern Australia ( $31^{\circ}49'S$ ,  $115^{\circ}44'E$ ). The seafloor in this region is characterised by high-relief rocky reefs comprised of aeolianite limestone interspersed with unconsolidated sand (Searle & Semeniuk 1985). The reefs host a diverse assemblage of macroalgae, which is dominated by the kelp *Ecklonia radiata* (Phillips et al. 1997, Wernberg et al. 2003), and rates of herbivory are typically low (Vanderklift et al. 2009). Water temperature varies seasonally, ranging from  $\sim 23^{\circ}\text{C}$  in the austral summer to  $\sim 16^{\circ}\text{C}$  in the austral spring (Smale & Wernberg 2009), and day length (time between sunrise and sunset) ranges from 10 to 14 h. We surveyed 19 sites within an area of approximately  $45\text{ km}^2$  (maximum distance between sites was 10.9 km); sites were selected to encompass a wide range of depths (4 to 17 m) and water clarity.

### Data collection

At each site, we measured productivity (defined as the rate of production of biomass, *sensu* Westlake 1965) of *Ecklonia radiata* following the method described by Mann & Kirkman (1981) and adapted by Fairhead & Cheshire (2004). We marked 20 individual kelps at each site by punching a hole into the central lamina 5 cm from the junction between the stipe and the lamina. (The meristem of *E. radiata* is

just above this junction, and so this method is intended to encompass the majority of growth; Mann & Kirkman 1981.) Marked kelps were collected between 30 and 54 d later and frozen until measurements were taken in the laboratory. This was repeated at 3 different times of the year, chosen to represent periods when productivity varies (Kirkman 1984, Bearham et al. 2013): December to January, May to June and September to October. For simplicity, we refer to these 3 different time periods as 'seasons'. One site could not be included in the May to June season because weather conditions were too poor. In all but 3 cases, at least 5 kelps were retrieved from each site. The average total length of individual kelps was  $56 \pm 15\text{ cm}$  (mean  $\pm$  SD).

In the laboratory, the distance of the hole from the junction between the stipe and the lamina was measured, and thallus extension (TE) was calculated by subtraction. The total length of the thallus was measured (cm), and the basal 20 cm of the thallus was cut into sections 5 cm long. Each section, and the remaining section of thallus, was weighed separately (g wet wt). Productivity was estimated as biomass accumulation (BA), following Fairhead & Cheshire (2004), as  $\text{BA} = xw/5d$  (in  $\text{g ind.}^{-1}\text{ d}^{-1}$ ), where  $x$  is the TE (cm),  $w$  is the wet wt (g) of the heaviest section and  $d$  is the number of days between punching the hole and collecting the kelp. Relative growth rate (RGR) was calculated as  $[\ln(W_e) - \ln(W_i)]/d$ , where  $W_e$  is the total weight of the entire thallus on final measurement and  $W_i$  is the initial weight of the thallus at tagging (calculated as  $W_e - \text{BA}$ ).

A section of tissue was removed from the 1st (youngest) and 7th fully developed lateral of 5 individual kelps from each site in each season, rinsed with deionised water and dried in an oven at  $60^{\circ}\text{C}$ . After drying, samples were ground using a mixer mill and then stored in microcentrifuge tubes in a desiccating cabinet.  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  were measured by continuous-flow isotope ratio mass spectrometry (using an ANCA-GSL elemental analyser and 20-20 isotope ratio mass spectrometer; Europa). The analytical precision derived from repeated measurements of an internal standard of *Fucus vesiculosus* (which had been previously calibrated against International Atomic Energy Agency or National Institute of Standards and Technology standards) was maximum  $\pm 0.07$  (SD) for  $\delta^{15}\text{N}$  and maximum  $\pm 0.17$  (SD) for  $\delta^{13}\text{C}$ .

At each site in each season, we deployed a single HOBO® Pendant Temperature/Light Data Logger (Model UA-002-08 or UA-002-64). Each logger was configured to record temperature ( $^{\circ}\text{C}$ ) and illuminance (lx) every 10 min. Illuminance was used as a

measure of light intensity; measures of irradiance are more directly relevant to mechanistic explanations of stable isotope fractionation during photosynthesis, but our primary interest was in determining patterns in stable isotope abundances at a wide range of sites with different light intensities. Measurements of illuminance and irradiance are typically highly correlated (Long et al. 2012, Bearham et al. 2013). On 5 occasions, we deployed 2 loggers close to each other to assess consistency of measurements; in each case, individual light and temperature measurements were highly correlated.

### Data analysis

To test whether measurements from the 2 different laterals (1st and 7th) followed similar spatial and temporal trends, we used ANCOVA to test for patterns in the stable isotope ratios of tissue from the 7th lateral among sites and seasons, with stable isotope values of the 1st lateral as the covariate.

To test for patterns of variation among sites (spatial variation) and among seasons (temporal variation), we used ANOVA. Because sites were specifically selected to represent known variations in water clarity and wave exposure, and seasons were specifically selected to represent times of the year with different water temperatures, we considered both to be fixed factors. We used variance components to determine the amount of variation left 'unexplained' (i.e. the residual term in the model) after accounting for patterns among sites and seasons. Although variance components for fixed terms in an ANOVA model are somewhat different conceptually than those for the residual term, they are still useful for determining the relative amount of variation accounted for by different terms within a model (Graham & Edwards 2001, Quinn & Keough 2002). Because there is no simple method for calculation of variance components for unbalanced data, we balanced our dataset by only including sites for which stable isotope data for 5 individual kelps were collected in every season. For these analyses, we used data yielded by the 1st and 7th fully formed laterals separately.

To test whether spatial and temporal variation might be due to differences in productivity, we also added measurements of growth (TE) and productivity (BA, RGR) as covariates to the ANOVA model. We first tested for differences among slopes, and if differences among slopes were not statistically significant (at  $\alpha = 0.05$ ), we fitted a common slope and tested whether productivity accounted for additional varia-

tion in  $\delta^{13}\text{C}$  or  $\delta^{15}\text{N}$ . We conducted separate analyses for each measure of growth and productivity. For these analyses, we only used data from the 1st fully formed lateral.

To test whether spatial and temporal patterns in  $\delta^{13}\text{C}$  or  $\delta^{15}\text{N}$  could be explained by patterns in light and temperature, we used multiple linear regressions. We used the average of the 1st fully formed lateral for each site-season combination, the average of the daily sum of the lux measurements for the first 10 d (after which measurements deteriorated as the sensors became fouled) and the average of the daily averages of all temperature measurements for the entire deployment. We also performed analyses with 2 other formats for the light data (the average lux hours per day and the average lux for the 2 h around solar noon); both yielded qualitatively similar results but poorer correlations and so are not included here. Loggers at 9 sites were dislodged during deployments in winter and were not retrieved. Because of this, we performed 2 sets of analyses. In the first set, we did separate analyses for each season. In the second set, we did a single analysis including all seasons for the subset of 8 sites for which all data were successfully collected in all seasons. When analyses yielded a statistically significant relationship (at  $\alpha = 0.05$ ) for both light and temperature, we used partial regression analyses to determine whether the relationships remained significant after accounting for the effect of the other variable. To account for the possibility that any relationships with light might be due to spatial autocorrelation with other variables influenced by depth, we used partial regression analyses to test whether relationships remained after accounting for the effect of depth.

## RESULTS

The range in  $\delta^{13}\text{C}$  (Fig. 1, Table A1 in the Appendix) of the 1st full lateral encompassed 8.9‰ in summer (range:  $-24.1$  to  $-15.2$ ‰), 9.7‰ in winter (range:  $-26.1$  to  $-16.4$ ‰) and 6.4‰ in spring (range:  $-23.9$  to  $-17.5$ ‰).  $\delta^{13}\text{C}$  of the 1st and 7th laterals were well correlated ( $r^2 = 0.47$ ), but the nature of differences between the 2 laterals varied among sites and seasons (significant heterogeneity among slopes:  $F = 1.71$ ,  $p = 0.02$ ). The difference in  $\delta^{13}\text{C}$  between the 2 laterals was significantly (but weakly) correlated with all measures of growth and productivity, with the highest correlation yielded by TE ( $r^2 = 0.07$ ,  $p < 0.001$ ).

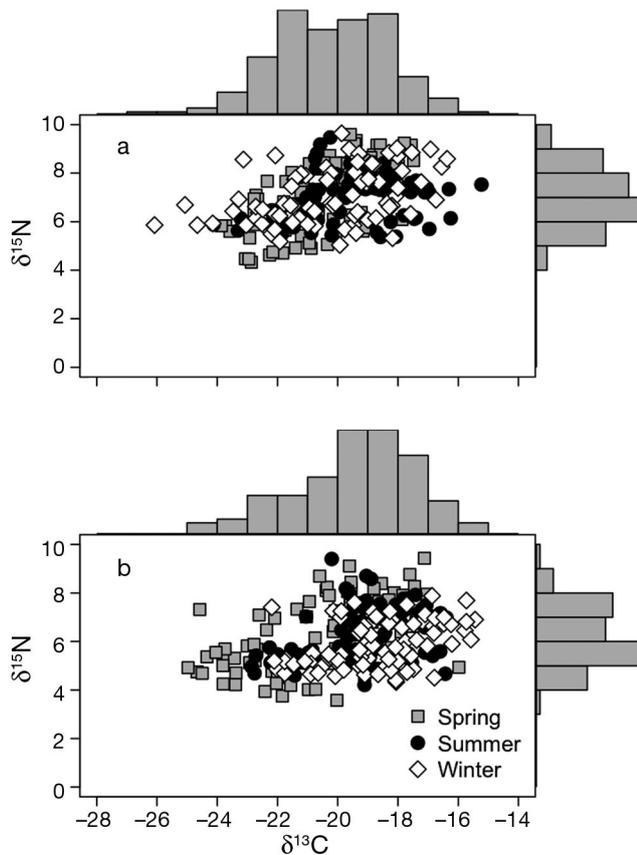


Fig. 1. Plots of  $\delta^{13}\text{C}$  versus  $\delta^{15}\text{N}$  for (a) the 1st fully formed lateral and (b) the 7th fully formed lateral in *Ecklonia radiata*. Histograms in the margins of each plot show counts in 1‰ increments.  $n = 268$  individuals

The range in  $\delta^{15}\text{N}$  (Fig. 1, Table A1) of the 1st full lateral encompassed 4.1‰ in summer (range: 5.4 to 9.5‰), 4.6‰ in winter (range: 5.0 to 9.6‰) and 5.3‰ in spring (range: 4.3 to 9.6‰).  $\delta^{15}\text{N}$  of the 1st and 7th laterals were highly correlated ( $r^2 = 0.76$ ,  $p < 0.001$ ), but as with  $\delta^{13}\text{C}$ , the nature of differences between the 2 laterals varied among sites and seasons (significant heterogeneity among slopes:  $F = 1.77$ ,  $p = 0.01$ ). The difference in  $\delta^{15}\text{N}$  between the 2 laterals was significantly (but weakly) correlated with BA ( $r^2 = 0.03$ ,  $p < 0.008$ ) but not with TE or RGR.

There were significant differences among sites and seasons for both  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$ , and all sources of variation in both analyses were statistically significant ( $p < 0.01$  in all cases; Table 1). Variation among individual samples accounted for a large proportion of the overall variation in  $\delta^{13}\text{C}$  (the variance component for the residual term accounted for 30% of the total variation). This was not the case for  $\delta^{15}\text{N}$ , for which variation among individuals accounted for a relatively minor proportion (8%) of the total varia-

tion.  $\delta^{13}\text{C}$  showed more overall variation than  $\delta^{15}\text{N}$  (variance components were higher for  $\delta^{13}\text{C}$  for all terms). The greatest amount of variation was accounted for by spatial patterns (i.e. by differences among sites) in each case, but the nature of the differences was not consistent in each season (statistically significant interaction between sites and seasons in Table 1). Results for the 7th lateral (not shown) were very similar.

All measures of growth and productivity explained a significant amount of the variation in  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  (Table 2). In each case, the slope of the relationship with TE, BA or RGR varied among sites and/or seasons, indicating that the nature of the relationship between productivity and stable isotope ratios was inconsistent. In addition, in each case the improvement in model fit was negligible ( $< 0.02$  improvement in the adjusted  $r^2$ ), or there was no overall improvement in the fit of the model (decrease in the adjusted  $r^2$ ).

Among-site patterns in mean  $\delta^{13}\text{C}$  were positively correlated with light in all seasons (Fig. 2), but this pattern was statistically significant only in spring (Table 3). Mean  $\delta^{13}\text{C}$  showed no trends related to temperature in any season (Table 3). Mean  $\delta^{15}\text{N}$  was positively correlated with light in all seasons (Fig. 2), but the pattern was weak and not significant in winter (Table 3). In addition, the correlation between light and  $\delta^{15}\text{N}$  in summer was not statistically significant after the effect of temperature was accounted for ( $p = 0.28$ ). Mean  $\delta^{15}\text{N}$  was significantly correlated with temperature in summer, when low mean  $\delta^{15}\text{N}$  was measured at sites with low temperatures (Fig. 3; this relationship remained after the effect of light was accounted for), but not in winter or spring (Table 3).

Table 1. Results of analyses of variance testing for spatial and temporal patterns of  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$ .  $\omega^2$  = relative magnitude of effects. **Bold** indicates significance at  $p < 0.05$ . VC = variance components

Source	df	MS	F	p	VC	95% CI	$\omega^2$
<b><math>\delta^{13}\text{C}</math></b>							
Site	15	26.53	20.96	<b>&lt;0.001</b>	1.68		40.4
Season	2	17.97	14.19	<b>&lt;0.001</b>	0.21		5.0
Site $\times$ Season	30	6.32	4.99	<b>&lt;0.001</b>	1.01		24.2
Residual	192	1.27			1.27	1.08	30.4
<b><math>\delta^{15}\text{N}</math></b>							
Site	15	14.45	130.35	<b>&lt;0.001</b>	0.96		70.3
Season	2	0.57	5.11	<b>0.007</b>	0.006		0.4
Site $\times$ Season	30	1.54	13.89	<b>&lt;0.001</b>	0.29		21.0
Residual	192	0.11			0.11	0.09	8.2

Table 2. Results of ANCOVA testing for spatial and temporal patterns, and relationships with productivity (BA), relative productivity (RGR) and growth (as thallus extension, TE) of  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$ . Blank rows indicate that source of variation has been pooled into the residual term. **Bold** indicates significance at  $p < 0.05$

Source	Covariate = BA				Covariate = RGR				Covariate = TE			
	df	MS	F	p	df	MS	F	p	df	MS	F	p
<b><math>\delta^{13}\text{C}</math></b>												
Covariate	1	9.22	6.99	<b>0.009</b>	1	6.65	5.44	<b>0.021</b>	1	11.87	10.55	<b>0.001</b>
Site	15	25.96	19.66	<b>&lt;0.001</b>	15	25.79	21.13	<b>&lt;0.001</b>	15	25.74	22.87	<b>&lt;0.001</b>
Season	2	18.23	13.81	<b>&lt;0.001</b>	2	22.64	18.54	<b>&lt;0.001</b>	2	20.07	17.83	<b>&lt;0.001</b>
Covariate $\times$ Site	15	3.33	2.52	<b>0.002</b>	15	3.56	2.92	<b>&lt;0.001</b>	15	2.90	2.58	<b>0.002</b>
Covariate $\times$ Season	2	5.13	4.20	<b>0.017</b>	2	5.13	4.20	<b>0.017</b>	2	0.77	0.68	0.507
Site $\times$ Season	30	4.97	3.76	<b>&lt;0.001</b>	30	4.84	3.96	<b>&lt;0.001</b>	30	5.50	4.88	<b>&lt;0.001</b>
Covariate $\times$ Site $\times$ Season									30	1.88	1.67	<b>0.025</b>
Residual	176	1.32			173	1.22			144	1.13		
<b><math>\delta^{15}\text{N}</math></b>												
Covariate	1	1.79	16.07	<b>&lt;0.001</b>	1	1.95	17.11	<b>&lt;0.001</b>	1	7.10	62.62	<b>&lt;0.001</b>
Site	15	14.48	129.68	<b>&lt;0.001</b>	15	14.33	125.86	<b>&lt;0.001</b>	15	13.98	123.26	<b>&lt;0.001</b>
Season	2	0.43	3.87	<b>0.023</b>	2	0.27	2.38	0.095	2	0.62	5.47	<b>0.005</b>
Covariate $\times$ Site	15	0.58	5.18	<b>&lt;0.001</b>	15	1.16	10.18	<b>&lt;0.001</b>	15	0.81	7.14	<b>&lt;0.001</b>
Covariate $\times$ Season	2	0.47	4.23	<b>0.016</b>	2	0.61	5.36	<b>0.005</b>	2	1.38	12.18	<b>&lt;0.001</b>
Site $\times$ Season	30	1.21	10.86	<b>&lt;0.001</b>	30	0.97	8.52	<b>&lt;0.001</b>	30	1.09	9.60	<b>&lt;0.001</b>
Covariate $\times$ Site $\times$ Season												
Residual	174	0.11			173	0.11			174	0.11		

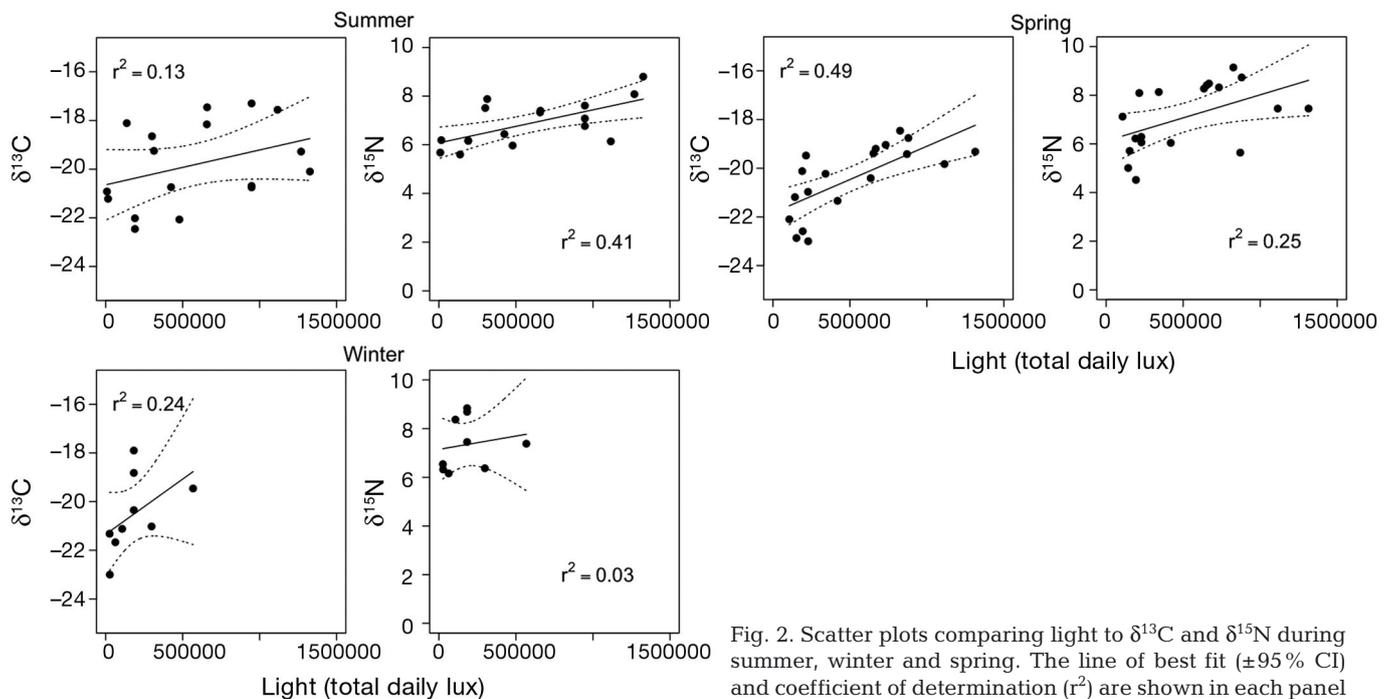


Fig. 2. Scatter plots comparing light to  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  during summer, winter and spring. The line of best fit ( $\pm 95\%$  CI) and coefficient of determination ( $r^2$ ) are shown in each panel

Analyses restricted to the 8 sites for which loggers were retrieved in all seasons yielded a significant and positive relationship between  $\delta^{13}\text{C}$  and light but not temperature (Table 4, Fig. 4). There was an indication of slight differences in mean  $\delta^{13}\text{C}$  among seasons ( $p = 0.075$ , Table 4), but the nature of the relationship between mean  $\delta^{13}\text{C}$  and light was consistent

(no statistically significant differences among slopes). Similarly, mean  $\delta^{15}\text{N}$  showed a significant and positive relationship with light (Fig. 4) but not with temperature; there was no evidence of differences among seasons (Table 4).

After accounting for depth, the relationship between  $\delta^{13}\text{C}$  and light remained (partial  $r^2 = 0.16$ ;  $p =$

Table 3. Results of multiple regressions testing for relationships between mean  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  of *Ecklonia radiata* and patterns in light and temperature. Separate analyses were done for each season. **Bold** indicates significance at  $p < 0.05$

Source	Summer				Winter				Spring				
	df	MS	F	p	df	MS	F	p	df	MS	F	p	
<b><math>\delta^{13}\text{C}</math></b>													
Light	1	6.30	2.26	0.155	1	4.75	1.92	0.215	1	17.96	16.90	<b>&lt;0.001</b>	
Temperature	1	2.03	0.73	0.407	1	0.09	0.04	0.853	1	1.58	1.49	0.241	
Residual	14	2.78			6	2.47			16	1.06			
<b><math>\delta^{15}\text{N}</math></b>													
Light	1	5.64	15.57	<b>0.001</b>	1	0.28	0.19	0.681	1	8.60	5.39	<b>0.034</b>	
Temperature	1	3.01	8.30	<b>0.012</b>	1	0.0002	0.0002	0.990	1	0.17	0.11	0.748	
Residual	14	5.08			6	1.48			16	1.60			

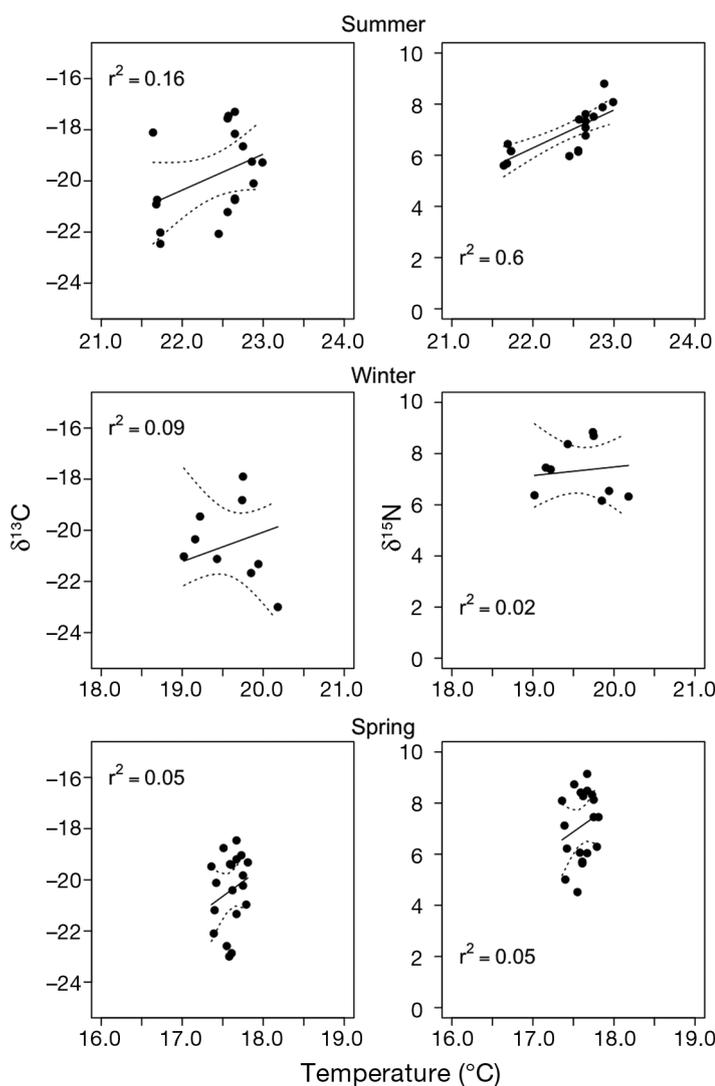


Fig. 3. Scatter plots comparing temperature to  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  during summer, winter and spring. The line of best fit ( $\pm 95\%$  CI) and coefficient of determination ( $r^2$ ) are shown in each panel

Table 4. Results of multiple regressions testing for relationships between mean  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  of *Ecklonia radiata* and patterns in light and temperature for the 8 sites for which loggers were retrieved each season. **Bold** indicates significance at  $p < 0.05$

Source	df	MS	F	p
<b><math>\delta^{13}\text{C}</math></b>				
Season	2	5.58	2.98	0.075
Light	1	9.31	4.97	<b>0.038</b>
Temperature	1	0.004	0.002	0.962
Residual	19	1.87		
<b><math>\delta^{15}\text{N}</math></b>				
Season	2	0.07	0.06	0.941
Light	1	7.40	6.77	<b>0.018</b>
Temperature	1	1.01	0.93	0.348
Residual	19	1.09		

0.05), but the relationship between  $\delta^{15}\text{N}$  and light was no longer present (partial  $r^2 = 0.008$ ;  $p = 0.67$ ). Conversely, the relationship between  $\delta^{15}\text{N}$  and depth was strong after accounting for light (partial  $r^2 = 0.50$ ;  $p < 0.001$ ). These results suggest that patterns in light intensity are able to account for the patterns in  $\delta^{13}\text{C}$  but that the patterns in  $\delta^{15}\text{N}$  might simply be due to other (unmeasured) variables that were correlated with depth.

## DISCUSSION

$\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  of the kelp *Ecklonia radiata* encompassed a wide range (10.9‰ for  $\delta^{13}\text{C}$  and 5.3‰ for  $\delta^{15}\text{N}$ ), demonstrating the considerable variability that can exist in stable isotope ratios of autotrophs. Most of this variation was due to spatial variation (differences among sites), but a large proportion of the variation in  $\delta^{13}\text{C}$  was not accounted for by spatial or tem-

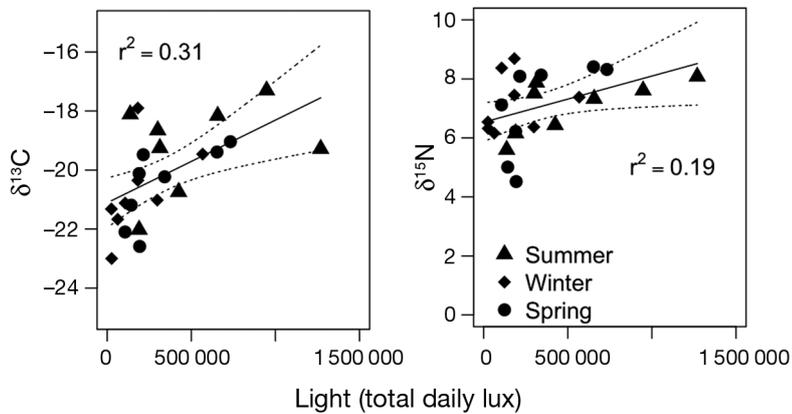


Fig. 4. Scatter plots comparing light to  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  for all seasons combined. The line of best fit ( $\pm 95\%$  CI) and coefficient of determination ( $r^2$ ) are shown in each panel

poral patterns. Spatial variation was in turn well explained by patterns in light intensity, with both  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  tending to be higher at places where there was more light (although this pattern was not found in all seasons). Positive correlations with light remained after accounting for differences in depth for  $\delta^{13}\text{C}$  but not for  $\delta^{15}\text{N}$ ; this suggests that inferences about light are robust for  $\delta^{13}\text{C}$  but that patterns in  $\delta^{15}\text{N}$  could also be due to other (unmeasured) variables that were correlated with depth. Some of the unexplained variation could be accounted for by patterns in growth or productivity, but these relationships were typically weak.

#### Spatial variation and relationships with light and temperature

The variation we found among sites in both  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  is consistent with the findings of other studies (Guest et al. 2010, Vanderklift & Wernberg 2010). We found that the proportion of variation that was accounted for by spatial patterns was highest for  $\delta^{15}\text{N}$ , for which there was relatively little variation attributable to temporal patterns (although it was statistically significant) and relatively little unexplained variation. In contrast, while  $\delta^{13}\text{C}$  also showed relatively little variation attributable to temporal patterns (although again it was statistically significant), a large proportion of the variation was not explained by either spatial or temporal patterns.

Such spatial patterns in  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  might be due to (1) variation in the rates at which the sources of C and N are used by the plant, (2) variation in the availability of different sources of C or N or (3) variation in

the stable isotope ratios of one or more of the sources.

Bearham et al. (2013) showed that spatial patterns of growth and productivity in *Ecklonia radiata* are strongly correlated with light and temperature in Marmion Lagoon, providing a plausible mechanism to explain patterns in  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$ . The nature of the relationship—higher  $\delta^{13}\text{C}$  at places that received more light—is consistent with the trend predicted if the rate of carbon fixation related to growth exceeds replacement, and faster growing plants would tend to use a greater proportion of the available carbon; this in turn should decrease fractionation as the  $\delta^{13}\text{C}$  of the plant approaches that of the carbon

source (Deuser et al. 1968, Korb et al. 1996). This expectation has been confirmed in several studies (e.g. Korb et al. 1996, Tanaka et al. 2008).

The relationships between the measurements of productivity and  $\delta^{13}\text{C}$  in this study are consistent with this expectation, but the patterns were very weak. The reasons for this are unclear, but one possibility is that the tissue that we used in the analyses (i.e. the 1st fully formed lateral) was outside the meristem, where most growth was occurring. If this was the case, then a weaker relationship would be expected because the  $\delta^{13}\text{C}$  will reflect metabolic turnover as well as newly fixed carbon.

Patterns in  $\delta^{15}\text{N}$  were qualitatively similar, and the same explanations might hold. However, patterns in  $\delta^{15}\text{N}$  could also be accounted for by depth alone, indicating the possibility that the patterns were due to spatial autocorrelation. This possibility, combined with the knowledge that N is not directly used during photosynthesis (Kirk 2011), indicate that other explanations might be plausible. Nevertheless, the significant (albeit weak) relationships between  $\delta^{15}\text{N}$  and growth and productivity, together with the knowledge that growth can be limited by the availability of N (Jackson 1977, Gerard 1982), do lend support to the indirect influence of light.

Variation in the availability, or in the stable isotope ratios, of different sources of C or N is plausible but less likely as an explanation for the magnitude of the variation observed. *E. radiata* is likely to use both  $\text{CO}_2$  and  $\text{HCO}_3^-$  (Raven et al. 2002), as well as the different forms of N ( $\text{NH}_4^+$ ,  $\text{NO}_3^-$ ,  $\text{NO}_2^-$ ). The relative availability of  $\text{CO}_2$  versus  $\text{HCO}_3^-$  varies according to temperature, pH and salinity (Raven et al. 2005), but the differences are not of a magnitude that could

explain a ~10‰ range in  $\delta^{13}\text{C}$ . The temperature range we recorded during our study was ~5°C, which will influence dissolved  $\text{CO}_2$  only slightly (Zeebe & Wolf-Gladrow 2001), and pH and salinity change very little in our study area (Greenwood et al. 2013), so these are unlikely to influence  $\delta^{13}\text{C}$ . Similarly, the relative availability of the different forms of N vary because of a number of influences, potentially causing variation of a magnitude we observed (~5‰), but off western Australia, these typically occur across broader spatial extents (hundreds of kilometres) and deeper depths (hundreds of metres) than those encompassed by our study (Lourey et al. 2006).

### Within-individual variation

The intra-individual differences between the 1st and 7th fully developed laterals are consistent with patterns reported by other researchers, who found substantial variation within the thallus of *Ecklonia radiata* (Fenton & Ritz 1989, Guest et al. 2010). Our data suggest that the intra-individual differences can be partly explained by growth and/or productivity (statistically significant correlations of the difference in stable isotope ratios between laterals with measurements of TE and BA).

Most of the growth of *E. radiata* occurs in the first 5 to 10 cm of the thallus, above the stipe (Mann & Kirkman 1981, Bearham et al. 2013), so we should expect longitudinal patterns in stable isotope ratios in the thallus (i.e. from the base to the apex) to reflect the growth history of the individual, unless there is significant metabolic turnover of C and N. Our data support this expectation, but the explanatory power of growth and productivity was weak ( $r^2 < 0.1$  in each case), indicating that there are other influences on patterns of distribution of stable isotope ratios within the thallus. It is possible that one such influence is the translocation of C and N within the thallus; such translocation is important for growth (Schmitz & Lobban 1976), and if it redistributes a large proportion of the C and/or N in the kelp tissue, this will tend to dampen any longitudinal patterns.

### CONCLUSIONS

We found high variability in the stable isotope ratios of *Ecklonia radiata*, but this variability was largely explicable by spatial and temporal patterns in light and to a lesser extent by individual growth histories. These insights provide ecologists with possi-

bilities to further enhance their understanding of food webs in several ways. For example, if light- or depth-related spatial gradients exist, then they could be used to distinguish the contribution of different basal food resources to consumers (e.g. Melville & Connolly 2003, Rasmussen 2010).

The results support the inference that light availability is an important determinant of spatial and temporal variation in  $\delta^{13}\text{C}$ . The results are consistent with a mechanism in which increased rates of photosynthesis (because of higher light availability) increase the demand for carbon so that a greater proportion of the available carbon is used, which in turn decreases fractionation. This inference is bolstered by the knowledge that there are strong proximate links between light and rates of carbon fixation. Despite this, the same trends were not strongly expressed by patterns in growth or production. Light was also able to predict patterns in  $\delta^{15}\text{N}$ , but we cannot discount the possibility that other (unmeasured) variables influenced this result; depth alone was able to account for patterns in  $\delta^{15}\text{N}$ , suggesting that other variables that co-vary with depth might be the ultimate cause of the patterns observed in  $\delta^{15}\text{N}$ .

*Acknowledgements.* We thank D. Thomson, F. Graham, R. Downie, K. Cook, S. Kondylas and R. Crossing for their assistance in the field and J. Tranter for the stable isotope analyses. We also thank K. McMahon, A. Reville and D. Walker for comments which helped improve the manuscript. This study was jointly funded by the Western Australian Marine Science Institution and the CSIRO Wealth from Oceans Flagship.

### LITERATURE CITED

- Bearham D, Vanderklift MA, Gunson JR (2013) Temperature and light explain spatial variation in growth and productivity of the kelp *Ecklonia radiata*. *Mar Ecol Prog Ser* 476:59–70
- Bearhop S, Adams CE, Waldron S, Fuller RA, MacLeod H (2004) Determining trophic niche width: a novel approach using stable isotope analysis. *J Anim Ecol* 73: 1007–1012
- Campbell JE, Fourqurean JW (2009) Interspecific variation in the elemental and stable isotope content of seagrasses in South Florida. *Mar Ecol Prog Ser* 387:109–123
- Cornelisen CD, Wing SR, Clark KL, Bowman MH, Frew RD, Hurd C (2007) Patterns in the  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  signature of *Ulva pertusa*: interaction between physical gradients and nutrient source pools. *Limnol Oceanogr* 52: 820–832
- Deuser WG, Degens ET, Guillard RRL (1968) Carbon isotope relationships between plankton and seawater. *Geochim Cosmochim Acta* 32:657–660
- Evans RD (2001) Physiological mechanisms influencing plant nitrogen isotope composition. *Trends Plant Sci* 6: 121–126

- Fairhead VA, Cheshire AC (2004) Rates of primary productivity and growth in *Ecklonia radiata* measured at different depths, over an annual cycle, at West Island, South Australia. *Mar Biol* 145:41–50
- Farquhar GD, Ehleringer JR, Hubick KT (1989) Carbon isotope discrimination and photosynthesis. *Annu Rev Plant Physiol Plant Mol Biol* 40:503–537
- Fenton GE, Ritz DA (1989) Spatial variability of  $^{13}\text{C}$ : $^{12}\text{C}$  and D:H in *Ecklonia radiata* (C.Ag) J. Agardh (Laminariales). *Estuar Coast Shelf Sci* 28:95–101
- Fiorini S, Gattuso JP, van Rijswijk P, Middleburg J (2010) Coccolithophores lipid and carbon isotope composition and their variability related to changes in seawater carbonate chemistry. *J Exp Mar Biol Ecol* 394:74–85
- Fourqurean JW, Escorcia SP, Anderson WT, Zieman JC (2005) Spatial and seasonal variability in elemental content,  $\delta^{13}\text{C}$ , and  $\delta^{15}\text{N}$  of *Thalassia testudinum* from South Florida and its implications for ecosystem studies. *Estuaries* 28:447–461
- Gerard VA (1982) In situ rates of nitrate uptake by giant kelp, *Macrocystis pyrifera* (L.) C. Agardh: tissue differences, environmental effects, and predictions of nitrogen-limited growth. *J Exp Mar Biol Ecol* 62:211–224
- Graham MH, Edwards MS (2001) Statistical significance versus fit: estimating the importance of individual factors in ecological analysis of variance. *Oikos* 93:505–513
- Greenwood JE, Symonds G, Zhong L, Lourey M (2013) Evidence of submarine groundwater nutrient supply to an oligotrophic barrier reef. *Limnol Oceanogr* 58:1834–1842
- Grice AM, Loneragan NR, Dennison WC (1996) Light intensity and the interactions between physiology, morphology and stable isotope ratios in five species of seagrass. *J Exp Mar Biol Ecol* 195:91–110
- Guest MA, Hirst AJ, Nichols PD, Frusher SD (2010) Multi-scale spatial variation in stable isotope and fatty acid profiles amongst temperate reef species: implications for design and interpretation of trophic studies. *Mar Ecol Prog Ser* 410:25–41
- Hu X, Burdige DJ, Zimmerman RC (2012)  $\delta^{13}\text{C}$  is a signature of light availability and photosynthesis in seagrass. *Limnol Oceanogr* 57:441–448
- Hurd CL, Hepburn CD, Currie KI, Raven JA, Hunter KA (2009) Testing the effects of ocean acidification on algal metabolism: considerations for experimental designs. *J Phycol* 45:1236–1251
- Hyndes GA, Hanson CE, Vanderklift MA (2013) The magnitude of spatial and temporal variation in  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  differs between taxonomic groups: implications for food web studies. *Estuar Coast Shelf Sci* 119:176–187
- Jackson GA (1977) Nutrients and production of giant kelp, *Macrocystis pyrifera*, off southern California. *Limnol Oceanogr* 22:979–995
- Kirk JTO (2011) Light and photosynthesis in aquatic ecosystems. Cambridge University Press, Cambridge
- Kirkman H (1984) Standing stock and production of *Ecklonia radiata* (C.Ag.): J. Agardh. *J Exp Mar Biol Ecol* 76:119–130
- Korb RE, Raven JA, Johnston AM, Leftley JW (1996) Effects of cell size and specific growth rate on stable carbon isotope discrimination by two species of marine diatom. *Mar Ecol Prog Ser* 143:283–288
- Loader NJ, McCarroll D, van der Knaap WO, Robertson I, Gagen M (2007) Characterizing carbon isotopic variability in *Sphagnum*. *Holocene* 17:403–410
- Long MH, Rheuban JE, Berg P, Zieman JC (2012) A comparison and correction of light intensity loggers to photosynthetically active radiation sensors. *Limnol Oceanogr Methods* 10:416–424
- Lourey MJ, Dunn JR, Waring J (2006) A mixed-layer nutrient climatology of Leeuwin Current and Western Australian shelf waters: seasonal nutrient dynamics and biomass. *J Mar Syst* 59:25–51
- Mann EH, Kirkman H (1981) Biomass method for measuring productivity of *Ecklonia radiata*, with the potential for adaptation to other large brown algae. *Aust J Mar Freshw Res* 32:297–304
- Melville AJ, Connolly RM (2003) Spatial analysis of stable isotope data to determine primary sources of nutrition for fish. *Oecologia* 136:499–507
- Mook WG (2001) Introduction: theory, methods, review. In: Mook WG (ed) Environmental isotopes in the hydrological cycle: principles and applications. International Hydrological Programme, Technical Documents in Hydrology, No. 39, Vol 1. International Atomic Energy Agency and United Nations Educational, Scientific and Cultural Organization, Paris
- Moore JW, Semmens BX (2008) Incorporating uncertainty and prior information into stable isotope mixing models. *Ecol Lett* 11:470–480
- Newsome SD, Martinez del Rio C, Bearhop S, Phillips DL (2007) A niche for isotopic ecology. *Front Ecol Environ* 5:429–436
- Newsome SD, Tinker MT, Monson DH, Oftedal OT and others (2009) Using stable isotopes to investigate individual diet specialization in California sea otters (*Enhydra lutris nereis*). *Ecology* 90:961–974
- Parnell AC, Inger R, Bearhop S, Jackson AL (2010) Source partitioning using stable isotopes: coping with too much variation. *PLoS ONE* 5:e9672
- Peterson BJ, Fry B (1987) Stable isotopes in ecosystem studies. *Annu Rev Ecol Syst* 18:293–320
- Phillips DL (2001) Uncertainty in source partitioning using stable isotopes. *Oecologia* 127:171–179
- Phillips JC, Kendrick GA, Lavery PS (1997) A test of a functional group approach to detecting shifts in macroalgal communities along a disturbance gradient. *Mar Ecol Prog Ser* 153:125–138
- Quinn GP, Keough MJ (2002) Experimental design and data analysis for biologists. Cambridge University Press, Cambridge
- Rasmussen JB (2010) Estimating terrestrial contribution to stream invertebrates and periphyton using a gradient-based mixing model for  $\delta^{13}\text{C}$ . *J Anim Ecol* 79:393–402
- Raven JA, Walker DI, Johnston AM, Handley LL, Kübler JE (1995) Implications of  $^{13}\text{C}$  natural abundance measurements for photosynthetic performance by marine macrophytes in their natural environment. *Mar Ecol Prog Ser* 123:193–205
- Raven JA, Johnston AM, Kübler JE, Korb R and others (2002) Mechanistic interpretation of carbon isotope discrimination by marine macroalgae and seagrasses. *Funct Plant Biol* 29:355–378
- Raven J, Caldeira K, Elderfield H, Hoegh-Guldberg O and others (2005) Ocean acidification due to increasing atmospheric carbon dioxide. The Royal Society, London
- Raven JA, Giordano M, Beardall J, Maberly SC (2011) Algal and aquatic plant carbon concentrating mechanisms in relation to environmental change. *Photosynth Res* 109:281–296

- Robinson D (2001)  $\delta^{15}\text{N}$  as an integrator of the nitrogen cycle. *Trends Ecol Evol* 16:153–162
- Rubenstein DR, Hobson KA (2004) From birds to butterflies: animal movement patterns and stable isotopes. *Trends Ecol Evol* 19:256–263
- Schmitz K, Lobban CS (1976) A survey of translocation in Laminariales (Phaeophyceae). *Mar Biol* 36:207–216
- Searle DJ, Semeniuk V (1985) The natural sectors of the inner Rottneest Shelf coast adjoining the Swan Coastal Plain. *J R Soc West Aust* 67:116–136
- Sigman DM, Karsh KL, Casciotti KL (2009) Nitrogen isotopes in the ocean. In: Steele JH, Turekian KK, Thorpe SA (eds) *Encyclopedia of ocean sciences*. Academic Press, London, p 40–54
- Smale DA, Wernberg T (2009) Satellite-derived SST data as a proxy for water temperature in nearshore benthic ecology. *Mar Ecol Prog Ser* 387:27–37
- Tanaka Y, Miyajima T, Yamada K, Hori M, Hasegawa N, Umezawa Y, Koike I (2008) Specific growth rate as a determinant of the carbon isotope composition of the temperate seagrass *Zostera marina*. *Aquat Bot* 89:331–336
- Vanderklift MA, Wernberg T (2010) Stable isotopes reveal a consistent consumer–diet relationship across hundreds of kilometres. *Mar Ecol Prog Ser* 403:53–61
- Vanderklift MA, Lavery PS, Waddington KI (2009) Intensity of herbivory on kelp by fish and sea urchins differs between inshore and offshore reefs. *Mar Ecol Prog Ser* 376:203–211
- Wernberg T, Kendrick GA, Phillips JC (2003) Regional differences in kelp-associated algal assemblages on temperate limestone reefs in south-western Australia. *Divers Distrib* 9:427–441
- West JB, Bowen GJ, Cerling TE, Ehleringer JR (2006) Stable isotopes as one of nature's ecological recorders. *Trends Ecol Evol* 21:408–414
- Westlake DF (1965) Theoretical aspects of the comparability of productivity data. In: Goldman CR (ed) *Proceedings of the IBP symposium on primary productivity in aquatic environments*. Mem Ist Ital Idrobiol 18:313–322
- Zeebe RE, Wolf-Gladrow D (2001) CO in seawater: equilibrium, kinetics, isotopes. In: Halpern D (ed) *Elsevier oceanography series 65*. Elsevier, Amsterdam

### Appendix.

Table A1. Depth (m), number of *Ecklonia radiata* analysed (n), average  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  of the 1st and 7th lateral, average temperature (Temp, °C) and light (lx h<sup>-1</sup>) at each of the sites surveyed in each of the seasons. Standard deviations are provided in parentheses. nd = no data

Site	Depth (m)	n	Summer (Dec 2008–Jan 2009)						Winter (May–June 2009)						Spring (Sept–Oct 2009)							
			1st $\delta^{13}\text{C}$	7th $\delta^{13}\text{C}$	1st $\delta^{15}\text{N}$	7th $\delta^{15}\text{N}$	Temp (°C)	Light (lx h <sup>-1</sup> )	n	1st $\delta^{13}\text{C}$	7th $\delta^{13}\text{C}$	1st $\delta^{15}\text{N}$	7th $\delta^{15}\text{N}$	Temp (°C)	Light (lx h <sup>-1</sup> )	n	1st $\delta^{13}\text{C}$	7th $\delta^{13}\text{C}$	1st $\delta^{15}\text{N}$	7th $\delta^{15}\text{N}$	Temp (°C)	Light (lx h <sup>-1</sup> )
M	11.3	5	-20.7 (0.52)	-20.3 (1.07)	6.4 (0.22)	5.3 (0.44)	21.7	71892	4	-23.0 (2.10)	-20.5 (1.24)	6.3 (0.35)	5.0 (0.40)	20.2	4616	5	-20.1 (1.03)	-21.5 (1.82)	6.2 (0.16)	5.0 (0.39)	17.4	13274
J	12.5	5	-18.1 (0.69)	-18.0 (1.03)	5.6 (0.26)	4.9 (0.50)	21.6	20475	5	-21.3 (1.00)	-19.7 (1.04)	6.5 (0.26)	5.1 (0.24)	19.9	4226	5	-21.2 (0.67)	-22.4 (1.27)	5.0 (0.25)	4.3 (0.43)	17.4	9940
G	17.3	5	-22.0 (0.92)	-21.3 (1.28)	6.2 (0.26)	5.4 (0.25)	21.7	31541	5	-21.7 (0.72)	-18.7 (0.60)	6.2 (0.59)	4.9 (0.33)	20.4	9987	5	-22.6 (0.71)	-22.2 (1.88)	4.5 (0.37)	4.1 (0.44)	17.5	16554
D	15.1	5	-22.5 (0.97)	-21.2 (0.83)	6.2 (0.28)	5.5 (0.21)	21.7	31541	5	-23.2 (0.93)	-20.9 (0.64)	6.1 (0.56)	5.0 (0.37)	20.4	4421	5	-23.0 (0.53)	-23.4 (0.71)	6.1 (0.24)	5.4 (0.18)	17.6	20473
A	14.7	5	-20.9 (0.77)	-19.0 (0.36)	5.7 (0.26)	5.2 (0.33)	21.7	1385	5	-23.0 (1.66)	-19.0 (1.28)	6.3 (0.30)	4.8 (0.38)	20.4	4421	5	-22.9 (0.65)	-23.8 (0.96)	5.7 (0.33)	5.1 (0.45)	17.6	13099
B	5.3	5	-22.1 (0.87)	-21.5 (1.29)	6.0 (0.36)	5.2 (0.39)	22.5	81667	5	-20.6 (1.35)	-21.4 (0.85)	5.9 (0.12)	5.2 (0.20)	nd	nd	5	-21.0 (0.49)	-19.3 (1.15)	6.3 (0.15)	5.5 (0.32)	17.8	18378
N	10.1	5	-18.2 (1.02)	-16.9 (0.26)	7.3 (0.07)	6.9 (0.22)	22.1	109512	5	-21.1 (1.59)	-17.7 (1.21)	8.4 (0.32)	6.5 (0.45)	19.4	17743	5	-19.5 (1.30)	-20.4 (1.33)	8.1 (0.20)	7.1 (0.35)	17.3	17926
K	2.5	5	-20.7 (0.88)	-19.1 (0.60)	6.8 (0.32)	6.4 (0.29)	22.7	158885	5	-18.5 (0.69)	-17.8 (0.57)	6.4 (0.27)	5.6 (0.30)	nd	nd	5	-19.8 (1.14)	-18.7 (0.75)	7.5 (0.37)	6.7 (0.54)	17.3	97389
LE	5.4	5	-20.8 (0.40)	-19.2 (0.75)	7.1 (0.24)	6.7 (0.19)	22.7	158885	5	-19.7 (2.34)	-18.9 (1.07)	6.5 (0.54)	5.6 (0.41)	nd	nd	5	-19.3 (0.74)	-18.1 (0.49)	7.5 (0.07)	7.1 (0.44)	17.8	112086
RD	7.1	5	-17.5 (0.93)	-17.4 (0.96)	7.4 (0.23)	7.0 (0.12)	22.6	109841	5	-19.4 (1.30)	-16.6 (1.01)	8.1 (0.41)	6.9 (0.47)	nd	nd	5	-18.5 (1.00)	-18.4 (0.89)	9.1 (0.33)	7.9 (1.06)	17.7	72143
H	9.7	5	-17.3 (1.21)	-18.2 (0.70)	7.6 (0.18)	7.2 (0.30)	22.2	158885	5	-21.0 (1.90)	-20.1 (1.44)	6.4 (0.52)	5.2 (0.27)	19.0	52137	5	-22.1 (0.84)	-20.5 (1.13)	7.1 (0.20)	6.8 (0.62)	17.4	19156
E	8.7	5	-21.2 (0.68)	-20.7 (1.23)	6.2 (0.22)	5.4 (0.28)	22.6	2611	0					nd	nd	5	-21.3 (0.51)	-22.4 (0.96)	6.0 (0.28)	5.3 (0.59)	17.7	74407
T	5.3	5	-17.6 (0.95)	-17.2 (0.44)	6.1 (0.09)	5.4 (0.18)	22.6	187660	5	-19.7 (1.02)	-18.3 (1.05)	5.5 (0.33)	4.9 (0.45)	nd	nd	5	-19.4 (1.06)	-19.6 (2.58)	5.6 (0.37)	5.1 (0.60)	17.6	78553
SR1	3.7	5	-19.3 (0.91)	-18.2 (0.79)	8.1 (0.27)	7.5 (0.31)	22.8	48502	5	-20.4 (1.07)	-19.3 (1.04)	7.5 (0.59)	7.2 (0.21)	19.2	30641	5	-20.2 (1.14)	-20.5 (2.51)	8.1 (0.42)	7.6 (0.49)	17.7	58494
SR2	3.9	5	-19.3 (0.93)	-17.5 (1.22)	7.9 (0.36)	7.4 (0.38)	22.9	210025	5	-19.5 (2.00)	-18.7 (2.17)	7.4 (0.42)	6.9 (0.36)	19.2	30641	5	-19.0 (0.86)	-18.1 (1.10)	8.3 (0.76)	7.7 (0.36)	17.7	78966
SR3	4.3	5	-20.1 (1.06)	-19.5 (0.53)	8.8 (0.60)	8.6 (0.55)	22.9	129263	1	-19.9	-19.4	9.6	7.6	19.2	nd	5	-19.2 (1.23)	-18.7 (0.78)	8.5 (0.72)	8.2 (0.80)	17.7	56821
W	4.3	5	-18.7 (0.61)	-18.3 (0.93)	7.5 (0.25)	7.2 (0.32)	23.0	215234	5	-17.9 (1.26)	-16.7 (0.96)	8.7 (0.30)	7.1 (0.47)	19.7	30012	5	-19.4 (1.10)	-19.3 (1.21)	8.4 (0.20)	8.0 (0.43)	17.6	28754
SL1	4.6	0					22.7	48444	5	-18.8 (0.88)	-18.0 (1.22)	8.8 (0.10)	6.7 (0.43)	19.6	93677	5	-18.8 (0.79)	-18.6 (1.03)	8.7 (0.25)	8.2 (0.38)	17.5	63202
SL2	4.9	5	-19.7 (1.07)	-19.1 (1.41)	7.3 (0.21)	7.1 (0.23)	22.9	224959	2	-18.3 (1.15)	-17.1 (1.63)	8.1 (0.30)	6.6 (0.32)	nd	nd	3	-20.4 (1.47)	-20.6 (1.45)	8.3 (0.42)	7.6 (0.56)	17.6	58368