Vol. 628: 55–71, 2019
https://doi.org/10.3354/meps13096

MARINE ECOLOGY PROGRESS SERIES
Mar Ecol Prog Ser

Published October 10

Variability in stable isotope values of South African Laminariales, *Ecklonia maxima* and *Laminaria pallida*, over different spatial and temporal scales

David C. Dyer[1,*], Mike J. Butler[2], Albertus J. Smit[3,4], Robert J. Anderson[1,5], John J. Bolton[1]

[1]Biological Sciences Department, University of Cape Town, Private Bag X3, Rondebosch, 7701, South Africa
[2]Environmental Isotope Laboratory, iThemba LABS, Private Bag 11, WITS, Johannesburg, 2050, South Africa
[3]Biodiversity and Conservation Biology Programme, University of the Western Cape, Private Bag X17, Bellville, 7535, South Africa
[4]South African Environmental Observation Network, Elwandle Coastal Node, Port Elizabeth, 6031, South Africa
[5]African Centre for Coastal Palaeoscience, Nelson Mandela University, PO Box 77000, Port Elizabeth, 6031, South Africa

ABSTRACT: Kelp forests are some of the most productive habitats in the oceans, supporting diverse, ecologically, and often commercially, important ecosystems. This study of 8 geographically separate sites in 2 seasons highlights the natural variability of stable isotope values ($\delta^{13}C$ and $\delta^{15}N$), at different spatial and temporal scales, within the 2 dominant kelp species along the west coast of South Africa. Additionally, stable isotope variability was investigated within and among different tissues within both kelp species. Within a kelp plant, $\delta^{13}C$ values had a variance of 1.7‰ for *Ecklonia maxima* and 1.5‰ for *Laminaria pallida*. The $\delta^{15}N$ values had a variance of 3.8‰ for *E. maxima* and 4.2‰ for *L. pallida*. There were also consistent variability patterns along the length of a single frond in both species, for both isotopes. Among the localities, *E. maxima* and *L. pallida* were highly variable with variances in $\delta^{13}C$ (9.4‰ and 11.2‰) and $\delta^{15}N$ (3.4‰ and 4.5‰) for the 2 species respectively. The $\delta^{13}C$ values of *L. pallida* and *E. maxima* displayed a clear pattern coinciding with depth, particularly for *L. pallida*. Within-site variability was a major contributor to the overall spatial variability for both species. This study provides further evidence for the importance of understanding basal variability of stable isotope values when determining the carbon sources of bottom-up controlled ecosystems.

KEY WORDS: $\delta^{13}C$ · $\delta^{15}N$ · Laminariales · South Africa

1. INTRODUCTION

Constructing food webs provides insight into the flow of energy within an ecosystem, particularly the routing of basal resources to higher-level consumers (Fry & Sherr 1989, Krumins et al. 2013). The analysis of food webs has been used to study structure and functioning of aquatic ecosystems globally, by providing an understanding of energy-flow pathways and the role of particular organisms within these habitats (Pimm 1982, Cabana & Rasmussen 1994, 1996, Vander Zanden & Rasmussen 1999). A key aspect of food web ecology is the characterisation of trophic levels (Vander Zanden et al. 1999, Post 2002a,b). The trophic level of constituent consumers can be used to assess the influence of natural or anthropogenic factors on the structure of food webs, such as those of coral reefs and kelp forests (Fredriksen 2003, Jack & Wing 2011). Therefore, accurate characterisation of the trophic interactions within an ecosystem is paramount to the understanding of its functioning (Pasquaud et al. 2010, Kadoya et al. 2012).

Stable isotope analysis, which has become an increasingly versatile tool for studies relating to trophic

*Corresponding author: dcdyer007@gmail.com

ecology, provides a more time-integrated depiction of complex interactions overlooked or under-represented by traditional methods (Peterson et al. 1985, Peterson & Fry 1987, Vinagre et al. 2008, Layman et al. 2012). Stable isotopes of carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) have been used to determine trophic sources and trophic interactions in many different ecosystems due to their reliable and predictable changes during trophic interactions (DeNiro & Epstein 1981, Fry 2006, Layman et al. 2012). Although largely lacking in studies from South Africa, stable isotope analysis has been successfully applied to numerous aspects of kelp forest ecology in other regions of the world (e.g. USA, Canada, Australia, New Zealand, Japan, France and Norway). These studies have focussed on various components of these systems, including, but not limited to, carbon acquisition by macroalgae (e.g. Raven et al. 1995, 2002, Raven & Giordano 2017), phytoplankton and primary production (reviewed by Miller & Page 2012, Ramshaw et al. 2017), the variability in macroalgal values (e.g. Stephenson et al. 1984, Simenstad et al. 1993, Dethier et al. 2013, Hyndes et al. 2013, Vanderklift & Bearham 2014, Mackey et al. 2015), producer–consumer relationships (e.g. Vanderklift & Ponsard 2003, Vanderklift et al. 2006, Vanderklift & Wernberg 2010, von Biela et al. 2016), and food web structure and functioning (e.g. Kaehler et al. 2000, 2006, Fredriksen 2003, Guest et al. 2010, Nadon & Himmelman 2010).

Traditionally, a key assumption of stable isotope studies was the consistency of primary producer stable isotope values over space and time, which allowed for traceability through the food web (Simenstad et al. 1993, Boon & Bunn 1994, Woodland et al. 2012, Dethier et al. 2013). For example, an estimate of the trophic position of consumers can be calculated relative to the nitrogen isotope value of the basal resource (i.e. primary producer) within the ecosystem (Post 2002a,b). However, variability in stable isotope values has been shown to operate at different spatial and temporal scales, both for basal resources as well as for consumers (Page et al. 2008, Guest et al. 2010, Hansen et al. 2012, Hyndes et al. 2013), and has been successfully used to trace animal migration (Hobson 1999) and construct isoscapes (West et al. 2009). The variability within marine macrophytes has been shown to be translated up the marine food web (Simenstad et al. 1993, O'Reilly et al. 2002, Vanderklift & Wernberg 2010, Hansen et al. 2012). However, the variability decreases toward apex consumers as the longer tissue-turnover rates in these organisms counter the short-term variability in lower-level organisms (Simenstad et al. 1993, Nordström et al. 2009, Hansen et al. 2012, Hyndes et al. 2013). Stable isotope values of basal resources, such as marine macrophytes and phytoplankton, are thus not consistent and hence can create erroneous conclusions when interpreting the flow of energy through the food web (Boon & Bunn 1994, Wing & Jack 2012, Dethier et al. 2013, Hyndes et al. 2013).

Kelp forest ecosystems provide an excellent case study for the importance of understanding basal resource variability. Kelps are brown macroalgae which form complex 3-dimensional habitats in near shore habitats in temperate and Arctic regions of the world (Steneck et al. 2002, Smale et al. 2013). Along the west coast of southern Africa, *Ecklonia maxima* (Osbeck) Papenfuss and *Laminaria pallida* Greville are the 2 most common and abundant kelp species, both forming dense and extensive kelp forests between Cape Agulhas (the southernmost point of Africa) and Rocky Point in northern Namibia (Field et al. 1980a, Field & Griffiths 1991, Bolton 2010). This area provides the optimal growing environment for kelps, as nutrient concentrations are high (due to coastal upwelling), light intensities are high, and there is continuous water movement (Andrews 1974, Field et al. 1980b). *E. maxima* is a large, canopy-forming species which extends to the surface in shallower areas (<9 m), whereas *L. pallida* is a smaller species, forming a sub-canopy subtidally (10 to 30 m) in the area south of Jacobsbaai (Fig. 1) but occurring with *E. maxima* in shallow inshore water northwards on the west coast (Stegenga et al. 1997, Rothman et al. 2017). Both species are typical aclonal macroalgae (*sensu* Santelices 2004), having fronds which originate from a single stipe that is attached to the substrate via a holdfast. The large sporophytes of both kelps are perennial and have fronds which constantly undergo erosion at the distal ends, matched by continuous growth in the meristematic regions (Dieckmann 1978, 1980, Mann et al. 1979).

The detrital fragments which are constantly dislodged and eroded from the distal tips of the kelp fronds enter the food web via the suspension-feeding organisms which inhabit these systems (Newell et al. 1982, Newell & Field 1983, Mann 1988, Stegenga et al. 1997). Phytoplankton is another important carbon source for these systems; however, concentrations are variable and depend greatly on the upwelling cycle (Carter 1982, Wulff & Field 1983, Fielding & Davis 1989). Together, these 2 sources represent the primary carbon sources for kelp forest food webs along the South African coastline. The relative importance of detritus and phytoplankton along the

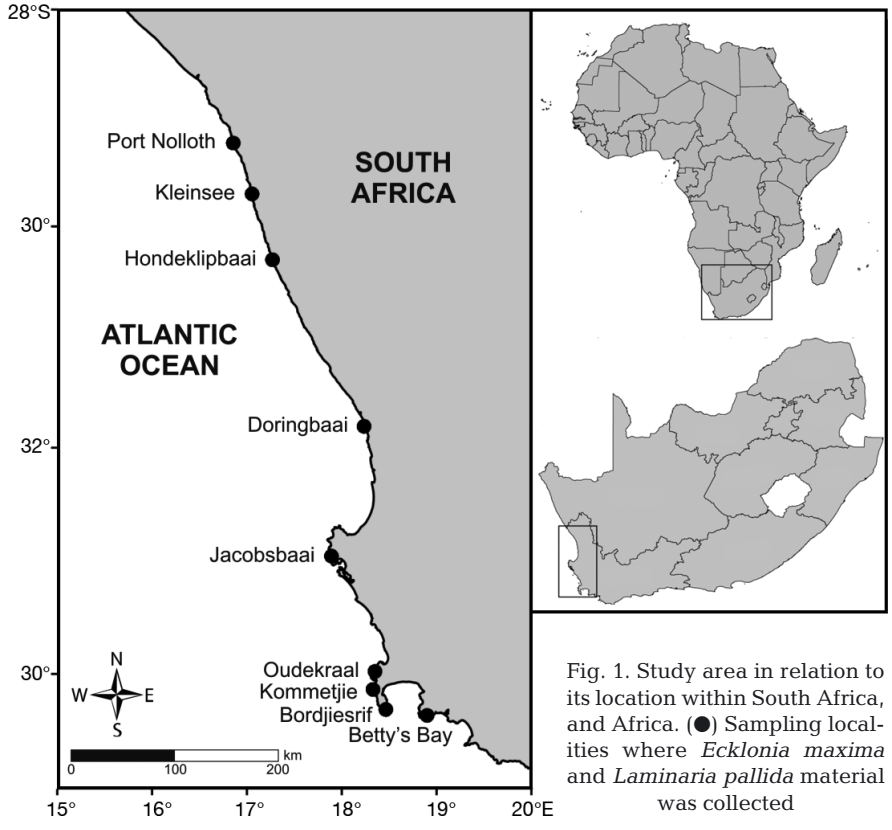

Fig. 1. Study area in relation to its location within South Africa, and Africa. (●) Sampling localities where *Ecklonia maxima* and *Laminaria pallida* material was collected

systems provide a bottom-up controlled trophic system, where changes in basal isotope values, and the associated variability, have the potential to be reflected in higher-level trophic organisms.

This study therefore aims to identify the variability in kelp (*Ecklonia maxima* and *Laminaria pallida*) stable isotope values in order to better understand the basal resource variation within these food webs. Specifically, the variability in stable isotope values ($\delta^{13}C$ and $\delta^{15}N$) was evaluated among the different tissues of kelp plants, the variability along the length of individual kelp fronds was investigated, and finally the temporal and spatial variability of a representative tissue (frond tip) was evaluated across geographic sampling localities and sampling occasions (seasons).

South African coast depends on the frequency of upwelling and on the rate of water movement in the kelp forest (Wulff & Field 1983, Mann 1988, Dyer et al. 2019).

Although stable isotope variability at the base of the food web is not likely to be observed at the same magnitude as in higher trophic organisms, there is evidence to suggest that $\delta^{13}C$ variability is translated to these organisms and can be localised to sites (Simenstad et al. 1993, O'Reilly et al. 2002). This is particularly evident in sessile or territorial organisms which utilise local primary production sources (Simenstad et al. 1993). As the faunal biomass of many South African kelp forests is dominated by sessile filter-feeders, such as mussels, sponges and ascidians (Field & Griffiths 1991), there is a strong possibility this variability will impact studies of kelp bed food webs. These organisms are the key link which couples the pelagic and benthic food webs together. Any variability in algal (or phytoplankton) stable isotope values will be translated through the food web to consumers, creating differences at each site. However, the magnitude and scale of this variability need to be determined. Nevertheless, kelp forest

2. MATERIALS AND METHODS

Two aspects of variability in kelp stable isotope values were examined: (1) inter-tissue variability (tissues and position on the frond) and (2) spatial and temporal variability.

Because the morphology and modes of frond development in *Laminaria* and *Ecklonia* are different (Fig. 2), we defined them as follows for the purpose of this study. *Laminaria* has a single flat frond with an undivided basal region (the lamina) that more distally splits into multiple blades. *Ecklonia* has a primary blade that gives rise along its margins to multiple secondary blades (Fig. 2).

2.1. Tissue comparisons

Nine whole kelp plants from each species (*Ecklonia maxima* and *Laminaria pallida*) were collected at Oudekraal, on the west coast of South Africa (Fig. 1), from a depth of 5 m by SCUBA divers. Each plant was split into distinct sections (holdfast, stipe, fronds) before transport back to the laboratory for further processing. Subsamples of the 4 primary tissues (holdfast,

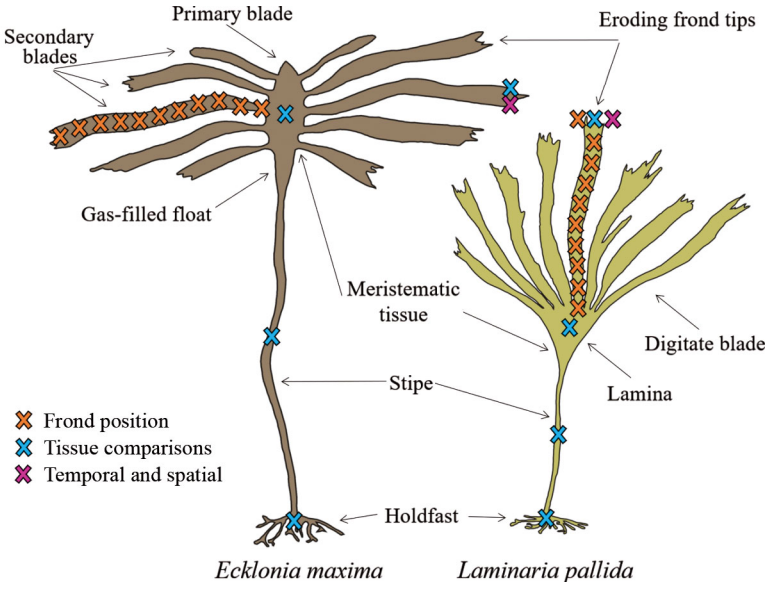

Fig. 2. Location of sample collection points on kelps *Ecklonia maxima* (left) and *Laminaria pallida* (right) for frond position, tissue comparisons and geographic sampling

stipe, primary blade [*Ecklonia*], lamina [*Laminaria*], and frond) were collected from each plant after thorough washing with distilled water. Tissue samples were collected from the same location on each kelp plant (Fig. 2). However, as *L. pallida* does not have a primary blade, this sample was collected in the centre of the main blade near the stipe (Fig. 2).

2.2. Frond position

Three whole fronds (primary blade and secondary blades for *Ecklonia*, lamina and blades for *Laminaria*) were collected from each kelp species (*E. maxima* and *L. pallida*) at Oudekraal from a depth of 5 m by SCUBA divers. A single frond was collected from a single plant; thus, each frond represents an individual in the population. Three fronds were used for each species of kelp, representing 3 individuals. The longest frond on kelp plants of a similar size was collected in order to rule out size-related bias. In *E. maxima*, this represents the longest secondary blade on the plant, whereas for *L. pallida*, this was the longest extension of the split digitate frond (lamina and longest blade). Fronds were kept whole and transported back to the laboratory for further processing. Each frond was thoroughly cleaned with distilled water, and the total length was measured. Subsamples were collected at pre-determined positions along the frond, with each position coinciding with 10% intervals of the total length (Fig. 2). At each position, a strip of frond was excised across the width of the frond. Therefore, frond position refers to these different positions along the frond of both *E. maxima* and *L. pallida* as defined above.

2.3. Spatial and temporal sampling

Ten whole kelp blades were collected from both *E. maxima* and *L. pallida* plants at 8 sites from Port Nolloth on the west coast to Betty's Bay, east of False Bay (see Fig. 1). Samples were collected at Port Nolloth, Kleinsee, Hondeklipbaai, Doringbaai, Jacobsbaai, Kommetjie, Bordjiesrif and Betty's Bay during both the austral summer and winter in 2015/16. Because the depth distribution of *L. pallida* changes moving northwards along the coast, it was not possible to keep the sampling depth constant at all sites. Therefore, at some sites (Port Nolloth, Kleinsee, Hondeklipbaai, Doringbaai and Jacobsbaai), both *E. maxima* and *L. pallida* samples were collected from plants at the surface in shallow (2–3 m) water. At the other sites, *E. maxima* samples were collected in the same position, but *L. pallida* samples were collected from deeper (5–8 m) water as they only occur there.

From each kelp frond, a portion of tissue was excised closest to the blade tip (Fig. 2) and frozen prior to laboratory processing. Once back at the laboratory, each sample was thawed and washed with distilled water.

2.4. Laboratory processing and analysis

All kelp tissue samples were dried in an air-circulated oven (60°C) for a period of 48 h. Once dried, samples were homogenized into a fine powder using a Retsch MM200 ball-mill. Powdered samples were then individually weighed out into tin capsules. Each capsule contained 1.2 mg of sample material, as specified by the analysis facility.

Stable isotope samples were analysed at iThemba LABS (Johannesburg) on a Flash HT Plus elemental analyser coupled to a Delta V Advantage isotope ratio mass spectrometer using a ConFlo IV interface (all equipment supplied by ThermoFisher).

Isotope values were expressed as the parts per mille deviation from the standard in delta (δ) notation according to:

$$\delta X = [(R_{sample}/R_{standard}) - 1] \times 1000 \quad (1)$$

where X is ^{13}C or ^{15}N, and R is the corresponding ratio of $^{13}C/^{12}C$ or $^{15}N/^{14}N$.

Carbon and nitrogen isotope values were corrected against an in-house standard (Merck Gel) as well as the Urea Working Standard (IVA Analysentechnik). Laboratory standards and blanks were run after every 20 samples. The overall precision of Merck Gel for nitrogen isotopes was 0.13‰, and 0.06‰ for carbon isotope measurements. For the urea standard, a precision of 0.12‰ was measured for carbon isotope ratios and a precision of 0.22‰ for nitrogen isotope standards. In-house standards were calibrated against National Institute of Standards and Technology (NIST) standard reference materials (1577, 2976 and 1547) and ultimately referenced against Vienna Pee Dee Belemnite (VPDB) and Air for $\delta^{13}C$ and $\delta^{15}N$, respectively.

2.5. Statistical analyses

Despite attempts at transforming the biomarker data ($\delta^{15}N$, $\delta^{13}C$, C:N ratio, C content and N content), it was not possible to satisfy the assumptions of normality and homoscedasticity of the analysis of variance (ANOVA) test. This was especially true for the stable isotope ($\delta^{15}N$ and $\delta^{13}C$) data. It was therefore decided to implement a permutational multivariate analysis of variance (PERMANOVA; Anderson 2001) to test for differences in biomarkers among the different kelp tissues for each species as well as to investigate differences among sites and between seasons for each of the kelp species. PERMANOVA tests are not constrained by the distribution of the data and are very robust to heterogeneity of multivariate dispersions, provided the study design is balanced (Anderson & Walsh 2013, Anderson 2017). These models return a Pseudo-F statistic which can be interpreted in the same way as the F statistic of a regular ANOVA. As part of the PERMANOVA test, variance components could also be determined, with R^2 values providing an estimate of the proportion of variance explained by each factor. Variance component tests provide estimates of the contribution of each factor to the variance observed in the response variable (Graham & Edwards 2001).

Untransformed biomarker data ($\delta^{15}N$, $\delta^{13}C$, C:N ratio, N content and C content) were used to construct a similarity matrix for each variable based on Euclidean distances. Tissue differences were analysed with tissue and species as factors using a 2-way PERMANOVA test ($n_{permutations}$ = 9999). Post hoc comparisons were made using pairwise comparison tests.

The frond position data was not analysed statistically, primarily because the patterns along the frond were not linear but also because sample sizes were too low to warrant meaningful analyses.

For the spatial and temporal data, the primary objective was to determine whether there is spatial and temporal variability in the biomarker data of the 2 kelp species. Therefore, a 2-way PERMANOVA test ($n_{permutations}$ = 9999) was conducted for each of the 5 biomarkers separately, with Site and Season being the main effects and the interaction term of Site × Season. As differences among sites were of particular interest, data were then separated by species and season, and post hoc pairwise comparisons were made among sites ($n_{permutations}$ = 9999) for each variable. Sites were then allocated to groups using the outcome of the pairwise tests, and groupings were displayed in the figures.

All statistical analyses were performed within R v. 3.5.2 (R Core Team 2018), with PERMANOVA analyses performed using the vegan package (Oksanen et al. 2019) and pairwise comparisons conducted with the pairwiseAdonis package (Martinez Arbizu 2019).

3. RESULTS

3.1. Tissues

The $\delta^{15}N$ value was significantly different ($p < 0.05$) among the different tissues (Pseudo-$F_{3,64}$ = 43.14, $p < 0.001$) and between the different kelp species (Pseudo-$F_{1,64}$ = 52.45, $p < 0.001$). However, the interaction of Tissue × Species was also significant (Pseudo-$F_{3,64}$ = 7.94, $p < 0.001$), indicating that the differences were not consistent for each species. The majority of the variability in $\delta^{15}N$ values was accounted for by tissue differences (48%), with species-level differences accounting for 19%. Post hoc tests revealed that holdfast values, for both species, were different from the other tissue types. Frond, stipe and primary blade values of *Laminaria pallida* were not different. In contrast, *Ecklonia maxima* frond values were the same as those of *L. pallida* but differed from *E. maxima* stipe and primary blade values.

The $\delta^{13}C$ values were significantly different among tissues (Pseudo-$F_{3,64}$ = 3.76, $p < 0.05$), but this only accounted for 10% of the variance. Significant differences were also identified between species (Pseudo-$F_{1,64}$ = 34.52, $p < 0.001$), which accounted for 31% of the variance in the data. The interaction term was not significant and was removed from the model. Most of the variance (59%) was thus explained by within-species measurements. Post hoc comparisons showed

that within the 2 species, all 4 tissues were the same in terms of their $\delta^{13}C$ values. However, between species, only the stipe values were the same, with the other 3 tissues being different from each other.

The $\delta^{13}C$ values of *E. maxima* and *L. pallida* varied (difference between minimum and maximum) by 1.7‰ and 1.5‰, respectively, among the 4 different tissues. The $\delta^{15}N$ values were more variable for both species across tissues: 3.8‰ and 4.2‰ for *E. maxima* and *L. pallida*, respectively (Table 1).

The C:N ratios were significantly different among the 4 tissues (Pseudo-$F_{3,64}$ = 37.28, $p < 0.001$), with among-tissue differences accounting for the majority (57%) of the variance in the data. Although there were significant differences identified between species (Pseudo-$F_{1,64}$ = 4.76, $p < 0.05$), these only accounted for 2% of the variance in the data. Similarly, the interaction term was significant (Pseudo-$F_{3,64}$ = 4.65, $p < 0.01$) but accounted for very little of the variance (7%). Thus, within-species variance accounted for 33% of the total variance in C:N ratio measurements. Post hoc testing showed that stipe values were the most different for both species, but frond and primary blade values were the same for both species.

3.2. Frond position

Consistent patterns emerged between frond position and both stable isotopes, $\delta^{13}C$ and $\delta^{15}N$, as well for the C:N ratio for both *E. maxima* and *L. pallida* (Fig. 3). Trends do not conform to linear patterns and thus were not analysed using correlation or regression methods. However, the consistency of the results across 3 replicate kelps, as evidenced by the error bars, indicates clear and consistent trends.

Table 1. PERMANOVA analysis of stable isotope ($\delta^{13}C$ and $\delta^{15}N$) values and C:N ratio of the 4 selected tissues from *Ecklonia maxima* and *Laminaria pallida*. MS: mean square. p-values are all significant (**bold**) at $p < 0.05$. Proportion of variance explained as indicated by magnitude of effect (R^2), with the largest value for each marker in *italics*

Source	df	MS	Pseudo-*F*	p	R^2
$\delta^{15}N$					
Tissue	3	48.41	43.14	**<0.001**	*0.48*
Species	1	58.86	52.45	**<0.001**	0.19
Tissue × Species	3	8.91	7.94	**<0.001**	0.09
Residual	64	1.12			0.24
$\delta^{13}C$					
Tissue	3	8.10	3.76	**<0.05**	0.10
Species	1	74.26	34.52	**<0.001**	0.31
Residual	67	2.15			*0.59*
C:N					
Tissue	3	372.07	37.28	**<0.001**	*0.57*
Species	1	47.50	4.76	**<0.05**	0.02
Tissue × Species	3	46.36	4.65	**<0.01**	0.07
Residual	64	9.98			0.33

The $\delta^{13}C$ value for both species became enriched with distance from the meristematic tissue at the base of the frond until about mid-way along the frond where it began to show depletion again toward the frond tip. The $\delta^{15}N$ values exhibit a similar trend for *E. maxima* with the frond tip being more depleted than the meristematic region at the base. The trend in *L. pallida* was less clear, with more variability in the data. The C:N ratio of both species was very consistent across the 3 replicates. For *E. maxima*, the C:N ratio dropped by almost 15 units along the length of the frond. However, *L. pallida* exhibited a different trend, with the C:N ratio rising towards the middle of the frond and then dropping steeply toward the tip, with the meristem and frond tip having similar values. The trends in the C:N ratio of *E. maxima* and *L. pallida* are better interpreted when viewed with the carbon and nitrogen content of each sampling point. Carbon and nitrogen content both increased along the *E. maxima* fronds, whereas an inverse relationship occurred in the *L. pallida* fronds.

The variance of $\delta^{13}C$ values within a single frond, from meristem to tip, was 2.6‰ for *E. maxima* and 3.1‰ for *L. pallida*. The $\delta^{15}N$ values showed a similar variance in both species, 2.9‰ for *E. maxima* and 2.7‰ for *L. pallida*.

3.3. Spatial and temporal variability

The $\delta^{15}N$ values of *E. maxima* (Pseudo-$F_{7,144}$ = 51.56, $p < 0.001$) and *L. pallida* (Pseudo-$F_{7,144}$ = 63.66, $p < 0.001$) were significantly different among the sampling sites (Table 2). The differences among sites accounted for the most variability in $\delta^{15}N$ values of both *E. maxima* (51%) and *L. pallida* (55%). The $\delta^{15}N$ values were significantly different between sampling occasions for both species; however, sampling occasion (season) accounted for very little of the variability observed (Table 2). The interaction of site and season was significant for both *E. maxima* and *L. pallida* and accounted for ~25% of the variability in $\delta^{15}N$ values (Table 2). This indicates that the differences among sites were not consistent in the 2 sampling occasions. Within-population variability (residuals) accounted for 20% of the $\delta^{15}N$ variability of *E. maxima* and 18% in *L. pallida* (Table 2). The summer $\delta^{15}N$ values were most enriched at Jacobsbaai (7.7‰) and Betty's Bay (6.1‰) for *L. pallida* and *E. maxima*,

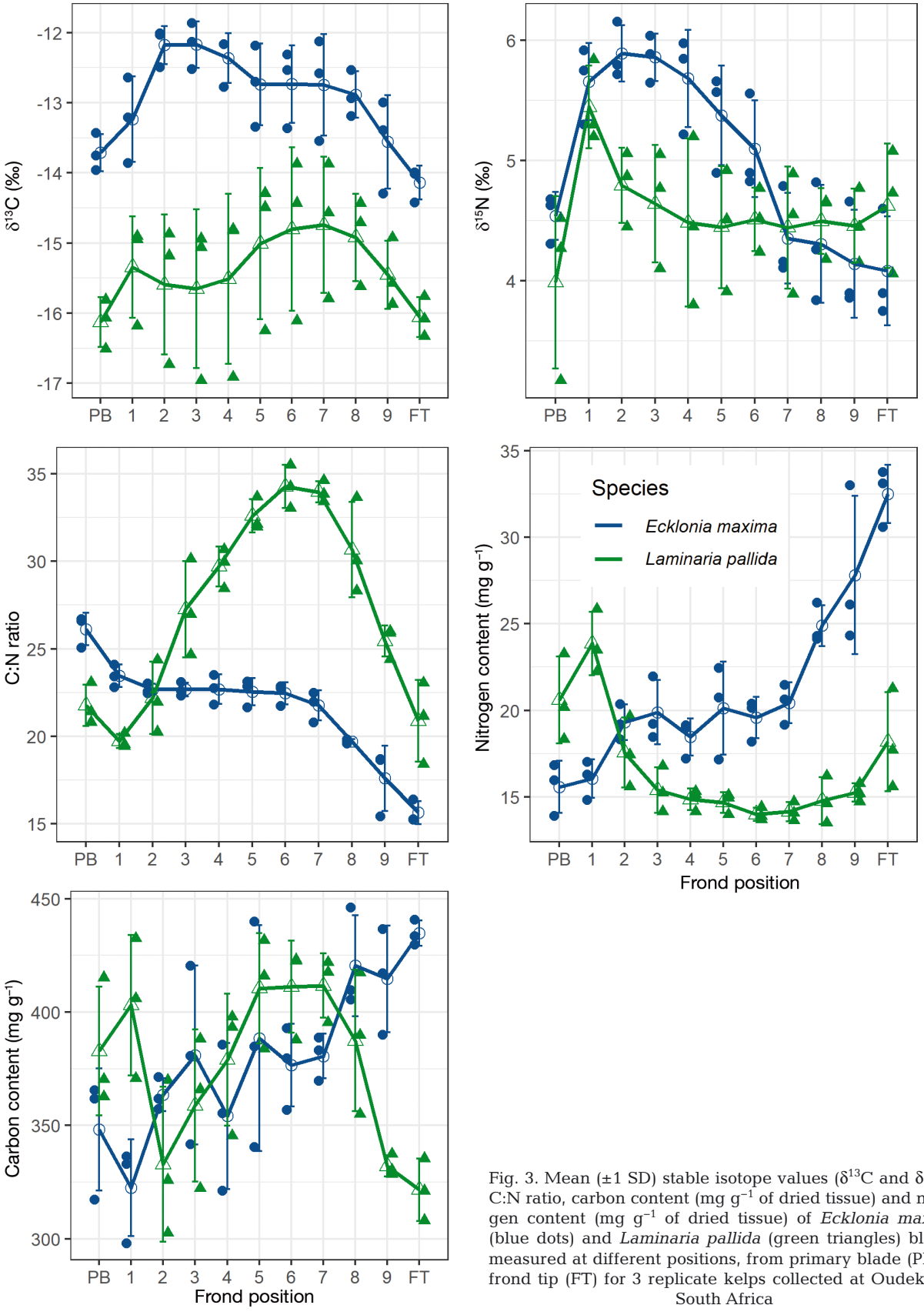

Fig. 3. Mean (±1 SD) stable isotope values ($\delta^{13}C$ and $\delta^{15}N$), C:N ratio, carbon content (mg g^{-1} of dried tissue) and nitrogen content (mg g^{-1} of dried tissue) of *Ecklonia maxima* (blue dots) and *Laminaria pallida* (green triangles) blades measured at different positions, from primary blade (PB) to frond tip (FT) for 3 replicate kelps collected at Oudekraal, South Africa

Table 2. Results of the PERMANOVA for *Ecklonia maxima* and *Laminaria pallida* for the 2 stable isotope values, C:N ratio and carbon and nitrogen content (%), testing for differences among sites and season. p-values are all significant (**bold**) at $p < 0.05$. Proportion of variance explained as indicated by magnitude of effects (R^2), with the largest value for each marker in *italics*

Source	df	*Ecklonia maxima*				*Laminaria pallida*			
		MS	Pseudo-*F*	p	R^2	MS	Pseudo-*F*	p	R^2
$\delta^{15}N$									
Site	7	20.27	51.56	**<0.001**	*0.51*	18.94	63.66	**<0.001**	*0.55*
Season	1	8.87	22.56	**<0.001**	0.03	4.58	15.38	**<0.001**	0.02
Site × Season	7	9.84	25.03	**<0.001**	0.25	8.89	29.87	**<0.001**	0.26
Residual	144	0.39			0.20	0.30			0.18
$\delta^{13}C$									
Site	7	12.28	4.62	**<0.01**	0.13	93.27	26.62	**<0.001**	*0.49*
Season	1	13.52	5.09	**<0.05**	0.02	18.38	5.25	**<0.05**	0.01
Site × Season	7	29.01	10.92	**<0.001**	0.30	23.65	6.75	**<0.001**	0.12
Residual	144	2.66			*0.56*	3.50			0.38
C:N									
Site	7	43.07	16.21	**<0.001**	0.34	220.51	39.31	**<0.001**	*0.61*
Season	1	49.20	18.52	**<0.001**	0.06	54.67	9.75	**<0.05**	0.02
Site × Season	7	21.72	8.18	**<0.001**	0.17	16.06	2.86	**<0.05**	0.04
Residual	144	2.66			*0.43*	5.61			0.32
%C									
Site	7	5922.5	23.26	**<0.001**	*0.35*	10764.30	25.45	**<0.001**	*0.34*
Season	1	4410	17.32	**<0.001**	0.04	29138.40	68.89	**<0.001**	0.13
Site × Season	7	5315.4	20.88	**<0.001**	0.31	7579.90	17.92	**<0.001**	0.24
Residual	144	254.6			0.31	423.00			0.28
%N									
Site	7	72.98	20.09	**<0.001**	0.34	110.51	44.60	**<0.001**	*0.57*
Season	1	128.88	35.48	**<0.001**	0.09	25.04	10.11	**<0.05**	0.02
Site × Season	7	48.00	13.21	**<0.001**	0.22	28.81	11.63	**<0.001**	0.15
Residual	144	3.63			*0.35*	2.48			0.26

respectively, and most depleted at Port Nolloth (3.2‰) and Kleinsee (2.6‰) for *L. pallida* and *E. maxima*, respectively. In winter, $\delta^{15}N$ was most enriched at Betty's Bay (7.4‰) for *L. pallida* and Kommetjie (7.6‰) for *E. maxima*. The $\delta^{15}N$ values were most depleted at Doringbaai for both *L. pallida* (0.9‰) and *E. maxima* (0.2‰). Post hoc comparisons indicated that the $\delta^{15}N$ values in summer could be generally grouped into 2 groups, with northern sites (Port Nolloth to Jacobsbaai) more similar to each other, and southern sites (Jacobsbaai to Betty's Bay) forming another group. There was some overlap among groups (see Fig. 4); however, the trend was similar for both species. The pattern during winter was substantially different, with Doringbaai being the most different for both species. However, the comparisons indicated that at all sites except Jacobsbaai and Kommetjie, the groupings for *E. maxima* and *L. pallida* were similar (see Fig. 5).

The $\delta^{13}C$ values of *E. maxima* (Pseudo-$F_{7,144} = 4.62$, $p < 0.01$) and *L. pallida* (Pseudo-$F_{1,7} = 26.62$, $p < 0.001$) were significantly different among sampling sites (Table 2). However, the variability explained by site was only 13% for *E. maxima* compared to 49% for *L. pallida* (Table 2). Although differences among sampling occasions (seasons) were significant, the variability explained by this factor was <2% for both species (Table 2). The interaction term (Site × Season) was statistically significant for both *E. maxima* and *L. pallida*, indicating the differences among sites were dependent on sampling occasion (Table 2). The residuals accounted for a large proportion of the variability explained for both *E. maxima* (56%) and for *L. pallida* (38%), indicating a large intra-population variability in $\delta^{13}C$ values. For the samples collected during austral summer (Fig. 4), *L. pallida* was most depleted in $\delta^{13}C$ at Jacobsbaai (−21.6‰) and most enriched in $\delta^{13}C$ at Doringbaai (−10.4‰). However, for the samples collected in the winter (Fig. 5), *L. pallida* was most depleted in $\delta^{13}C$ at Jacobsbaai (−24.0‰) and most enriched in $\delta^{13}C$ at Hondeklipbaai (−11.6‰). *E. maxima* was most depleted in $\delta^{13}C$ at Port Nolloth (−19.5‰) and most enriched in $\delta^{13}C$ at Kommetjie (−10.2‰) during summer. Similarly, in winter, *E. maxima* was most depleted in $\delta^{13}C$ at Kleinsee (−22.1‰) and most enriched in $\delta^{13}C$ at Jacobsbaai (−13.6‰). Post hoc comparisons revealed that the most interesting trend in the data, which was evident in both sampling occasions but more pronounced in winter, was the break at Jacobsbaai in the mean $\delta^{13}C$ values of *L. pallida* creating 2 distinct groups (Figs. 4 & 5). The northern group consisted of the sites between Port Nolloth and Doringbaai, whereas the southern group consisted of the 4 remaining sites. Interestingly, *E. maxima* data did not follow this trend, with all sites generally grouping together. At this point, *E. maxima* and *L. pallida* mean values also diverge from each other, with *L. pallida* being more depleted at all subsequent (southward) sites.

Across the sampling localities, the variance of stable carbon isotope values ($\delta^{13}C$) was higher in *L. pallida*

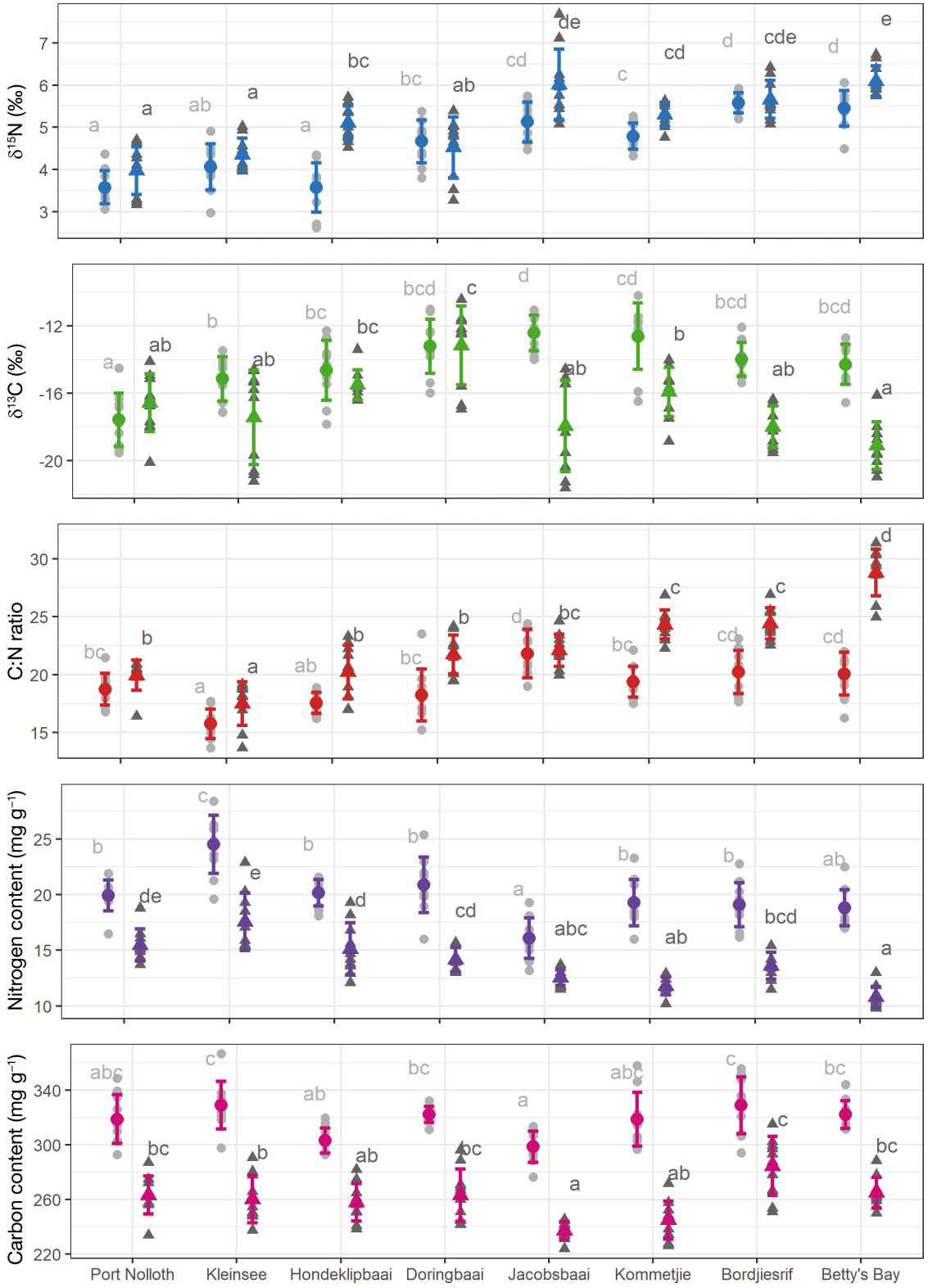

Fig. 4. Summer stable isotope ($\delta^{13}C$ and $\delta^{15}N$) values, C:N ratio and nitrogen and carbon content (%) for kelps *Ecklonia maxima* (light grey circles) and *Laminaria pallida* (dark grey triangles) at 8 sampling localities (north to south from left to right). Coloured points and error bars indicate mean and standard deviation. Light (*E. maxima*) and dark (*L. pallida*) grey letters indicate post hoc groupings for each species. Note different scale to Fig. 5

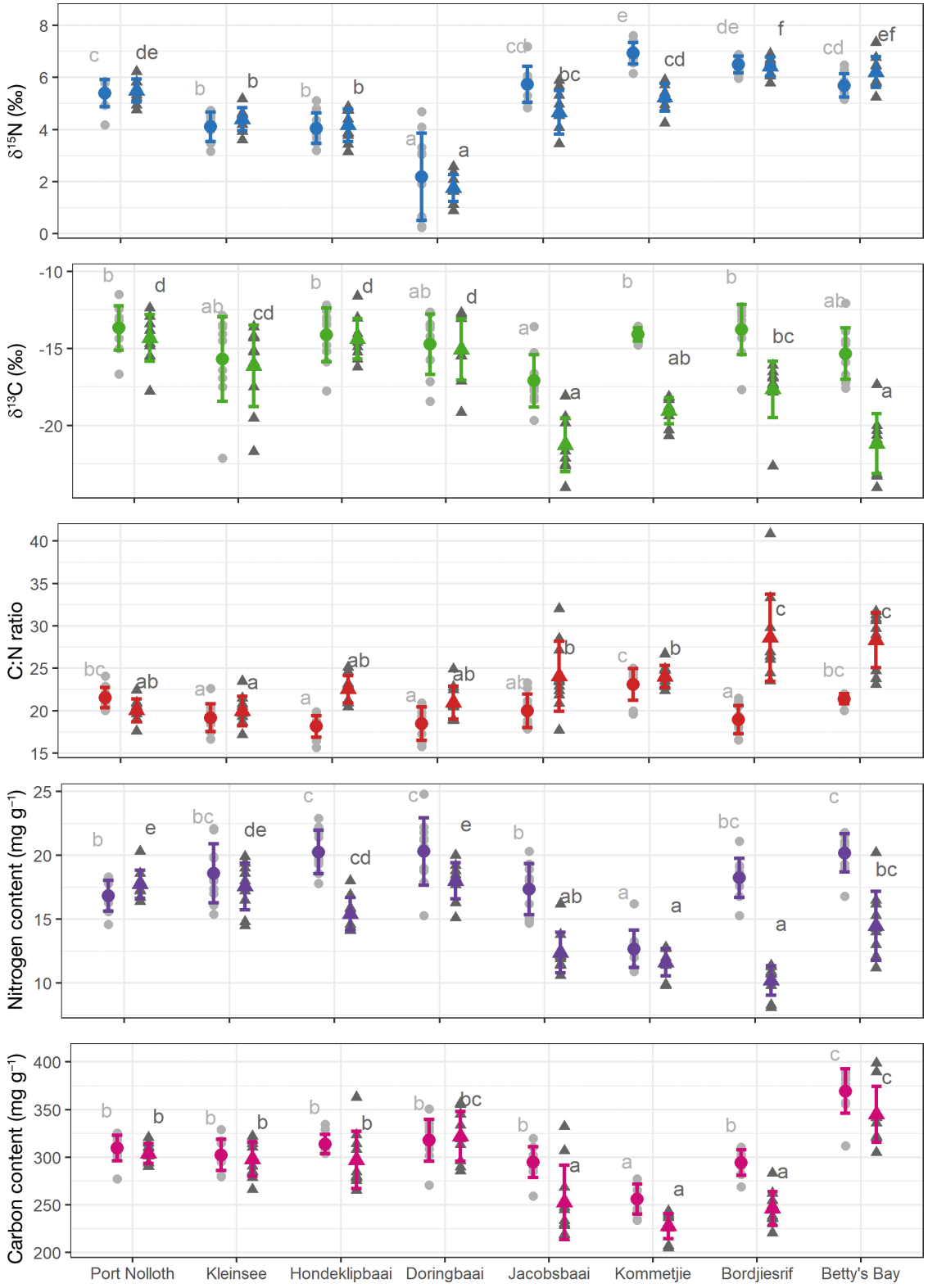

Fig. 5. Winter stable isotope ($\delta^{13}C$ and $\delta^{15}N$) values, C:N ratio and nitrogen and carbon content (%) for kelps *Ecklonia maxima* (light grey circles) and *Laminaria pallida* (dark grey triangles) at 8 sampling localities (north to south from left to right). Coloured points and error bars indicate mean and standard deviation. Light (*E. maxima*) and dark (*L. pallida*) grey letters indicate post hoc groupings for each species. Note different scale to Fig. 4

(11.2‰) compared to *E. maxima* (9.4‰) for the summer sampling occasion. However, in winter, this pattern was reversed: *E. maxima* (15.0‰) had a greater variance compared to *L. pallida* (12.4‰). The nitrogen isotope values ($\delta^{15}N$) also displayed the same pattern, with *L. pallida* having a greater variance in values (4.5‰) relative to *E. maxima* (3.4‰) in the summer, and *E. maxima* having a greater variance in values (6.5‰) relative to *L. pallida* (7.4‰) in the winter.

The C:N ratio of *E. maxima* (Pseudo-$F_{7,144}$ = 16.21, $p < 0.001$) and *L. pallida* (Pseudo-$F_{7,144}$ = 39.31, $p < 0.001$) was significantly different among the sampling sites (Table 2). The variability explained by among site differences was, however, substantially greater in *L. pallida* (61%) in comparison to *E. maxima* (34%). Sampling occasion (season) was statistically significant for both species; however, it only accounted for a small proportion of the variability in C:N ratios (*E. maxima*: 6% and *L. pallida*: 2%). The interaction term was significant for both *E. maxima* and *L. pallida* and accounted for 17 and 4% of the variability in C:N ratio, respectively (Table 2). Intra-population variability, as explained by the residual values, accounted for a large proportion of the variability in C:N ratio of *E. maxima* (31%) and *L. pallida* (32%). Post hoc comparisons also revealed a similar pattern to the $\delta^{13}C$ values, with sites south of Jacobsbaai showing distinct differences between *E. maxima* and *L. pallida* values, for both sampling occasions but again more apparent during the winter sampling occasion. Geographically, the general trend in the data shows an increase in C:N ratio moving from north to south, being more apparent in summer months (Figs. 4 & 5).

The nitrogen content (%) of the tissue was significantly different among sites for both *E. maxima* (Pseudo-$F_{7,144}$ = 20.09, $p < 0.001$) and *L. pallida* (Pseudo-$F_{7,144}$ = 44.60, $p < 0.001$). However, the variability explained by site was substantially higher for *L. pallida* (57%) compared to *E. maxima* (34%). Although sampling occasion (season) was significant for both species, the variability explained by season was low for both *E. maxima* (9%) and *L. pallida* (2%). The interaction term (Site × Season) was significant for both *E. maxima* and *L. pallida* (Table 2), indicating the among-site differences were dependent on the season. The variability explained by the interaction term was larger for *E. maxima* (22%) than for *L. pallida* (15%). Intra-population variability, as explained by the residual values, accounted for a large proportion of the variability in tissue nitrogen content of *E. maxima* (35%) and *L. pallida* (26%). The post hoc comparisons showed 2 distinct patterns in nitrogen content for the 2 sampling occasions. In summer, tissue nitrogen content of *E. maxima* was consistently higher than that of *L. pallida* at all 8 sampling sites (Fig. 4). Nitrogen content of *L. pallida* was lower in the southern sites compared to the sites north of Jacobsbaai. In contrast, *E. maxima* nitrogen content was more similar across the sampling sites. However, in winter, the nitrogen content of the 2 species was more similar at a number of the sites, with the 4 southern sites showing a different trend to those north of Jacobsbaai (Fig. 5).

The tissue carbon content (%) of *E. maxima* (Pseudo-$F_{7,144}$ = 23.26, $p < 0.001$) and *L. pallida* (Pseudo-$F_{7,144}$ = 25.45, $p < 0.001$) was significantly different among sampling sites (Table 2). The variability explained by among-site differences was similar for both species (35%). Sampling occasion (season) was significant for both species but only accounted for a small fraction of the variability in tissue carbon content, particularly for *E. maxima* (Table 2). The interaction of site and season was significant for both species and accounted for a similar proportion of the variability in tissue carbon content of *E. maxima* (31%) and *L. pallida* (24%). The variability explained by intra-population differences (among individuals collected at the same time) accounted for 31% of the variability in *E. maxima* and 28% of the variability in *L. pallida* (Table 2). Similar to the nitrogen content, post hoc comparisons of carbon content revealed 2 very different patterns in summer and winter, with summer *E. maxima* values being consistently higher than *L. pallida* values at all sites (Fig. 4). Values for each species were more consistent among the sampling sites, not showing any geographical groupings. In winter, however, carbon content of the 2 species was more similar at all sites apart from Jacobsbaai and Bordjiesrif (Fig. 5). For *L. pallida*, the northern sites again grouped together, but here Jacobsbaai, Kommetjie and Bordjiesrif group together, with Betty's Bay being distinct from the northern and southern groups.

The results in Table 2 indicate a similar pattern across all the biomarkers for *E. maxima*, with the exception of $\delta^{15}N$ values and carbon content. For all other markers ($\delta^{13}C$, C:N ratio and %N) the largest proportion of the variability was explained by intra-population variability. This is the variability among individuals collected at the same place within a single sampling occasion. *Laminaria pallida* exhibited a different trend, with site explaining the largest proportion of the variability in all 5 biomarkers used (Table 2).

4. DISCUSSION

The results of the present study show that stable isotope values of both *Ecklonia maxima* and *Laminaria pallida* vary among different tissues of a single plant, within a single lamina within each species, among sampling occasions, and among sites across a broad spatial scale. This was largely to be expected, as the stable isotope signatures of kelps from elsewhere in the world are variable over space and time (Stephenson et al. 1984, Simenstad et al. 1993, Fredriksen 2003, Vanderklift & Bearham 2014, Mackey et al. 2015, Buchholz et al. 2019). However, no study has yet revealed the scale and magnitude of the variability in kelps along the South African coastline. Within-site variability was a large contributor to the variance in most of the biomarkers measured for *E. maxima*, which is consistent with the findings of Mackey et al. (2015) for *E. radiata* along the Australian coastline. In contrast, the variance in *L. pallida* biomarkers could predominantly be traced back to differences among sampling sites, which is likely an artefact of the depth distribution of this species along the coastline. These findings highlight an important consideration which needs to be made when designing ecological studies that depend on stable isotope analysis.

4.1. Scales of variability

The identification of variability in macrophyte (macroalgae and seagrasses) stable isotope values has become increasingly common in the literature, but still remains poorly understood (Dethier et al. 2013). When looking at variability in stable isotope values, the scale (spatial and temporal) becomes an important consideration. Spatial variability of marine macrophyte stable isotope values has been shown to operate at different geographical scales, from among sites separated by small distances (10s of km) (Raven et al. 1995, Dethier et al. 2013) to larger regional distances of 100s of km (Simenstad et al. 1993, Vanderklift & Wernberg 2010, Mackey et al. 2015, Stepien 2015). Additionally, Stephenson et al. (1984) identified the variability of $\delta^{13}C$ within a single lamina of the kelp *Saccharina latissima* (as *L. longicruris*; see McDevit & Saunders 2010) and reported significant variation between the meristem and distal portions of the fronds, as well as among different tissues. Similarly, Fredriksen (2003) found that the distal parts of the fronds of the kelp, *L. hyperborea* (Gunnerus) Foslie, were isotopically lighter, in ^{13}C and ^{15}N, than the basal sections.

Both *L. pallida* and *E. maxima* showed spatial variability over the scale of the west coast of South Africa (a distance of ~700 km), with statistically different $\delta^{13}C$ and $\delta^{15}N$ values among sites. Significant differences were also identified among the different tissues of both kelps, particularly for $\delta^{15}N$. Even at the smallest scale, within a single lamina, the results for both *E. maxima* and *L. pallida* showed considerable variation along the length of the blade from primary blade to frond tip. However, variability among replicates was larger for *L. pallida* than *E. maxima*, and thus these patterns could benefit from further investigation.

Temporal variability has also been recorded for marine and aquatic macrophytes in general (Boon & Bunn 1994, Dethier et al. 2013) and specifically for kelps such as *E. radiata* (Vanderklift & Bearham 2014, Mackey et al. 2015) and *L. hyperborea* (Fredriksen 2003). The results of this study, however, indicate a very different trend, as sampling occasion (season) accounted for a very small fraction of the variability in stable isotope values of both *E. maxima* and *L. pallida*. As the interaction of site and season accounted for a larger proportion of the variability, it is likely that this is related to the seasonal nature of coastal upwelling along the coastline (discussed in Sections 4.2 and 4.4).

4.2. Variability in $\delta^{15}N$ values

The variability of nitrogen stable isotope signatures in marine macroalgae is primarily due to changes in nitrogen source, with algal $\delta^{15}N$ values reflecting those of the sources (Dudley et al. 2010). Marine dissolved inorganic nitrogen (DIN) is known to range in $\delta^{15}N$ between 6 and 8‰ (Miyake & Wada 1967, Liu & Kaplan 1989, Sigman et al. 1997, 2000), and thus many temperate algae have $\delta^{15}N$ values within this range (Monteiro et al. 1997, Cornelisen et al. 2007, Dudley & Shima 2010). Therefore, unsurprisingly, the $\delta^{15}N$ values of both *E. maxima* and *L. pallida* fall within the expected 6 to 8‰ range.

Seasonal differences in $\delta^{15}N$ were also detected in both *E. maxima* and *L. pallida*, which were collected during summer and winter sampling occasions. However, season alone accounted for much less of the observed variability compared to the interaction of site and season. Nevertheless, the most likely explanation for this variability is the seasonal nature of coastal upwelling, which is a key factor controlling the influx of nitrate into South African kelp forest systems. During the summer months, upwelling intensity is at its highest, whereas winter months see a large reduction in

both frequency and intensity (Andrews & Hutchings 1980). Additionally, a gradient in upwelling intensity is known to exist along the west coast, with lower-intensity upwelling cells in the southern regions (see Lutjeharms & Meeuwis 1987). Therefore, the spatial and temporal variability in upwelling intensity and frequency is most likely influencing the observed patterns in $\delta^{15}N$ of the kelp tissue.

However, the magnitude of seasonal variability in South African systems is much lower than those in other regions, such as the North Atlantic. In the northern hemisphere, where growth is often limited by nutrient (C and N) or light availability, kelps have adopted a mechanism to store nitrogen and carbon (see Chapman & Craigie 1977, 1978, Chapman & Lindley 1980). For example, *S. latissima* (as *L. longicruris*) has been shown to accumulate nitrate (NO_3^-) during winter and supply this to meristematic tissue for 6 to 8 wk during the growing season in spring (Chapman & Craigie 1977). In contrast, South African kelps, particularly *E. maxima*, do not store carbon or nitrogen compounds to the same extent (Smith 2007). However, short-term changes in tissue nitrogen content can be linked to changes in the nitrogen content of the surrounding water (e.g. upwelling), as *E. maxima* is known to take up more nitrogen (as NO_3^-) under upwelling conditions (Probyn & McQuaid 1985). Therefore, the seasonal trends observed in the $\delta^{15}N$ values, nitrogen content and C:N ratios could be linked to upwelling, as the trends match the spatial and temporal patterns in upwelling intensity. Additionally, although not within the scope of this study, the influence of geographical variability in upwelling intensity (see Lutjeharms & Meeuwis 1987) on kelp stable isotope values needs further investigation along the southern African coastline.

Vanderklift & Bearham (2014) also show that light availability can be a contributor to variability in the $\delta^{15}N$ values of kelps, specifically for *E. radiata*. However, the results of this study did not provide any evidence of this influence. Despite *E. maxima* and *L. pallida* growing at different depths, and thus having different light availability, their $\delta^{15}N$ values were very similar at the sites south of Jacobsbaai. Similarly, Buchholz et al. (2019) show that light availability had little effect on the $\delta^{15}N$ values of *Alaria esculenta*.

4.3. Variability in $\delta^{13}C$ values

Variability in the $\delta^{13}C$ values of marine macrophytes and in particular marine algae has received the attention of several studies. Stephenson et al. (1984) summarised the factors which influence $\delta^{13}C$ values into 5 main topics, viz. (1) the isotopic composition of source carbon, (2) the proportional utilisation of bicarbonate (HCO_3^-) and carbon dioxide (CO_2), (3) the photosynthetic pathway used (C3 vs. C4), (4) the influence of isotopically distinct epibionts, and (5) the differential storage of biochemical compounds. These factors operate at different scales, both spatial and temporal, and therefore create a complex landscape where variability is inevitable.

In the present study, the most interesting pattern that emerged from the spatial analysis was the deviation in $\delta^{13}C$ values that occurred between the means of *E. maxima* and *L. pallida* south of Jacobsbaai. This is also the point where *E. maxima* and *L. pallida* begin to diverge in terms of depth habitat occupied (Field et al. 1980a, Rothman et al. 2017). When *L. pallida* is restricted to deeper water, the $\delta^{13}C$ value becomes more depleted than that of *E. maxima*. From the factors reviewed by Stephenson et al. (1984), the most likely factor resulting in this trend is light availability.

Light availability has been identified as an important factor which aids in the absorption of HCO_3^- from the water column, as this requires more energy via carbon concentrating mechanisms (Simenstad et al. 1993, Stepien 2015, Drobnitch et al. 2017). *E. radiata* is known to require more energy, gathered from irradiance, to assimilate HCO_3^- preferentially over CO_2 (Cornelisen et al. 2007). Consequently, light availability was shown to be the primary cause of variability in $\delta^{13}C$ values of *E. radiata* along the Australian coastline (Vanderklift & Bearham 2014). Similarly, the $\delta^{13}C$ of *A. esculenta* was greatly influenced by depth (light availability) in Kongsfjorden, Svalbard (Buchholz et al. 2019). From our results, it is evident that when *L. pallida* is found at the surface with *E. maxima* (north of Jacobsbaai), the 2 species have more similar $\delta^{13}C$ values. This suggests that they use the same source of carbon and the same carbon metabolism. However, when the 2 species are separated by depth (south of Jacobsbaai), with *L. pallida* in deeper water, there is a marked difference in values, with *L. pallida* being severely depleted in $\delta^{13}C$. Light availability would certainly correlate with this pattern, and therefore, it is likely to have resulted in the observed difference in $\delta^{13}C$ values.

4.4. Variability in C:N ratio and tissue C and N content

The C:N ratio and carbon and nitrogen content of both species showed a predictable trend along the

coastline, matching the geographical and seasonal patterns of upwelling frequency and intensity. Kelps in the northern sites (north of Jacobsbaai) had higher tissue nitrogen content in both seasons which correlates well to the increased upwelling frequency and intensity along this part of the coastline (Andrews & Hutchings 1980, Lutjeharms & Meeuwis 1987). Consequently, the C:N ratio of both kelps was also lower at these sites during both sampling occasions. Conversely, the general southward decrease in tissue nitrogen and the increase in C:N ratio follows the decrease in upwelling intensity documented along the coastline. The carbon content of both species was fairly stable along the coastline during both seasons, which corroborates the findings of Smith (2007) in showing that South African kelps do not store carbon compounds in their tissues.

4.5. Implications for food web studies

Although poorly understood, stable isotope variability, such as that which has been identified in this study, must be taken into account in order to gather accurate information about the food web (Nordström et al. 2009, Dethier et al. 2013, Hyndes et al. 2013). This is especially pertinent when determining the ultimate carbon sources of the food web in systems which potentially rely on several plant or algal components (Boon & Bunn 1994). Hadwen et al. (2010) illustrated how temporal variability of algal $\delta^{13}C$ values can lead to differences in source contributions of up to 11% when determining the diet of stream consumers. Additionally, if systems have overlapping carbon sources, it becomes very difficult to distinguish which source is driving the food web.

The influence of temporal and spatial variability can be mitigated through careful site- and time-specific sample collection. However, intra-population variability poses more of a problem and thus needs further attention when designing ecological studies. A common way of dealing with variability involves isotopic baselining, where consumer isotope signatures are corrected against those of longer-lived species near the base of the food web (see Post 2002a). Additionally, modern Bayesian mixing models allow for the incorporation of variability in basal signatures when calculating the proportion of sources within a mixture (e.g. diet) (Woodland et al. 2012, Stock & Semmens 2016). Therefore, it is possible to use the natural variability in stable isotope signatures to add information to stable isotope analyses, but doing so does require some knowledge about the scale and magnitude of this variability in the study system.

5. CONCLUSIONS

The stable isotope values of South African kelp species are highly variable across various scales of space and time. *Ecklonia maxima* and *Laminaria pallida* values are variable at the scale of centimetres along the length of the frond as well as at the scale of hundreds of kilometres among sampling sites. Several authors have shown that using a single point sample to assign a stable isotope value to a source of production is fundamentally flawed, as it does not take into account the variability which has been demonstrated to operate at different spatial and temporal scales (Fenton & Ritz 1989, Fry & Sherr 1989, Boon & Bunn 1994, Dethier et al. 2013).

By highlighting the variability in stable isotope signatures of the 2 kelp species along the South African coastline, we hope to provide important information to ecologists trying to understand the trophic importance of kelp. Incorporating this variability into stable isotope mixing models and designing studies which take this variability into account is therefore recommended. Using values from one site, one sampling occasion or literature values and applying these values to studies across temporal and/or spatial scales is strongly discouraged. Instead, kelp stable isotope values should be evaluated, with sufficient replicates, for each species at each study site and within different seasons in order to account for the natural variability which may exist. The addition of different stable isotope markers (e.g. $\delta^{34}S$) and/or fatty acid composition could also provide useful information when studying the trophic ecology of these ecosystems.

Acknowledgements. We thank Chris Boothroyd and Derek Kemp from the Seaweed Research Unit (Department of Agriculture, Forestry and Fisheries), as well as Mark Noffke, for their assistance with the collection of kelp samples. Maggie Reddy is thanked for her assistance with the processing of samples in the laboratory. Two anonymous reviewers are thanked for their comments which substantially improved the manuscript. Funding for this research was provided by the National Research Foundation (NRF), grant number 111719.

LITERATURE CITED

Anderson MJ (2001) A new method for non-parametric multivariate analysis of variance. Austral Ecol 26:32–46

Anderson MJ (2017) Permutational multivariate analysis of variance (PERMANOVA). In: Balakrishnan N, Colton T,

Everitt B, Piegorsch W, Ruggeri F, Teugels JL (eds) Wiley StatsRef: statistics reference online. John Wiley & Sons, Chichester, p 1–15

Anderson MJ, Walsh DC (2013) PERMANOVA, ANOSIM, and the Mantel test in the face of heterogeneous dispersions: What null hypothesis are you testing? Ecol Monogr 83:557–574

Andrews WRH (1974) Selected aspects of upwelling research in the southern Benguela Current. Tethys 6: 327–340

Andrews WRH, Hutchings L (1980) Upwelling in the southern Benguela Current. Prog Oceanogr 9:1–81

Bolton JJ (2010) The biogeography of kelps (Laminariales, Phaeophyceae): a global analysis with new insights from recent advances in molecular phylogenetics. Helgol Mar Res 64:263–279

Boon PI, Bunn SE (1994) Variations in the stable isotope composition of aquatic plants and their implications for food web analysis. Aquat Bot 48:99–108

Buchholz CM, Lebreton B, Bartsch I, Wiencke C (2019) Variation of isotope composition in kelps from Kongsfjorden (Svalbard). Mar Biol 166:71

Cabana G, Rasmussen JB (1994) Modelling food chain structure and contaminant bioaccumulation using stable nitrogen isotopes. Nature 372:255–257

Cabana G, Rasmussen JB (1996) Comparison of aquatic food chains using nitrogen isotopes. Proc Natl Acad Sci USA 93:10844–10847

Carter RA (1982) Phytoplankton biomass and production in a southern Benguela kelp bed system. Mar Ecol Prog Ser 8:9–14

Chapman A, Craigie J (1977) Seasonal growth in *Laminaria longicruris*: relations with dissolved inorganic nutrients and internal reserves of nitrogen. Mar Biol 40:197–205

Chapman A, Craigie J (1978) Seasonal growth in *Laminaria longicuris*: relations with reserve carbohydrate storage and production. Mar Biol 46:209–213

Chapman A, Lindley J (1980) Seasonal growth of *Laminaria solidungula* in the Canadian High Arctic in relation to irradiance and dissolved nutrient concentrations. Mar Biol 57:1–5

Cornelisen CD, Wing SR, Clark KL, Hamish Bowman M, Frew RD, Hurd CL (2007) Patterns in the $\delta^{13}C$ and $\delta^{15}N$ signature of *Ulva pertusa*: interaction between physical gradients and nutrient source pools. Limnol Oceanogr 52:820–832

DeNiro MJ, Epstein S (1981) Influence of diet on the distribution of nitrogen isotopes in animals. Geochim Cosmochim Acta 45:341–351

Dethier MN, Sosik EA, Galloway AWE, Duggins DO, Simenstad CA (2013) Addressing assumptions: variation in stable isotopes and fatty acids of marine macrophytes can confound conclusions of food web studies. Mar Ecol Prog Ser 478:1–14

Dieckmann GS (1978) Aspects of growth and production of *Laminaria pallida* (Grev.) J. Ag. off the Cape Peninsula. MSc thesis, University of Cape Town

Dieckmann GS (1980) Aspects of the ecology of *Laminaria pallida* (Grev). J. Ag. off the Cape Peninsula (South Africa) I. Seasonal growth. Bot Mar 23:579–585

Drobnitch ST, Pochron T, Miranda C (2017) Patterns and drivers of $\delta^{13}C$ variation in the giant kelp, *Macrocystis pyrifera.* Limnol Oceanogr 63:871–885

Dudley BD, Shima JS (2010) Algal and invertebrate bioindicators detect sewage effluent along the coast of Titahi Bay, Wellington, New Zealand. N Z J Mar Freshw Res 44:39–51

Dudley BD, Barr NG, Shima JS (2010) Influence of light intensity and nutrient source on $\delta^{13}C$ and $\delta^{15}N$ signatures in *Ulva pertusa.* Aquat Biol 9:85–93

Dyer DC, Butler MJ, Smit AJ, Anderson RJ, Bolton JJ (2019) Kelp forest POM during upwelling and downwelling conditions: using stable isotopes to differentiate between detritus and phytoplankton. Mar Ecol Prog Ser 619:17–34

Fenton GE, Ritz DA (1989) Spatial variability of ^{13}C:^{12}C and D:H in *Ecklonia radiata* (C. Ag.) J. Agardh (Laminariales). Estuar Coast Shelf Sci 28:95–101

Field JG, Griffiths CL (1991) Littoral and sublittoral ecosystems of southern Africa. In: Mathieson AC, Nienhuis P H (eds) Ecosystems of the world. Elsevier, Amsterdam, p 323–346

Field JG, Griffiths CL, Griffiths RJ, Jarman NG, Zoutendyk P, Velimirov B, Bowes A (1980a) Variation in structure and biomass of kelp communities along the south-west Cape coast. Trans R Soc S Afr 44:145–203

Field JG, Griffiths CL, Linley EAS, Carter RA, Zoutendyk P (1980b) Upwelling in a nearshore marine ecosystem and its biological implications. Estuar Coast Mar Sci 11: 133–150

Fielding PJ, Davis CL (1989) Carbon and nitrogen resources available to kelp bed filter feeders in an upwelling environment. Mar Ecol Prog Ser 55:181–189

Fredriksen S (2003) Food web studies in a Norwegian kelp forest based on stable isotope ($\delta^{13}C$ and $\delta^{15}N$) analysis. Mar Ecol Prog Ser 260:71–81

Fry B (2006) Stable isotope ecology. Springer, New York, NY

Fry B, Sherr EB (1989) $\delta^{13}C$ measurements as indicators of carbon flow in marine and freshwater ecosystems. Contrib Mar Sci 27:13–47

Graham MH, Edwards MS (2001) Statistical significance versus fit: estimating the importance of individual factors in ecological analysis of variance. Oikos 93:505–513

Guest MA, Hirst AJ, Nichols PD, Frusher SD (2010) Multiscale 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

Hadwen WL, Spears M, Kennard MJ (2010) Temporal variability of benthic algal $\delta^{13}C$ signatures influences assessments of carbon flows in stream food webs. Hydrobiologia 651:239–251

Hansen JH, Hedeholm RB, Sünksen K, Christensen JT, Grønkjær P (2012) Spatial variability of carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) stable isotope ratios in an Arctic marine food web. Mar Ecol Prog Ser 467:47–59

Hobson KA (1999) Tracing origins and migration of wildlife using stable isotopes: a review. Oecologia 120:314–326

Hyndes GA, Hanson CE, Vanderklift MA (2013) The magnitude of spatial and temporal variation in $\delta^{15}N$ and $\delta^{13}C$ differs between taxonomic groups: implications for food web studies. Estuar Coast Shelf Sci 119:176–187

Jack L, Wing S (2011) Individual variability in trophic position and diet of a marine omnivore is linked to kelp bed habitat. Mar Ecol Prog Ser 443:129–139

Kadoya T, Osada Y, Takimoto G (2012) IsoWeb: a Bayesian isotope mixing model for diet analysis of the whole food web. PLOS ONE 7:e41057

Kaehler S, Pakhomov EA, McQuaid CD (2000) Trophic structure of the marine food web at the Prince Edward Islands (Southern Ocean) determined by $\delta^{13}C$ and $\delta^{15}N$

analysis. Mar Ecol Prog Ser 208:13–20
Kaehler S, Pakhomov EA, Kalin RM, Davis S (2006) Trophic importance of kelp-derived suspended particulate matter in a through-flow sub-Antarctic system. Mar Ecol Prog Ser 316:17–22
Krumins JA, van Oevelen D, Bezemer TM, De Deyn GB and others (2013) Soil and freshwater and marine sediment food webs: their structure and function. Bioscience 63: 35–42
Layman CA, Araújo MS, Boucek R, Hammerschlag-Peyer CM and others (2012) Applying stable isotopes to examine food-web structure: an overview of analytical tools. Biol Rev Camb Philos Soc 87:545–562
Liu K, Kaplan IR (1989) The eastern tropical Pacific as a source of ^{15}N enriched nitrate in seawater off southern California. Limnol Oceanogr 34:820–830
Lutjeharms JRE, Meeuwis JM (1987) The extent and variability of South-East Atlantic upwelling. S Afr J Mar Sci 5:51–62
Mackey AP, Hyndes GA, Carvalho MC, Eyre BD (2015) Physical and biogeochemical correlates of spatio-temporal variation in the $\delta^{13}C$ of marine macroalgae. Estuar Coast Shelf Sci 157:7–18
Mann KH (1988) Production and use of detritus in various freshwater, estuarine, and coastal marine ecosystems. Limnol Oceanogr 33:910–930
Mann KH, Jarman NG, Dieckmann GS (1979) Development of a method for measuring the productivity of the kelp *Ecklonia maxima* (Osbeck) Papenfuss. Trans R Soc S Afr 44:27–41
Martinez Arbizu P (2019). pairwiseAdonis: Pairwise multilevel comparison using adonis. R package version 0.3. https://github.com/pmartinezarbizu/pairwiseAdonis
McDevit DC, Saunders GW (2010) A DNA barcode examination of the Laminariaceae (Phaeophyceae) in Canada reveals novel biogeographical and evolutionary insights. Phycologia 49:235–248
Miller RJ, Page HM (2012) Kelp as a trophic resource for marine suspension feeders: a review of isotope-based evidence. Mar Biol 159:1391–1402
Miyake Y, Wada E (1967) The abundance ratio of $^{15}N/^{14}N$ in marine environments. Rec Oceanogr Works Jpn 9: 37–53
Monteiro PMS, Anderson RJ, Woodbourne S (1997) $\delta^{15}N$ as a tool to demonstrate the contribution of fish-waste-derived nitrogen to an *Ulva* bloom in Saldanha Bay, South Africa. S Afr J Mar Sci 18:1–9
Nadon MO, Himmelman JH (2010) The structure of subtidal food webs in the northern Gulf of St. Lawrence, Canada, as revealed by the analysis of stable isotopes. Aquat Living Resour 23:167–176
Newell RC, Field JG (1983) The contribution of bacteria and detritus to carbon and nitrogen flow in a benthic community. Mar Biol Lett 4:23–36
Newell RC, Field JG, Griffiths CL (1982) Energy balance and significance of micro-organisms in a kelp bed community. Mar Ecol Prog Ser 8:103–113
Nordström M, Aarnio K, Bonsdorff E (2009) Temporal variability of a benthic food web: patterns and processes in a low-diversity system. Mar Ecol Prog Ser 378:13–26
O'Reilly CM, Hecky RE, Cohen AS, Plisnier PD (2002) Interpreting stable isotopes in food webs: recognizing the role of time averaging at different trophic levels. Limnol Oceanogr 47:306–309
Oksanen J, Blanchet FG, Friendly M, Kindt R and others (2019) vegan: community ecology package. R package version 2.5-6. https://CRAN.R-project.org/package=vegan
Page HM, Reed DC, Brzezinski MA, Melack JM, Dugan JE (2008) Assessing the importance of land and marine sources of organic matter to kelp forest food webs. Mar Ecol Prog Ser 360:47–62
Pasquaud S, Pillet M, David V, Sautour B, Elie P (2010) Determination of fish trophic levels in an estuarine system. Estuar Coast Shelf Sci 86:237–246
Peterson BJ, Fry B (1987) Stable isotopes in ecosystem studies. Annu Rev Ecol Syst 18:293–320
Peterson BJ, Howarth RW, Garritt RH (1985) Multiple stable isotopes used to trace the flow of organic matter in estuarine food webs. Science 227:1361–1363
Pimm SL (1982) Food webs. In: Pimm SL (ed) Food webs. Springer, Dordrecht, p 1–11
Post DM (2002a) Using stable isotopes to estimate trophic position: models, methods, and assumptions. Ecology 83: 703–718
Post DM (2002b) The long and short of food-chain length. Trends Ecol Evol 17:269–277
Probyn TA, McQuaid CD (1985) In-situ measurements of nitrogenous nutrient uptake by kelp (*Ecklonia maxima*) and phytoplankton in a nitrate-rich upwelling environment. Mar Biol 88:149–154
R Core Team (2018) R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna. www.r-project.org
Ramshaw BC, Pakhomov EA, Markel RW, Kaehler S (2017) Quantifying spatial and temporal variations in phytoplankton and kelp isotopic signatures to estimate the distribution of kelp-derived detritus off the west coast of Vancouver Island, Canada: variation in primary production detritus. Limnol Oceanogr 62:2133–2153
Raven JA, Giordano M (2017) Acquisition and metabolism of carbon in the Ochrophyta other than diatoms. Philos Trans R Soc B 372:20160400
Raven JA, Walker DI, Johnston AM, Handley LL, Kübler JE (1995) Implications of ^{13}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
Rothman MD, Bolton JJ, Stekoll MS, Boothroyd CJT, Kemp FA, Anderson RJ (2017) Geographical variation in morphology of the two dominant kelp species, *Ecklonia maxima* and *Laminaria pallida* (Phaeophyceae, Laminariales), on the west coast of Southern Africa. J Appl Phycol 29:2627–2639
Santelices B (2004) A comparison of ecological responses among aclonal (unitary), clonal and coalescing macroalgae. J Exp Mar Biol Ecol 300:31–64
Sigman D, Altabet M, Michener RH, McCorkle D, Fry B, Holmes R (1997) Natural abundance-level measurement of the nitrogen isotopic composition of oceanic nitrate: an adaptation of the ammonia diffusion method. Mar Chem 57:227–242
Sigman D, Altabet M, McCorkle D, Francois R, Fischer G (2000) The $\delta^{15}N$ of nitrate in the Southern Ocean: nitrogen cycling and circulation in the ocean interior. J Geophys Res Oceans 105:19599–19614
Simenstad CA, Duggins DO, Quay PD (1993) High turn-

over of inorganic carbon in kelp habitats as a cause of $\delta^{13}C$ variability in marine food webs. Mar Biol 116: 147–160

Smale DA, Burrows MT, Moore PJ, O'Connor N, Hawkins SJ (2013) Threats and knowledge gaps for ecosystem services provided by kelp forests: a northeast Atlantic perspective. Ecol Evol 3:4016–4038

Smith MJ (2007) Seasonal variation in nutritional content of the kelp *Ecklonia maxima* on the west and south west coasts of South Africa, with reference to its use as abalone feed. MSc thesis, University of Cape Town

Stegenga HS, Bolton JJ, Anderson RJ (1997) Seaweeds of the South African west coast. Creda Press, Cape Town

Steneck RS, Graham MH, Bourque BJ, Corbett D, Erlandson JM, Estes JA, Tegner MJ (2002) Kelp forest ecosystems: biodiversity, stability, resilience and future. Environ Conserv 29:436–459

Stephenson RL, Tan FC, Mann KH (1984) Stable carbon isotope variability in marine macrophytes and its implications for food web studies. Mar Biol 81:223–230

Stepien CC (2015) Impacts of geography, taxonomy and functional group on inorganic carbon use patterns in marine macrophytes. J Ecol 103:1372–1383

Stock BC, Semmens BX (2016) Unifying error structures in commonly used biotracer mixing models. Ecology 97: 2562–2569

Vander Zanden MJ, Rasmussen JB (1999) Primary consumer $\delta^{13}C$ and $\delta^{15}N$ and the trophic position of aquatic consumers. Ecology 80:1395–1404

Vander Zanden MJ, Casselman JM, Rasmussen JB (1999) Stable isotope evidence for the food web consequences of species invasions in lakes. Nature 401:464–467

Vanderklift MA, Bearham D (2014) Variation in $\delta^{13}C$ and $\delta^{15}N$ of kelp is explained by light and productivity. Mar Ecol Prog Ser 515:111–121

Vanderklift MA, Ponsard S (2003) Sources of variation in consumer-diet $\delta^{15}N$ enrichment: a meta-analysis. Oecologia 136:169–182

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, Kendrick GA, Smit AJ (2006) Differences in trophic position among sympatric sea urchin species. Estuar Coast Shelf Sci 66:291–297

Vinagre C, Salgado JP, Costa MJ, Cabral HN (2008) Nursery fidelity, food web interactions and primary sources of nutrition of the juveniles of *Solea solea* and *S. senegalensis* in the Tagus estuary (Portugal): a stable isotope approach. Estuar Coast Shelf Sci 76:255–264

von Biela VR, Newsome SD, Bodkin JL, Kruse GH, Zimmerman CE (2016) Widespread kelp-derived carbon in pelagic and benthic nearshore fishes suggested by stable isotope analysis. Estuar Coast Shelf Sci 181:364–374

West JB, Bowen GJ, Dawson TE, Tu KP (eds) (2009) Isoscapes: understanding movement, pattern, and process on Earth through isotope mapping. Springer, New York, NY

Wing SR, Jack L (2012) Resource specialisation among suspension-feeding invertebrates on rock walls in Fiordland, New Zealand, is driven by water column structure and feeding mode. Mar Ecol Prog Ser 452:109–118

Woodland RJ, Rodríguez MA, Magnan P, Glémet H, Cabana G (2012) Incorporating temporally dynamic baselines in isotopic mixing models. Ecology 93:131–144

Wulff FV, Field JG (1983) Importance of different trophic pathways in a nearshore benthic community under upwelling and downwelling conditions. Mar Ecol Prog Ser 12:217–228

Editorial responsibility: Stephen Wing, Dunedin, New Zealand

Submitted: October 9, 2018; Accepted: August 4, 2019
Proofs received from author(s): September 21, 2019