Multi-scale spatial variation in stable isotope and fatty acid profiles amongst temperate reef species: implications for design and interpretation of trophic studies

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ABSTRACT: Stable isotopes of carbon and nitrogen and fatty acid analyses are increasingly being used in combination to determine the trophic structure of marine systems. For stable isotopes, the variability in carbon and nitrogen isotopic signatures has long been recognised and has been characterised for some taxa. Whilst it is known that metabolic processes may influence fatty acid profiles, the spatial variability of fatty acid profiles has not been documented. Understanding at what scale these 2 biochemical tracers vary, and if the scale of variability corresponds between tracers, is crucial for the correct design and interpretation of combined tracers in trophic studies. This study is the first to examine spatial variability in fatty acid profiles per se, and in combination with stable isotope ratios in the same organisms at multiple spatial scales. We used a spatially hierarchical design which sampled across broad geographic regions, reefs within regions, and also between different parts of macroalgal plants common on temperate reefs. For stable isotopes of carbon and nitrogen, variability was greatest at intermediate spatial scales (between locations within regions, and sites within locations). In contrast, fatty acid profiles showed the greatest variation amongst individual replicates of lobster, abalone and macroalgae. This study demonstrates that for the increasing number of trophic studies using combined biochemical tracers, sampling design should cater to the differences in the variability of each tracer technique and allocate sampling accordingly.

KEY WORDS: Stable isotopes · Fatty acid analysis · Nested hierarchical design · Multivariate variance components · Tasmania

INTRODUCTION

Biochemical tracers such as stable isotopes of carbon and nitrogen, and more recently, fatty acid analyses, are widely used to examine the diet of consumers and the trophic structure of marine systems. The basis of these approaches is that a consumer incorporates the ‘marker’ or ‘signature’ of its food source into its somatic tissue with minimal or predictable changes (Corraze 1999, Peterson 1999). Variation in the ratio of the rare to heavy isotope is referred to as fractionation, and is due to differential discrimination of the rare to heavy isotope during chemical reactions associated with metabolic processes, and differential diffusion of isotopes during uptake (Peterson & Fry 1987). As carbon changes very little between successive trophic levels (0–1‰, McCutchan et al. 2003), the carbon isotope can often indicate the ultimate source of primary production at the base of a consumer diet. The nitrogen isotope experiences greater fractionation per trophic level (3–4‰) and is thus used to infer the trophic status of a consumer (McCutchan et al. 2003). Signature fatty
acids include individual fatty acids that are rare, and unique ratios of commonly occurring fatty acids, both of which can be reflected in the fatty acid profile of a consumer (Dalsgaard et al. 2003). Variation in the fatty acid profiles of organisms is associated with the metabolic processes of the organism (Dalsgaard et al. 2003).

Environmental factors have been identified as the primary mechanism influencing the fractionation rates (variability) of isotopic ratios. For example, light intensity (Hemminga & Mateo 1996, Alcoverro et al. 2001), temperature (Hemminga & Mateo 1996), CO2 availability (Burkhardt et al. 1999), water depth (Grice et al. 1996), and nutrient source (Marguillier et al. 1997, Waser et al. 1998, Finlay 2004) influence isotope ratios of autotrophs by altering the rate of productivity. Such factors differ across a range of spatial scales, potentially altering the isotopic ratio of the consumers that depend upon them. As variations in fatty acid profile are associated with the metabolic processes of an organism, factors that have the capacity to alter metabolic processes arguably have the capacity to influence the variation in fatty acid profiles of autotrophs and consumers. The extent to which fatty acids may be altered may also depend on the role of the fatty acid in the target organism metabolism (Chandrapavan et al. 2009). To date, the impact of these factors on fatty acid profiles has primarily been limited to laboratory or captive studies and can vary amongst species (Dalsgaard et al. 2003).

Examination of the spatial scale of tracer variation has only been conducted for stable isotopes. For example, kelp plants collected from across the Aleutian Island chain, Alaska (USA), were more variable in δ13C signatures between sites (i.e. separated by 100s of metres and nested within islands) than islands (separated by 10s of km; Simenstad et al. 1993). Several other studies have also shown that carbon isotope ratios vary at a range of spatial scales (Boon & Bunn 1994, Jennings et al. 1997, Boyce et al. 2001). Whilst this research is useful in highlighting the importance of quantifying the variance of stable isotope signatures at a range of scales, the lack of precise information about the scale investigated (either because it is not reported or is difficult to discern with any accuracy from figures) limits the utility of these results in guiding the design of experiments in contemporary trophic studies.

Typically, studies that aim to determine trophic structure of natural systems use surveys that sample a range of consumers and their potential food sources. Correlations between the tracer signature of a consumer and a potential food source provide an indication of likely dietary contributions, and reduce the potential complexity of dietary relationships which may then be verified more explicitly using experimental means. However, as many questions of trophic interest incorporate a range of spatial scales (e.g. inshore versus offshore movement of carbon, Odum et al. 1979, Deegan & Garratt 1997; contribution of marine versus terrestrial carbon to coastal food webs, Andrews et al. 1998, Dittmar et al. 2001), high variability may obscure, and potentially confound, interpretation of trophic relationships where scales of variation are not sufficiently understood.

Fatty acids are increasingly the tracer of choice to complement the use of stable isotope analysis to clarify the trophic structure of marine systems (e.g. Kharlamenko et al. 2001, Alfaro et al. 2006, Guest et al. 2008, 2009, Jaschinski et al. 2008, Soreide et al. 2008, Stevens et al. 2008, Tucker et al. 2008, Jack et al. 2009). This is primarily due to the potentially greater resolving power of the combined approach in discriminating among food sources. However, no studies have examined the spatial variability of stable isotopes and fatty acids when combined to address trophic questions. Such studies are done with the implicit assumption that an experiment designed to suit one technique will also suit the other, despite no knowledge of the relative scales of variability in the biochemical tracer ratios of the target organisms. Moreover, there are no examples wherein the spatial variability of fatty acid profiles has been quantified despite their wide application. In part, this is because fatty acid dietary analysis involves quantifying the variance amongst many fatty acids simultaneously (often >100 fatty acid profiles), using multivariate statistical techniques such as discriminant function analysis (Phillips et al. 2003a) or classification trees (Iverson et al. 1997, 2007). However, statistical methods for partitioning variance among multivariate data are now available (McArdle & Anderson 2001, Anderson et al. 2005). In this study we quantified spatial variance in fatty acid profiles within nested-hierarchical sampling designs using a nonparametric test, permutational multivariate analysis of variance (PERMANOVA; Anderson 2005).

Globally, temperate reef systems are dominated by structurally complex and diverse macroalgal assemblages (Steneck et al. 2002). Brown algae dominate these systems and provide habitat for a diversity of fish and invertebrates including several of major economic importance. In Tasmania, Australia, the sea urchin _Heliocidaris erythrogramma_, the purple wrasse _Noto- labrus fucicola_, the southern rock lobster _Jasus edwardsii_ and the black lip abalone _Haliothis rubra_ are common residents of algal-dominated reefs, the latter 2 being important commercial fishery species. _N. fucicola_ feeds on small crustaceans and molluscs (Denny & Schiel 2001), whilst _J. edwardsii_ is considered opportunistic, feeding on urchins, ascidians or abalones (Guest et al. 2009). _H. rubra_ and _H. erythrogramma_ are herbivorous grazers (Sanderson et al. 1996, Guest et al.
Guest et al.: Spatial variation of biochemical tracers (2008). The dominant brown algal species in these systems include *Ecklonia radiata* (Laminariales) and *Phyllospora comosa* (Fucales), although *Durvillaea potatorum* (Durvillaeales) is also common in the upper sublittoral along exposed coastlines. Little is known about the contribution of these autotrophs to the dominant consumers in these systems, and biochemical tracer techniques are increasingly being considered as a means to resolve their trophic status in Tasmania and elsewhere (Guest et al. 2008).

Here we examined the spatial variability of stable isotope ratios of carbon and nitrogen, and the fatty acid profiles of brown algal, invertebrate (including rock lobster and abalone) and fish reef species at 3 spatial scales (between regions, between reefs within a region, and between parts of plants) that are commonly of interest in trophic studies. Specifically, we addressed: (1) at what spatial scales do stable isotope ratios and fatty acid profiles of target organisms vary, (2) does this level of variability correspond between tracers, and (3) what are the implications for the design and interpretation of trophic studies, particularly when comparing across larger spatial scales?

**MATERIALS AND METHODS**

**Survey design and field methods.** Subtidal marine primary producers (autotrophs) and consumers were sampled at 2 distinct spatial scales to assess the level of variance expressed at spatial scales relevant to the design, analysis and interpretation of diet studies. All samples were collected between June and late August (Austral winter) in cooler water temperatures and outside the peak growing season for the species examined. Samples were collected at (1) a broad regional scale encompassing the east, southeast and west coasts of Tasmania (reefs separated by 100s of km), (2) a smaller (meso) scale (reefs separated by 100 to 1000 m) and (3) between the parts of macroalgal plants. Ideally we would have included each of these scales within a single survey design; however, this would have been logistically impractical and beyond the resources of this study. We see the multi-scale approach as a reasonable compromise given these limitations.

Variation at regional and meso spatial scales was examined using a nested, spatially-hierarchical design. At the broad regional scale, 5 regions were selected that corresponded with biogeographic regions identified by Edgar et al. (1997) based on the faunal and floristic assemblage structure of subtidal reefs (Fig. 1). Within each region, 3 locations separated by a distance of approximately 1 km were selected, and fish, invertebrates and macroalgae were sampled for stable isotope and fatty acid analyses (≥3 replicate individuals were collected where possible). At the meso-scale, 3 locations adjacent to Maria Island (Fig. 1), each separated by a distance of 1 km, were selected at random, and within these locations, invertebrates and macroalgae were sampled at 3 sites each separated by 100 m. The sizes of individuals within a species were similar. The mean size of *Jasus edwardsii* was 99.56 (±1.32 SE) mm carapace length; *Haliotis rubra*, 102.85 (±2.94) mm shell width; and *Notolabrus fucicola* 231 (±3.41) mm fork length. There was no relationship between the size of a species and tracer signatures (analyses not shown, but see Guest et al. 2009 for *J. edwardsii*), so individuals within a species from all size classes were pooled for all analyses.

The final spatial scale examined differences in stable isotope signatures between different parts of 3 brown macroalgal species common on Tasmanian subtidal reefs. Replicate algal samples were collected at random at a single location, and then sections of tissue were collected from designated parts of the algae (e.g. stipe, frond and apex). For *Ecklonia radiata*, the distal parts of the alga such as the apex, upper frond, lower frond and stipe were collected, and additional samples...
were taken from the central blade of the alga. For *Durvillaea potatorum*, the same distal regions were sampled but the midstem was taken at a point midway along the length of the central frond. Parts of *Phyllospora comosa* sampled were the frond, bladder and stipe. With the exception of the lobster *Jasus edwardsii*, abalone *Haliotis rubra* and kelp *E. radiata*, analyses incorporating all species across all locations were not possible due to natural limits to species ranges and/or the absence of species at all sites visited (Table 1).

All samples were frozen after collection, then thawed and rinsed in distilled water prior to processing. Leg muscle from the second or third walking leg of lobsters was removed from the exoskeleton, and a 2 cm³ section of muscle was removed from the fish and from the abalone foot for later analysis. All samples were then freeze-dried for 24 to 48 h and ground using a mortar and pestle. Samples were then partitioned for fatty acid and stable isotope analyses.

**Chemical analyses.** The ratios of ¹³C/¹²C and ¹⁵N/¹⁴N for all samples were calculated as the relative per mille (‰) difference between the sample and the recognised international standard (Pee Dee belemnite carbonate for carbon; air for nitrogen) and analysed on a Micro-mass Isochro-continuous flow isotope ratio mass spectrometer. Precision of the mass spectrometer calculated from duplicate samples was 0.2‰.

Dried animal and algal samples (15 mg of each) were trans-methylated to produce fatty acid methyl esters (FAME) using methanol:chloroform:concentrated hydrochloric acid (10:1:1, 80°C, 2 h). Direct trans-methylation of samples has previously been validated against conventional methods (Christie 1982) for a microheterotroph (Lewis et al. 2000) and for striped trumpeter larvae and rotifers (M. Bransden & G. Dunstan unpubl. data). FAME were extracted into hexane–chloroform (4:1, 3 x 1.5 ml). Gas chromatographic (GC) analyses were performed with an Agilent Technologies 6890N GC equipped with an HP-5 capillary column (50 m x 0.32 mm i.d.), a flame ionisation detector (FID), a split/splitless injector and an Agilent Technologies 7683 auto sampler, using GC operating conditions previously described (Phillips et al. 2003b). Individual components were identified using mass spectral data (Finnigan Thermoquest GCQ GC-mass spectrometer) and by comparing retention time data with those obtained for authentic and laboratory standards.

**Data analysis.** Stable isotope signatures and fatty acid profiles at the regional- and meso-scale were analysed using a 2-way nested design in which variance was partitioned into region, location and site components using univariate and recently developed multivariate (PERMANOVA; Anderson 2005) statistical tests. All factors were treated as random. The objective was to identify the spatial scales at which chemical dietary markers varied and link these patterns to diet-related questions addressed at a number of different spatial scales (i.e. broad regional, intermediate and within producers). Variance components for stable isotope signatures of δ¹³C and δ¹⁵N were calculated using restricted maximum likelihood (REML) estimation (linear mixed-effects model, SYSTAT 12). The residual variance (error term) component in this design relays the unexplained variation expressed principally between replicate samples. Variance components for each of the model terms were expressed as a percentage of the total variance. As there was no correlation between the size of lobster and abalone and δ¹³C and δ¹⁵N stable isotope ratios, irrespective of the spatial scale examined, size was not included as a covariate in these analyses.

**Fatty acid profiles** (comprising 107 individual fatty acids) were analysed using nested 2-way PERMANOVA (Anderson 2005) based on Euclidean distances between samples. PERMANOVA is a multivariate statistical routine for testing the simultaneous response of 1 or more variables within an analysis of variance (ANOVA) experimental design on the basis of any distance measure, using permutation methods (Anderson 2005). A triangular matrix summarising similarity in fatty acid profiles between samples in multi-dimensional space was generated using a Euclidean distance measure on the untransformed percent composition of all 107 fatty acids. Variance components for each of the terms were calculated by equating the PERMANOVA mean squares to their expected values using a multivariate analogue of the ANOVA approach (Searle et al. 1992, Anderson et al.

### Table 1. Species analysed at each of 3 spatial scales: regional, meso and intra-plant for stable isotope (SI) and fatty acid (FA) chemical tracers. n: number of replicates

<table>
<thead>
<tr>
<th>Scale/species</th>
<th>n</th>
<th>SI</th>
<th>FA</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Regional</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Jasus edwardsii</em> (lobster)</td>
<td>3</td>
<td>x</td>
<td>x</td>
</tr>
<tr>
<td><em>Haliotis rubra</em> (abalone)</td>
<td>3</td>
<td>x</td>
<td>x</td>
</tr>
<tr>
<td><em>Ecklonia radiata</em> (brown alga)</td>
<td>3</td>
<td>x</td>
<td>x</td>
</tr>
<tr>
<td><em>Notolabrus fucicola</em> (wrasse)*</td>
<td>3</td>
<td>x</td>
<td></td>
</tr>
<tr>
<td><em>Phyllospora comosa</em> (brown alga)</td>
<td>3</td>
<td>x</td>
<td></td>
</tr>
<tr>
<td><strong>Meso</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>J. edwardsii</em></td>
<td>3</td>
<td>x</td>
<td>x</td>
</tr>
<tr>
<td><em>H. rubra</em></td>
<td>3</td>
<td>x</td>
<td>x</td>
</tr>
<tr>
<td><em>E. radiata</em></td>
<td>3</td>
<td>x</td>
<td>x</td>
</tr>
<tr>
<td><em>Helicocidaris erythrogramma</em> (urchin)</td>
<td>3</td>
<td>x</td>
<td></td>
</tr>
<tr>
<td><strong>Intra-plant</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Durvillaea potatorum</em> (brown alga)</td>
<td>5</td>
<td>x</td>
<td></td>
</tr>
<tr>
<td><em>E. radiata</em></td>
<td>9</td>
<td>x</td>
<td></td>
</tr>
<tr>
<td><em>P. comosa</em></td>
<td>9</td>
<td>x</td>
<td></td>
</tr>
</tbody>
</table>

*Collected using baited fish traps*
2005). The statistical significance of each term was tested using unrestricted permutations of the raw data (n = 999 permutations; Anderson 2005).

Differences in $\delta^{13}$C and $\delta^{15}$N stable isotope signatures between parts of 3 macroalgal species were analysed using a randomised block ANOVA design in which differences between plant blocks were treated as a fixed factor and replicate algae as a blocking factor. This design allowed us to adjust for high variability in stable isotope signatures between individual algal plants (see ‘Results’) whilst comparing plant parts. Where treatment effects were detected, algal parts were contrasted using Tukey’s HSD post hoc tests.

RESULTS

Broad regional scale

The greatest proportion of variance was expressed at the location, followed by the residual (i.e. between replicate samples) spatial scale (Table 2, Figs. 2 & 3). Regional differences by comparison were minor (i.e. many variance components were 0) and the $\delta^{13}$C signature of Ecklonia radiata was the only isotope signature that varied substantially at the regional scale.

The $\delta^{13}$C signatures of all species across regional and locational scales were wide but greatest for Haliotis rubra, ranging from $-24.3$ to $-12.6\%$ (mean ± SE = $-18.6 ± 0.4\%$), followed by Ecklonia radiata ($-24.4$ to $-14.9\%$, mean $= -18.5 ± 0.5\%$), Jasus edwardsii ($-20.2$ to $-13.5\%$, mean $= -15.6 ± 0.2\%$), and Notolabrus fucicola ($-19.0$ to $-14.3\%$, mean $= -16.0 ± 0.2\%$). The range of $\delta^{15}$N signatures was greatest for N. fucicola (16.9 to 7.9\%, mean $= 14.5 ± 0.2\%$), followed by H. rubra (16.1 to 7.3\%, mean $= 8.9 ± 0.3\%$), J. edwardsii (16.2 to 7.9\%, mean $= 13.9 ± 0.2\%$), and E. radiata (9.9 to 5.3\%, mean $= 7.7 ± 0.4\%$).

By comparison, multivariate variance components of fatty acid profiles for Jasus edwardsii and Haliotis rubra were greatest at the residual scale (i.e. 61 and 74\% of the total variance for J. edwardsii and H. rubra, respectively; Table 3). Similar amounts of variance were expressed at the location, residual, and to a lesser extent regional scales for the algae Ecklonia radiata and Phyllospora comosa, whereas variation was greatest between replicate samples for J. edwardsii and H. rubra.

The fatty acids 16:0 (8.7–22.3\% of total), 20:4ω6 (10.6–18.7\%) and 20:5ω3 (4.6–14.0\%) commonly occurred in all species (Table 4). For Jasus edwardsii, 18:1ω9c (16.1 ± 2.5\%) was the most abundant, with relatively high levels of 22:6ω3 (8.5 ± 2.0\%), 18:0 (6.6 ± 0.8\%) and 16:1ω7c (4.0 ± 0.5\%). Haliotis rubra was characterised by relatively high levels of 22:5ω6 (8.6 ± 0.2\%), which was low or absent in all other species. 14:0 (4.7–7.5\%), 18:1ω9c (17.7–21.5\%) and 18:4ω3 (4.6–5.4\%) were common in both brown algal species.
Fig. 2. δ¹³C ratios, displaying variance at regional and location (nested within region) spatial scales for *Jasus edwardsii* (lobster), *Haliotis rubra* (abalone), *Ecklonia radiata* (brown alga) and *Notolabrus fucicola* (wrasse). Horizontal lines are medians, boxes are inter-quartile ranges, and vertical bars are ranges excluding outliers (*x* = outlier, *○* = far outlier).
Fig. 3. $\delta^{15}$N ratios. Other details as in Fig. 2
The greatest variation in stable isotope signatures was again found at smaller spatial scales (i.e. the nested spatial and residual terms; Table 5, Figs. 4 & 5). The only exception to this pattern was recorded for the $^{13}$C signature of *Haliotis rubra*, which displayed considerable variation between locations (Fig. 5), in turn reducing the relative amount of variance expressed at the site level.

The range of $^{13}$C signatures of all species across meso- and site scales was greatest for the urchin *Heliocidaris erythrogramma* ($-20.7$ to $-13.6\%$, mean $= -16.6 \pm 0.3\%$), followed by *Haliotis rubra* ($-24.3$ to $-16.2\%$, mean $= -20.1 \pm 0.4\%$), *Ecklonia radiata* ($-21.2$ to $-15.5\%$, mean $= -18.1 \pm 0.3\%$) and *Jasus edwardsii* ($-17.9$ to $-13.5\%$, mean $= -16.6 \pm 0.2\%$). The range of $^{15}$N signatures was also greatest for *H. erythrogramma* ($14.7$ to $7.9\%$, mean $= 11.2 \pm 0.3\%$), with the range of $^{15}$N signatures across meso- and site scales similar for *J. edwardsii* ($15.7$ to $9.3\%$, mean $= 14.1 \pm 0.2\%$) followed by *E. radiata* ($10.7$ to $5.3\%$, mean $= 8.8 \pm 0.3\%$) and lastly, *H. rubra* ($10.4$ to $7.3\%$, mean $= 9.0 \pm 0.4\%$).
The pattern for the fatty acid profiles was also similar to that found at the regional scale, with the greatest proportion of variance explained by the residual term (Table 6), indicating high variation between samples. In the case of Jasus edwardsii, this is likely to be because location and site did not vary significantly (p > 0.05) explaining little of the overall variance. However, for Haliotis rubra and Ecklonia radiata, spatial terms did explain significant amounts of variance (p < 0.05), but still the proportion of variance unexplained by these terms (and thus by the residual) remained high (>58%).

### Intra-algal differences (within-plant scale)

The most variable algal species in terms of intra-algal differences was Phyllospora comosa, whilst the least variable species was Durvillaea potatorum (Table 7, Fig. 6). The δ¹³C and δ¹⁵N signatures of D. potatorum did not vary significantly between the stipe, mid-stem, mid- and upper blade and apex. In contrast, P. comosa fronds (mean = 10.1 ± 0.4‰) were more enriched in δ¹⁵N than the bladders (mean = 8.5 ± 0.5‰) and stipes (mean = 9.0 ± 0.5‰), and the fronds (mean = –17.2 ± 1.0‰) were more enriched in δ¹³C than the stipes (mean = –20.2 ± 0.4‰; Tukey HSD post hoc test, Table 7). The δ¹³C and δ¹⁵N signatures also varied between plant parts for Ecklonia radiata. The upper

<table>
<thead>
<tr>
<th>Source</th>
<th>df</th>
<th>MS</th>
<th>F ratio</th>
<th>p</th>
<th>Post hoc tests</th>
</tr>
</thead>
<tbody>
<tr>
<td>D. potatorum δ¹³C</td>
<td>Plant part</td>
<td>5</td>
<td>2.292</td>
<td>2.807</td>
<td>0.029</td>
</tr>
<tr>
<td>Block (alga)</td>
<td>8</td>
<td>15.396</td>
<td>18.855</td>
<td>&lt;0.001</td>
<td>(p = 0.015)</td>
</tr>
<tr>
<td>Residual</td>
<td>40</td>
<td>0.817</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>δ¹⁵N</td>
<td>Plant part</td>
<td>5</td>
<td>5.835</td>
<td>3.995</td>
<td>0.005</td>
</tr>
<tr>
<td>Block (alga)</td>
<td>8</td>
<td>0.313</td>
<td>0.214</td>
<td>0.987</td>
<td>(p = 0.03), &gt;Apex</td>
</tr>
<tr>
<td>Residual</td>
<td>40</td>
<td>1.46</td>
<td></td>
<td></td>
<td>(p = 0.004)</td>
</tr>
<tr>
<td>E. radiata  δ¹³C</td>
<td>Plant part</td>
<td>2</td>
<td>19.953</td>
<td>4.578</td>
<td>0.028</td>
</tr>
<tr>
<td>Block (alga)</td>
<td>8</td>
<td>6.408</td>
<td>1.47</td>
<td>0.248</td>
<td>(p = 0.031)</td>
</tr>
<tr>
<td>Residual</td>
<td>15</td>
<td>4.358</td>
<td></td>
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<td></td>
</tr>
<tr>
<td>δ¹⁵N</td>
<td>Plant part</td>
<td>2</td>
<td>6.342</td>
<td>7.325</td>
<td>0.006</td>
</tr>
<tr>
<td>Block (alga)</td>
<td>8</td>
<td>3.248</td>
<td>3.751</td>
<td>0.013</td>
<td>(p = 0.006), &gt;Stipe</td>
</tr>
<tr>
<td>Residual</td>
<td>15</td>
<td>0.866</td>
<td></td>
<td></td>
<td>(p = 0.047)</td>
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</table>
Fig. 4. δ¹³C ratios, displaying variance at location and site (nested with location) spatial scales for *Jasus edwardsii* (lobster), *Haliotis rubra* (abalone), *Ecklonia radiata* (brown alga) and *Heliocidaris erythrogramma* (urchin) at Maria Island. Horizontal lines are medians, boxes are inter-quartile ranges, and vertical bars are ranges excluding outliers (*= outlier, o= far outlier).
Fig. 5. δ¹⁵N ratios. Other details as in Fig. 4.
frond (mean = $-19.4 \pm 0.7\%$) was more enriched in $\delta^{13}C$ than the stipe (mean = $-20.3 \pm 0.6\%$), although this difference was <1% (Table 7). The lower frond of $E. radiata$ (mean = $9.7 \pm 0.5\%$) was also more enriched in $\delta^{15}N$ than the stipe (mean = $7.9 \pm 0.1\%$) and the apex (mean = $7.4 \pm 0.4\%$).

**DISCUSSION**

**At what spatial scales do biochemical tracers vary?**

This study is the first to explicitly partition (1) fatty acid profiles into their constituent spatial components,
and (2) fatty acid profiles in combination with stable isotope ratios for the same organisms at multiple spatial scales. Stable isotope and fatty acid profiles were found to vary at different spatial scales. The greatest amount of variation for stable isotopes was recorded at the intermediate spatial scale (i.e. 100 to 1000 m scale), whereas fatty acid profiles varied most between replicate samples. Whilst this pattern of tracer variation appeared to be generally consistent within a species across scales for stable isotopes and fatty acids (i.e. regional and meso-scales), the pattern did vary between species, such that invertebrate and macroalgal fatty acid profiles displayed differing patterns of variance across the spatial scales considered (Table 3).

One of the most interesting findings of this study was that biochemical tracers were least variable at the largest spatial scales considered. Where regional patterns were observed, the differences were not as anticipated. For example, the most isolated coastline sampled, the west coast, could rarely be distinguished from the other regions (with the exception of δ15N ratios for Ecklonia radiata). In comparison to the east and southeast coasts of Tasmania, the west coast is largely unpopulated and is exposed to heavy oceanic swell from the Southern Ocean (falling within the latitudes of the ‘Roaring 40s’). Whereas the east coast of Tasmania is primarily influenced by the southward flowing East Australian Current (EAC) in summer, the west coast is influenced by the weaker Zeehan Current and receives freshwater and terrigenous input from a number of major freshwater systems. This finding implies that broadscale processes in Tasmania can be characterised without the need to sample intensively at this larger, regional scale.

Intra-plant variation in carbon and nitrogen stable isotope signatures was also detected in this study, notably for the alga Phyllospora comosa, but was not consistent between species or elements. Intra-plant variability in δ15N and δ13C is attributed to the source, storage and metabolism of plant nitrogen and/or carbon (Schmitz & Loban 1976, Vizzini & Mazzola 2003). Understanding intra-plant variability in stable isotope signatures of autotrophs provides a useful guide of how to process autotrophs for trophic studies. No differences were found for δ13C and δ15N for Durvillaea potatorum and δ13C signatures for Ecklonia radiata. For these algal species, small sections of the alga may be considered to represent the entire plant, and for large cumbersome algae such as D. potatorum, thus will reduce the difficulty associated with the transport and storage of whole-alga samples. By contrast, the δ15N of E. radiata and both δ13C and δ15N signatures of P. comosa varied significantly between plant parts. Consequently, it is difficult to provide general advice about the variability of algal plant structures. Additionally, as the part of the plant that is eaten directly by the consumer is often unknown, or contributes to the diet of the consumer via the detrital pathway (e.g. Jaramillo et al. 2003, Guest et al. 2004, Tala & Edding 2005, Decottignies et al. 2007), a composite sample that provides an average isotope signature of the autotroph is recommended.

Intra-plant variability of carbon isotopes has previously been reported for macroalgae. The distal parts of Ecklonia radiata (Fenton & Ritz 1989) and Laminaria longicruris (Stephenson et al. 1984) were more depleted (i.e. more negative) in δ13C (by ~3–7‰ and 2.1–8‰, respectively) than the remainder of the plant. No such pattern was recorded for parts of E. radiata in the current study. The δ13C signatures of all algal parts in the current study were also generally more negative (mean ± SE = –19.4 ± 0.2‰) than that reported for E. radiata previously (mean = –16.1 ± 0.2‰, Fenton & Ritz 1989), suggesting that other factors such as the size/age of the algal fronds (e.g. Stephenson et al. 1984) or the time of collection may provide a better explanation for the observed homogeneity of isotope signatures. No previous studies have examined the intra-plant variability of δ15N signatures in macroalgae. For the seagrass Posidonia oceanica, rhizomes were more enriched in δ15N than leaves, leaf litter and aegragrophiles in all seasons except in spring, where there was no difference between δ15N signatures of rhizomes and leaf litter (Vizzini & Mazzola 2003).

Implications of spatial variation amongst chemical tracers for the design of trophic studies using biochemical tracers

Ecologists have long been aware that spatial variability has important implications for the design, and subsequent analysis, of experiments and surveys (Morrissey et al. 1992, Downes et al. 1993, Underwood & Petraitis 1993). This is because the results of experiments depend critically upon the spatial scale at which they are undertaken. Where the scale of sampling is incongruous with the hypotheses proposed, spatial variation may confound conclusions, making logical interpretation impossible (Underwood 1997). Moreover, where scales of spatial variability are poorly understood, incorrect allocation of sampling effort may act to decrease the probability of detecting differences between samples and treatments (i.e. influence the power of statistical tests).

The potential for spatial variability in stable isotope signatures to confound interpretation of trophic studies was first illustrated by Simenstad et al.’s (1993) study of organic matter derived from kelp production in the Aleutian Islands, Alaska. Simenstad et al. (1993) found

Table 3
that δ¹³C signatures were more variable between sites (i.e. locations separated by 100s of metres and nested within islands) than islands (separated by 10s of km). Such variability may confound interpretation where only a single site or small number of sites are used to characterise the stable isotope signature of islands at the scale of the entire Aleutian island system. Similarly, Jennings et al. (1997) found that high variability in δ¹³C signatures between sites for a range of reef species obscured attempts to determine the importance of alternate trophic pathways. In the current study, we also detected significant and high variation for stable isotope signatures at the level of sites relative to the broader regional and the smaller replicate sample (i.e. residual term) scales sampled. Failure to incorporate sufficient sampling at this smaller spatial scale may therefore hinder attempts to adequately characterise and understand trophic patterns at larger geographic spatial scales.

In contrast to stable isotopes, little is known about the spatial scales at which fatty acid chemical tracers vary. Currently, there is limited information upon which to base the allocation of sampling effort across a range of spatial scales pertinent to the trophic questions studied. Moreover, when used in conjunction with other chemical tracers, such as stable isotopes, it is currently not possible to know whether the design used for one chemical tracer is also appropriate for another. In the current study, spatial variation in fatty acid profiles for all taxa was generally greatest among individual replicate samples irrespective of the spatial scale examined. This finding implies that high sample variability is a potential source of confounding where insufficient replicates are used to characterise the fatty acid profiles of target species. As consequence, future application of fatty acid profiles to examine trophic relationships should maximise replication among individuals within treatments to determine dietary relationships.

The results of this study suggest that broadscale comparisons are likely to be confounded by insufficient sampling at smaller spatial scales. This is evident in the variation in stable isotope signatures that was greatest between sites (and to a lesser extent between samples), whereas variation in fatty acid profiles was greatest between replicate samples. In the case of fatty acid profiles of consumers such as abalone and rock lobster, residual variance accounted for the majority of the variance expressed (>60%). In trophic surveys, high variation in chemical tracers at small to intermediate spatial scales may lead to the incorrect attribution of a food source to a consumer’s diet, and/or incorrect assignation of trophic status. For example, shifts of ~3‰ in δ¹⁵N (e.g. Minagawa & Wada 1984, McCutchan et al. 2003) between a producer and each higher trophic level are considered to reflect the trophic status of a consumer. Variation in δ¹⁵N that is due to the spatial scale of sampling may therefore lead to incorrect assignation of trophic status. Similarly, high variability among replicate samples in fatty acid profiles, or insufficient replication among individuals may hinder the clarification of trophic relationships at larger spatial scales. We emphasise that appropriate allocation of effort does not necessarily mean increasing the number of replicates across the design, but that replicates are correctly assigned to the scale at which most variability occurs to better characterise that variation in biochemical tracers in order to avoid erroneous conclusions.

**Scales of variability: causal mechanisms**

Biochemical tracer signatures of consumers may be influenced by the age, size and sex of an individual (i.e. for fatty acids, those characteristics that influence consumer behaviour and/or metabolism, thus altering consumer fatty acid profiles). In the current study, there was no relationship between consumer size and fatty acid profile, although this may be due to the homogeneous size distribution of the populations sampled. The diverse diets of lobsters and wrasses may partly explain the high variability in fatty acid profiles among individuals even of the same size class. Moulting or reproductive stage may also influence lobster fatty acid profiles (Ritar et al. 2003). Where possible, it would be advantageous to standardise factors such as the size, sex and moult stage of individuals to reduce variability between samples.

Small-scale spatial variation can often be misconstrued for temporal change between sampling events if the variance at these 2 scales is not explicitly and carefully partitioned in the sampling design (Thrush et al. 1996, Underwood 1997). Such variation, due to small-scale patchiness and spatial heterogeneity, may often exceed temporal variation in marine systems (Hirst & Kilpatrick 2007) and is a common source of experimental confounding in field studies. Alternately, as environmental and physiological factors may change over time/season, temporal variation in biochemical profiles may be interpreted as spatial variation where spatial and temporal variation are not considered explicitly. In the current study, the potential influence of temporal variability was minimised by sampling all spatial scales within a 3 mo period (and for most, within a 2 mo period) during winter months that displayed colder water temperatures and slower growth rates, thus reducing the likelihood of rapid physiological and/or environmental change across the period considered.
Nested sampling designs may also provide important clues about the scale at which ecological processes operate (Underwood 1997), by allowing identification of the spatial scales at which chemical tracers vary. In this study, stable isotope signatures were found to vary principally between sites (and to a lesser extent between samples), whereas fatty acid profiles varied primarily between replicate samples and, depending on the species examined, to a lesser degree between sites. This finding supports the notion that δ13C and δ15N stable isotope signature variation is primarily influenced by environmental factors and secondarily by metabolism. By contrast, fatty acid profiles may primarily be influenced by metabolism and secondarily by environmental factors. Metabolism is influenced by food availability and quality, growth rate, the reproductive stage of the organism and the functional role of the tissues analysed (Chandrapavan et al. 2009). Such insights allow us both to allocate sampling effort accordingly for future survey designs, but also allow explicit hypothesis testing of the potential causes of tracer variability in experimental studies, thereby improving the application of biochemical tracers to understanding dietary relationships.

CONCLUSIONS

This study is one of the first to explicitly partition spatial variance for chemical tracers and the first to examine spatial variance simultaneously for stable isotopes and fatty acids. Stable isotopes varied principally between sites nested within locations sampled at broader spatial scales. In comparison, fatty acid profiles varied principally between replicate samples nested within sites. In general, little variance was expressed in fatty acid profiles at the broad geographic scale in comparison to the smaller scales sampled. The differing spatial variance structure for stable isotopes and fatty acids may suggest that these chemical tracers are influenced to a varying degree by factors that operate at different spatial scales (i.e. environmental versus physiological factors). However, these differences may potentially strengthen their application when used in combination because they allow studies to measure alternate aspects of food web structure. The results indicate that sampling designs that are tailored for one chemical tracer may not necessarily be suitable for another. Our results indicate that whilst sampling at the scale of sites is important when using stable isotopes, greater emphasis should be placed on collecting more individual replicate samples when using fatty acid techniques. In most cases, this can be achieved by allocating differing levels of sampling intensity for different chemical tracers within the overall design, without compromising the sensitivity of the design. The results of this study also clearly indicate that failure to undertake replicate sampling at smaller to intermediate spatial scales may lead to potentially confounded interpretations about patterns at broader spatial scales.

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