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Short communication

Within and among-site variability in $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ for three estuarine producers, *Sporobolus virginicus*, *Zostera capricorni*, and epiphytes of *Z. capricorni*

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Abstract

Carbon and nitrogen stable isotope ratios of three estuarine autotrophs (the seagrass, *Zostera capricorni* and its epiphytic algae, and the saltcouch grass *Sporobolus virginicus*) were measured within and among sites in Southern Moreton Bay, Southeast Queensland. For all taxa, isotope ratios were significantly affected by the position of a plant within a site. Carbon signatures of *S. virginicus* were more enriched at upper elevations (-14.3‰), and more depleted at the lower edge (-15.0‰). Small but significant differences for *S. virginicus* were also found between edge (-14.7‰) and interior (-14.9‰) positions. *Z. capricorni* was more depleted in ^{15}N at edge (5.9‰) positions than the interior (6.2‰). The seagrass epiphytes varied along the elevation gradient, being more depleted in ^{13}C at the upper (-19.7‰) than the lower (-19.3‰) edge. This small within-site variation ($< 1\text{‰}$) may result from differences in the physical characteristics among the sites that influence the productivity of plants and thereby their isotope ratios, but would not preclude the use of carbon and nitrogen stable isotopes in small-scale food web studies. At a larger scale, isotope ratios differed significantly among sites separated by several kilometres and the range of this variation was greater for all taxa than at the within-site scale. Differences among sites are probably due to variation in nutrient source and hydrodynamics.

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1. Introduction

Naturally occurring stable isotope ratios of carbon and nitrogen are useful tools for tracing the fate of assimilated nutrients (France, 1995), and clarifying the structural dynamics of food webs (Michener and Schell, 1994). The success of stable isotope ratios in food web ecology, however, depends upon isotope ratios changing in predictable ways as elements cycle through the biosphere (Peterson and Fry, 1987). Isotope ratios of a single producer may be influenced by two broad means, firstly, changes in productivity, and secondly by differences in nutrient source. Changes in productivity may in turn be affected by differences in the physical characteristics of a plant's position (e.g. Lei and Lechowicz, 1997). Differences in nutrient source may also be influenced by plant position (e.g. Marguillier et al., 1997) but can result from the spatial variation of hydrological processes (Hughes et al., 1998).

In terrestrial systems, physical characteristics such as light intensity have been shown to vary amongst positions within a site (e.g. Ehleringer et al., 1986). In aquatic systems, differences in physical characteristics including light intensity (Hemminga and Mateo, 1996), water turbulence (Finlay et al., 1999), water depth (Grice et al., 1996), day-length and pH (Thompson and Calvert, 1994) and nutrient source (Marguillier et al., 1997) have been demonstrated to influence plant isotope ratios. Of the few studies that explicitly examine the influence of plant position on isotope ratios, the spatial scale within sampling sites is often unspecified (e.g. Boyce et al., 2001), or at the scale of several kilometres (e.g. Jennings et al., 1997). Hence, the small-scale variability of stable isotope ratios is often neglected.

Understanding the spatial variability of stable isotope ratios has important implications for their use in clarifying food web dynamics for two reasons. Firstly, given that physical characteristics may vary within a site, the scale of isotopic variation must be determined before the chosen sampling regime can be assumed to be representative of that habitat. Where the spatial variation of isotope ratios for a single primary producer remains undetected, spurious conclusions regarding the food web dynamics of that system may result (Boon and Bunn, 1994). Secondly, where the spatial variation of isotope ratios for primary producers is known, it can be used to track the food source of a consumer over different spatial scales (e.g. Melville and Connolly, 2003).

This study examined the small and large-scale variability in isotope ratios of primary producers located at different positions within replicate sites of estuarine habitat. The primary producers of interest were the saltcouch grass, *Sporobolus virginicus*, the seagrass *Zostera capricorni*, and the epiphytic algae growing on *Z. capricorni*. These plants were selected because they are abundant and conspicuous primary producers along the subtropical coast of Eastern Australia (Abal and Dennison, 1996). As such, they are the subject of many studies examining both food web processes and habitat use in estuarine and off-shore habitats in this region (e.g. Laegdsgaard and Johnson, 1995; Thomas and Connolly, 2001).

2. Methods

The study location was at the Southern end of Moreton Bay, Queensland, Australia (27°50'S, 153°23'E), and sampling was done in April 2001. Five sites of *S. virginicus*,

and five of *Z. capricorni* and its associated epiphytic algae were selected. Sites were defined and selected based on discrete habitat boundaries denoted by an abrupt change in vegetation type. *S. virginicus* sites were defined by a distinct mangrove boundary on three sides and the water's edge on the fourth. *Z. capricorni* sites were defined by a distinct absence of vegetation as the seagrass habitat gave way to adjacent mudflat or deep-water habitat. Sites were between 2 and 30 km apart. The average size of a saltmarsh site was 4.9 ha (± 0.7 ha, 1 S.E.), seagrass sites averaged 3.8 ha (± 0.9 ha) of continuous seagrass.

Within each site, five positions were chosen: upper, lower, left, right and interior. The positions within each site were determined using a theodolite where the upper edge was defined as the point of highest elevation. The average height difference between the upper and lower positions amongst sites for seagrass and seagrass epiphytes was 18.8 ± 7.2 and 23.0 ± 3.6 cm for saltmarsh. 'Edge' literally meant the outer most plants along the site perimeter. Three samples of *S. virginicus* or six samples of *Z. capricorni* (three to be used for analysis of epiphyte signatures) were taken at randomly selected sites at each of the five positions (i.e. 15 samples per site). All samples were immediately frozen until processing.

All samples were thawed and rinsed to remove sediments prior to processing. Only green leaves of saltcouch grass and seagrass were chosen. Seagrass leaves were cleaned free of epiphytes using a scalpel. Epiphytes were obtained from separate seagrass samples by gently scraping a scalpel along seagrass blades taking care not to remove the seagrass epidermis. Epiphyte samples included a mix of calcareous encrusting algae, coralline red algae, small filamentous algae and numerous diatom species. All samples were dried at 60 °C for 24 h, ground, placed into tin capsules and their isotope ratios analysed on an Isoprime mass spectrometer. The ratios of $^{13}\text{C}/^{12}\text{C}$ and $^{15}\text{N}/^{14}\text{N}$ were calculated as the relative per mil (‰) difference between the sample and recognised international standards (PeeDee belemnite limestone carbonate for carbon; air for nitrogen).

Differences in isotope ratios of primary producers located along the elevation gradient within a site, and in the edge and interior positions, were tested separately for each primary producer using planned contrasts. Planned contrasts allow for the comparison of means between groups or combinations of groups determined *a priori* where each contrast tests specific hypotheses (Quinn and Keough, 2002). In this case, differences between elevations were tested using a set of three contrasts comparing mean isotope ratio between lower and middle (right, left and interior positions combined), middle and upper, and lower and upper elevations. When all three contrasts were significant, and differences were in a linear pattern (e.g. upper > middle > lower), a significant elevation gradient was considered to have been demonstrated. A separate contrast was used to compare mean isotope ratios between edge (lower, upper, left and right combined) and interior positions.

Differences in isotope ratios among sites were also examined separately for each primary producer using a two-way analysis of variance. The two factors were site (random, five levels) and position (fixed, five levels). The position factor was not directly of interest (position being fully analysed by contrasts) but was included in the analysis of variance to allow the variation due to sites to be more precisely partitioned.

3. Results

Primary producer isotope ratios differed significantly among positions within a site. However, the pattern of variation in isotope ratios differed among primary producers and between elements (Table 1).

Carbon ratios of *S. virginicus* differed significantly with elevation gradient, and between interior and edge positions (Fig. 1a). The gradient was significantly higher at the upper position than the middle or lower positions, and higher in the interior than the edge. However, as this value lies within the precision of the mass spectrometer ($\pm 0.2\%$), the difference between edge to interior isotope ratios of this producer must be viewed with caution. Differences amongst isotope ratios along the elevation gradient for this producer, though statistically significant, were less than 1‰. The isotope ratios for nitrogen of *S. virginicus* did not differ significantly among positions (overall mean = $5.5 \pm 0.7\%$).

The carbon isotope ratios of *Z. capricorni* did not differ significantly among positions within a site (overall mean = $-11.7, \pm 0.1\%$). However, the isotope ratios of nitrogen for *Z. capricorni* were significantly higher in the interior than at the edge of a site (Fig. 1b), but not along the elevation gradient.

The $\delta^{13}\text{C}$ ratios of seagrass epiphytes were significantly higher at the lower than the middle or upper positions in a site (Fig. 1c), and the range of isotopic variation along this gradient was larger than that for the other producers examined. However, the $\delta^{13}\text{C}$ ratios did not differ significantly between interior and edge positions. Nitrogen isotope ratios did not differ among any positions within a site for seagrass epiphytes (overall mean = $4.6 \pm 0.2\%$).

The isotope ratios differed significantly among sites but only for some primary producer/element combinations (Table 1). The variation among sites was least for *S. virginicus* for both carbon (range = 0.35%) and nitrogen (range = 2.37%). Seagrass epiphyte

Table 1
Mean squares and statistical significance for planned contrasts and ANOVA

Source of variation	d.f.	<i>S. virginicus</i>		<i>Z. capricorni</i>		Seagrass epiphytes	
		$\delta^{13}\text{C}$	$\delta^{15}\text{N}$	$\delta^{13}\text{C}$	$\delta^{15}\text{N}$	$\delta^{13}\text{C}$	$\delta^{15}\text{N}$
(a) Contrasts							
Edge: interior	1	0.51 ^a	<0.01	0.16	0.95 ^c	0.34	0.67
Upper: lower	1	3.99 ^a	36.45 ^a	0.70	0.24	14.20 ^a	0.71
Upper: middle	1	3.94 ^a	41.20 ^a	0.50	0.79 ^c	4.33	0.39
Lower: middle	1	0.21 ^b	0.95	0.01	0.010	6.42 ^c	0.18
(b) Main effects							
Position	4	1.60	15.45 ^b	0.57	0.37	3.98	0.36
Site	4	0.25	1.81	9.88 ^a	16.30 ^a	42.57 ^a	15.90 ^a
Position x site	16	0.73 ^a	3.10 ^a	0.39	0.31	2.91 ^b	1.74
Residual		<0.01	0.98	0.25	0.19	1.13	1.40

(a) Planned contrasts edge: interior and elevation and (b) ANOVA to partition the variance attributed to site. For contrasts, an elevation gradient was considered present where all three contrasts (upper:lower; upper:middle; and lower:middle) were significant.

^a $P < 0.001$.

^b $P < 0.01$.

^c $P < 0.05$.

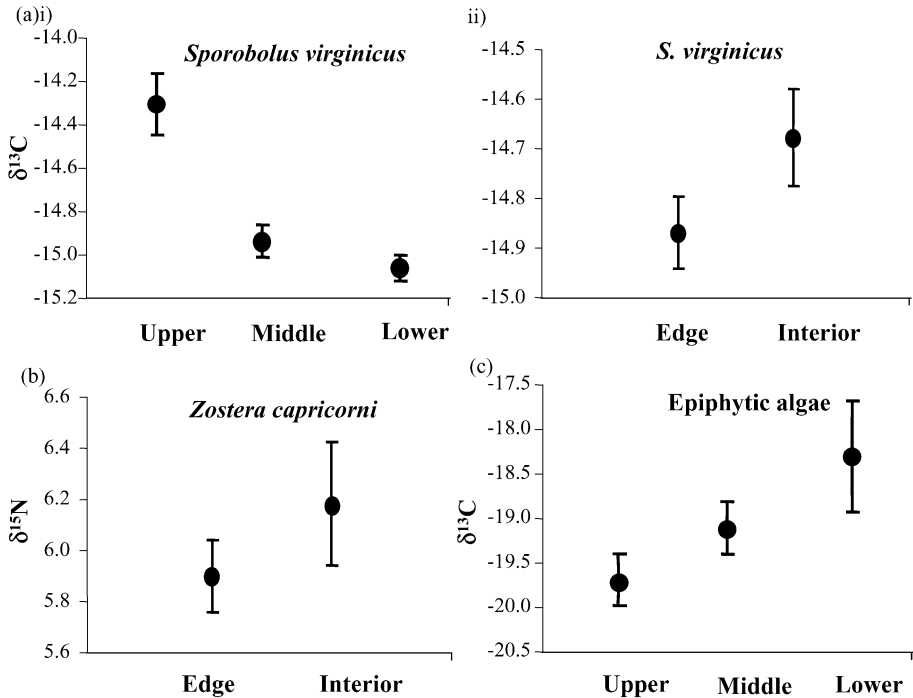


Fig. 1. (a) Mean carbon isotope ratios (± 1 S.E.) of *S. virginicus* from, (i) upper, middle (interior, left and right) and lower and, (ii) edge (upper, lower, left and right) and interior positions for carbon. (b) Mean nitrogen isotope ratios (± 1 S.E.) of *Z. capricorni* from edge (upper, lower, left and right) and interior positions. (c) Mean carbon isotope ratios (± 1 S.E.) of epiphytic algae from upper, middle (interior, left and right) and lower positions. All means and standard error are of the five sampling sites.

isotope ratios were most variable among sites for both carbon (range = 4.36‰) and nitrogen (range = 2.56‰). The variability of both carbon (range = 1.94‰) and nitrogen (range = 2.53‰) isotope ratios for *Z. capricorni* among sites was intermediate between the *S. virginicus* and the seagrass epiphyte.

4. Discussion

The carbon and nitrogen stable isotope ratios of the primary producers varied among positions within a site. However, the pattern of variation differed among primary producers and elements. Although the mechanisms driving this variation were not tested here, such differences are most likely due to differences in productivity (pre-dominantly affecting carbon), or sources of nutrient (affecting carbon or nitrogen) for primary producers lying at different positions within a site, or throughout the estuary.

Previous studies have shown primary productivity in aquatic systems to be influenced by changes in light conditions (e.g. Grice et al., 1996), nutrient availability (e.g. Silbertstein

et al., 1986), and inundation frequency (e.g. Lugo et al., 1980). Changes in productivity may in turn influence the isotope ratio of primary producers by altering the demand for carbon (Grice et al., 1996; Hemminga and Mateo, 1996). Where the rate of productivity is high, carbon demand is high and the ability of the plant to discriminate against the heavier isotope (^{13}C) is reduced (Udy and Dennison, 1997) resulting in isotope ratios enriched (less negative) in ^{13}C . In the present study, carbon isotope ratios were more enriched in ^{13}C along an elevation gradient for *S. virginicus* and the epiphyte of *Z. capricorni*, and in the interior of the site for *S. virginicus*. The enriched ratios of *S. virginicus* at upper elevations compared to lower may be due to differences in inundation frequency along the elevation gradient influencing soil chemistry and thus productivity. The change in carbon isotopes along the elevation gradient for epiphytes of *Z. capricorni*, are more likely to be a result of differences in light conditions.

The isotope ratio of the primary producer may also be influenced by the assimilation of nutrients from different sources that have distinct isotope ratios. Differences in nutrient source for primary producers at various positions within a site may be due to small-scale changes in hydrology, or proximity to adjacent habitat (and therefore an alternative source of nutrients, e.g. Marguillier et al., 1997). Proximity to adjacent habitat and therefore an alternative nutrient source, may provide another explanation for the isotopic gradient in carbon ratios for *S. virginicus*, and in nitrogen ratios for *Z. capricorni*. For the seagrass, those plants positioned at the edge of the site may rely on leaf rather than root uptake of nitrogen (Pederson et al., 1997) due higher wave energy (Eckman and Duggins, 1993) causing a reduction in the leaf boundary layer (Fonseca and Fisher, 1986) making water column sources of nitrogen more available. Equally, isotopic differences observed between sites within this study may be due to differences in nutrient source at a larger scale resulting from geomorphological and hydrological characteristics of the estuary and the transportation of nutrients (Lee, 1995).

Despite the observed within-site variability of isotope ratios of carbon and nitrogen, it is important to note the small range of variability measured, which is consistent among primary producers. The range of within-site isotopic variability was always less than 2‰ and mostly less than 1‰. Such variation is negligible in terms of food web studies, and does not preclude the use of stable isotope ratios for discrimination between potential food items at the small (~200 m) scale. Among-site variation of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ ratios for each producer however, was larger than that observed at the within-site scale. Whilst the among-site variation recorded in this study (<5‰) is small compared to previous large-scale studies (>10‰) (e.g. Boon and Bunn, 1994), capturing any large-scale variation in isotope ratios remains important when the data are used to understand large-scale food web processes. Additionally, such among-site variation may be useful for spatial correlation of food webs for more mobile species (e.g. Melville and Connolly, 2003).

The aim of this study was to examine the small and large-scale variability of carbon and nitrogen isotope ratios of three estuarine primary producers for use in food web studies. The results demonstrated that there is small but significant within-site variation for some primary producers and some elements. Most variation, however, is present among sites at different locations throughout the estuary. Given that few food web studies using stable isotope analysis examine the spatial variability in isotope ratios of autotrophs, this information places greater confidence in the utility of stable isotopes for small-scale studies.

For large-scale food web studies, this information provides evidence to support the need for an examination of spatial variability of stable isotope ratios. Finally, this study provides an opportunity for further work into understanding the mechanisms by which the spatial variability of primary producer isotope ratios is determined.

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References

- Abal, E.G., Dennison, W.C., 1996. Seagrass depth range and water quality in Southern Moreton Bay, Queensland, Australia. *Mar. Freshwater Res.* 47, 763–771.
- Boon, P.I., Bunn, S.E., 1994. Variations in the stable isotope composition of aquatic plants and their implications for food web analysis. *Aquat. Bot.* 48, 99–108.
- Boyce, M.C., Lavery, P., Bennett, I.J., Horwitz, P., 2001. Spatial variation in the $\delta^{13}\text{C}$ signature of *Ruppia megacarpa* (Mason) in coastal lagoons of Southwestern Australia and its implication for isotopic studies. *Aquat. Bot.* 71, 83–92.
- Eckman, J.E., Duggins, D.O., 1993. Effects of flow speed on growth of benthic suspension feeders. *Biol. Bull.* 185, 28–41.
- Ehleringer, J.R., Field, C.B., Lin, Z.F., Kuo, C.Y., 1986. Leaf carbon isotope and mineral composition in subtropical plants along an irradiance cline. *Oecologia* 70, 520–526.
- Finlay, J.C., Power, M.E., Cabana, G., 1999. Effects of water velocity on algal carbon isotope ratios for river food web studies. *Limnol. Oceanogr.* 44, 1198–1203.
- Fonseca, M.S., Fisher, J.S., 1986. A comparison of canopy friction and sediment movement between four species of seagrass with reference to their ecology and restoration. *Mar. Ecol. Prog. Ser.* 29, 15–22.
- France, R.L., 1995. Differentiation between littoral and pelagic foodwebs in lakes using stable carbon isotopes. *Limnol. Oceanogr.* 40, 1310–1313.
- Grice, A.M., Loneragan, N.R., Dennison, W.C., 1996. Light intensity and the interactions between physiology, morphology and stable isotope ratios in five species of seagrass. *J. Exp. Mar. Biol. Ecol.* 195, 91–110.
- Hemminga, M.A., Mateo, M.A., 1996. Stable carbon isotopes in seagrasses: variability in ratios and use in ecological studies. *Mar. Ecol. Prog. Ser.* 140, 285–298.
- Hughes, C.E., Binning, P., Wilgoose, G.R., 1998. Characterisation of the hydrology of an estuarine wetland. *J. Hydrol.* 211, 34–49.
- Jennings, S., Renones, O., Morales-Nin, B., Polunin, N.V.C., Moranta, J., Coll, J., 1997. Spatial variation in the ^{15}N and ^{13}C stable isotope composition of plants, invertebrates and fishes on Mediterranean reefs: implications for the study of trophic pathways. *Mar. Ecol. Prog. Ser.* 146, 109–116.
- Laegdsgaard, P., Johnson, C.R., 1995. Mangrove habitats as nurseries: unique assemblages of juvenile fish in subtropical mangroves in Eastern Australia. *Mar. Ecol. Prog. Ser.* 126, 67–81.
- Lee, S.Y., 1995. Mangrove outwelling: a review. *Hydrobiologia* 295, 203–212.
- Lei, T.T., Lechowicz, M.J., 1997. The photosynthetic response of eight species of *Acer* to simulated light regimes from the centre and edges of gaps. *Funct. Ecol.* 11, 16–23.
- Lugo, A.E., Cintron, G., Geonaga, C., 1980. The mangrove ecosystem under stress. In: *Memoirs of the seminar on the scientific study and human impact on the mangrove ecosystem*, UNESCO, Montevideo (Uruguay), ROSTLAC. pp. 261–285.

- Marguillier, S., van der Velde, G., Dehairs, F., Hemminga, M.A., Rajagopal, S., 1997. Trophic relationships in an interlinked mangrove-seagrass ecosystem as traced by $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$. *Mar. Ecol. Prog. Ser.* 151, 115–121.
- Melville, A.J., Connolly, R.M., 2003. Spatial analysis of stable isotope data to determine primary sources of nutrition for fish. *Oecologia* 136, 499–507.
- Michener, R.H., Schell, D.M., 1994. Stable isotope ratios as tracers in marine aquatic food webs. In: Lajtha, K., Michener, R.H. (Eds.), *Stable isotopes in ecology and environmental science*. Blackwell Science Publications, Oxford, pp. 1–21.
- Pederson, M.F., Paling, E.I., Walker, D.I., 1997. Nitrogen uptake and allocation in the seagrass *Amphibolus antarctica*. *Aquat. Bot.* 56, 105–117.
- Peterson, B.J., Fry, B., 1987. Stable isotopes in ecosystem studies. *Ann. Rev. Ecol. Syst.* 18, 293–320.
- Quinn, G.P., Keough, M.J. 2002. *Experimental Design and Data Analysis for Biologists*. Cambridge University Press, Cambridge.
- Silbertstein, K., Chiffings, A.W., McComb, A.J., 1986. The loss of seagrass in Cockburn Sound, Western Australia. III. The effect of epiphytes on productivity of *Posidonia australis* (Hook F.). *Aquat. Bot.* 24, 355–371.
- Thomas, B.E., Connolly, R.M., 2001. Fish use of subtropical saltmarshes in Queensland, Australia: relationships with vegetation, water depth and distance onto the marsh. *Mar. Ecol. Prog. Ser.* 209, 275–288.
- Thompson, P.A., Calvert, S.E., 1994. Carbon-isotope fractionation by a marine diatom: the influence of irradiance, day-length, pH and nitrogen source. *Limnol. Oceanogr.* 39, 1835–1844.
- Udy, J.W., Dennison, W.C., 1997. Growth and physiological responses of three seagrass species to elevated nutrients in Moreton Bay, Australia. *J. Exp. Mar. Biol. Ecol.* 217, 253–277.