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Application of isotope mixing models to discriminate dietary sources over small-scale patches in saltmarsh

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ABSTRACT: Intertidal grazing crabs play an important role in estuarine ecosystems, transforming carbon fixed by autotrophs into forms available to a wide range of consumers. Whether the autotrophic carbon is derived primarily from intertidal vegetation or microalgae is an important question to be resolved, as the modification of estuaries alters the balance between these potential food sources, and restoration efforts are best guided by an understanding of the primary drivers of ecosystem energy flow. We utilised the mosaic of C_3 and C_4 vegetated patches in a temperate saltmarsh to clarify the relative contributions of potential sources of carbon and nitrogen to the diet of 2 species of grapsid crabs: *Paragrapsus laevis* and *Helograpsis haswellianus*. The 2 vegetation communities occupied the same position in relation to tidal elevation. We analysed stable isotopes of carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) to discriminate 3 potential sources of dietary carbon using an IsoSource mixing model: microphytobenthos (MPB); fine benthic organic matter (FBOM); and fresh plant material. We found enrichment of $\delta^{13}C$ and depletion of $\delta^{15}N$ in crabs sampled from patches of the C_4 grass *Sporobolus virginicus*, consistent with the use of C_4 derived carbon compared to those sampled in the C_3 chenopod *Sarcocornia quinqueflora*. However, microphytobenthos was similarly depleted within large patches of *S. virginicus*, implying uptake of dissolved inorganic carbon originating from plant respiration. Multiple-source mixing (IsoSource) models indicated a primary role for MPB and FBOM in crab diets, with locally derived plant material making little contribution to crab diet. The result contrasts with those of studies from subtropical and tropical systems.

KEY WORDS: Microphytobenthos · Grapsid crab · Saltmarsh · Intertidal · Stable isotopes · Carbon · Nitrogen

INTRODUCTION

Autotrophs occupying estuarine intertidal environments play an important role in fixing atmospheric and dissolved inorganic carbon, subsequently utilised in estuarine ecosystems (Kneib 1997, 2002) or sequestered and stored in estuarine soils (Chmura et al. 2003). A range of autotrophs contribute to the productivity of intertidal flats, including macrophytes (mangrove, saltmarsh and seagrass), phytoplankton, and microphytobenthos adhering to the surface sediment. Marine macroalgae may also be locally important (Karsten & West 1993).

Early studies emphasised the importance of plant detritus to estuarine and nearshore ecosystems, derived from both mangrove (Giddens et al. 1986,

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Robertson 1986) and saltmarsh (Odum 1968). Observations of feeding behaviour of organisms (Robertson & Daniel 1989, Micheli 1993), and estimates of carbon mass balance led to conceptual models positioning macrophytic plant production as foundational to productive estuarine and nearshore ecosystems (Teal 1962, Lee 1999). However, 2 lines of evidence prompted a revision of sources of heterotrophic carbon. First, the high C:N ratio of mangrove plant detritus suggested that sole dependence on mangrove leaf material for nutrition was unlikely, even amongst leaf-shredding grapsid crabs (Skov & Hartnoll 2002). Secondly, the application of stable isotope ecology in intertidal environments demonstrated a disparity between grazing herbivore isotope signatures and macrophytes highly depleted in $\delta^{13}C$ (France 1998, Bouillon et al. 2002, Mazumder & Saintilan 2010). Attention has therefore turned to microphytobenthos (MPB) as an important contributing source of dietary carbon for intertidal heterotrophs (Bouillon et al. 2008).

The importance of MPB to the diet of intertidal crabs has been demonstrated by exclusion experiments (Reinsel 2004) and observation of naturally occurring and labelled isotopes. Oakes et al. (2010) used labelled isotopes to clarify the contribution of MPB and mangrove leaf material to the diet of grazing crabs and foraminifera. They found strong differences between species, with the grapsid crab *Parasesarma erythrodactyla* consuming predominantly leaf material and up to 33% MPB, while the ocypodid crab *Australoplax tridentata* consumed mostly MPB and minimal leaf material. These observations concurred with other studies indicating grapsids in mangrove relying more heavily on leaf litter (Lee 1998), while ocypodids in the same settings characteristically consume bacteria and MPB (Rodelli et al. 1984, Dye & Lasiak 1986).

The presence of the C_4 grass *Sporobolus virginicus* (salt couch) as an important component of the saltmarshes of eastern Australia provides an opportunity to test hypotheses concerning the relative contribution of saltmarsh plant material and MPB to the diet of saltmarsh heterotrophs. This is made possible by the narrow range of movement of crabs feeding in the saltmarsh — less than 10 m in studies in Australia and the USA (Guest et al. 2004, Morgan et al. 2006). Saintilan & Mazumder (2010) utilised the mosaic pattern of saltmarsh plants to compare the diet of crabs in the relatively $\delta^{13}C$-enriched *S. virginicus* patches with those in the $\delta^{13}C$-depleted *Sacrocornia quinqueflora* (samphire) patches. They found a consistent enrichment in crab $\delta^{13}C$ signatures within *S. virginicus* patches, suggesting a contribution of saltmarsh plant detritus to crab diet. In the present study, we sampled the obvious available carbon sources in replicate *S. virginicus* and *S. quinqueflora* patches to determine, through analysis of stable isotopes of carbon and nitrogen, the relative contribution of autotrophic sources to the diet of the 2 dominant species of saltmarsh crab; *Paragrapsus laevis* and *Helograpsus haswellianus*. We utilised a dual isotope mixing model (Phillips & Gregg 2003) to test probable sources of dietary carbon: the cellulose of plant material (the C_4 saltmarsh grass *S. virginicus* and the C_3 chenopod herb *S. quinqueflora*); MPB; and fine benthic organic material (FBOM) incorporating mostly decomposed detritus. Both species of crab were sampled in both habitats. Therefore, the sampling of crabs and probable food sources across habitats and sites allowed the testing of the following hypotheses:

(1) that crabs derive their dietary requirements from food sources available within small habitat patches,

(2) that the 2 common saltmarsh crab species are selective in their utilisation of plant material as a food source, and

(3) that MPB contributes significantly to crab diet in all patches.

MATERIALS AND METHODS

Study locations

The study was conducted at 2 separate locations containing saltmarsh habitat within Brisbane Water (Fig. 1), the marine entrance of the Hawkesbury-Nepean estuary to the north of Sydney, Australia. The locations included the saltmarsh areas of Empire Bay and Davistown.

Each of these locations were chosen for the study based on their vegetation coverage, types of saltmarsh vegetation present, their inundation characteristics and ease of access to each site. Generally, each location was dominated by the saltmarsh vegetation *Sarcocornia quinqueflora* and *Sporobolus virginicus*, with each generally present as homogenous patches separate from one another. The patches of saltmarsh at Empire Bay were larger (ca. 0.3–1 ha) than those at Davistown (ca. 0.1 ha). Adjacent fringing mangrove stands, which consisted primarily of *Avicennia marina*, were also present within both areas. Each location was generally inundated once a month for 9 mo of the year during spring tide events.

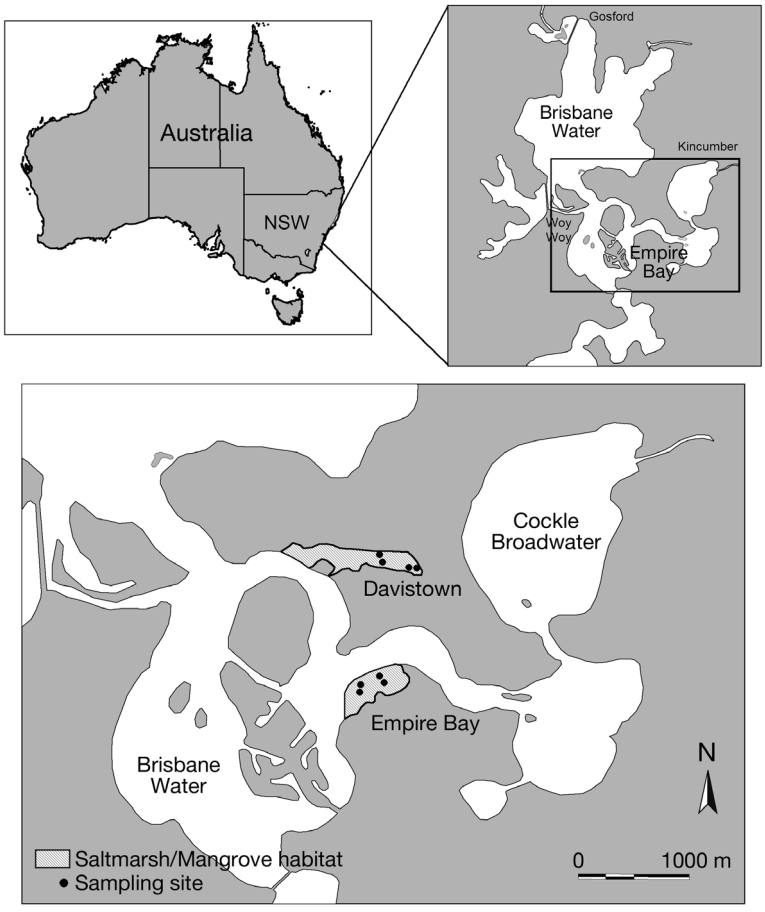

Fig. 1. Saltmarsh areas within Brisbane Water sampled during the study

Sampling methods

Pit traps (Salmon & Hyatt 1983) were used to sample shore crabs residing in saltmarsh habitat during the spring high tide in August 2009. Four traps were placed within each of 4 sites at each location (i.e. Empire Bay and Davistown), giving a total of 8 sites with 32 traps. To gain an understanding of the differences in isotopic signatures of shore crabs residing within different vegetation types, 2 of the 4 sites at each location were positioned within *Sporobolus virginicus*, whilst the other 2 sites were situated within *Sarcocornia quinqueflora*. On collection, crabs from the 4 pit traps at each site were combined and approximately 5 individuals of each of the 2 common species (*Paragrapsus laevis* and *Helograpsus haswellianus*) were collected where possible, placed into labelled jars and put into an ice slurry for further processing.

In addition to the collection of shore crab samples, potential food sources that consisted of various autotrophs present within saltmarsh habitat were collected for isotopic analyses. As it has been shown that plant species from saltmarsh habitat (i.e. *Sacrocornia quinqueflora*, *Sporobolus virginicus* etc.) have relatively low variability in $\delta^{13}C$ values over a range of spatial scales (Guest & Connolly 2005, Guest et al. 2006), only 3 replicate plant samples were taken of both *S. quinqueflora* and *S. virginicus* at each of the 2 locations (Empire Bay and Davistown). Three replicate sediment samples (which were later partitioned into FBOM and MPB) were collected from each of the 8 sites that were sampled for shore crabs. The plant samples were collected by cutting the vegetation at the base of the plant, whilst the sediment was collected by scraping off the top 1 cm layer of sediment (Melville & Connolly 2003) in an area approximately 20 × 20 cm from the saltmarsh surface.

Laboratory processing

Each shore crab was thoroughly rinsed with de-ionised water prior to processing. Muscle tissue was collected from the claws of the shore crabs, with care being taken not to include any hard shell fragments. Where possible, each replicate sample was taken from a single crab, although on some occasions where individual crabs were small, 2 crabs were used to make a single replicate. In general, 3 replicate crab samples of each species (i.e. *Paragrapsus laevis* and *Helograpsus haswellianus*) were obtained for each of the 8 sites, although at some sites a full complement of crab samples was unable to be achieved due to the low numbers of crabs collected.

Plant samples were thoroughly rinsed with de-ionised water prior to processing. Leaves were cut from the plant and placed into labelled Petri dishes ready for drying. Only leaf matter from each plant was used in each replicate.

Sediment samples were processed by separating out the FBOM and MPB from the entire sediment. Each sediment sample was firstly halved, with one half being used for FBOM samples and the other half used for MPB samples.

FBOM was separated from the entire sample by firstly rinsing the sediment through a series of sieves (0.25, 0.5, 1 and 4 mm) and then elutriating only the

0.25 mm sieve fraction to obtain the FBOM samples similar to the method of Saintilan & Mazumder (2010). A small quantity of 1 M hydrochloric acid was added to each FBOM sample and left for 3 h to remove any inorganic carbon present in the sediment (Polunin et al. 2001). The samples were then rinsed with de-ionised water and placed into labelled jars ready for drying. Because this acid-washing process results in enrichment of $\delta^{15}N$ (Pinnegar & Polunin 1999), untreated FBOM samples were analysed for $\delta^{15}N$. The treated acid-washed samples of FBOM were analysed for $\delta^{13}C$.

MPB was separated by first rinsing the sediment through 63 µm mesh to remove any infauna present within the sediment. Material passing through this mesh was then placed into jars and left to settle out. Once this had occurred, the top layer of water in each jar was decanted and 15 ml of the remaining sediment slurry was transferred to a centrifuge tube with 15 ml of colloidal silica (LUDOX™ AM30, density = 1.21) and centrifuged at 10 000 rpm (11 963 × *g*) for 10 min similar to the method of Guest et al. (2004). The band of materials (MPB) that formed at the top of the tube was then rinsed onto a glass fibre filter with de-ionised water. Any MPB material retained on the filter was carefully scraped into a labelled Petri dish ready for drying. It should be noted that not all MPB samples were able to be obtained as some samples, especially those from Davistown, generally had very little MPB present within the sediment.

All samples obtained (crab, plant, FBOM and MPB) were dried for 48 h at 50°C and ground to a fine powder before being weighed and placed into tin capsules ready for isotopic analyses. Samples were analysed using a continuous-flow stable isotope mass spectrometer (CF-IRMS, IsoPrime GV Instruments). The standard for carbon was BDH AnalaR Sucrose from cane sugar calibrated against Australian National University Sucrose (IAEA-C6). The standard for nitrogen was BDH AnalaR Ammonium Sulphate calibrated Ambient air and IAEA-Stable isotope values were reported in delta (δ) units in parts per thousand (‰) relative to the international standard and determined as follows:

$$X(‰) = \left(\frac{R_{sample}}{R_{standard}} - 1 \right) \times 1000 \quad (1)$$

where $X = \delta^{13}C$ or $\delta^{15}N$, and $R = {}^{13}C/{}^{12}C$ or ${}^{15}N/{}^{14}N$, respectively.

The full experimental design of the study, including the number of replicates that were collected/processed, is shown in Fig. 2.

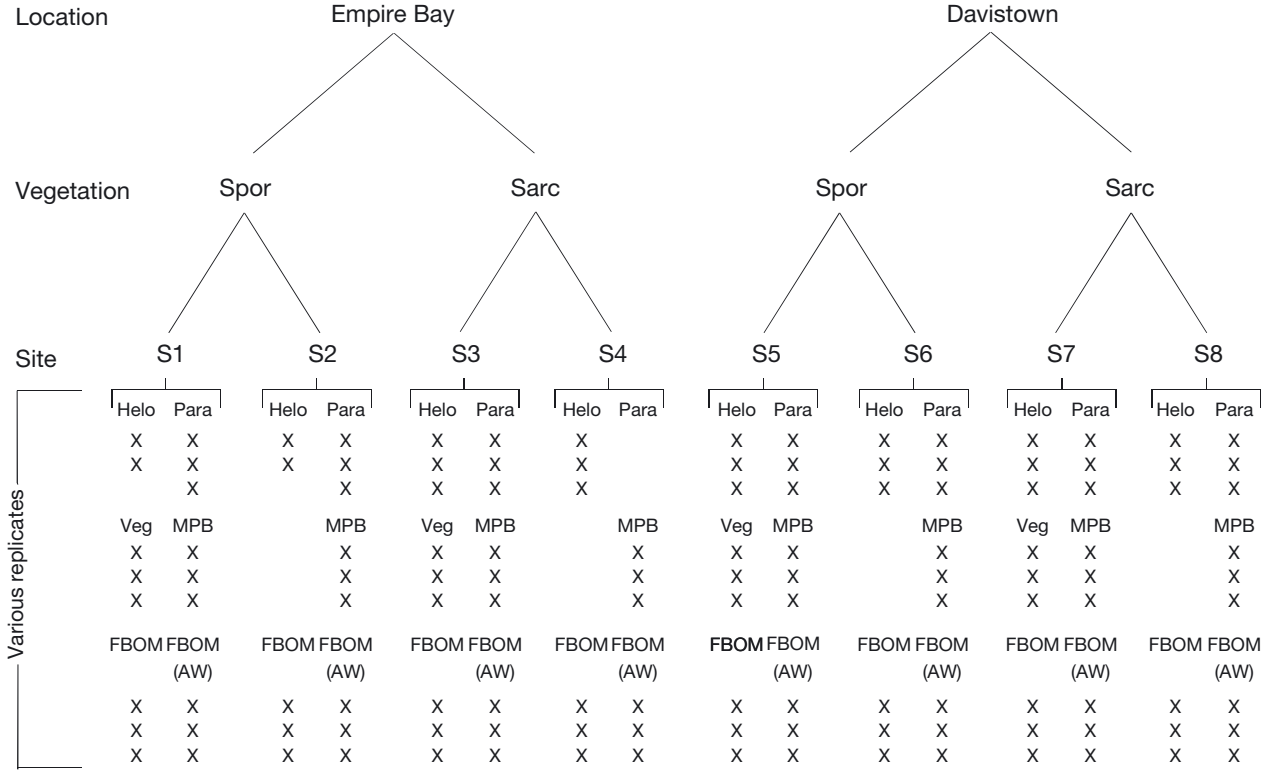

Fig. 2. Sampling design used throughout the study. Spor: *Sporobolus virginicus*; Sarc: *Sarcocornia quinqueflora*; Helo: *Helograpsus haswellianus*; Para: *Paragrapsus laevis*; Veg: vegetation; MPB: microphytobenthos; FBOM: fine benthic organic matter; AW: acid-washed

Statistical methods

Multifactorial permutational analysis of variance (PERMANOVA) was used to investigate the spatial variability of the various isotopic signatures obtained during the study. The advantages of this approach over traditional ANOVA are that there is no assumption that the data are normally distributed, and the test is not affected by unbalanced designs (i.e. different numbers of sites/replicates in different factors of the design). Furthermore, these analyses provide exact tests (i.e. the probability of rejecting the null hypothesis is the same as the value for alpha, which is conventionally 0.05) for each level in an experimental design and do not rely on interpolations from tabled values of *F* (Anderson et al. 2008).

Preliminary analyses of the data suggested that the factor of Site showed little variability in many of the variables analysed, often showing $p > 0.25$. In addition, not all compliments of samples could be obtained at each site for crabs and MPB. Therefore, to allow for a simpler interpretation of the analyses, it was decided that replicates from each site be effectively 'pooled' together and the factor of Site excluded from the analyses for all variables (i.e. crabs, MPB and FBOM). As vegetation samples were only collected from 1 site per vegetation type within each location, the factor of Site was already redundant in the analyses involving vegetation.

For crab isotope signatures, a 3-factor PERMANOVA was used to examine differences in crab isotopic signatures between locations, vegetation types and different crab species. For all autotrophs collected during the study (i.e. vegetation, FBOM and MPB), a 2-factor PERMANOVA was used to statistically compare their isotopic signatures between locations and vegetation types. Each factor used in the experimental design and their various attributes for the analyses were:

- Location (fixed, orthogonal factor consisting of 2 levels: Empire Bay and Davistown)
- Vegetation (fixed, orthogonal factor consisting of 2 levels: *Sacrocornia quinqueflora* and *Sporobolus virginicus*)
- Crab (fixed, orthogonal factor consisting of 2 levels: *Paragrapsus laevis* and *Helograpsus haswellianus*)

All analyses were univariate and were based on Euclidean distance matrices calculated by PRIMER v6 for Windows (PRIMER-E) using untransformed data. The variables analysed included $\delta^{13}C$ and $\delta^{15}N$ for crabs, vegetation, FBOM and MPB.

Where the p-value for a particular factor or interaction term was non-significant at a level greater than 0.25 (i.e. $p > 0.25$), the test was made more powerful by post-hoc pooling procedures (Winer et al. 1971, Anderson et al. 2008). When a significant result was achieved for an interaction term, post-hoc permutational *t*-tests were used (via PERMANOVA) to determine at which levels within the term these differences occurred at. Note that no pairwise tests were performed when Location, Vegetation or Crab were significant, as these factors only contained 2 levels, and thus no pairwise tests were required.

IsoSource mixing model

In addition to the above statistical analyses, a multiple-source mixing model (IsoSource; Phillips & Gregg 2003) was used to examine the relative contributions of potential food sources (autotrophs) to the diet of shore crabs (consumers) taken from Empire Bay and Davistown. This model attempts to calculate all possible combinations of the various potential food source contributions (as a percentage) in 1% increments, with combinations adding to within a pre-defined tolerance level of the consumer signature considered as feasible solutions.

Prior to any analyses, isotope signatures for autotrophs were corrected to allow for enrichment fractionation effects between trophic levels. $\delta^{13}C$ values were corrected for an enrichment of 0.4‰ and $\delta^{15}N$ values were corrected with a 2.3‰ enrichment value (McCutchan et al. 2003). All models were initially run with a tolerance level of 0.1‰, although when a solution was not obtained for a particular model, the tolerance level was increased incrementally by 0.05‰ until a solution was attained. This increase in the tolerance level was to help account for any uncertainty in the fractionation values used during each model run and the variability within consumer and producer isotope values (Currin et al. 2011).

Models were run for both crab species (*Paragrapsus laevis* and *Helograpsus haswellianus*) within each vegetation type (*Sacrocornia quinqueflora* and *Sporobolus virginicus*) at the 2 locations (Empire Bay and Davistown). For each of the 8 models, 3 potential food sources were included: the major vegetation from a particular patch (either *S. quinqueflora* or *S. virginicus* plant material depending on the type of habitat sampled), FBOM and MPB.

RESULTS

As expected, mean $\delta^{13}C$ values for *Sporobolus virginicus* plant material were significantly more enriched compared to those of *Sacrocornia quinqueflora* for both locations (Fig. 3); however, post-hoc pairwise tests detected significant differences in $\delta^{13}C$ for *S. quinqueflora* between Empire Bay and Davistown ($p = 0.0128$), which indicated isotope signatures for various vegetation types can vary within an estuary. *S. virginicus* was found to be significantly depleted in $\delta^{15}N$ compared to *S. quinqueflora* for both locations sampled (Table 1, Fig. 3).

A significant Location × Vegetation interaction was detected for FBOM $\delta^{13}C$ values (Table 1). FBOM and MPB collected from *Sporobolus* patches at Empire Bay were always more enriched in $\delta^{13}C$ than those collected from *Sarcocornia* patches at the same location (post-hoc pairwise $p = 0.0021$ for FBOM). This was in contrast to FBOM and MPB sampled from Davistown, with no distinct separation in $\delta^{13}C$ signatures between either patch types (Fig. 3).

Patterns for $\delta^{15}N$ values of FBOM and MPB were more consistent, with Location and Vegetation factors both showing a significant effects (Table 1). FBOM and MPB collected within *Sarcocornia* patches was always significantly enriched in $\delta^{15}N$ compared to FBOM and MPB in *Sporobolus* patches regardless of location (Fig. 3). In addition, mean $\delta^{15}N$ values for FBOM and MPB were always significantly enriched at Davistown compared to that at Empire Bay, irrespective of vegetation type (Fig. 3).

Helograpsus haswellianus was always significantly more enriched in $\delta^{13}C$ and $\delta^{15}N$ compared with *Paragrapsus laevis* sampled at the same location (Table 1, Fig. 2). Crabs of both species collected from Davistown were also always more enriched with $\delta^{15}N$ compared to Empire Bay (Table 1, Fig. 3). In addition, crabs sampled from *Sporobolus* patches were always more enriched in $\delta^{15}N$ compared to those collected from *Sarcocornia* patches regardless of location (Table 1, Fig. 3).

IsoSource mixing model results are presented in Table 2 for both locations. Both crab species collected from Empire Bay appeared to have similar diets, although the dominant source of food depended on the habitat type they were sampled from. MPB was clearly the dominant food source for both crab species residing within the *Sporobolus virginicus* habitat at Empire Bay, whereas FBOM contributed majorly to the diets of both crab species collected from *Sacrocornia quinqueflora* from the same location (Table 2).

In contrast, contributions from the various sources taken from Davistown were not as definitive as those from Empire Bay, with FBOM and MPB both contributing simultaneously to the diets of both crab species (Table 2). In addition, no clear patterns could be ascertained from the IsoSource results for either crab spe-

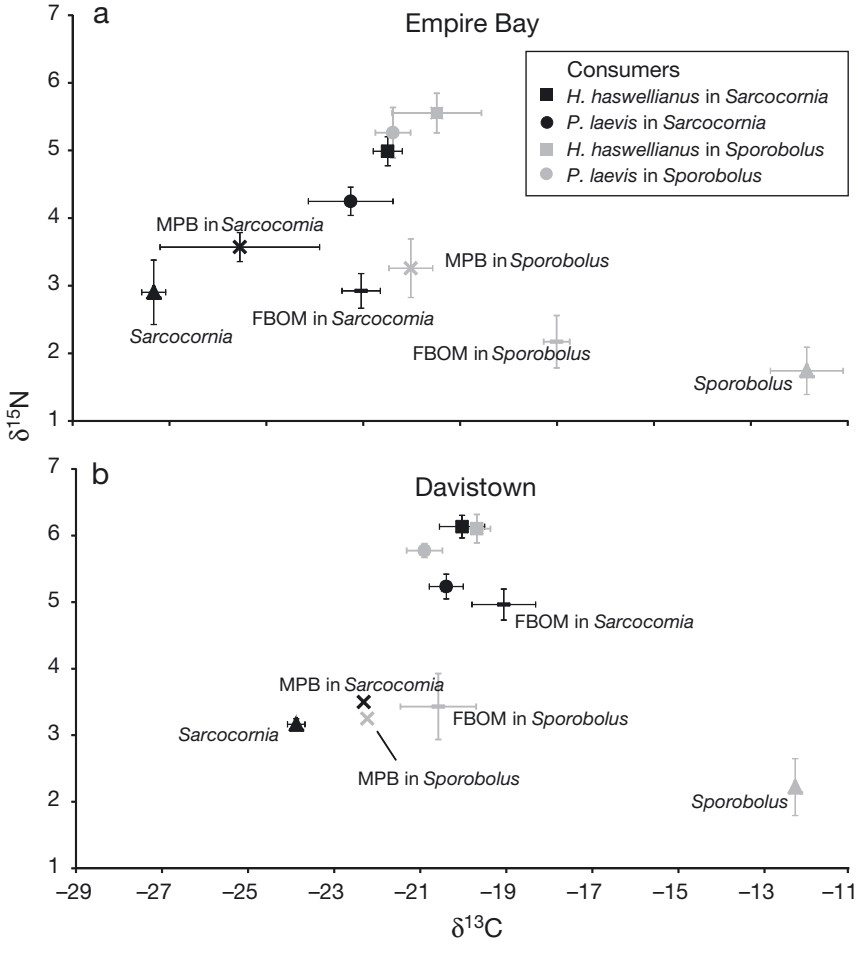

Fig. 3. Mean (±SE) $\delta^{13}C$ and $\delta^{15}N$ values for 2 species of shore crab (*Helograpsus haswellianus and Paragrapsus laevis*), 2 dominant vegetation types (*Sporobolus virginicus and Sarcocornia quinqueflora*), fine benthic organic matter (FBOM) and microphytobenthos (MPB) sampled from the (a) Empire Bay and (b) Davistown, Australia, saltmarshes

Table 1. Univariate PERMANOVA results for various isotope signatures sampled during the study. Significant factors ($p < 0.05$) are in **bold**. FBOM: fine benthic organic matter; MPB: microphytobenthos; LoxVe: Location/Vegetation interaction; VexCr: Vegetation/Crab interaction

(a) $\delta^{13}C$ Source of variation	df	MS	Pseudo-*F*	P(perm)
Crab				
Location	1	0.277	0.241	0.628
Vegetation	1	1.925	1.679	0.204
Crab	**1**	**6.857**	**5.980**	**0.020**
LoxVe	1	2.714	2.367	0.135
Pooled	38	1.147		
Vegetation				
Location	1	0.750	1.503	–
Vegetation	1	471.540	945.690	–
LoxVe	**1**	**2.667**	**5.348**	**0.046**
Residual	8	0.499		
FBOM				
Location	1	0.025	0.006	–
Vegetation	1	13.544	3.480	–
LoxVe	**1**	**59.023**	**15.166**	**0.001**
Residual	20	3.892		
MPB				
Location	1	7.444	2.699	0.132
Vegetation	**1**	**17.819**	**6.461**	**0.020**
Pooled	12	2.758		

(b) $\delta^{15}N$ Source of variation	df	MS	Pseudo-*F*	P(perm)
Crab				
Location	**1**	**6.721**	**23.782**	**0.000**
Vegetation	**1**	**2.935**	**10.386**	**0.002**
Crab	**1**	**3.419**	**12.099**	**0.001**
LoxVe	1	0.799	2.826	0.106
VexCr	1	0.715	2.529	0.123
Pooled	37	0.283		
Vegetation				
Location	1	0.410	1.130	0.309
Vegetation	**1**	**3.320**	**9.149**	**0.023**
Pooled	9	0.363		
FBOM				
Location	**1**	**16.319**	**20.903**	**0.000**
Vegetation	**1**	**7.825**	**10.024**	**0.005**
Pooled	21	0.781		
MPB				
Location	**1**	**43.402**	**9.015**	**0.011**
Vegetation	**1**	**40.840**	**8.483**	**0.016**
LoxVe	1	7.282	1.513	0.234
Residual	11	4.815		

Table 2. Range of feasible contributions (%) of various potential food sources to *Helograpsus haswellianus* and *Paragrapsus laevis* sampled from different vegetation types at Empire Bay and Davistown, Australia, based on $\delta^{13}C$ and $\delta^{15}N$ isotope values as determined by IsoSource (Phillips & Gregg 2003). Minimum tolerance values that provided a solution for each model run are also indicated. FBOM: fine benthic organic matter; MPB: microphytobenthos

Habitat	Producer	Empire Bay				Davistown			
		H. haswellianus		*P. laevis*		*H. haswellianus*		*P. laevis*	
		Median	1^{st}–99^{th} percentile	Median	1^{st}–99^{th} percentile	Median	1^{st}–99^{th} percentile	Median	1^{st}–99^{th} percentile
Sarcocornia	*S. quinqueflora*	0	0–2	19	0–36	5	0–15	4	0–12
	FBOM	98	97–100	79	61–99	50	46–53	23	20–25
	MPB	1	0–3	2	0–5	45	32–54	73	63–80
	Tolerance (‰)	0.25		1		0.4		0.9	
Sporobolus	*S. virginicus*	1	0–2	0	0–0	2	2–2	2	0–4
	FBOM	3	0–7	0	0–1	96	95–99	46	29–67
	MPB	96	93–99	99	99–100	1	0–3	52	32–67
	Tolerance (‰)	0.10		0.80		0.40		0.20	

cies taken from the 2 types of saltmarsh vegetation from this location.

Modelling results suggested that fresh plant material contributed very little to the diets of crabs sampled from both locations, although results suggest that *Sacrocornia quinqueflora* did contribute nearly 20% to the diet of *Paragrapsus laevis* collected from this vegetation type at Empire Bay (Table 2).

DISCUSSION

Previous studies have demonstrated the limited foraging range of crabs in intertidal environments (Guest et al. 2006). A capture-recapture experiment demonstrated that grapsid crabs rarely moved more than 2 m from their burrow, a behaviour possibly associated with burrow defence, and common

amongst grapsids (Fratini et al. 2000, Skov & Hartnoll 2002). Given this behaviour, it is possible to utilise the fine-scale mosaic of C_3 and C_4-dominated vegetated habitats in the intertidal saltmarsh to test hypotheses concerning the relative importance of plant and algal sources to crab diets. The study tests both the proportional contribution of macrophytic and microphytic nutritional sources, and the spatial scale over which these sources are likely to be accessed for 2 species of intertidal burrowing crab.

The consistent enrichment of crab signatures where collected from patches of the C_4 grass *Sporobolus virginicus* suggests the contribution of carbon fixed by this species. The importance of *S. virginicus* to saltmarsh crab diet has been previously argued by Guest et al. (2006), and Saintilan & Mazumder (2010). However, the presence of *S. virginicus* as a dominant vegetation type could be influencing the isotope signature of the microphytobenthos, by contributing to the local pool of dissolved inorganic carbon (DIC). This hypothesis has been suggested by Guest et al. (2006) in explaining the slight depletion of MPB signatures in mangrove forests in Queensland. Given that both *S. virginicus* and FBOM were too highly enriched in $\delta^{13}C$ to be contributing directly to crab diet at Empire Bay, the enrichment observed in crab $\delta^{13}C$ might have been due to the enrichment of MPB with carbon originally fixed by *S. virginicus*. This was not the case in the smaller *Sporobolus* patches at Davistown, suggesting that a patch size threshold exists before the enriched DIC can be distinguished in MPB from the background DIC originating from the relatively depleted *Sarcocornia* and the mangrove *Avicennia marina*, which fringes the estuary.

The possibility of a secondary influence of macrophyte isotopic signatures mediated through aquatic microphytes incorporating DIC should present a note of caution in the interpretation of aquatic foodwebs, and in this case, depletion of crab signatures across *Sporobolus viriginicus* saltmarsh is primarily due to MPB according to our IsoSource modelling.

In general, crabs were selecting the food source of greatest nutritional value, as indicated by C:N ratios. The contribution of living plant material was minimal at all sites reflecting the high C:N ratio of *Sacrocornia quinqueflora* and *Sporobolus virginicus* (average 22.9 and 25.1, respectively). In the *S. virginicus* habitat at Empire Bay, crabs fed on MPB (mean C:N 7.6, compared with mean FBOM C:N 12.5). In the *S. quinqueflora* zone, preference switched to FBOM, which had the lower C:N ratio on average. In all other situations, MPB and FBOM C:N ratios were equivalent, and crabs utilised both sources.

However, we cannot exclude differences in the availability of MPB as a potential explanation in the utilisation of this source. Several of our sites had little measurable MPB, and while crabs do not by their grazing exhaust availability of MPB (Ribeiro & Iribarne 2011), they may encounter fluctuating density of MPB in the upper intertidal zone with seasonal, tidal and diurnal phases (Reinsel 2004, Ribeiro & Iribarne 2011) that may differ between habitats and sites. Regional differences in the relative contribution of algal and detrital sources to crab diet have been recorded elsewhere, and are a notable distinction between east and west coast US coastal ecosystems (Kwak & Zedler 1997). The result suggests that in temperate Australian locations crabs feed primarily on FBOM and MPB rather than fresh plant material in saltmarsh, in common with mangrove (Mazumder & Saintilan 2010), and in contrast to crabs in mangrove and saltmarsh in subtropical and tropical estuaries (Lee 1998, Guest et al. 2004, Nordhaus et al. 2011). For example, Oakes et al. (2010) used isotope enrichment to demonstrate that MPB contributed one-third of the nutrition requirements of *Parasesarma erythrodactyla* and mangrove leaf detritus up to 80% in a subtropical Australian mangrove forest.

The 2 crab species sampled play an important role in the saltmarsh and associated estuarine ecosystem, transforming autotrophic carbon into forms available to a range of consumers (Mazumder et al. 2009). Larval outwelling from the saltmarsh during spring tidal phases contributes substantially to the diet of several species of estuarine fish (Hollingsworth & Connolly 2006, Mazumder et al. 2006) including several of commercial importance. The relative importance of macrophytic and microphytic carbon to these ecosystem processes is an important consideration in ecosystem management and the setting of restoration targets, and is likely to vary between geographic settings (Tue et al. 2012). We propose that assessment and modelling of these contributions will depend on an improved understanding of transfers of dissolved inorganic carbon between autotrophs, aided by the application of additional tracers (Melville & Connolly 2003, Fry 2013).

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