Spatial analysis of carbon isotopes reveals seagrass contribution to fishery food web

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Abstract. Despite the widespread use of carbon stable isotopes to distinguish among potential energy pathways in food webs, their usefulness is limited where potential basal carbon sources are numerous and diverse. We measured carbon isotope values of the major fisheries species, the mostly carnivorous *Scylla serrata* (giant mud crab), and potential basal, autotrophic sources supporting the food web. Conventional mixing modelling of autotroph and crab isotope data could not differentiate contributions from different sources. Pooling of modelled contributions from sources with similar isotope values indicated a role for organic matter from seagrass meadows or saltmarshes, but still did not define contributions well. Crab isotope data from a subsequent, spatially explicit survey of 14 sites, selected to represent different distances from key habitats, were analyzed using multiple regression. Crab isotope values showed a significant relationship with distance from seagrass ($R^2 = 0.87$), but not with distance from mangroves or saltmarsh grass. Alongside seagrass meadows, crabs had very enriched isotope values, demonstrating their reliance on sources with enriched isotope values (seagrass and algae epiphytic on seagrass, 65–90% of their energy intake). At the site furthest from seagrass (21 km), crabs assimilated carbon primarily from depleted sources such as mangroves and terrestrial organic matter from coastal catchments (70–85%). Explicit spatial analysis of isotope data following a comprehensive survey revealed energy pathways not evident in conventional analyses.

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INTRODUCTION

Elucidation of energy pathways in food webs remains a central component of ecology, important in its own right for understanding ecosystem function, and an essential element underpinning disciplines such as habitat conservation and restoration (Howe and Simenstad 2015), animal migration (Hobson et al. 2012), biotic uptake of contaminants (Jardine et al. 2012) and resilience research (Rooney et al. 2006).

Stable isotope analysis, particularly of carbon, continues to provide a useful avenue for tracing energy pathways. Different photosynthetic pathways in vegetated habitats can give rise to marked differences in carbon isotope ratios

(Middelburg 2014). Carbon isotopes can therefore be used to determine the ultimate autotrophic sources of nutrition supporting food webs (see reviews by Layman et al. 2012, Ramos and Gonzáles-Solís 2012). The usefulness of carbon isotopes, however, is limited where the numbers and variety of potential basal sources for a food web are large. This situation arises frequently enough to have been one of the key drivers behind the rapid development and proliferation of mathematical mixing models to analyze isotope data (Phillips and Gregg 2003, Moore and Semmens 2008, Parnell et al. 2010). Yet ultimately even the most sophisticated models struggle to distinguish unequivocally among potential pathways where food webs have numerous sources, often with overlapping isotope signatures (Fry 2013).

The difficulty in resolving energy pathways when potential sources are numerous and diverse is encountered in terrestrial (e.g., Blumenthal et al. 2012), freshwater (e.g., Rasmussen 2010) and marine ecosystems (e.g., Baker et al. 2013). It is particularly common at the boundary of aquatic and terrestrial systems, where there is a range of potential terrestrial, littoral, benthic macrophyte and microalgal sources (Bunn et al. 2003, Connolly 2003, Bouillon and Connolly 2009). Carbon isotope data are typically collected at numerous locations during modern surveys to discern food webs, and one of the most promising opportunities for distinguishing energy pathways is statistical analysis of spatially explicit isotope data (Bouillon et al. 2011, Olsen et al. 2011, Layman et al. 2012, Ruiz-Cooley and Gerrodette 2012). The possibility of analysis of spatial isotope data has been enhanced through a reduction in costs for mass spectrometry leading to the use of greater numbers of survey sties. In aquatic ecosystems, graphical and statistical methods taking advantage of explicit spatial patterns have been used on food webs supporting river fauna (Rasmussen 2010) and production of shrimp (Kitting et al. 1984) and fish in coastal waters (Melville and Connolly 2003).

We used carbon isotopes of potential basal food sources and of a predominantly carnivorous fisheries species, *Scylla serrata* (Forskal) (giant mud crab), to examine further the usefulness of spatially explicit isotope surveys. *S. serrata* is widely distributed throughout the Indo-west Pacific, on the coasts of south and east Africa, the Middle East, southeast Asia to Japan, the western Pacific islands and northern Australia, and has been introduced to Hawaii (FAO 2014). It is an exemplary species for our purposes for four reasons: (1) It lives on and can be caught over shallow (<2 m deep), unvegetated mudflats, which occur alongside major vegetated habitats such as mangrove, saltmarsh and seagrass (Webley et al. 2009); (2) individuals do not move far on a daily basis (Hill 1978, Bonine et al. 2008), so their isotope signatures can show site-specific variation; (3) it grows rapidly, and therefore reflects the isotope ratio of its diet quickly; and (4) its ecology is of broad interest due to its importance commercially (~175,000 t/yr globally, wild and aquaculture; FAO 2014) and culturally and for subsistence fishing in southeast Asia and western Pacific islands (Demopoulos et al. 2008, Meynecke et al. 2012). Baseline isotope surveys of crabs in one of the major fishing areas in Queensland, Australia, showed a typical lack of resolution. We therefore undertook a more comprehensive survey and used spatially explicit analysis to test for relationships between crab isotope values and distances from key habitats. This improved resolution of carbon pathways, revealing substantial contributions from seagrass meadows.

Materials and Methods

Baseline surveys

Adult crabs (>90 mm carapace width) were collected from unvegetated habitat in two baseline surveys (Fig. 1), the first in summer (smaller scale: $n = 5$ crabs at each of 6 sites, total area approximately 20×10 km) and the second in winter (broader scale: $n = 5$ crabs at each of 6 sites, total area approximately 100×20 km) in Moreton Bay, Queensland, Australia (153°42' E, 27°93' S). Muscle tissue was excised from crab chelipeds. In Moreton Bay, within-site crab $\delta^{13}C$ variability is not influenced by sex (paired t test, $P = 0.12$; $n = 7$) or size ($R^2 = 0.12$, $P = 0.36$, $n = 6$; Waltham 2009). Six autotrophs were collected ($n = 5$ samples from each site, in both baseline surveys: mangroves (*Avicennia marina*, MAN), seagrass (*Zostera muelleri*, SG), C_3 saltmarsh succulents (*Suaeda australis*, SMS), C_4 saltmarsh grass (*Sporobolus virginicus*, SMG), seagrass epi-
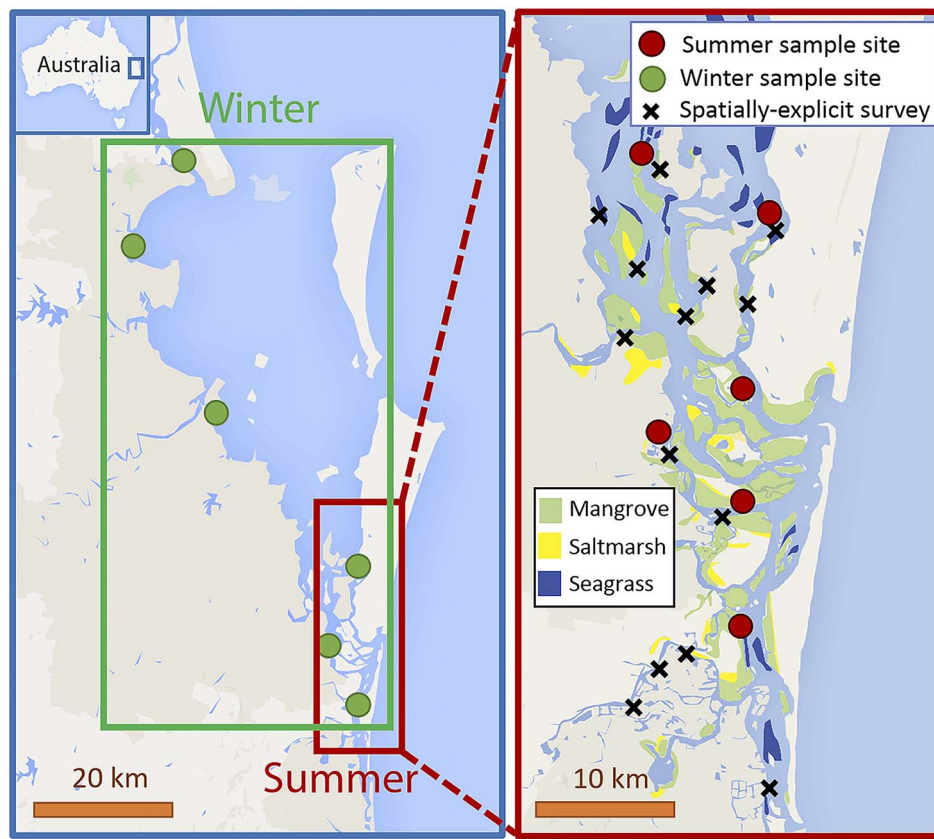
Fig. 1. Survey locations. Left panel: inset shows location of study area within Australia. Main map shows location of Baseline Survey 1 (Summer) and location of survey and sites for the larger Baseline Survey 2 (Winter). Right panel: detail of Baseline Survey 1, showing sites, and the sites and habitats for the spatially explicit survey.

phytes (EPI, separated from seagrass blades in the laboratory following Guest et al. (2004)), and microphytobenthos (MPB, predominantly diatoms, collected by centrifuging surficial sediment with colloidal silica following Connolly et al. (2005b)). Phytoplankton densities were very low relative to the high load of sediment and particulate detrital material in the water. We were therefore unable to obtain a phytoplankton sample pure enough to represent this autotroph. Crab and autotroph samples were dried, ground and analyzed on an Isoprime isotope ratio mass spectrometer. Ratios of $^{13}C/^{12}C$ were expressed as the relative difference (‰) between the sample and the conventional standard, Pee Dee Belemnite limestone carbonate. Analytical precision of the mass spectrometer was 0.2‰.

The relative contribution of different basal carbon sources to crabs was assessed separately for the two surveys using the Bayesian mixing model stable isotope analysis in R (SIAR; Parnell et al. 2010, and see Ramírez et al. 2014). Based on this species diet of grazing and filter-feeding invertebrates (Hill 1979), and an assumed enrichment of C isotopes (McCutchan et al. 2003), we used a trophic enrichment factor (TEF) for crabs of 1‰ (SD 0.1). Although we know the diet of the crabs in broad terms, TEFs are notoriously difficult to be precise about given that, within a single species, they vary with factors such as growth rate and food quality (McCutchan et al. 2003). We therefore tested the sensitivity of our SIAR modelling by running the models again using adjusted TEF values of 0 and 2‰, and found that this had little effect on the distributions and rank order of autotroph contributions. SIAR was run for 500,000 iterations with the first 50,000 discarded. As expected, the breadth of

Table 1. Carbon isotope values of potential basal energy sources during Baseline Survey 1, grouped into those with enriched, intermediate and depleted values, and of giant mud crabs (*Scylla serrata*) during Baseline Surveys 1 and 2.

| Taxon | Carbon isotope values (‰) | |
	Mean	SE
Enriched autotrophs		
Seagrass	−12.5	1.0
Epiphytic algae	−17.2	1.4
Saltmarsh grass	−15.9	1.5
Intermediate autotrophs		
Microphytobenthos	−22.5	1.4
Depleted autotrophs		
Mangroves	−29	0.9
Saltmarsh succulents	−28	0.8
Crabs, Survey 1	−18.4	0.7
Crabs, Survey 2	−17.9	0.6

distributions for different autotrophs prevented clear conclusions about relative contributions, so we proceeded to pool autotrophs with similar isotope values (see Phillips 2012) to provide greater resolution of contributions (enriched: SG, EPI, SMG; intermediate: MPB; depleted: MAN, SMS).

Explicit spatial survey

The baseline surveys suggested that sources with enriched carbon isotopes (seagrass, epiphytes and saltmarsh grass) were important to crabs. We surveyed crabs again, using a more explicit spatial survey at 14 sites from across the same approximate area used in Baseline Survey 1 (Fig. 1). The locations of sites were selected based on maps of aquatic vegetation to provide a spread of distances from the three habitats of interest: seagrass (*Zostera muelleri*), saltmarsh grass (*Sporobolus virginicus*) and mangroves (*Avicennia marina*). Distance was measured as the most direct route, via water, to the nearest habitat patch. We used a multiple regression with all three habitats in the model to test how well distances to different habitats (log transformed) explained crab isotope values; Akaike information criteria were used to examine model fit, and partial regressions were used to determine the amount of variation explained by individual habitats. Distance to seagrass was found to be important, so we then ran SIAR modeling for each of the 14 sites, using the site mean for crab isotopes and the average value for each auto-

troph (pooling baseline surveys to give the most reliable representation of average autotroph values). The same settings and procedures were used for SIAR as described for Baseline Surveys, above. This spatially explicit design was surveyed once only, but carbon isotope ratios of crabs collected at the same two sites twice more over the subsequent year showed that site-specific ratios were consistent through time (the three values at one site: −23.1, −22.3, −22.2‰; and at another site: −17.0, −16.0, −16.7‰).

RESULTS

Baseline surveys

Carbon isotope values of the six autotrophs were well spread in both surveys, with mangroves and saltmarsh succulents having the most depleted $\delta^{13}C$ values, seagrass, epiphytes and saltmarsh grass the most enriched, and MBP intermediate (Table 1). Crab isotope values were similar in the two surveys (Survey 1: mean −18.4‰, SE 0.7; Survey 2: −17.9‰, 0.6). The ranges of feasible contributions for different autotrophs in SIAR were broad, and the median contributions low and similar to each other (Fig. 2). This prevented clear conclusions being drawn. Source contributions were better defined by combining feasible autotroph contributions into three groups. Over the whole bay, the contribution of carbon from enriched autotrophs was considerable: 95% of values between 45–80% in Survey 1 and 35–80% in Survey 2 (Fig. 2). The remaining carbon was assimilated from MPB and depleted sources (mangroves and saltmarsh succulents).

Explicit spatial survey

Carbon isotope values of crabs in the spatially explicit survey varied more (site means from −14.9 to −24.9‰) than in the baseline surveys, and the overall mean was slightly more depleted (−20.1‰, SE 3.3). Only distance from seagrass showed a significant relationship with crab isotope values (Fig. 3; $R^2 = 0.87$, $P < 0.001$). Crab isotope values were more enriched near seagrass, particularly within 600 m, but were not related to distances from saltmarsh grass or mangroves (neither habitat added significantly to the multiple regression model, and R^2 values were also low and non-significant when tested
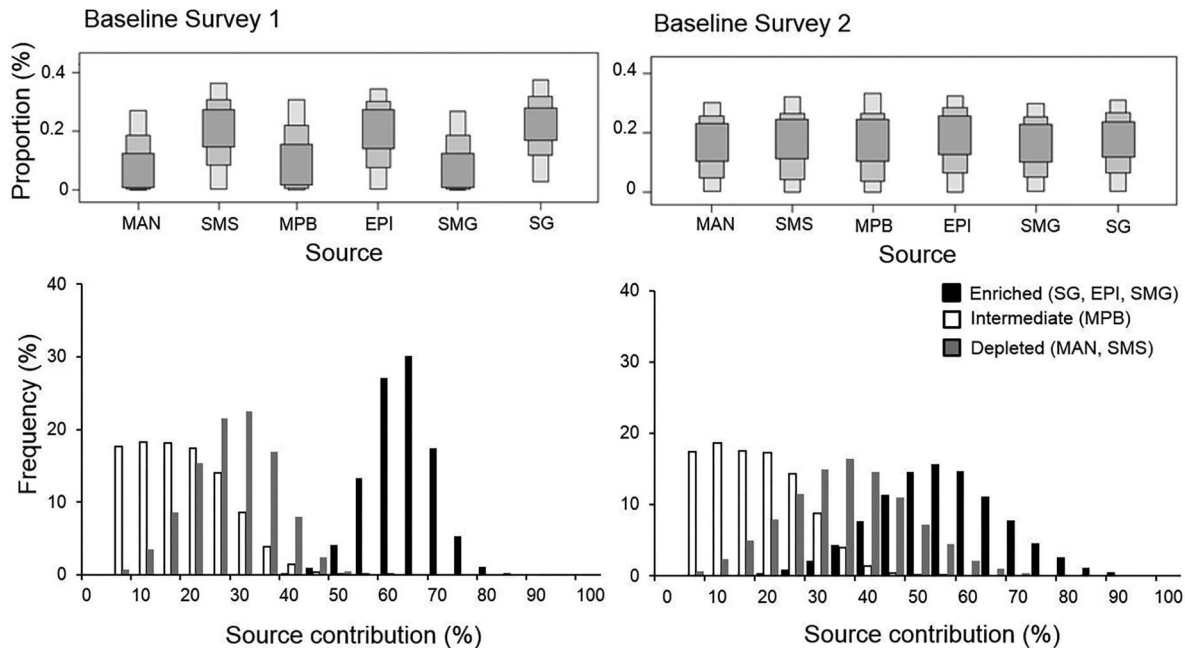
Fig. 2. Top panels: distributions of possible source mixtures explaining giant mud crab isotope values (from SIAR modeling) for the two baseline surveys, averaged across locations. Proportions are credibility intervals plotted at 95%, 75%, and 25%. MAN, mangroves; SMS, saltmarsh succulents; MPB, microphytobenthos; EPI, epiphytic algae; SMG, saltmarsh grass; SG, seagrass. Lower panels: combined contributions from the three autotroph groups to crabs from the same two baseline surveys.

individually; data shown graphically in the Appendix). Results of SIAR modelling at each of the 14 sites showed, once sources were pooled into the three categories, that the median contribution of enriched sources decreased with distance from seagrass (this pattern was almost identical to that for crab isotopes against distance from seagrass, with a similar $R^2 = 0.90$, so is not shown again). To illustrate the shift from enriched to depleted source contributions with increasing distance from seagrass, we have displayed the SIAR results for three sites: the site closest to seagrass (Point A on Fig. 3), the site furthest from seagrass (Point C on Fig. 3), and one mid-way between those two extremes (Point B on Fig. 3). The site closest to seagrass, which also had the most enriched crab isotope value (−14.9‰), demonstrated substantial assimilation of carbon from enriched sources (95% of values between 65–90%), with the remainder assimilated from MPB or depleted sources (Fig. 3). We interpret this contribution as largely coming from seagrass meadows (seagrass and/or its epi-

phytes), since some of the sites are far (several kilometres) from saltmarsh grass, the other potential enriched source. The relative contribution from depleted carbon sources increased with distance from seagrass: at 5.2 km from seagrass (crab average = −20.1‰), enriched sources provided 30–50% and depleted sources 20–70%. At the site furthest from seagrass (21 km), with the most depleted isotope average (−24.9‰), crabs primarily assimilated carbon from depleted sources (70–85%).

DISCUSSION

Carbon sources

Our key conclusion is that sources of carbon at the base of the food web supporting giant mud crabs in Queensland vary with location. The strong spatial pattern matches the specificity of carbon sources in relation to habitat boundaries previously recorded in small intertidal crab species (Guest et al. 2006). For giant mud crabs living near seagrass meadows, seagrass and its epiphytes provide the majority of carbon. Sea-
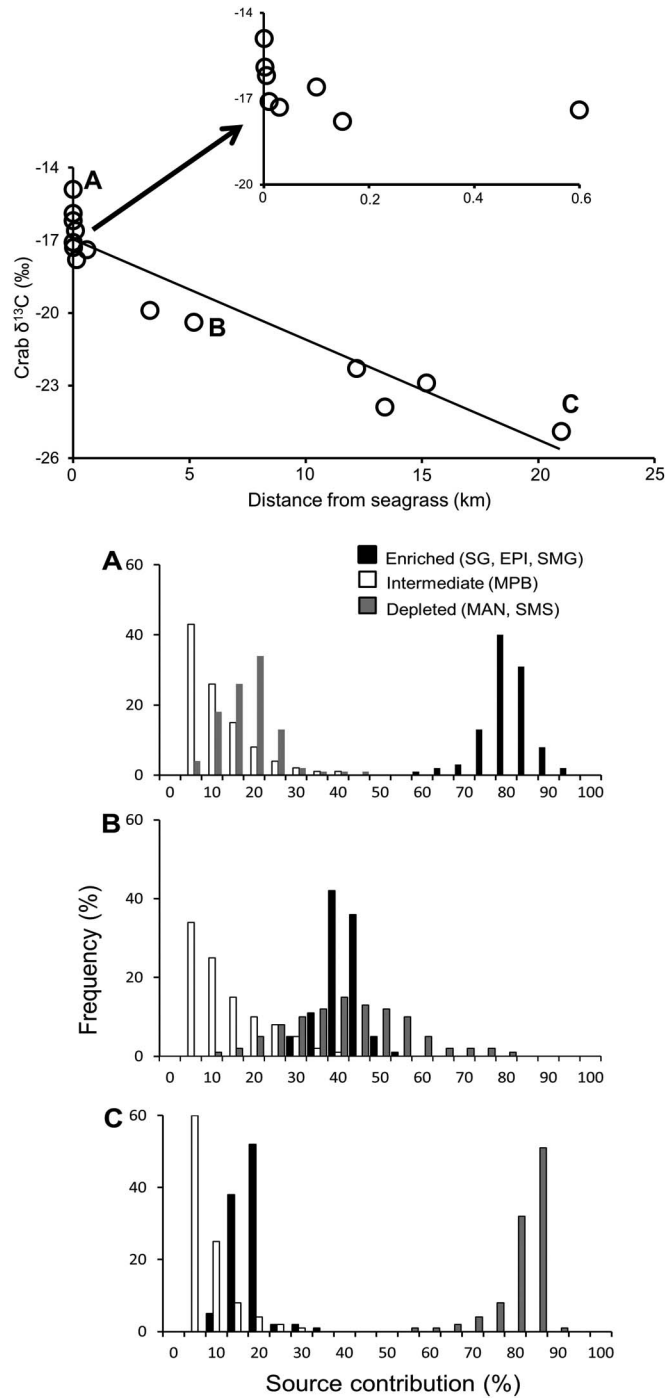
Fig. 3. Upper graph with inset: significant relationship between giant mud crab isotope values (means per site, $n = 3$ crabs per site) and distance from seagrass meadows (distances shown as raw values, tested statistically as log transformed data). Upper inset shows detail within 0.6 km of seagrass. Lower histogram bar graphs show combined source contributions for the three pooled autotroph groups at 3 sites: A, closest to seagrass; B, mid distance; C, furthest from seagrass (see lettering on main upper panel; letter is shown *immediately* alongside the site for which SIAR modeling was run).

grass meadows are important sites of carbon sequestration (Fourqurean et al. 2012, Macreadie et al. 2014), but typically produce more carbon than is stored in sediments or used by consumers inside the meadow (Fourqurean et al. 2012). This excess production is potentially available for use in food webs in adjacent habitats (reviewed by Heck et al. 2008), and has been shown to be important for fishes occurring over unvegetated mudflats in Moreton Bay (Melville and Connolly 2005). Mud crabs consume a variety of food types but in Moreton Bay predominantly forage for slow moving or sessile macro-invertebrates, such as bivalves, gastropods and small crabs (Hill 1979). Although the mechanism for transfer of carbon from seagrass meadows to crabs in adjacent habitat cannot be determined definitively from our study, it therefore seems likely that it might involve waterborne transport of particulate matter and uptake by intermediary detritivorous invertebrates, as shown in a study of another omnivorous crustacean in Australian waters (Connolly et al. 2005a).

SIAR modelling of source mixtures was unable to distinguish clearly between contributions from seagrass and its epiphytic algae; the crabs might assimilate carbon from epiphytic algae, seagrass, or a mixture of the two. The contributions of seagrass and associated algae to coastal food webs are usually pooled together in studies using carbon isotopes because of a lack of source separation (e.g., Spiller et al. 2010). Improving the resolution of contributions of epiphytes and seagrass is an area for further research and techniques showing promise include manipulative enrichment experiments (Winning et al. 1999, Mutchler et al. 2004) and the use of sulfur isotopes (Connolly et al. 2004). Regardless of whether seagrass or epiphytes are nutritionally more important to consumers, the results reinforce the need to conserve seagrass meadows in Queensland and protect them from adverse anthropogenic influences (Maxwell et al. 2014). Managing fisheries requires an ecosystem-based approach, where energy and nutrient sources are also protected from degradation by human activities, even where they are spatially segregated from the habitat where the fishery species lives. This projects is the first step towards that goal for giant mud crabs.

Unlike in North American marshes, detritus from saltmarshes in Australian waters has little impact on food webs beyond their borders (Hyndes et al. 2014). Australian marshes are located high in the intertidal zone and are inundated only infrequently (Hollingsworth and Connolly 2006), potentially limiting detrital export (Connolly 1999). Although saltmarsh grass is likely to be highly productive, the total aerial cover of saltmarsh in the study area (7 km^2; Waltham 2009) is only 17% of that of seagrass (Maxwell et al. 2015).

The potential contribution of MPB (the chief intermediate source) to crab nutrition was modest. This might partly reflect the way SIAR handles multiple sources at either end of the carbon isotope range with a single potential source in the middle (near to the consumer value, in this case crabs). But it is also true that for crabs with a strongly enriched carbon isotope values, the contribution from intermediate sources must be relatively minor. This inference also includes any role that phytoplankton plays. Although we could not isolate enough phytoplankton to obtain an isotope signature, typical phytoplankton isotope values in the region are −21.9‰ (Schlacher et al. 2009), approximately similar to those of MPB.

At greater distances from seagrass meadows, crabs rely on a pool of carbon from relatively depleted sources. This carbon pool is probably a mixture of different sources, but one key contributor is likely to be mangroves. The concept of mangrove carbon "outwelling" on currents to coastal waters and driving productivity has received considerable attention. A carbon isotope study of energy sources of *S. serrata* in the western Pacific also concluded that mangroves were important in some circumstances (Benstead et al. 2006). Isotope analysis in small tropical estuaries also shows a mangrove contribution to fish (Abrantes et al. 2015). Nevertheless many studies find little if any contribution from mangroves to food webs beyond the forest (reviews by Bouillon et al. 2008, Igulu et al. 2013). The low nutritive status of mangrove leaves (high C:N ratio) is considered the primary reason behind their apparent lack of contribution to adjacent food webs. New evidence is emerging, however, that the refractory mangrove carbon may be more widely utilised once it has been further decomposed and perhaps also

colonised microbially (Bui and Lee 2014). This could explain the role of mangrove carbon in Moreton Bay in locations far from seagrass meadows.

The indication of a background carbon pool with depleted isotope values might also suggest a contribution from the terrestrial habitats in coastal catchments (Gaston et al. 2006). Terrestrial forest plants typically have isotope values similar to mangroves (Abrantes et al. 2013), so any contribution of terrestrial matter to the food webs, for example via riverine input (Wissel and Fry 2005), cannot be separated from that of mangroves. This possibility could be important for management of land-uses in coastal catchments and could be pursued in future with additional biomarkers capable of separating mangroves from terrestrial plants (e.g., Dittmar et al. 2006).

The value of spatially explicit surveys

The important general concept demonstrated here is that explicit spatial analysis can reveal energy pathways in food webs not easily elucidated in other ways. We see this as part of a wider trend towards fuller use of spatial (or temporal) variability in isotope surveys (Layman et al. 2012). With the increasingly automated chemical analysis of biological samples, and thus more comprehensive isotope survey designs, several studies have shown the importance of capturing variation in isotope values of autotrophs and consumers (e.g., Galvan et al. 2012, Hyndes et al. 2014). There was an attempt at using variability in isotope values to advantage early in the development of isotope ecology, when Kitting et al. (1984) compared variation in shrimp isotope values from site to site with that of seagrass and epiphytic algae. Later, a more rigorous statistical approach to this type of correlation analysis was developed that could handle isotopes of multiple elements (Melville and Connolly 2003). A related approach correlating producer and consumer isotope values along the length of a river was developed by Rasmussen (2010), who was able to separate the contributions from riparian and in-stream vegetation, two sources with similar average isotope values but having different patterns of variability along the river gradient.

Alternative strategies exist for overcoming the lack of resolution in situations with numerous and diverse potential sources. Isotopes of additional elements can be used for source determination. Nitrogen is frequently utilised but we examined this for Moreton Bay and found it unhelpful for two reasons. First, variation of TEFs within a species is greater for nitrogen than carbon, yet the overall range of nitrogen values for potential sources is far less. Together, these facts mean that modeling output is sensitive to even quite minor errors in TEF values. Second, in Moreton Bay there is a gradient in nitrogen isotopes of some producers across the bay (an effect of treated sewage inputs and urbanisation on the western side of the bay; Pitt et al. 2009). Because this gradient is independent of habitat type it confounds attempts to separate the habitat influence on crab nutrition. Sulfur isotopes have been shown to separate potential sources more frequently (Connolly et al. 2004) and should be considered in future studies. Another alternative is labeling of specific sources with artificially altered isotope ratios (e.g., Oakes et al. 2010), although this approach is usually limited to animals occupying smaller areas than the giant mud crab. A further option is to use alternative and additional tracers (e.g., metal concentrations, Demopoulos et al. 2008; fatty acids, Mortillaro et al. 2011). Each of these strategies has advantages and disadvantages. We suggest, in any case, that their value will often be greater when used in conjunction with explicit spatial analysis of isotope data.

Some of the longstanding debates in trophic ecology about the relative contributions of different sources to food webs have continued unresolved because of the difficulty in differentiating among numerous and diverse carbon isotope signatures. Our revelations about carbon sources for the food web supporting giant mud crabs show how explicit analysis of spatial isotope data might help to answer some of the outstanding questions ecologists have about food webs.

Acknowledgments

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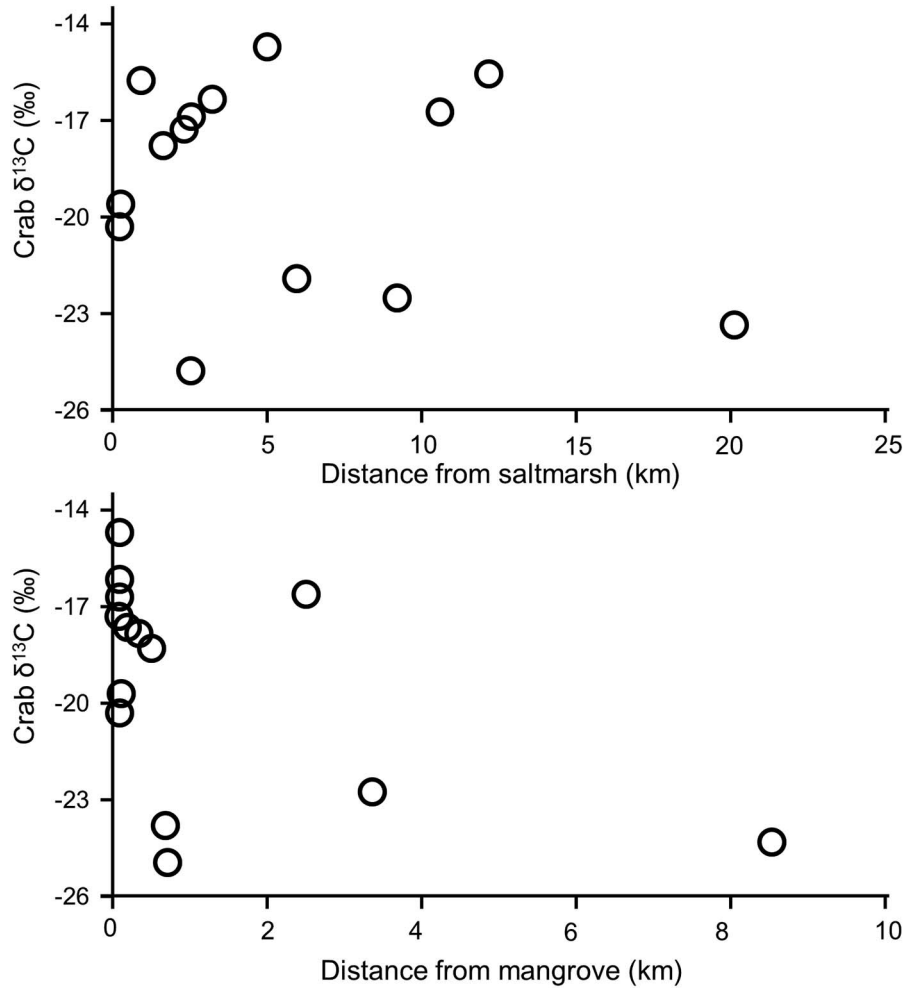
SUPPLEMENTAL MATERIAL

APPENDIX

Fig. A1. Non-significant relationships between crab carbon isotope values and distances from saltmarsh (top panel) and mangroves (bottom panel).