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STABLE ISOTOPE ECOLOGY

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Spatial analysis of stable isotope data to determine primary sources of nutrition for fish

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Abstract Carbon and nitrogen stable isotopes were used to determine the ultimate autotrophic sources supporting production of three commercially important fish species over unvegetated mudflats in a subtropical estuary. Mean isotope values over the whole estuary for fish and autotroph sources were modeled to indicate feasible combinations of sources. Variability in isotope values among nine locations (separated by 3–10 km) was then used as a further test of the likelihood that sources were involved in fish nutrition. A positive spatial correlation between isotope values of a fish species and an autotroph indicates a substantial contribution from the autotroph. Spatial correlations were tested with a newly developed randomization procedure using differences between fish and autotroph values at each location, based on carbon and nitrogen isotopes combined in two-dimensional space. Both whole estuary modeling and spatial analysis showed that seagrass, epiphytic algae and particulate organic matter in the water column, including phytoplankton, are likely contributors to bream (*Acanthopagrus australis*) nutrition. However, spatial analysis also showed that mangroves were involved (up to 33% contribution), despite a very low contribution from whole estuary modeling. Spatial analysis on sand whiting (*Sillago ciliata*) demonstrated the importance of two sources, mangroves (up to 25%) and microalgae on the mudflats, considered unimportant based on whole estuary modeling. No spatial correlations were found between winter whiting (*Sillago maculata*) and autotrophs, either because fish moved among locations or relied on different autotrophs at different locations. Spatial correlations between consumer and source isotope values provide a useful analytical tool for identifying the role of autotrophs in foodwebs, and demonstrated here that both in situ production of microalgae and organic matter from adjacent habitats were important to fish over mudflats.

Keywords Estuary · Mangroves · Microphytobenthos · Seagrass · Spatial variability

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Introduction

Understanding the role of autotrophs in estuarine foodwebs has important implications for management and conservation. The relative conservation value of habitats has been determined largely by estimating the diversity and abundance of species present (Beck et al. 2001). Evidence demonstrating which autotrophs constitute the ultimate source of nutrition for estuarine animals provides additional data for an objective determination of the relative value of different habitats. Given the potential for extensive movement of energy (carbon) and nutrients in estuarine systems (Odum 1984), consumers may be segregated from the autotrophs upon which they rely (Kneib 2000).

Early foodweb studies attempted to use gut content analysis of organisms at higher trophic levels to clarify trophic dynamics. This method has difficulties, however, as not all ingested material is assimilated (Michener and Schell 1994), and some ingested animals such as nematodes are assimilated very quickly and are therefore rarely found in the stomach (Gee 1989). All animals ultimately rely on autotrophic sources, but for carnivorous fish, gut content analysis of their prey and all intermediate levels would be required to determine which autotroph(s) are at the base of the trophic pathway. One method that allows measurement of assimilated, and therefore nutritionally important, materials is stable isotope analysis. The stable isotope ratios of carbon ($^{13}C/^{12}C$) and nitrogen ($^{15}N/^{14}N$) differ among autotrophs (Fry 1984; Boon et al. 1997; Bouillon et al. 2002). This ratio, the stable isotope signature, is taken on by consumers and reflected in their tissues at whatever trophic level they occur (Peterson 1999).

Large spatial or temporal variations in the isotopic signatures of primary producers can potentially confound attempts to establish the major dietary sources of consumers (Boon and Bunn 1994). Hence, it is essential to quantify these variations before conclusions can be drawn regarding the relative importance of various allochthonous and autochthonous sources of carbon and nutrients (Stephenson et al. 1984). Considerable variation (>10‰) has been found in both the carbon and nitrogen isotopic signatures between individuals of the same species of aquatic plant collected from different sites at the same time of year (Boon and Bunn 1994). Instead of treating this variation in autotroph values as a difficulty to be overcome, variation among locations can be used to determine their importance to consumers. If an autotroph is of high nutritional importance to a consumer, then the isotopic signature of that consumer will shift in the same direction as the autotroph. For example, in a situation where $\delta^{13}C$ signatures of seagrass were the same in two bays but those of algal epiphytes differed, it was shown that $\delta^{13}C$ signatures of shrimp matched those of epiphytes and not seagrass (Fry 1984). Similarly, Kitting et al. (1984) showed that $\delta^{13}C$ signatures of shrimp matched those of epiphytic algae more closely than those of seagrass as the autotroph signatures varied among several sites. This spatial tracking of the isotopic signature of autotrophs by shrimp was used as evidence that the shrimp were assimilating mainly algae. Temporal variation in animal and source isotope values has also been used to indicate assimilation of certain sources (McCutchan and Lewis 2002).

Even the most recently developed isotope mixing models leading to a unique solution (e.g., Phillips and Gregg 2001) are restricted to analyzing only one more autotroph than elements used. These models are of little use in systems such as estuaries where the number of potential autotroph sources (seven in the current study) is much greater than the number of elements potentially able to be used (in estuaries, three: C, N, S). This weakness led Phillips and Gregg (2003) to develop a model calculating feasible combinations of sources that can potentially explain consumer isotope signatures where sources are numerous. Even this latest model, however, cannot reliably distinguish sources making a major contribution to foodwebs. We present a spatial analysis technique that can potentially identify autotrophs involved in the nutrition of consumers in such systems. We take a significant positive relationship between the isotope signatures of a consumer and an autotroph from location to location as indicating very strongly that the autotroph is contributing substantially to the nutrition of the consumer. Previous spatial analyses have determined autotroph importance qualitatively. We not only test for spatial relationships probabilistically, but have also developed a statistical procedure that can operate on data from multiple elements simultaneously. Results of the spatial technique are compared with feasible combinations from the model of Phillips and Gregg (2003).

Whilst there have been many isotope studies attempting to determine which autotrophs are involved in the nutrition of animals found over seagrass meadows (Fry et al. 1986), fewer have assessed this for animals found over unvegetated habitats (Herman et al. 2000; Middelburg et al. 2000), and none have done so for fish. Past research in seagrass ecosystems indicates that algal epiphytes may be more important than seagrass in the nutrition of animals (Fry 1984; Kitting et al. 1984; Nichols et al. 1985; Moncrieff and Sullivan 2001). Benthic microalgal production has been found to be an important component of food webs on saltmarshes (Sullivan and Moncrieff 1990) and in intertidal mangrove forests (Bouillon et al. 2002). Given the high productivity of microphytobenthos (Dennison and Abal 1999), it is possible that algal production is important to fish caught over unvegetated mudflats. Here we use spatial analysis of stable isotope signatures to attempt to determine which autotrophs provide nutrition to three species of fish found over unvegetated habitats in southeast Queensland, Australia.

Methods

Sample collection and processing

Autotrophs and fish were collected in March 2000 at nine locations in southern Moreton Bay, southeast Queensland (Fig. 1). All samples were frozen immediately upon collection. Three species of fish, *Acanthopagrus australis* (Sparidae, yellowfin bream, 45–263 mm, 7 sites), *Sillago ciliata* (Sillaginidae, sand whiting, 15–337 mm, 6 sites) and *S. maculata* (Sillaginidae, winter whiting, 19–103 mm, 7 sites), were collected from unvegetated mudflats using seine nets. Samples of white muscle were taken for processing.

Mangrove leaves were collected from three species (*Aegiceras corniculatum*, *Avicennia marina* and *Rhizophora stylosa*), where present, at each of the nine locations. Isotope signatures of these three species were pooled because they were similar. Three species of seagrass (*Zostera capricorni*, *Halophila ovalis* and *H. spinulosa)* were also collected, and again the isotope signatures were pooled because they were similar. Seagrass epiphytes were separated from seagrass in the laboratory by scraping them off with a scalpel. Saltmarsh plants were collected, where present, and pooled into two groups, saltmarsh succulents (*Sarcocornia quinqueflora* and *Suaeda australis*) and saltmarsh grass (*Sporobolus virginicus*). Particulate organic matter (POM) was collected by filtering 100–800 l of water through 37-μm mesh.

Microphytobenthos (MPB) was collected by scraping the surface 1 cm of sediment from mudflats near where collections of fish were made. One hundred milliliters of sediment was washed through 53-μm mesh to remove infauna. Material passing through the mesh was then washed through 5-µm mesh. Material retained on this mesh was added (9 ml) to a centrifuge tube containing 21 ml colloidal silica (Ludox AM30, density =1.21) and centrifuged at 10,000 rpm for 10 min. A band of diatoms, some organic matter and silica particles formed at the top of the centrifuge tube. This band was removed and again washed through 5-μm mesh to remove the silica and any remaining microbes.

All samples were dried to constant weight at 60°C. After processing, samples were placed in tin capsules and analysed on an Isoprime isotope ratio mass spectrometer. The ratios of $^{15}N/^{14}N$ and $^{13}C/^{12}C$ were expressed as the relative per mil (‰) difference between the sample and conventional standards (air for nitrogen; Pee Dee belemnite limestone carbonate for carbon).

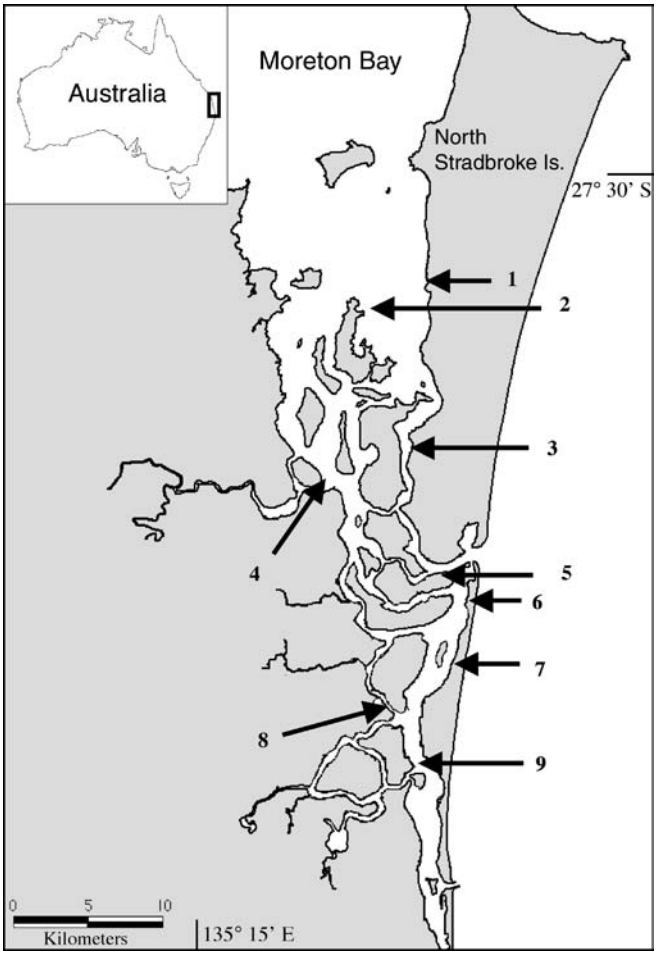

Fig. 1 Location of the nine study sites in southern Moreton Bay

Whole estuary analysis

Data were first analyzed using the isotope values of fish and autotrophs averaged across all locations. With many more potential sources than elements analyzed, mixing models cannot provide a unique solution. Instead we used the Isosource model to calculate feasible combinations of autotrophs that could explain the consumer signature (Phillips and Gregg 2003). This method examines all possible combinations of each autotroph potential contribution (0–100%) in small increments (here 1%). Combinations that summed to within 0.01‰ of the consumer signature were considered feasible solutions. As recommended by Phillips and Gregg (2003), results are reported as the distribution of feasible solutions for each autotroph. The 1%ile and 99%ile range is also given, rather than the full range which is sensitive to small numbers of observations on the tails of the distribution.

Previous studies have shown that nitrogen isotopes in organisms are enriched relative to their diet (Peterson et al. 1986). This fractionation is much larger for ^{15}N than ^{13}C, and nitrogen isotopes have been used to gather information about the trophic level of animals and foodweb structure. To account for fractionation of nitrogen we subtracted the assumed 3‰ per trophic level increase from the nitrogen isotope signature of the fish (De Niro and Epstein 1981; Minagawa and Wada 1984). The number of trophic levels above autotrophs for each fish species was assigned using published dietary information for each species. $\delta^{13}C$ fractionation is close to zero (Peterson and Fry 1987), so no adjustment was made for this element.

Spatial analysis

To determine if spatial tracking was occurring, mean isotope values were calculated for each fish species and autotroph taxon at each location. Using $\delta^{13}C$ and $\delta^{15}N$ signatures as Cartesian coordinates, Euclidean distances were calculated for any one fish species between the value for fish and an autotroph taxon at all locations at which they both occurred. These distances were averaged (D) to produce a measure of correlation in two-dimensional space (tracking). To obtain a distribution of potential fish/autotroph distances, location labels of autotrophs were changed and Euclidean distances were recalculated. The observed D of the fish/utotroph combination was then compared to this distribution of possible D values, giving a probabilistic significance test. If the D value was small relative to the distribution of possible values, then the fish species was said to be tracking that particular autotroph. This was done for all possible combinations of autotrophs against the observed fish data. Each fish species was tested against each autotroph.

Size-dependent isotopic signatures

The relationship between fish length and isotope values was tested for each fish species using regression analysis, on carbon and nitrogen separately. Where a significant relationship existed, raw stable isotope values were adjusted for length using the following formula:

$$\delta X' = \delta X - (a.FL)$$

where δX'= adjusted isotope signature, δX= raw isotope value, a= regression coefficient and FL= fork length of fish (mm).

Results

Autotroph isotope signatures

Stable isotope signatures of the seven taxa of autotrophs were generally well separated using both carbon and nitrogen (Fig. 2). Mangroves and saltmarsh succulents had the most depleted $\delta^{13}C$ signatures while seagrass, seagrass epiphytes and saltmarsh grass had the most enriched signatures. Saltmarsh grass had the most depleted $\delta^{15}N$ signature and seagrass epiphytes and

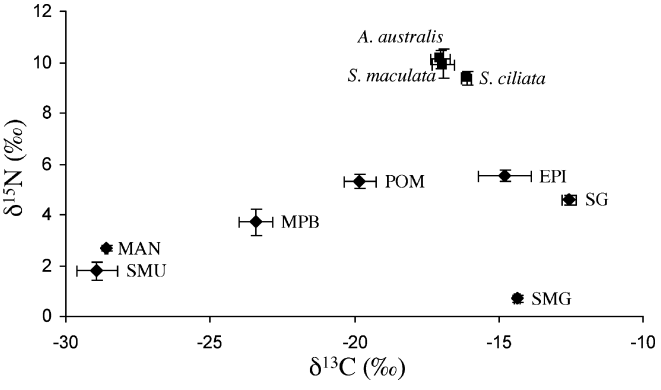

Fig. 2 Mean (±SE) carbon and nitrogen isotope values of *Acanthopagrus australis*, *Sillago ciliata* and *S. maculata* and seven autotrophs. *SG* Seagrass; *EPI* seagrass epiphytes; *MAN* mangroves; *MPB* microphytobenthos; *POM* particulate organic matter; *SMG* saltmarsh grass; *SMU* saltmarsh succulents

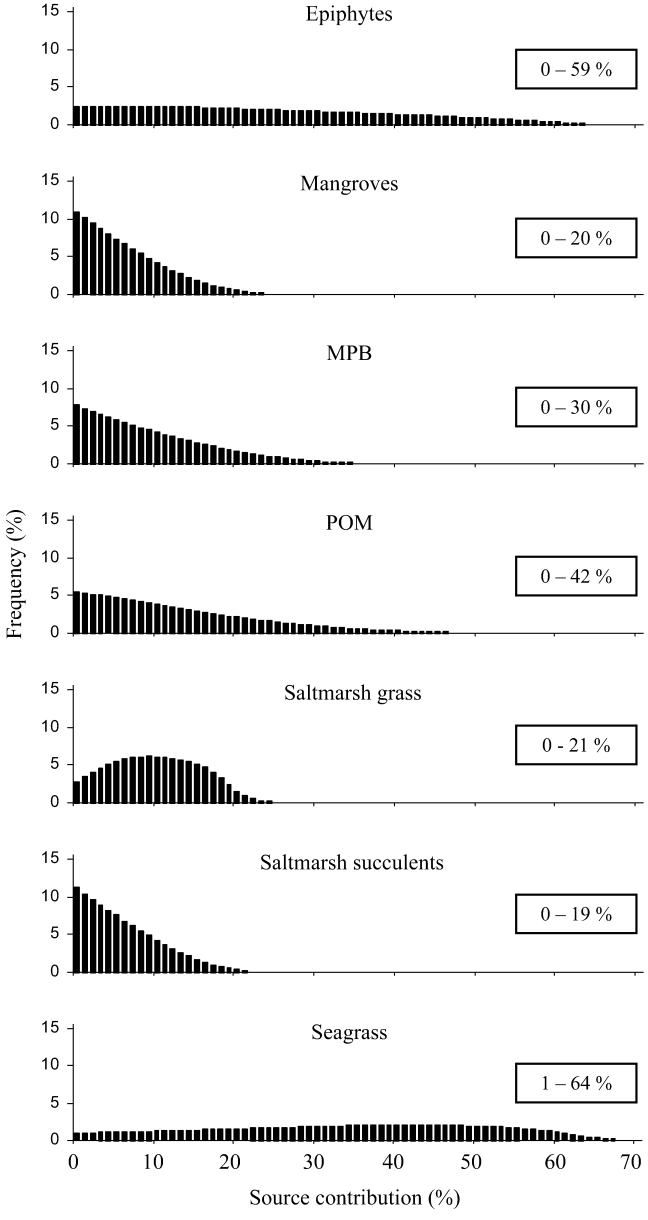

Fig. 3 Histograms of the distribution of feasible contributions of the seven autotrophs for *Acanthopagrus australis*, after correcting fish values for ^{15}N trophic level fractionation. Values in *boxes* are 1%ile and 99%ile ranges for these distributions

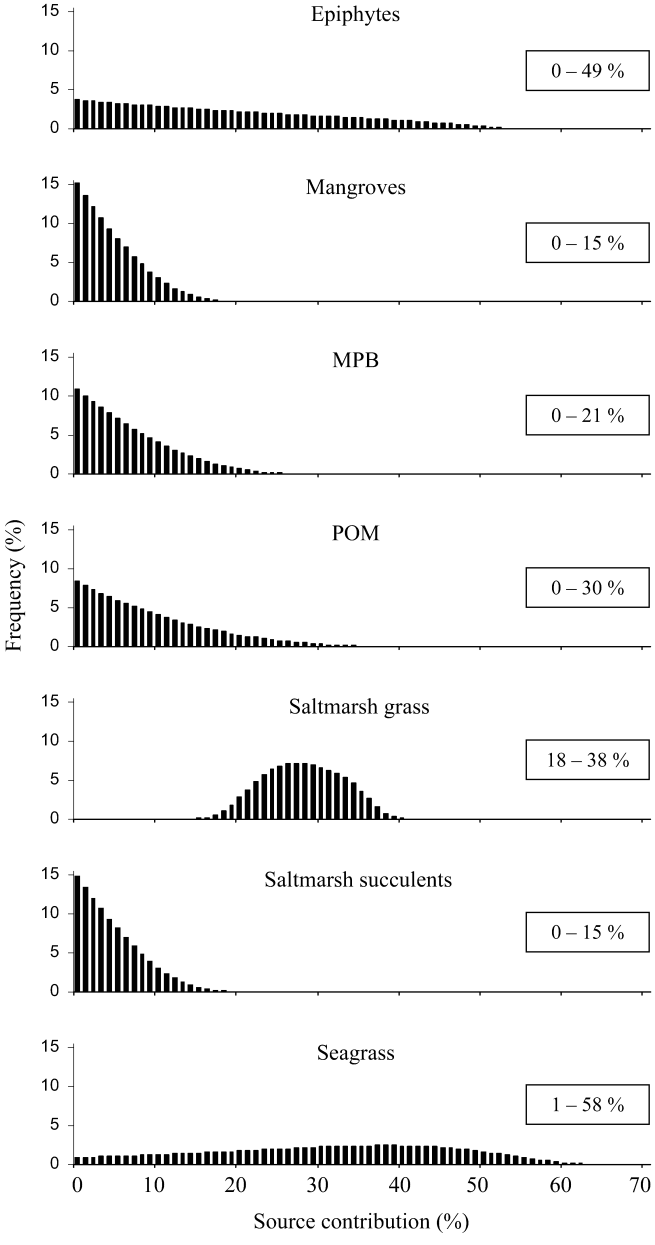

Fig. 4 Histograms of the distribution of feasible contributions of the seven autotrophs for *Sillago ciliata*, after correcting fish values for ^{15}N trophic level fractionation. Values in *boxes* are 1%ile and 99%ile ranges for these distributions

POM had the most enriched signatures. There is a greater range in values for $\delta^{13}C$ (−28.9 to −12.5‰) than $\delta^{15}N$ (0.7–5.5‰; Fig. 2).

Fish isotope signatures

The three species of fish had very similar $\delta^{13}C$ and $\delta^{15}N$ signatures (Fig. 2): *Acanthopagrus australis* (−17.0±0.3‰ and 10.1±0.4 ‰, respectively), *Sillago ciliata* (−16.1±0.2 ‰ and 9.4±0.3‰, respectively) and *S. maculata* (−16.9±0.4 ‰ and 9.9±0.6‰, respectively).

Whole estuary analysis

Modeling of isotope values averaged over all locations gave similar results for the three fish species, although the distributions of feasible contributions varied slightly (Figs. 3, 4, 5). Seagrass and epiphytic algae ranked highly for each species, as did saltmarsh grass for both *Sillago* species, with POM ranking slightly lower. Feasible contributions of mangroves and saltmarsh succulents were very small, and those of MBP were intermediate (Figs. 3, 4, 5).

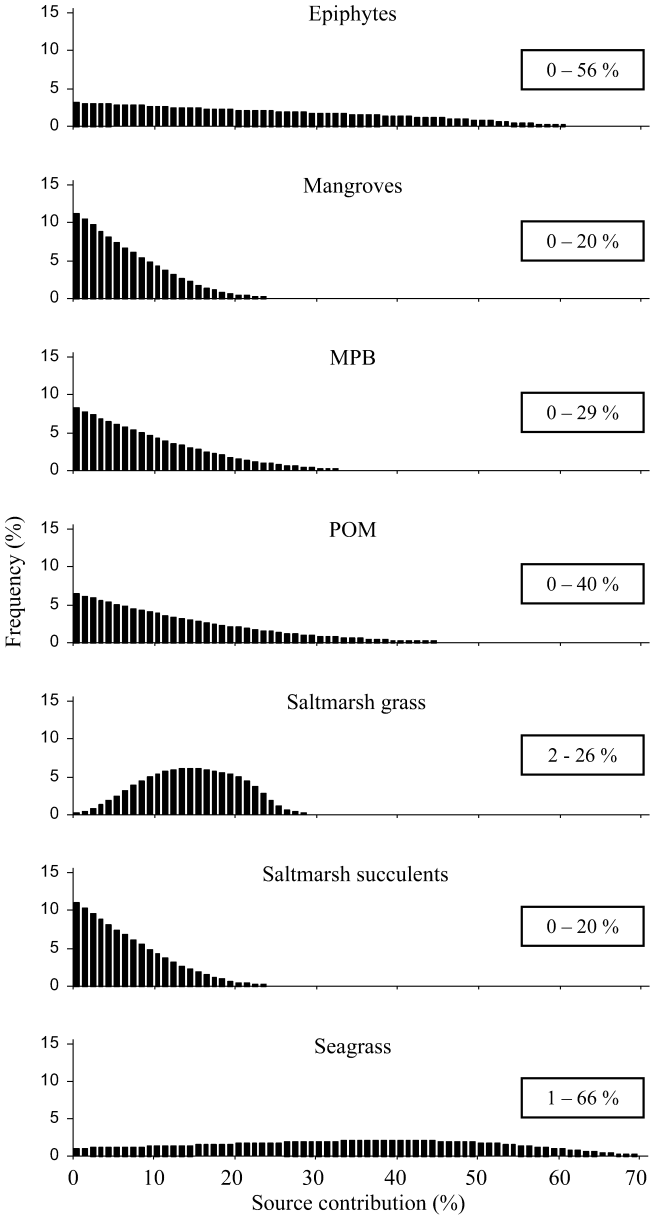

Fig. 5 Histograms of the distribution of feasible contributions of the seven autotrophs for *Sillago maculata*, after correcting fish values for ^{15}N trophic level fractionation. Values in *boxes* are 1%ile and 99%ile ranges for these distributions

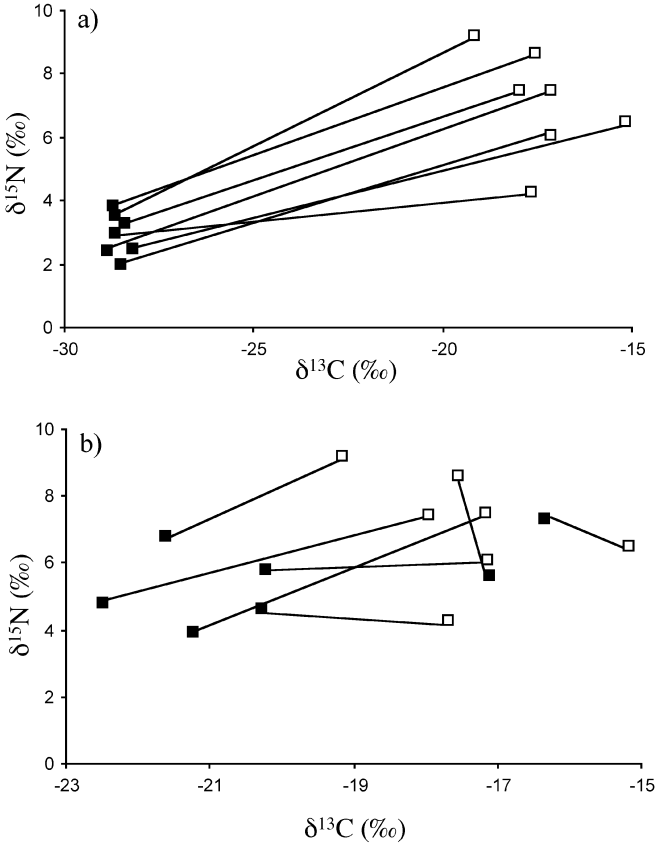

Fig. 6 δ^{13}C and δ^{15}N values at seven joint locations for **a** *Acanthopagrus australis* and mangroves, and **b** *A. australis* and POM. *Lines* join *A. australis* and autotroph from same location. ❑ *A. australis*, ■ mangroves or POM. *A. australis* values adjusted for fractionation

Spatial analysis

There was no correlation between length and δ^{13}C for any fish species (P>0.05), nor was there for length and δ^{15}N for *S. ciliata* or *S. maculata*. However, there was a positive relationship between length and δ^{15}N for *A. australis*. δ^{15}N signatures of *A. australis* were therefore adjusted using the correction equation above (a=0.02) prior to the spatial analysis.

If there is a consistent pattern in the magnitude and direction of the difference between the isotope signature of an autotroph and fish from location to location, observed D will be small relative to possible values, and fish can be said to be tracking the autotroph (e.g., *A. australis* and mangroves; Fig. 6a). Note that the test is independent of the average distance between autotroph and consumer values. Where the pattern in the magnitude and direction of the differences is inconsistent (e.g., *A. australis* and POM; Fig. 6b), no spatial correlation of autotroph and fish isotope signatures exists.

Results of the spatial analysis differed markedly for the three species. Those autotrophs which were more closely tracked by *A. australis* and *S. ciliata* (P<0.10) were well separated from those less closely tracked (P>0.13; Table 1). The significance level was determined post hoc as there was a clear separation among autotrophs at this level. A fish was considered to be tracking an autotroph if <10% of the possible distances (D) was shorter than the observed distance. *A. australis* most closely tracked mangroves, seagrass, POM and saltmarsh grass. *S. ciliata* tracked mangroves, POM and MPB while *S. maculata* did not track the isotope signature of any autotroph (Table 1).

Table 1 Results of spatial analysis for *Acanthopagrus australis*,*Sillago ciliata* and *S. maculata*. Numbers are the percentage of possible *D* values smaller than observed *D*. Low numbers indicate locational tracking of autotroph isotope signatures by that fish species. Values in bold are significant ($P<0.1$).*na* Fish occurred at insufficient locations ($n<4$) where autotroph was present. *MAN* Mangrove leaves,*SG* seagrass, *EPI* epiphytes,*POM* particulate organic matter, *MPB* microphytobenthos, *SMG* saltmarsh grass, *SMU* saltmarsh succulents

	A. australis	*S. ciliata*	*S. maculata*
MAN	**7.6**	**4.5**	13.3
SG	**6.7**	28.3	20.8
EPI	18.3	75.9	66.1
POM	**5.1**	**6.3**	55.5
MPB	14.4	**7.7**	69.9
SMG	**4.2**	na	66.7
SMU	62.3	na	79.2

Discussion

Autotroph isotope signatures

Stable isotope signatures of carbon and nitrogen for mangroves, seagrass, seagrass epiphytes, saltmarsh grass and saltmarsh succulents are similar to those reported in previous studies (Fry 1984; Harrigan et al. 1989; Lee 1995; Boon et al. 1997; Bouillon et al. 2002). The $\delta^{13}C$ values of MPB were similar to the most depleted values reported in the literature (Deegan and Garritt 1997). $\delta^{13}C$ values of POM were within the range of previously reported values (Ogawa and Ogura 1997), representing either a mixture of detritus particles of several autotrophs and/or phytoplankton values.

Autotroph sources for fish

Spatial analysis highlighted the role of four autotrophs in the nutrition of *Acanthopagrus australis*. Three of these, seagrass, saltmarsh grass and POM, also had a high likelihood of contribution based on whole estuary modeling. However, the fourth autotroph showing spatial tracking, mangroves, would be considered unlikely to contribute substantially based on whole estuary modeling. The spatial analysis has in this case provided important additional information unavailable in whole estuary modeling. *A. australis* individuals caught over unvegetated mudflats apparently rely on autotroph sources from adjacent habitats. Stomach content analysis of *A. australis* in Moreton Bay has shown this species to be carnivorous, feeding mainly upon benthic crustaceans and other invertebrates (Morton et al. 1987). The incorporation of organic matter from adjacent habitats might occur through transport of detrital particulate matter to mudflats (Odum 1984) or through a series of predator-prey interactions, in a process known as trophic relay (Kneib 2000). The contribution from in situ MPB production appears relatively minor, but the involvement of POM might include a contribution from phytoplankton, which occurs ubiquitously in estuaries and could there be considered a partially in situ source.

For *Sillago ciliata*, spatial analysis indicated a contribution from three autotrophs, of which only POM had even a medium likelihood of contribution based on whole estuary modeling. The other two autotrophs, mangroves and MPB, would have been considered unlikely to contribute based on whole estuary modeling. In situ MPB production, and possibly phytoplankton as part of the POM, do contribute to the nutrition of *S. ciliata*. Although it has been shown that MPB on mudflats is assimilated by meiofauna (Herman et al. 2000; Middelburg et al. 2000), its importance to animals at higher trophic levels has been little studied. The only previous demonstration of the role of MPB in the nutrition of such animals is for macroinvertebrates in mangroves forests, which were shown to assimilate MPB from the mangrove sediments (Bouillon et al. 2002). For *S. ciliata*, autotroph sources from adjacent habitats also make a contribution. This species is a benthic carnivore (Burchmore et al. 1988) and, as for *Acanthopagrus australis*, the transfer of organic matter from adjacent habitats to mudflats might occur through transport of detrital particulate matter or via trophic relay. The mean $\delta^{13}C$ value for *S. ciliata* is more enriched than any of the autotrophs with which it had a spatial relationship. One or more of the $\delta^{13}C$ enriched autotrophs must be involved in *S. ciliata* nutrition, despite the lack of spatial relationships. It might be that, of the autotrophs with enriched $\delta^{13}C$ signatures, different ones are important to *S. ciliata* at different locations, so that no relationship is discernible across all locations.

S. maculata was the only species not to track the isotope signature of any autotroph. This could be a result of differences among sites in factors other than isotope values, such as food availability or trophic structure. We consider the most likely explanations for this result are site-specific diet selection or movement of individuals among sites. This species is a benthic carnivore, feeding mainly on crustaceans and polychaetes (Burchmore et al. 1988). It has been shown in southern Australian waters that the diet of this species can vary with location (Burchmore et al. 1988), and it is possible that site-specific diet selection in Moreton Bay could mean that different autotrophs are important from location to location. Such a pattern of dependence upon different autotrophs at different locations within an estuary has previously been shown for prawns (Loneragan et al. 1997). There is little information on the movement of *S. maculata*, although small-scale spawning migrations have been recorded (Kerby and Brown 1994). Whilst movement among sites remains a possibility for this species, more detailed studies of movements among locations separated by several kilometers would be needed to evaluate this possibility fully.

Table 2 Summary of results of a single element (carbon) mixing model for *Acanthopagrus australis*and *Sillago ciliata*, using mangroves and seagrass. *CL* Confidence limit

	Mangroves			Seagrass		
	Lower 95% CL	Mean	Upper 95% CL	Lower 95% CL	Mean	Upper 95% CL
A. australis	0.24	0.28	0.33	0.67	0.72	0.76
S. ciliata	0.19	0.22	0.25	0.75	0.78	0.81

Importance of mangroves

In assessing the contribution of estuarine autotrophs from a whole estuary perspective (Figs. 3, 4, 5), mangroves appeared unlikely to be a substantial contributor for any of the fish species. However, two out of the three species show locational tracking of the mangrove isotope signature. Hence mangroves appear to have some importance as a nutrition source for fish found over unvegetated habitats. In addition to the feasibility modeling, to determine the potential of mangroves as a source for these two fish species, a single element (carbon) mixing model was run, using just two sources, mangroves and seagrass. Seagrass was chosen because it has the most enriched carbon isotopic signature. These isotopically distinct autotrophs therefore represent the maximum contribution mangroves could have made to the diet of the two fish species. Mangroves could comprise up to 33% (upper 95% confidence limit) of the carbon used by *A. australis* and up to 25% used by *S. ciliata* (Table 2). Although the whole estuary approach indicated mangroves to be an unlikely autotroph source for fish species, spatial analysis has revealed its potential importance for fish in unvegetated habitats.

Mangrove detritus has been found to contribute up to 84% of the total assimilated carbon by prawns found in mangrove areas (Chong et al. 2001). However, mangrove carbon contribution decreased downstream from the vegetated areas as tidal influence increased production and the contribution of phytoplankton. Even then, the contribution of mangrove detritus amounted to between 16% and 24% for prawns. Rodelli et al. (1984) found that consumers in mangrove creeks assimilated on average 65% mangrove carbon, but this dependency gradually decreased with distance offshore. However, significant assimilation of mangrove-derived carbon was only detectable in a limited number of species, with local and imported algal sources a major contributor of carbon to benthic consumers in intertidal mangrove forests (Bouillon et al. 2002).

Where a fish species shows a positive spatial relationship with more than one autotroph, it is possible that not all of the autotrophs are actually contributing. One autotroph may be contributing to the fish species, while other autotrophs may simply happen to show the same pattern of variability across locations as this first autotroph. This possibility can be excluded if tests of spatial correlations amongst the autotrophs involved show that they vary in different ways across the locations. We made multiple pairwise comparisons amongst each of the autotrophs showing a spatial relationship with a fish species, using the spatial analysis described above to compare fish with autotrophs. Where two autotroph taxa had enough joint locations to make an effective test, the patterns of variability among locations differed for all relevant taxa for both *A. australis* and *S. ciliata*. For example, for *S. ciliata*, this shows that fish isotope values tracked those of POM, MPB and mangroves even though these three autotrophs themselves had a different pattern.

Size-dependent isotopic signatures

There was a positive relationship between length and δ^{15}N of *A. australis*. Similar results have been found for other fish species and are often attributed to either ontogenetic change in diet or differential metabolic fractionation of nitrogen with age (Beaudoin et al. 1999; Overman and Parrish 2001). However, other studies have found no correlation between length and isotope values (Rau et al. 2001; Vander Zanden and Rasmussen 2001). Since there was no correlation between length and δ^{13}C of *A. australis* it seems more likely that there is differential metabolic fractionation of nitrogen with age and not an ontogenetic shift in diet. Given the absence of a correlation between length and δ^{13}C or δ^{15}N values of *S. ciliata* and *S. maculata*, it is clear that any adjustment factor required to avoid invalidating dietary reconstruction based on stable isotopes will be species-specific. If possible, isotope-based interpretations of diet should be limited to individuals of the same size to avoid any potential confounding effects (Branstrator et al. 2000). However, in this study, although *A. australis* individuals could potentially be separated into two size classes, neither class was caught at enough sites to analyse on its own.

The differential metabolic fractionation of nitrogen with size (age) in *A. australis* could lead to incorrect dietary reconstruction when using stable isotopes, particularly if different size classes dominate different locations. For example, congregation at particular sites of larger (older) fish, with greater fractionation over their autotroph source, would obscure the relative contribution of autotrophs overall. The use of corrected values in the spatial analysis removed this effect due to size of the organism.

Spatial analysis versus mixing models

There have been previous attempts at using locational tracking to evaluate the importance of autotrophs. Kitting et al. (1984) noted that consumer isotopic signatures responded to shifts in algal epiphyte values rather than seagrass values. However, this trend was only examined graphically; the two-dimensional significance test used in this study provides a more rigorous, quantifiable measure of locational tracking.

Isotope analyses often correct for fractionation using a mean value of 3‰ per trophic level for nitrogen. However, levels of ^{15}N fractionation have been shown to vary considerably about this mean (Vander Zanden and Rasmussen 2001), being affected by starvation (Hesslein et al. 1993), age (Overman and Parrish 2001) and food quality (Adams and Sterner 2000); having to correct for fractionation based on an assumption of 3‰ per trophic level is therefore a weakness of mixing models. One advantage of the spatial analysis technique used here is that correction for fractionation based on trophic level assignation is unnecessary. Although values can be corrected for size-isotope relationships (as above), the actual distance between fish and autotroph values is irrelevant and there is no need to attempt to adjust fish values for trophic level. Only the pattern of differences from location to location between fish and the autotroph being tested is of interest.

Selection of sampling sites is an important factor in the success of using the spatial analysis presented here. Sites should be far enough apart to avoid substantive movement of individual consumers or of organic matter among sites. Furthermore, the analytical position is improved if ecological information is available about any potential differences among sites in food availability, diet selection or other aspects of trophic structure. Nevertheless, once it is shown that consumer isotope values are correlated spatially with one or more autotrophs, these concerns are minimal.

We argue that when spatial analysis finds a consistent offset between consumer and autotroph values there is a strong logical link indicating a contribution substantial enough to result in the spatial correlation. Furthermore, the strength of this relationship can be tested probabilistically, and the test can be repeated in time. On the other hand, basing likely contributions simply on proximity of a consumer to autotroph value is unable to resolve any situation where the combination of two or more sources gives the same result as another single source. Unfortunately this is almost always a possibility in estuarine work where sources are numerous. The spatial test on its own cannot resolve everything, and is best used in conjunction with the whole estuary feasibility modeling.

Conclusion

Explicit spatial analysis helped determine the importance of autotrophic sources. Several different autotroph taxa were shown to be important sources of nutrition for fish found in unvegetated habitats. Both in situ and outwelled organic matter was important for fish species. Spatial analysis showed that, for *A. australis*, mangroves, seagrass, POM and saltmarsh contribute to their nutrition. For *S. ciliata*, mangroves, MPB and POM contribute. The contribution of mangroves to these two species is particularly surprising given the low likelihood of substantial contribution based on whole estuary analysis. Spatial analysis did not further our understanding of *S. maculata* sources, either because fish move among locations or because they utilize different sources at different locations. The combination of spatial analysis and modeling of feasible sources can be used together to help resolve situations in which numerous potential sources are available to consumers.

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