Stable isotope tracing of the contribution of seagrass production to subtropical fisheries species occurring outside seagrass areas

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OBJECTIVES:

1. Quantify the contribution of seagrass meadows to fisheries species found not in seagrass but elsewhere in estuaries or offshore.
2. Determine the ultimate source of primary (plant) productivity sustaining fisheries production of several key species of fish and crustaceans in subtropical Australian waters.
3. Ensure that information about the relative importance of seagrass to production in different fisheries is taken to fisheries and other coastal managers to influence future management decisions.

Non-technical Summary

Outcomes

Results from this project affect the relative importance coastal managers will place on different estuarine habitats. Until now primary production from mangrove forests has been ranked highly for its presumed contribution to fisheries species occurring seaward of mangroves. This project has shown, however, that in subtropical Australian estuaries and bays, fish and crustaceans caught over shallow mudflats are much more likely to obtain substantial nutrition from seagrass meadows and *in situ* production of microalgae. Mudflats lacking conspicuous vegetation not only provide habitat for certain key fish and crustacean species but also seem to play an important trophic role. The project also developed quantitative techniques for analysing stable isotope data. These have already been taken up by other scientists, and will help them answer big picture questions about fisheries foodwebs that have appeared intractable.

Need

The conservation and protection of estuarine habitats such as mangroves, seagrass, saltmarsh and unvegetated mudflats remain under constant threat from human activities. In the face of loss of habitat, coastal managers are forced to choose among different habitats as to their importance, especially to fisheries sustainability. The relative importance of different estuarine habitats has been too reliant on the occurrence of fisheries species in the habitat itself. Energy and nutrients (as organic matter) are mobile in aquatic systems, so plant production (including algae) in one habitat might be at the base of foodwebs supporting fisheries in another habitat. These potential trophic links between plants in one place and animals in another should be taken into account in deciding the merits of different habitats. Stable isotope analysis was used as a chemical tracer of the role plants from different habitats have in supporting foodwebs sustaining production of fish and crustaceans caught over shallow mudflats and offshore. The emphasis was on determining the importance of seagrass production, but to put that in context we also examined the contribution of plants from other habitats.

Objective 1. Quantify the contribution of seagrass meadows to fisheries species found not in seagrass but elsewhere in estuaries or offshore.

- We overcame previous weaknesses in isotope analysis by developing a mixing model capable of including numerous autotrophs and their spatial variance, which provided a quantitative platform from which to determine likely contributions.
- In Moreton Bay, southeast Queensland, 22 fish species caught over unvegetated mudflats were analysed using carbon and nitrogen isotopes. Seagrass had the most enriched carbon isotope signature of any plant, and all fish had values within the enriched half of the range of plant values. The likely contribution of seagrass and microalgae on seagrass was very high for most of the 22 species.
- For specialist fish species such as sea garfish, seagrass was clearly the major source of nutrition. For some species with more generalist feeding habits, seagrass was also the major source at the base of their foodweb, for example contributing up to 72% of bream nutrition and 78% of summer whiting nutrition.
- This seagrass material is transported either via export of particulate matter from seagrass to mudflats or via a series of predator-prey interactions (trophic relay).
- Seagrass also made a substantial contribution to fisheries foodwebs in Port Curtis, central Queensland, with *Zostera* seagrass the main contributor to fish, and Halophila seagrass the main contributor to crustaceans such as mud crabs and banana prawns.
- The contribution of seagrass to snapper nutrition was examined using isotopes of sulfur, in addition to carbon and nitrogen. Macroalgae was the most likely source at the base of the foodweb supporting snapper, although seagrass may also contribute.
- A model of decreasing contribution of estuarine plants (such as seagrass) to snapper with distance offshore was tested. Any differences in contributions with distance offshore were masked by movement among locations by snapper over periods of months (the time taken for tissue in adult fish to reflect plant source).

Objective 2. Determine the ultimate source of plant productivity sustaining fisheries production of key species of fish and crustaceans in subtropical Australian waters.

- For fish and crustaceans in Moreton Bay and Port Curtis, we modeled the importance of *in situ* sources (e.g. microalgae on mudflats, or phytoplankton production in the water column) versus sources transported to mudflats (e.g. seagrass, mangroves). Seagrass and its epiphytic algae (transported autotrophs) were the most likely sources of nutrition for most fish species, but most species also had an *in situ* source within the three most likely sources.
- Apart from seagrass, saltmarsh grass had a high likelihood of contributing to the nutrition of many fish species in Moreton Bay and Port Curtis. We suspect this is a spurious result, reflecting the similarity in saltmarsh grass signatures to those of seagrass. This should be tested in future using alternative chemical biomarkers.
- Mangrove contributions to fisheries foodwebs are small but detectable (e.g. 28% for bream and 22% for summer whiting). This small contribution is surprising given the proximity of mudflats to extensive mangrove forests in both study areas.
- Snapper caught inside and offshore of southern Moreton Bay all had isotopic signatures consistent with either: a) a macroalgae source, or b) a mixture of carbon isotope enriched macrophyte sources such as seagrass with a smaller amount of a carbon isotope depleted source such as mangroves, with or without a macroalgae contribution. Alternative chemical biomarkers will be needed to distinguish between these possibilities.

Objective 3. Ensure that information about the relative importance of seagrass to production in different fisheries is taken to fisheries and other coastal managers to influence future management decisions.

- It is important to disseminate information at two levels: isotope methodological advances and scientific findings about the role of estuarine habitats in fisheries foodwebs, and the management implications flowing from them.
- This project developed quantitative techniques that increase the scientific rigour of isotope studies, and which are being used in subsequent isotope studies. Ultimately the increased scientific rigour will help to more quickly answer management questions about foodwebs and fisheries that have to date been nearly intractable. The new methods have been presented at scientific conferences.
- The scientific findings being disseminated are that: a) seagrass is important as an ultimate source of energy and nutrients to fish that are not actually caught over seagrass, and b) mudflats not only act as habitat for certain fish and crustacean species but also support autotrophic production that sustains fish production.
- Management implications are that removal of seagrass or mudflats (e.g. by dredging) will therefore disrupt trophic pathways.
- Results from Moreton Bay have been presented directly to fish habitat managers, and results from Port Curtis to the State of Port Curtis conference.

KEYWORDS: benthic microalgae, banana prawns, bream, estuaries, food webs, garfish, mangroves, mud crabs, saltmarsh, seagrass, snapper, stable isotopes, whiting

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1.0 Background

Seagrass meadows provide habitat for juveniles of some economically important fish and crustacean species (Bell & Pollard 1989). Some fish species are found almost exclusively in seagrass (e.g. trumpeters, *Pelates octolineatus*; rock (weedy) whiting, family Odacidae; and several pipefish species, family Syngnathidae). Most fisheries species found in seagrass, however, are also found in adjacent habitats, either of unvegetated soft bottom, mangrove forest or in deeper waters. The importance of coastal habitats for sustaining fisheries production was recognised by FRDC in funding two major reviews: Cappo et al. (1997) "A review & synthesis of Australian fisheries habitat research", and Butler & Jernakoff (1999) "Seagrass in Australia: strategic review and development of an R & D plan". The first report highlighted the need to examine indirect links between habitat and fisheries production, and the second gave high priority to research on determining how dependent on seagrass are fisheries species that do not actually occur in seagrass.

Fisheries species are in many cases more abundant in unvegetated patches adjacent to seagrass than in seagrass itself (e.g in Victoria: juvenile flounder, and (sometimes) juvenile King George whiting, *Sillaginodes punctata* (Jenkins et al. 1997); in northern NSW: whiting, *Sillago* spp. (Gray et al. 1996)). Production of fish species of economic importance in southern Australia is often as high in unvegetated areas as in seagrass beds (Edgar & Shaw 1995a). Halliday (FRDC 91/041) found that in Tin Can Bay, Qld, abundances of eastern king prawns (*Penaeus plebejus*) and whiting (*Sillago* spp.) increased where seagrass died off during his 2 year survey. Experimental removal of seagrass on a small scale has also failed to demonstrate that fisheries species are adversely affected (Connolly 1994). An argument can be developed from these results that seagrass is overrated as a habitat supporting fisheries. It is likely, however, that the failure of researchers to demonstrate a link between seagrass and many species in adjacent waters is that there has been enough seagrass remaining in close proximity to provide primary (plant) production to drive the food web that supports the species. That is, energy (carbon) and nutrients (e.g. nitrogen) are moving from seagrass beds to other habitats (e.g. unvegetated mud flats) and indirectly supporting the fisheries species there. Even fisheries species living in deeper parts of estuaries or offshore, and having no obvious link with seagrass, might derive nutrition indirectly from seagrass beds. Larval blue grenadier (*Macruronus novaezelandiae*) on the west coast of Tasmania, for example, apparently benefit from detritus of seagrass from the Australian mainland (Thresher et al. 1992).

The question of how important seagrass is in sustaining fisheries species not actually occurring in seagrass is best answered using stable isotope analysis. This is the method recommended in Butler & Jernakoff (1999) because it circumvents many of the limitations of traditional methods of assessing diet. Major food pathways in several different aquatic ecosystems have been traced using stable isotope analysis (e.g. Peterson & Fry 1988, Bunn & Boon 1993). Elements such as carbon and nitrogen have more than one naturally occurring isotope. The method relies on different sources of food (plants) having different ratios of the rare and common isotopes (e.g. $^{13}C/^{12}C$); these ratios are transferred to the tissue of animals consuming the plant source (e.g. small crustaceans), and thence on to secondary consumers (e.g. fisheries species).

The study of blue grenadier (cited above) used stable isotope analysis, and Loneragan et al. (1997) used the method very effectively to demonstrate that juvenile tiger prawns in the Gulf of Carpentaria rely mostly on seagrass rather than mangroves as an energy source. Indeed,

recent stable isotope work in Australia and Asia suggests that, in general, mangrove production is important to species occurring in mangrove forests but does not contribute to food webs in other parts of estuaries (Lee 1995).

The dominant paradigm during the 1970s and 1980s was that plant material from habitats such as seagrass was exported on currents to other parts of estuaries and offshore, where it decomposed and formed the basis of detrital food webs that led to fisheries production (Odum 1984). In a major review of estuarine food webs, Kneib (1997) pointed out that large amounts of energy and nutrients might also be transferred from shallow vegetated habitats to deeper waters by a process known as trophic relay. That is, small fish in very shallow waters, having eaten small herbivorous invertebrates, are themselves eaten by larger fish that range occasionally into shallow water. These larger fish are in turn eaten by even larger predatory fish that spend most of their time further offshore. Energy and nutrients are thus moved from shallow to deeper waters in the bodies of animals. This process may well be important in the context of Australian seagrass beds, which have a fish fauna dominated by vast numbers of small species of no direct economic value (e.g. perchlets – Chandidae; gobies – Gobiidae, pipefish - Syngnathidae). Stable isotope analysis is able to detect energy/nutrient transfer by either of these processes.

Structure of report

The main body of this report consists of 4 chapters.

Chapter 4. This chapter reports stable isotope results from a major survey of 22 fish species and the seven major autotroph taxa in Moreton Bay. It also describes and employs a mixing model we developed to determine the putative contribution of the numerous autotroph sources available in estuaries.

Chapter 5. Isotope results of a bay-wide survey in Port Curtis are reported here, for nine fish species and four crustacean species.

Chapter 6. Results from the Moreton Bay survey were re-analysed in this chapter, using a spatially explicit test developed by us in this study. We used this novel analytical method to further test the importance of the numerous autotroph sources potentially available, to three common estuarine fisheries species.

Chapter 7. This chapter examines the contribution of estuarine and offshore reef autotroph sources to snapper, measured over two years.

A major limitation in previous stable isotope work in estuarine systems has been the absence of data on inconspicuous or little known autotroph sources. During this project, with the assistance of A/Prof Lee from Griffith University and with additional financial support from other sources, we developed and tested a new technique for separating microalgae from estuarine sediment. This technique is fully described in Appendix 3. We also examined in detail several aspects of mangrove isotope measurement, results of which are reported in Appendix 4.

2.0 Need

An examination of which fisheries species are sustained by seagrass plant production was highlighted as a major research priority in the reviews of fisheries habitat research gaps by Cappo et al. (1997) and Butler & Jernakoff (1999). The recommended method in Butler & Jernakoff for tracing seagrass production to fisheries species is stable isotope analysis. Coastal and fisheries managers currently consider seagrass to be valuable, nevertheless there are many seagrass meadows under threat and still being lost. An argument can be developed, supported by current scientific evidence, that many important fisheries species are not reliant on seagrass and that their numbers actually increase upon the decline of seagrass. Estuarine and offshore fisheries species that do not appear to be dependent on seagrass might actually be so, but indirectly; they may be deriving their food from animals in a trophic web that is sustained by energy (carbon) and nutrients (e.g. nitrogen) transported from seagrass meadows. Another estuarine habitat, mangrove forest, has previously been touted as generating plant production that drives food webs elsewhere in estuaries and offshore. Recent evidence from Australia and Asia suggests this is not so; mangroves may only sustain species living in mangrove forests (Lee 1995). Still other habitats involve very significant quantities of plant production; nothing is known of the importance of these other sources to fisheries production in subtropical Australian waters. The question whether seagrass production is the major source of primary production sustaining fisheries production needs answering. The only method used successfully for tracing the ultimate autotrophic source at the base of foodwebs supporting fisheries species is stable isotope analysis.

The study areas of Moreton Bay and Port Curtis are good test cases for research of this kind. These bays are of extraordinary importance to Queensland fisheries, with Moreton Bay alone comprising up to 30% of the total Queensland catch of inshore recreational and commercial species (Tibbetts & Connolly 1998). There are also important fisheries in deeper waters adjacent to these bays. Both bays have extensive areas of seagrass, but also mangroves, saltmarsh and very large areas of mudflats without conspicuous vegetation. Both bays have also suffered ongoing loss of estuarine habitats (Walker 1997, Dennison & Abal 1999). This loss is not evenly spread across the different habitats. Saltmarshes have suffered particularly severely in Moreton Bay, for example, with 90% of saltmarsh habitat being lost in southern Moreton Bay since 1955 (SKM 2001). The need, therefore, is for information on the importance of specific habitats to estuarine foodwebs and fisheries production. This need has also been identified by the CRC for Coastal Zone, Estuary and Waterway Management, which is currently developing projects aimed at protection and even restoration of estuarine habitats in Moreton Bay and Port Curtis.

3.0 Objectives

The objectives in the original project proposal were to:

1. Quantify the contribution of seagrass meadows to fisheries species found not in seagrass but elsewhere in estuaries or offshore.

2. Determine the ultimate source of primary (plant) productivity sustaining fisheries production of several key species of fish and crustaceans in subtropical Australian waters.

3. Ensure that information about the relative importance of seagrass to production in different fisheries is taken to fisheries and other coastal managers to influence future management decisions.

No changes were made to these objectives during the project.

4.0 Whole estuary analysis of the contribution of estuarine autotrophs to fish over unvegetated mudflats in Moreton Bay

4.1 Introduction

Fish that occupy mudflats in estuarine systems must ultimately obtain their carbon from one of two potential sources, 1) *in situ* production, or 2) material transported (outwelled) from nearby autotrophs to the mudflats. *In situ* production occurs in the water column or on the surface (microphytobenthos) of the mudflats. Organic matter can be transported to mudflats either by direct movement of plant material from external sites of production, or in the bodies of animals as a series of predator-prey interactions (Kneib 2000).

The outwelling hypothesis was developed to explain high secondary productivity near the extensive areas of the saltmarsh plant *Spartina alterniflora* on the east coast of the USA (Odum 1984). While there are substantial saltmarshes on the subtropical east coast of Australia, mangroves dominate the mid-intertidal fringes of estuaries there. Forests of mangroves fix approximately 600 g carbon m^{-2} y^{-1} in Moreton Bay (Dennison & Abal 1999). As yet, however, there is little evidence that carbon fixed by mangroves moves far out of these forests (Lee 1995). Seagrasses represent another potential source of carbon in subtropical estuarine systems. Seagrasses form large beds in the estuaries of subtropical Australia and fix approximately 200 g carbon m^{-2} y^{-1} in Moreton Bay (Dennison & Abal 1999). While some fish that are common over mudflats directly consume seagrass (Clements & Choat 1997), most seagrass is consumed directly by crustaceans or enters the detrital food web (Edgar & Shaw 1995a). Seagrass epiphytes, a mixture of diatoms, fine filamentous algae and encrusting coralline algae, may fix as much carbon as the seagrass they grow on (Keough & Jenkins 1995). Seagrass epiphytes have been shown to contribute carbon to many invertebrates that feed in seagrass beds (Moncreiff & Sullivan 2001) and represent a potential source of carbon for fish that occur over mudflats. *In situ* production by microphytobenthos and phytoplankton may be an important source of carbon for fish that occur over mudflats. Microphytobenthos, the most productive autotroph in Moreton Bay, fixes approximately 1700 g carbon m^{-2} y^{-1} (Dennison & Abal 1999). Phytoplankton, which is ubiquitous in estuarine systems, fixes approximately 175 g carbon m^{-2} y^{-1} in Moreton Bay (Dennison & Abal 1999).

High rates of anthropogenic development in the coastal zone mean managers are often faced with choosing which habitats to preserve. Seagrass beds, saltmarshes and mangrove forests are considered to be of high conservation value (Edgar & Shaw 1995b) and as such, are preserved at the cost of mudflats. Some fish species occur more often over mudflats than other areas in estuaries (Gray et al. 1998), indicating that mudflats contribute to biodiversity. If *in situ* production supplies a substantial proportion of the nutrition to fish that occur over mudflats, managers should also be preserving this habitat for its trophic contribution to fisheries production.

Stable isotopes are increasingly being used to determine which autotrophs supply energy to food webs. Early stable isotope studies used only one element (generally carbon) and data were analysed by visually estimating the proximity of the consumer of interest to potential sources on a line (e.g. Nichols et al. 1985). Later, to overcome the lack of separation of the carbon isotope signatures of some autotrophs, biplots were constructed using two elements (generally carbon and nitrogen) (e.g. Peterson et al. 1986, Fry 1988). More recently, mixing models have been used to assign percent contributions by different autotrophs to the diet of

the consumer of interest (e.g. Szepanski et al. 1999). The most recent models use variances about autotroph and consumer mean isotope values to calculate variances about mean contributions by autotrophs (Phillips & Gregg 2001).

Mixing models used in previous studies (e.g. Phillips & Gregg 2001, Phillips & Koch 2001) have been restricted to analysing one more autotroph than elements used. For example, a study using isotopes of carbon and nitrogen could only analyse the contribution of three autotrophs. However, in the current study there are seven taxa of autotrophs, and so isotope ratios of six elements would be needed. To overcome this problem we have developed a Euclidean mixing model that determines, for any consumer, the mean putative contribution, and variance about this contribution, for multiple autotrophs. We use "putative contribution" to describe results from the Euclidean mixing model, as there are multiple solutions for each analysis.

There have been many studies that attempt to determine which autotrophs fix carbon used by consumers that are found over seagrass meadows (Fry 1984, Kitting et al. 1984, Nichols et al. 1985, Fry et al. 1986, Moncreiff & Sullivan 2001). Although there have been studies that examine gut contents of fish found over unvegetated mudflats (Moriarty 1976, Robertson 1977, Connolly 1995, Edgar & Shaw 1995c), there have been no studies that attempt to determine which autotrophs fix carbon for these fish. Here, we use stable isotope analysis of carbon and nitrogen to determine whether outwelling of carbon to mudflats, or *in situ* production, contributes most nutrition to fish that occur over mudflats in a large estuarine system. The Euclidean mixing model developed is used to assess the putative contribution of autotrophs, including seagrass, to estuarine fish.

4.2 Methods

Sample collection and processing

Southern Moreton Bay, southeast Queensland, is characterised by intertidal and shallow subtidal seagrass beds interspersed with extensive mudflats. The coastline comprises islands and the mainland, fringed with mangroves, which are often backed by saltmarsh. Autotrophs and fish were collected in March 2000 at nine locations in Moreton Bay (Fig. 1). All samples were frozen immediately upon collection.

Fish were collected from mudflats using several sizes of seine nets. Samples of white muscle were taken for processing.

Mangrove leaves (MAN) were collected from 3 species (*Aegiceras corniculatum*, *Avicennia marina* and *Rhizophora stylosa*), where present, at each location. All samples of mangrove leaves were green, as we have shown that the stable isotope ratios of green and yellow mangrove leaves do not differ (Appendix 4), and green mangrove leaves are more easily and efficiently collected. Values from the three species were pooled as their isotopic signatures were similar.

Where present, three species of seagrass (SG; *Zostera capricorni*, *Halophila ovalis* and *H. spinulosa)* were collected from each location. Seagrass epiphytes (EPI) were separated from seagrass in the laboratory by scraping them off with a scalpel.

Saltmarsh plants used for stable isotope analysis comprised three species; the C_3 saltmarsh succulents (SMU; *Sarcocornia quinqueflora* and *Suaeda australis*) and the C_4 saltmarsh grass (SMG; *Sporobolus virginicus*).

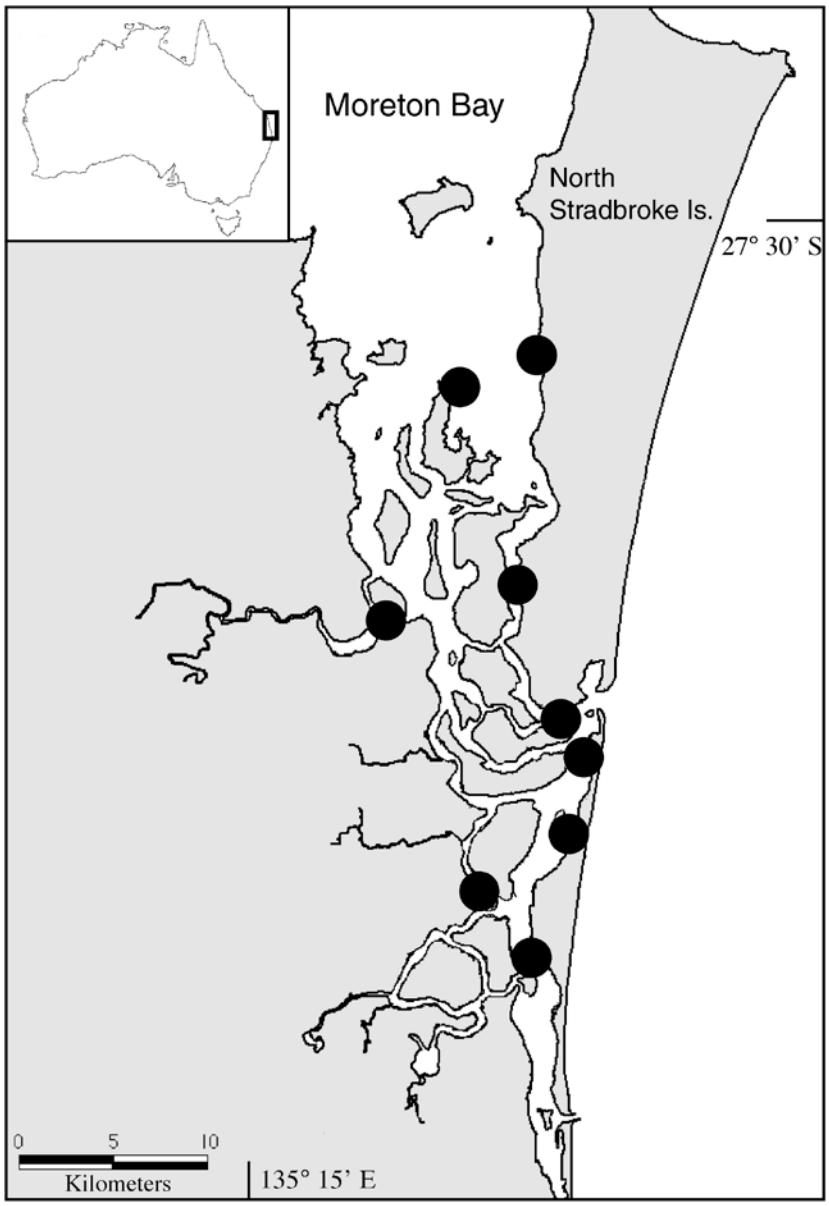


Figure 1. Map of southern Moreton Bay indicating sampling sites.

Microphytobenthos (MPB) were collected by scraping the surface 1 cm of sediment from mudflats near where collections of fish were made. 100 mL 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 minutes. 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 a 5 μm mesh to remove the silica and any remaining microbes.

Particulate organic matter (POM) was defined as that fraction retained after filtering 100-800 litres of water through 53 μm mesh.

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; PeeDee belemite limestone carbonate for carbon).

Fractionation and trophic level

Previous studies have shown that nitrogen isotopes in organisms are enriched relative to their diet (e.g. Peterson & Fry, 1987). This fractionation is much larger for ^{15}N than ^{13}C, hence nitrogen isotopes can provide useful information about the trophic level of animals and the food web 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 & Epstein, 1981; Minagawa & Wada, 1984). The number of trophic levels above autotrophs for each fish species was assigned using published dietary information for each species (Table 1). $\delta^{13}C$ fractionation is close to zero (Peterson & Fry 1987), so no adjustment was made for this element.

Euclidean mixing model

Autotrophs were pooled into seven taxa: mangroves, seagrass, seagrass epiphytes, POM, MPB, the C_3 saltmarsh succulents and the C_4 saltmarsh grass. Mean $\delta^{13}C$ and $\delta^{15}N$ values were calculated for each fish and autotroph taxon, after adjusting fish $\delta^{15}N$ values for trophic fractionation. Using the $\delta^{13}C$ and $\delta^{15}N$ values as Cartesian coordinates, Euclidean distances (E) between fish values and each of the autotroph categories were calculated according to:

$$E = [(\delta^{13}C_{autotroph} - \delta^{13}C_{fish})^2 + (\delta^{15}N_{autotroph} - \delta^{15}N_{fish})^2]^{½}$$

Variances were calculated about these Euclidean distances as follows:

$$s^2 = a \times s^2 (\delta^{13}C_{autotroph}) + a \times s^2 (\delta^{13}C_{fish}) + b \times s^2 (\delta^{15}N_{autotroph}) + b \times s^2 (\delta^{15}N_{fish})$$

where s^2 = variance, $a = ((\delta^{13}C_{autotroph} - \delta^{13}C_{fish}) / \text{distance})^2$ and
$b = ((\delta^{15}N_{autotroph} - \delta^{15}N_{fish}) / \text{distance})^2$

A small Euclidean distance between a fish and an autotroph indicates a large putative dietary contribution, so distances were inverted to make the measure more intuitive. The inverted distance for each autotroph was then calculated as a percentage of the total of the inverted distances for all autotrophs for a particular fish species.

Table 1. List of fish species analysed and trophic levels used for correction of fractionation for each species.

Species	Common name	Family	Trophic level above autotrophs	Source for trophic level assignation
Acanthopagrus australis	Yellowfin bream	Sparidae	2	Blaber & Blaber (1980), Morton et al. (1987)
Ambassis jacksoniensis	Yellow perchlet	Chandidae	1.5	Morton et al. (1987)
Arrhamphus sclerolepis	Snub-nosed garfish	Hemiramphidae	1.5	Blaber & Blaber (1980)
Girella tricuspidata	Luderick	Girellidae	1.5	Clements & Choat (1997)
Herklotsichthys castelnaui	Southern herring	Clupeidae	2	Kuiter (1996)
Hyporhamphus australis	Sea garfish	Hemiramphidae	1.5	Robertson & Klumpp (1983)
Hyporhamphus quoyi	Short-nosed garfish	Hemiramphidae	1.5	Robertson & Klumpp (1983)
Liza argentea	Tiger mullet	Mugilidae	1	Morton et al. (1987)
Lutjanus russelli	Moses perch	Lutjanidae	2.5	Kuiter (1996)
Mugil cephalus	Sea mullet	Mugilidae	1.5	Coleman & Mobley (1984), Moriarty (1976)
Myxus elongatus	Silver mullet	Mugilidae	1	Morton et al. (1987)
Platycephalus arenarius	Sand flathead	Platycephalidae	3	Klumpp & Nichols (1983)
Platycephalus fuscus	Dusky flathead	Platycephalidae	3	Kuiter (2000)
Pomatomus saltatrix	Tailor	Pomatomidae	2	Blaber & Blaber (1980)
Pseudorhombus arsius	Large-toothed flounder	Paralichthyidae	3	Kuiter (2000)
Pseudorhombus jenynsii	Small-toothed flounder	Paralichthyidae	3	Kuiter (2000)
Rhabdosargus sarba	Tarwhine	Sparidae	2	Kuiter (2000)
Scomberoides lysan	Double-spotted queenfish	Carangidae	3	Blaber (1986)
Sillago ciliata	Sand whiting	Sillaginidae	2	Burchmore et al. (1988)
Sillago maculata	Winter whiting	Sillaginidae	2	Burchmore et al. (1988)
Tylosurus gavialoides	Stout longtom	Belonidae	3	Kuiter (2000)
Valamugil georgii	Fantail mullet	Mugilidae	1	Morton et al. (1987)

4.3 Results

Autotroph isotope signatures

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 succulents and saltmarsh grass had the most depleted $\delta^{15}N$ signatures and seagrass epiphytes and POM had the most enriched signatures.

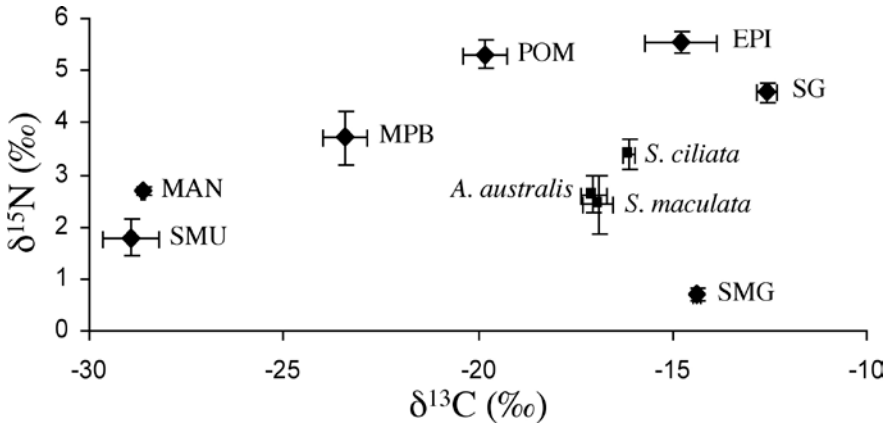


Figure 2. Mean (± SE) carbon and nitrogen isotope values of autotrophs (seagrass – SG; seagrass epiphytes – EPI; mangroves – MAN; microphytobenthos – MPB; particulate organic matter – POM; saltmarsh grass – SMG; saltmarsh succulents – SMU) and *Acanthopagrus australis*, *Sillago ciliata* and *S. maculata*. Fish $\delta^{15}N$ signatures have been adjusted for fractionation.

Fish isotope signatures

Isotope signatures varied among fish species (Table 2). The variability among fish species is less than that for autotrophs for $\delta^{13}C$ signatures yet similar for $\delta^{15}N$ signatures (Fig. 3 and 4, respectively). All fish species had $\delta^{13}C$ signatures lying within the enriched half of the range of autotroph values (Fig. 3), although *Hyporhamphus australis* was slightly more enriched than the most enriched autotroph. Predators (e.g. *Pomatomus saltatrix* and *Platycephalus arenarius*) had the most enriched $\delta^{15}N$ signatures whereas detritivores (e.g. *Myxus elongatus*) and omnivores (e.g. *H. australis* and *Arrhamphus sclerolepis*) had the most depleted $\delta^{15}N$ signatures (Table 2). After correction for fractionation the $\delta^{15}N$ signatures of all but one species lay within the range of autotroph $\delta^{15}N$ signatures (Fig. 4).

Table 2: Size range and $\delta^{13}C$ and $\delta^{15}N$ values for all fish species.

Species	n	Size range (mm)	$\delta^{13}C$ (‰)		$\delta^{15}N$ (‰)	
			Mean	SE	Mean	SE
Acanthopagrus australis	30	45-263	-17.0	0.3	10.1	0.4
Ambassis jacksoniensis	13	26-30	-21.2	0.2	9.6	0.1
Arrhamphus sclerolepis	4	32-124	-18.9	0.9	6.6	1.2
Girella tricuspidata	6	263-342	-19.8	1.1	12.5	1.2
Herklotsichthys castelnaui	35	59-115	-19.0	0.3	11.4	0.5
Hyporhamphus australis	6	192-235	-11.3	0.2	6.1	0.5
Hyporhamphus quoyi	18	44-130	-17.3	0.4	9.9	0.5
Liza argentea	8	73-250	-19.1	0.2	6.8	1.5
Lutjanus russelli	2	56-62	-16.6	0.3	10.1	1.3
Mugil cephalus	13	246-303	-17.0	0.8	9.1	0.8
Myxus elongatus	12	99-124	-14.8	0.2	4.4	0.2
Platycephalus arenarius	2	66-112	-17.3	1.5	11.1	0.5
Platycephalus fuscus	9	289-532	-16.9	0.3	12.4	0.3
Pomatomus saltatrix	8	290-373	-18.8	0.5	13.7	0.3
Pseudorhombus arsius	18	31-110	-16.0	0.2	11.4	0.2
Pseudorhombus jenynsii	4	34-54	-17.2	0.0	8.5	0.3
Rhabdosargus sarba	8	108-288	-16.3	0.8	10.7	0.5
Scomberoides lysan	13	50-115	-15.7	0.4	11.8	0.2
Sillago ciliata	136	15-337	-16.1	0.2	9.4	0.3
Sillago maculata	26	19-103	-16.9	0.4	9.9	0.6
Tylosurus gavialoides	6	248-630	-16.2	0.6	11.8	0.4
Valamugil georgii	8	123-280	-13.8	0.8	10.0	0.4

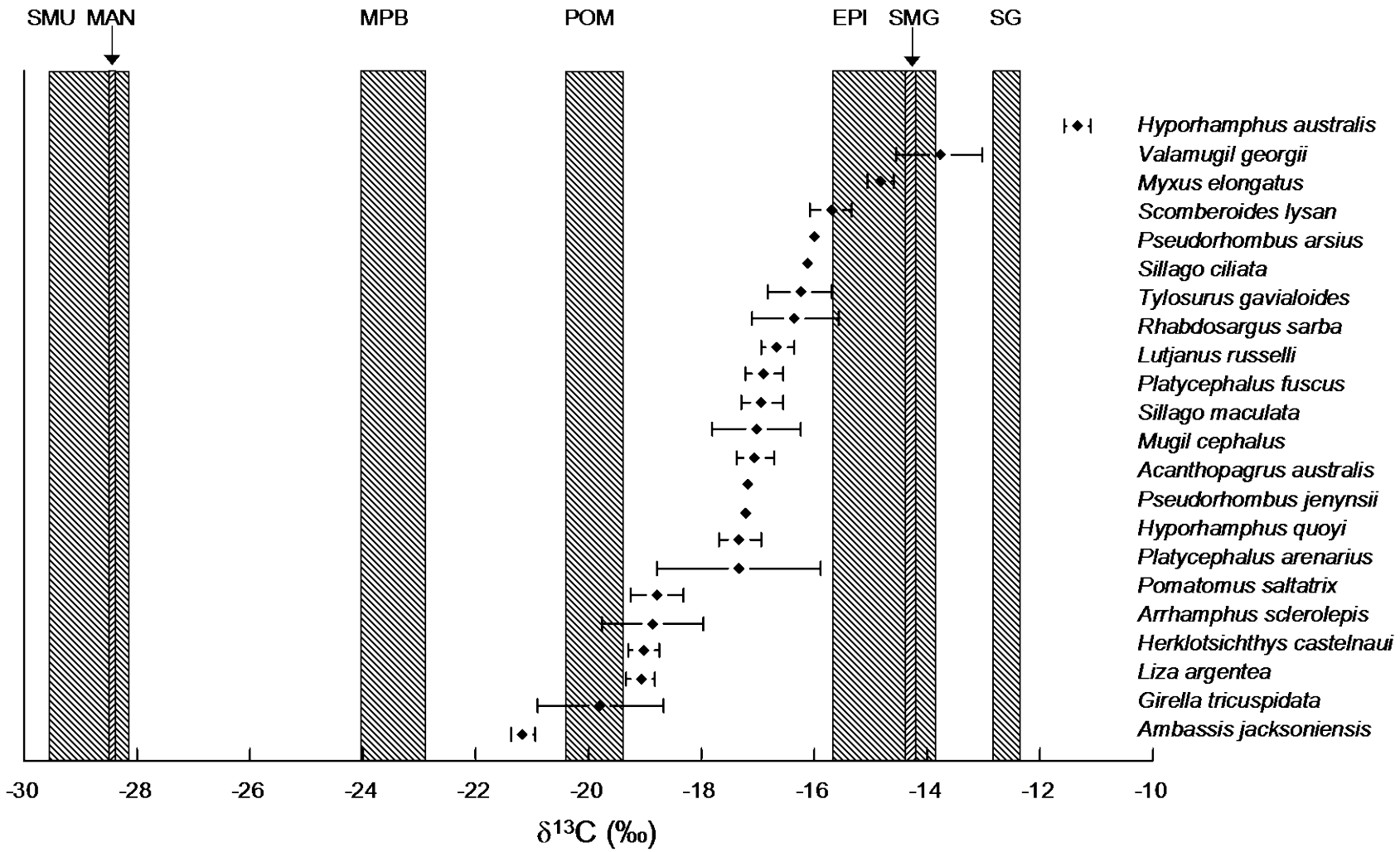


Figure 3: Mean $\delta^{13}C$ (‰) values of fish overlaid on autotroph values. Values are mean ± SE for fish and autotrophs.

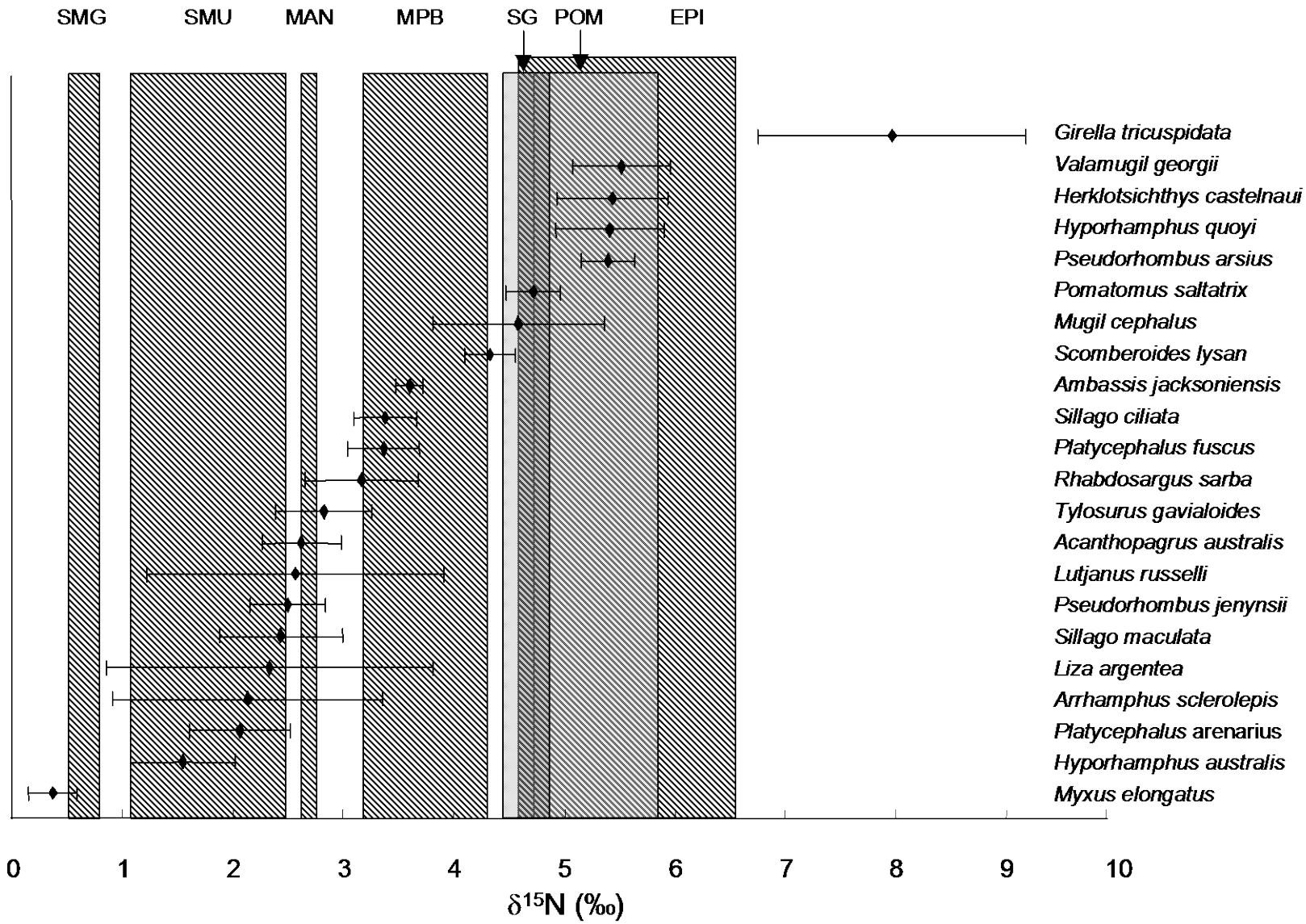


Figure 4: Mean $\delta^{15}N$ (‰) values of fish (adjusted for fractionation) overlaid on autotroph values. Values are mean ± SE for fish and autotrophs.

Table 3: Results of the Euclidean mixing model. Autotrophs are ranked by putative contribution (1, 2 and 3). SMG – saltmarsh grass, POM – particulate organic matter, EPI – seagrass epiphytes, MPB – microphytobenthos, SG – seagrass.

Species	Putative contribution – rank			Putative contribution (%)			SD about putative contribution		
	1	2	3						
Acanthopagrus australis	SMG	EPI	POM	22	20	19	2	4	3
Ambassis jacksoniensis	POM	MPB	EPI	30	28	10	2	3	4
Arrhamphus sclerolepis	POM	SMG	MPB	24	17	16	2	1	3
Girella tricuspidata	POM	EPI	MPB	24	17	16	2	4	3
Herklotsichthys castelnaui	POM	EPI	MPB	57	11	10	3	5	3
Hyporhamphus australis	SMG	SG	EPI	28	27	17	1	2	3
Hyporhamphus quoyi	POM	EPI	SG	26	26	14	3	5	2
Liza argentea	POM	MPB	SMG	25	17	16	2	3	1
Lutjanus russelli	SMG	EPI	POM	24	21	17	1	3	2
Mugil cephalus	EPI	POM	SG	27	23	15	4	3	2
Myxus elongatus	SMG	SG	EPI	59	11	10	1	2	1
Platycephalus arenarius	SMG	POM	EPI	24	19	18	1	2	3
Platycephalus fuscus	EPI	POM	SMG	23	20	19	3	2	1
Pomatomus saltatrix	POM	EPI	MPB	46	14	12	3	5	3
Pseudorhombus arsius	EPI	SG	POM	44	15	14	5	2	3
Pseudorhombus jenynsii	SMG	EPI	POM	22	19	19	1	3	2
Rhabdosargus sarba	EPI	SMG	POM	24	22	17	3	1	2
Scomberoides lysan	EPI	SG	SMG	38	18	15	3	2	1
Sillago ciliata	EPI	SMG	SG	26	21	18	3	2	2
Sillago maculata	SMG	EPI	POM	24	20	18	1	3	3
Tylosurus gavialoides	SMG	EPI	SG	24	22	17	1	2	2
Valamugil georgii	EPI	SG	SMG	44	30	9	5	2	1

Euclidean mixing model

Only five of seven autotrophs appear in the listing of the top three contributing autotrophs for the 22 species of fish (Table 3): seagrass epiphytes, saltmarsh grass, POM, seagrass and MPB. Mangroves did not appear as one of the top three contributing autotrophs, and had the least putative contribution in the detailed analysis for the economically important species of fish (Table 4; *Acanthopagrus australis*, *S. ciliata* and *S. maculata*). *Ambassis jacksoniensis* and *Hyporhamphus australis* had the most extreme $\delta^{13}C$ values among fish and hence were closer to different autotrophs (Fig. 3). The highest putative contribution to *A. jacksoniensis* was by POM, MPB, and seagrass epiphytes, whereas that to *H. australis* was by saltmarsh grass, seagrass, and seagrass epiphytes. Putative contributions of autotrophs to fish species in different functional groups are shown in Fig. 5.

Table 4: Detailed results of Euclidean mixing model for the three most abundant species of fish.

	A. australis		*S. ciliata*		*S. maculata*	
	% Contribution	SD	% Contribution	SD	% Contribution	SD
EPI	20	4	26	3	20	3
MAN	6	3	5	2	6	2
MPB	11	3	9	3	11	3
POM	19	3	16	3	18	3
SG	15	3	18	2	15	2
SMG	22	2	21	2	24	3
SMU	6	4	5	3	6	3

Relative importance of in situ *production versus outwelled carbon*

91% of species have a high putative contribution from seagrass epiphytes to their diet (Table 5). POM is also a high putative source of carbon for 73% of species surveyed in this study. Saltmarsh grass is important for 68%, seagrass for 41% and MPB for 27% of species (Table 5). The median difference in the putative contribution of the 1st and 3rd ranked autotrophs is 9% (Table 3). This indicates a high similarity in the importance of the top three ranked autotrophs. Six of the 22 fish species had all of the top three ranked autotrophs being purely outwelled sources (seagrass epiphytes, seagrass and saltmarsh grass).

Table 5. Summary of Euclidean mixing model results for each autotroph. Values represent the number of fish species out of 22 in total for which the putative contribution of a particular autotroph is important, ranked by putative contribution (1, 2 or 3).

Autotroph	Source	Rank 1	Rank 2	Rank 3	Total	%*
EPI	outwelled	7	9	4	20	91
POM	*in situ*, outwelled	7	3	6	16	73
SMG	outwelled	8	3	4	15	68
SG	outwelled	-	5	4	9	41
MPB	*in situ*	-	2	4	6	27

* percentage (%) values are representative of the rankings combined

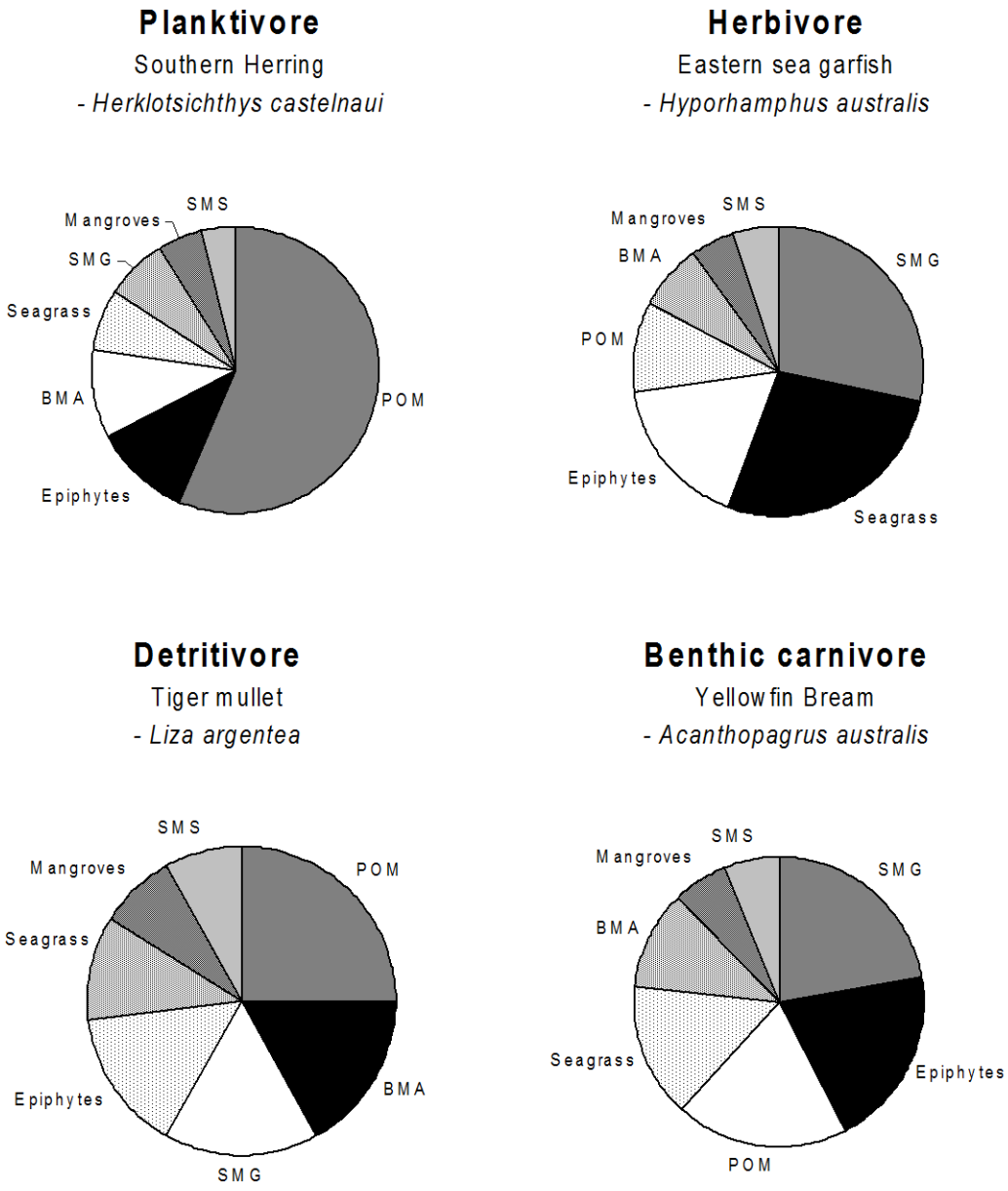


Figure 5: Putative contributions of autotrophic sources to the diet of fish species in Moreton Bay displaying different life history types.

4.4 Discussion

Autotroph isotope signatures

Stable isotope signatures for mangroves (Lee 1995, Newell et al. 1995, Loneragan et al. 1997, Bouillon et al. 2002), seagrass (Fry 1984, Harrigan et al. 1989, Boyce et al. 2001, Davenport & Bax, 2002), seagrass epiphytes (Fry 1984, Boon et al. 1997, Harrigan et al. 1989, Moncreiff & Sullivan 2001), and the carbon isotope signature of one of the saltmarsh succulents, *Sarcocornia quinqueflora* (Boon et al. 1997), are similar to those reported in previous studies. We could find no previous reports of stable isotope signatures for *Suaeda australis* or *Sporobolus virginicus*, however, *S. virginicus* is a C_4 plant (King et al. 1990) and has a $\delta^{13}C$ signature characteristic of C_4 plants (Farquhar et al. 1989). Carbon isotope signatures of POM, which includes phytoplankton, were also within the range of previously reported values (Fry 1984, Harrigan et al. 1989, Bouillon et al. 2002, Davenport & Bax 2002) and were central in the spread of the autotroph carbon isotope signatures. This is consistent with the theory that POM is ultimately derived from a variety of plant sources, including phytoplankton and decomposing components of the other autotrophs (Riley 1970, Richard et al. 1997, Bouillon et al. 2000). The mean carbon isotope signature of MBP was at the extreme limit of reported values, being similar to the most depleted values (Currin et al. 1995, Newell et al. 1995, Deegan & Garritt 1997, Middelburg et al. 2000, Sauriau & Kang 2000).

Isotope variability

The variability in isotope values among fish species and autotroph groups differed between elements. The variability of carbon isotope signatures among fish species was less than that among autotrophs, with values for fish species lying exclusively in the enriched half of the range of autotroph values. For all fish species this demonstrates that the contribution from the two depleted autotroph sources, mangroves and saltmarsh succulents, is minor. For fish species with very enriched signatures (e.g. *Hyporhamphus australis*), the carbon source must be one or more of the very enriched autotrophs (seagrass, epiphytes, saltmarsh grass). The variability of nitrogen isotope signatures among fish was greater than that among autotrophs. This is not unexpected as estuarine fish in this study span several trophic levels due to their feeding modes (Table 1). The range of trophic levels ensured a wide spread of $\delta^{15}N$ for fish species. For example, carnivorous fish such as *Platycephalus fuscus* had higher $\delta^{15}N$ values than detritivorous species such as *Myxus elongatus*.

Mixing models

Results from two element mixing models that have more than three sources should be interpreted with caution. If material from one or more sources contributes nothing to the foodweb, values for other sources will be higher than those reported here. If the non-contributing endmember(s) have a stable isotope signature similar to that of the heterotroph being analysed, the remaining endmembers contribute significantly more than is reported by the model (Phillips & Gregg, 2001). For example, the removal of seagrass epiphytes from putative contributors for *Sillago ciliata* results in an increase of putative contribution for the remaining autotrophs (Table 6). It does not, however, change the order of putative contribution of the remaining autotrophs, and this component of the mixing model is robust.

Table 6 Results of the Euclidean mixing model for *Sillago ciliata* with and without seagrass epiphytes. All values are % contribution.

Autotroph	All autotrophs included	Epiphytes removed
MAN	5	7
SG	18	24
POM	16	21
EPI	26	removed
MPB	9	12
SMG	21	28
SMU	5	7

A further problem with mixing models (of all types) is the quality of the data used to correct for fractionation. In studies such as these, trophic level must be assigned based on independent information, such as gut content analysis. We used a correction factor of 3 ‰ per trophic level; however, this is a mean (De Niro & Epstein 1981, Minagawa & Wada 1984, Peterson & Fry 1987), around which fractionation levels have been shown to vary considerably (e.g. Vander Zanden & Rasmussen 2001). The adjusted $\delta^{15}N$ signature for *Girella tricuspidata* was more enriched in ^{15}N than all autotroph $\delta^{15}N$ signatures (Fig. 4), suggesting that an erroneous correction factor was used for this species, despite having good published information about its diet. Fractionation values as high as 4.9‰ have been reported for fish muscle (Sholto-Douglas et al. 1991). Levels of fractionation have been shown to be affected by starvation (Hesslein et al. 1993), age (Overman & Parrish 2001) and food quality (Adams & Sterner 2000). Only quite detailed experimental work can demonstrate how these factors influence fractionation levels for any one species, and this is not practicable where numerous species are being analysed as in the present study. Such experiments will be necessary, however, to take the work further for key species, since mixing model results would be sensitive to incorrect trophic fractionation adjustments.

The Euclidean mixing model indicates that across all fish species, seagrass epiphytes, POM, saltmarsh grass, seagrass and MPB have the highest putative contribution (in order of decreasing importance), whereas mangroves and saltmarsh succulents contribute little (Table 5). As such, production that contributes to fish from mudflats is both *in situ* and outwelled from adjacent vegetated areas. Given the extent of mangroves in the study area (76 km^2; Sinclair Knight Mertz 2000), it is surprising that they contribute little. While early studies used the high productivity of mangrove forests to argue that they must be important contributors to food webs (Odum & Heald 1972; Rodelli et al. 1984), more evidence is accumulating that indicates they contribute little (Lee 1995, Newell et al. 1995, Lee 2000, Bouillon et al. 2002). Much of the carbon from mangroves is consumed by invertebrates *in situ* (Boto & Bunt 1981, Bouillon et al. 2002) and may be predominantly recycled within the mangrove forest.

While the Euclidean mixing model indicated a high putative contribution by the saltmarsh grass, it is possible that this is a result of its carbon isotope signature being similar to that of seagrass (Fig. 2). Although saltmarsh grass is a C_4 plant and is likely to be highly productive, the total areal cover of saltmarsh (7 km^2) is only 17 % of that covered by seagrass in the study area (Sinclair Knight Mertz 2000). Saltmarsh consists of both succulents and grass, therefore the actual areal cover of saltmarsh grass will be less than this. Also, it is situated on higher ground than mangroves and is only inundated on the highest tides. It has been shown that

infrequent inundation leads to little material produced by upper intertidal autotrophs entering the main waterways of an estuary (Lee 1995). If the contribution of saltmarsh grass is less than that indicated by its high putative contribution, as shown above the putative contributions of other sources would be higher than reported, but in the same rank order. We recommend the use of alternative chemical biomarkers to distinguish the importance of saltmarsh grass from that of seagrass.

Many of the fish caught over mudflats in this area are also caught over seagrass beds (Gray et al. 1996). Seagrass beds in the study area cover some 41 km^2 (Sinclair Knight Mertz 2000). Some of the carbon that constitutes their nutrition may be ingested in these seagrass habitats, or they may consume invertebrates that feed in seagrass beds but have moved to mudflats. As such, the fish may be part of a trophic relay from seagrass beds to mudflats (Kneib 2000). Seagrass epiphytes made a consistently large putative contribution to fish nutrition (Table 5). Combining the likely contributions of seagrass and seagrass epiphytes, primary production from seagrass beds appears to be the major food source of outwelled material for fish caught over mud flats.

The most recent mixing models use variances about autotroph and consumer mean isotope values to calculate variances about mean contributions by autotrophs (e.g. Phillips & Gregg 2001). Sensitivity analyses of previous models have shown that uncertainty around proportion estimates is strongly affected by three factors: the size of the differences in isotope signatures among sources, source and fish variability, and sample size (Phillips & Gregg 2001). In this study there is a large range in isotope signatures between sources, low source and fish isotope signature variability and large sample size (Fig. 2, Table 2). Hence, there is only a small amount of variation (SD) about putative contribution calculated by the Euclidean mixing model (Table 3), and the putative contributions can be regarded with a high degree of confidence.

Autotroph importance for common species

Juvenile and adult *Sillago ciliata* occur in seagrass and unvegetated habitats (Burchmore et al. 1988, Kerby & Brown 1994), although they are more common over unvegetated flats than seagrass (Gray et al. 1996). They are benthic carnivores, with polychaetes and crustaceans comprising 98% of their diet (Burchmore et al. 1988). Results of the Euclidean mixing model indicate that this species most likely obtains substantial nutrition from seagrass epiphytes, saltmarsh grass and seagrass. As argued above, contribution of saltmarsh grass to fish in unvegetated areas may be less than indicated by the mixing model results. Organic material from seagrass beds, either from epiphytic algae or the seagrass itself, remains as the most likely source supporting *S. ciliata* production. This may occur through movement of particulate matter or via trophic relay.

Hyporhamphus australis is generally herbivorous, although it also eats planktonic crustaceans (Robertson & Klumpp 1983). Setting aside once again the potential contribution from saltmarsh grass, *H. australis* also apparently obtains substantial nutrition from seagrass beds. Although it has a carbon isotope ratio that is enriched in ^{13}C relative to seagrass and seagrass epiphytes, *H. australis* may be selectively assimilating seagrass components (Tibbetts 1997), or there is an increase in autotroph isotopic signature due to fractionation between diet and consumer. In any case, this species also demonstrates the importance of outwelled carbon to organisms inhabiting unvegetated areas.

In situ production is also important to organisms found over unvegetated mudflats. *Liza argentea*, for example, has high putative contributions from both MPB and POM. This species is known to ingest mud (Morton et al. 1987). The high putative contribution from MPB in mixing model results demonstrates the likelihood that this autotroph is being assimilated by *L. argentea*, either directly or via meiofaunal grazers.

Results of the mixing model indicated high putative contribution from saltmarsh grass, seagrass epiphytes and POM for *Acanthopagrus australis*. However, as argued above, contribution of saltmarsh grass to fish in unvegetated areas may be less than indicated by the mixing model results. *A. australis* feed mainly upon benthic crustaceans and other invertebrates (Morton et al. 1987), which are known to feed upon seagrass epiphytes (Fry 1984, Kitting et al. 1984). Therefore, trophic relay appears to be important in the supply of outwelled nutrition to *A. australis* in unvegetated areas. The high putative contribution of POM, to the extent that it includes phytoplankton, also indicates the importance of *in situ* production to *A. australis*.

Sillago maculata is a benthic carnivore, feeding mainly on crustaceans and polychaetes (Burchmore et al. 1988). As for *Acanthopagrus australis*, the mixing model indicated a high putative contribution from saltmarsh grass, seagrass epiphytes and POM. Again, both *in situ* and outwelled material most likely make a substantial contribution to the nutrition of this species.

Conclusion

The development of a Euclidean mixing model has provided a platform to assess the relative importance of outwelling and *in situ* carbon production to estuarine fish in unvegetated areas. Outwelled carbon from seagrass beds is the major contributor, either directly from seagrass or via seagrass epiphytes. Mangroves and saltmarsh succulents do not make substantial contributions to the species studied, and saltmarsh grass has a high putative contribution but needs to be considered cautiously because its contribution is unable to be separated from that of seagrass. *In situ* production of MPB and possibly phytoplankton also appears to make a substantial contribution to the nutrition of the fish occurring over unvegetated mudflats.

5.0 Whole estuary analysis of the contribution of estuarine autotrophs to fish and crustaceans over unvegetated mudflats in Port Curtis

5.1 Introduction

Animals occurring over unvegetated mudflats in estuaries must ultimately obtain nutrition either from *in situ* autotrophic sources or from organic matter transported from elsewhere. *In situ* sources are microalgae either in the water column (phytoplankton) or on the surface of the mudflats (microphytobenthos). Organic matter can be transported to mudflats either by direct movement of plant material from external sites of production, or in the bodies of animals as a series of trophic interactions (Kneib 2000).

Port Curtis is, like Moreton Bay (Chapter 4), a marine-dominated estuarine embayment with very extensive areas of estuarine habitat. Although industrial development since the 1960s has resulted in large-scale loss of estuarine habitats (Walker 1997), the bay is nevertheless still dominated by extensive forests of mangroves (56 km^2) backed by saltmarsh (49 km^2). Seagrasses occur lower in the intertidal and shallow subtidal zone (no area estimate available) but the most obvious feature of the bay is the enormous areas of shallow mudflats (77 km^2). Mudflats in subtropical east Australian bays are occupied by several fish and crustacean species, most of which also occur over vegetated habitats but less frequently (Gray et al. 1998). Far less science has been done in Port Curtis than in Moreton Bay, and we were unable to find estimates of productivity of different estuarine plants in the bay. Although fin-fish are of interest in Port Curtis, important fisheries also exist here for crustacean species. For example, over 40% of mud crabs (*Scylla serrata*) caught in Queensland come from the central Queensland region around Port Curtis (Walker 1997). Banana prawns (*Fenneropenaeus merguiensis*) are another important fisheries species in and around Port Curtis. The aim here was to determine likely autotrophic sources at the base of foodwebs supporting fish and crustacean production over unvegetated mudflats in Port Curtis.

We used stable isotope analysis of carbon and nitrogen to determine whether outwelling of organic matter to mudflats, or *in situ* production, contributes most nutrition to fish that occur over mudflats in a large estuarine system. The Euclidean mixing model developed in Chapter 4 was used here to estimate putative contributions from different estuarine autotrophs to fish and crustaceans in Port Curtis.

5.2 Methods

Sample collection and processing

Port Curtis in central Queensland is characterised by intertidal and shallow subtidal seagrass beds interspersed with extensive mudflats. The coastline comprises islands and the mainland, fringed by very extensive mangrove forests backed by saltmarsh, including unvegetated saltpans. Autotrophs and fish were collected in May 2000 at three locations in Port Curtis (Fig. 1). All samples were frozen immediately upon collection.

Fish and crustaceans were collected from mudflats using seine nets. Nine fish species and four crustacean species were collected. Samples of muscle tissue were taken for processing.

Mangrove leaves (MAN) were collected from 3 species (*Avicennia marina*, *Ceriops tagal* and *Rhizophora stylosa*), where present, at each location. All samples of mangrove leaves were green, as we have shown that the stable isotope ratios of green and yellow mangrove leaves do not differ (Appendix 4), and green mangrove leaves are more easily and efficiently collected. Values from the three species were pooled as their isotopic signatures were similar.

Where present, two species of seagrass (SG; *Zostera capricorni*, *Halophila ovalis)* were collected from each location. The mean $\delta^{13}C$ signatures of these two species were > 5 ‰ apart, so they were not pooled, and were treated as separate taxa when modelling. Not enough seagrass epiphyte material could be obtained to do isotope analysis.

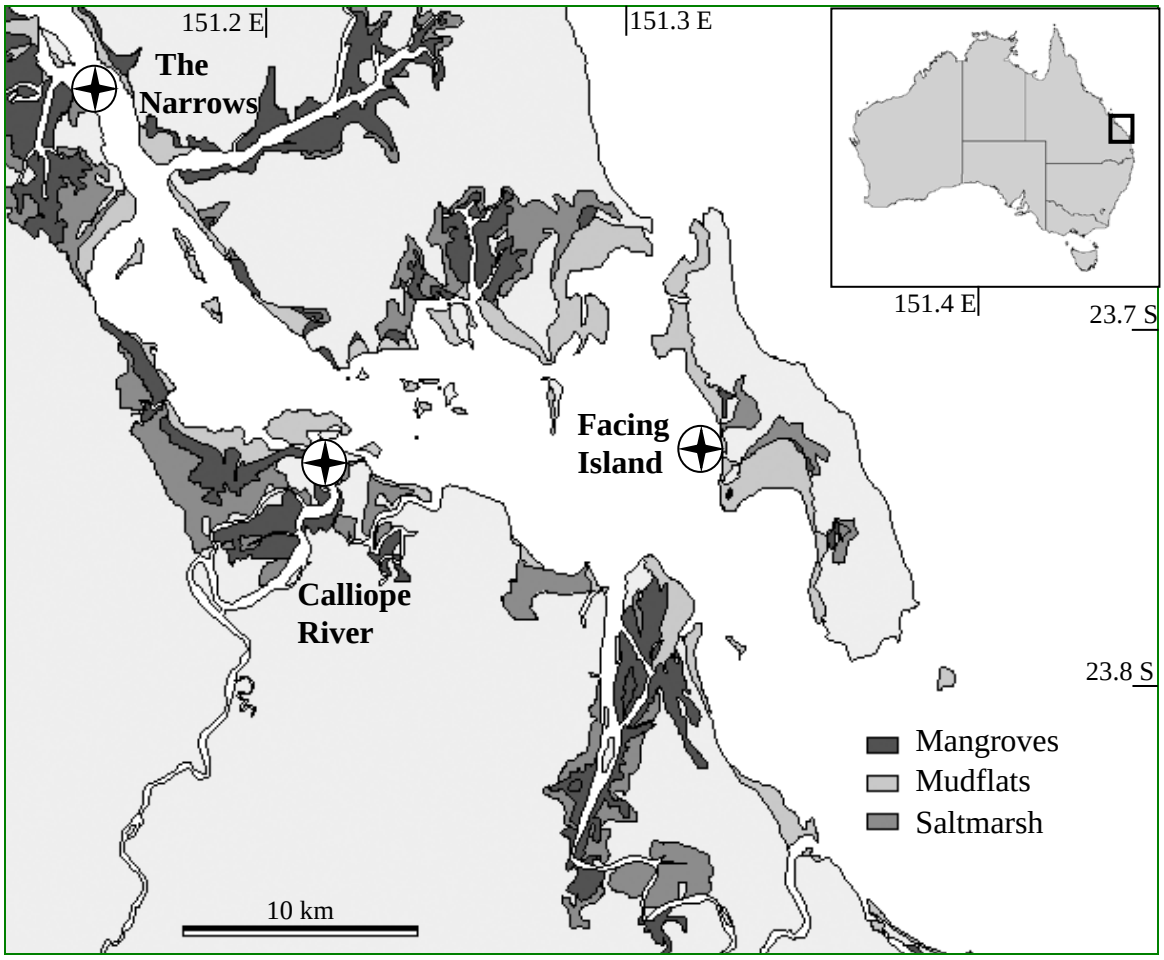


Figure 1. Map of Port Curtis indicating sampling sites.

Saltmarsh plants used for stable isotope analysis comprised three species; the C_3 saltmarsh succulents (SMU; *Sarcocornia quinqueflora* and *Suaeda australis*) and the C_4 saltmarsh grass (SMG; *Sporobolus virginicus*).

Microphytobenthos (MPB) was collected by scraping the surface 1 cm of sediment from mudflats near where collections of fish were made. 100 mL 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 minutes. 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 a 5 μm mesh to remove the silica and any remaining microbes.
Dense algal mats (AM) consisting predominantly of cyanobacteria covered quite large areas of unvegetated pans on saltmarshes. These were scraped from the sediment surface and washed clean in the laboratory prior to processing.

Particulate organic matter (POM) was defined as that fraction retained after filtering 100-800 litres of water through 53 μm mesh.

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; PeeDee belemite limestone carbonate for carbon).

Fractionation and trophic level

Previous studies have shown that nitrogen isotopes in organisms are enriched relative to their diet (e.g. Peterson & Fry, 1987). This fractionation is much larger for ^{15}N than ^{13}C, hence nitrogen isotopes can provide useful information about the trophic level of animals and the food web structure. To account for fractionation of nitrogen we subtracted the assumed 3 ‰ per trophic level increase from the nitrogen isotope signature of the animals (De Niro & Epstein, 1981; Minagawa & Wada, 1984). The number of trophic levels above autotrophs for each animal species was assigned using published dietary information for each species (Table 1). $\delta^{13}C$ fractionation is close to zero (Peterson & Fry 1987), so no adjustment was made for this element.

Table 1. List of fish and crustacean species analysed and trophic levels used for correction of fractionation for each species.

Species	Common name	Trophic level(s) above autotrophs	References
Fish			
Acanthopagrus australis	Yellowfin bream	2	Blaber & Blaber 1980 Morton et al. 1987
Arrhamphus sclerolepis	Snub-nosed garfish	1.5	Blaber & Blaber 1980
Drepane punctata	Sicklefish	2.5	Kuiter 1996
Gerres subfasciatus	Common Silverbiddy	2	Middleton et al. 1984
Herklotsichthys castelnaui	Southern herring	2	Kuiter 1996
Hyporhamphus quoyi	Short-nosed garfish	1.5	Robertson & Klumpp 1983
Leiognathus equulus	Pony fish	2	Amesbury & Myers 1982
Sillago ciliata	Sand whiting	2	Burchmore et al. 1988
Valamugil georgii	Fantail mullet	1	Morton et al. 1987
Crustaceans			
Fenneropenaeus merguiensis	Banana prawn	1.5	Pers. Obs.
Oratosquilla stephensoni	Stephenson's Mantis Prawn	2.5	Grant 1997
Penaeus esculentus	Tiger Prawn	1.5	Wassenberg & Hill 1987
Scylla serrata	Mud Crab	2	Grant 1997

Euclidean mixing model

Autotrophs were pooled into eight taxa: mangroves, *Zostera* seagrass, *Halophila* seagrass, POM, MPB, algal mats, the C_3 saltmarsh succulents and the C_4 saltmarsh grass. Mean $\delta^{13}C$ and $\delta^{15}N$ values were calculated for each animal and autotroph taxon. Only animal species for which more than one specimen was obtained were modelled (six fish and three crustacean species). Using the $\delta^{13}C$ and $\delta^{15}N$ values as Cartesian coordinates, Euclidean distances (E) between fish values and each of the autotroph categories were calculated according to:

$$E = [(\delta^{13}C_{autotroph} - \delta^{13}C_{fish})^2 + (\delta^{15}N_{autotroph} - \delta^{15}N_{fish})^2]^{½}$$

Variances were calculated about these Euclidean distances as follows:

$$s^2 = a \times s^2 (\delta^{13}C_{autotroph}) + a \times s^2 (\delta^{13}C_{fish}) + b \times s^2 (\delta^{15}N_{autotroph}) + b \times s^2 (\delta^{15}N_{fish})$$

where s^2 = variance, $a = ((\delta^{13}C_{autotroph} - \delta^{13}C_{fish}) / distance)^2$ and $b = ((\delta^{15}N_{autotroph} - \delta^{15}N_{fish}) / distance)^2$

A small Euclidean distance between a fish and an autotroph indicates a large putative dietary contribution, so distances were inverted to make the measure more intuitive. The inverted distance for each autotroph was then calculated as a percentage of the total of the inverted distances for all autotrophs for a particular fish species.

5.3 Results

Autotroph isotope signatures

Isotope signatures of the eight taxa of autotrophs were generally well separated using both carbon and nitrogen (Fig. 2 & 3). Autotrophs fell into 3 groups based on $\delta^{13}C$ signatures: 1) enriched sources of *Zostera*, saltmarsh grass and MPB, 2) sources with middle values, consisting of *Halophila*, algal mats, and POM, and 3) depleted sources of mangroves and saltmarsh succulents. MPB had the most depleted $\delta^{15}N$ signatures and algal mats, POM and seagrass (both species) had the most enriched signatures.

Fish and crustacean isotope signatures

Isotope signatures varied among fish and crustacean species (Table 2). The variability among fish and crustacean species is less than that for autotrophs for $\delta^{13}C$ signatures yet similar for $\delta^{15}N$ signatures (Fig. 2 and 3, respectively). All fish species had $\delta^{13}C$ signatures lying within the enriched half of the range of autotroph values (Fig. 2). Crustacean $\delta^{13}C$ signatures were very closely grouped, all lying in the centre of the range of autotroph values, with means between –18 and –20 ‰. Crustacean values were depleted relative to all but one fish species (*Herklotsichthys castelnaui*). Carnivores (e.g. *Acanthopagrus australis* and *Sillago ciliata*) had the most enriched $\delta^{15}N$ signatures whereas detritivores (e.g. *Valamugil georgii*) and omnivores (e.g. *Hyporhamphus quoyi* and *Arrhamphus sclerolepis*) had the most depleted $\delta^{15}N$ signatures (Table 2). After correction for fractionation the $\delta^{15}N$ signatures of all species lay within the range of autotroph $\delta^{15}N$ signatures (Fig. 3).

Table 2: Size range and $\delta^{13}C$ and $\delta^{15}N$ values for all fish and crustacean species.

Species	n	Size Range (mm)	$\delta^{13}C$		$\delta^{15}N$	
			Mean	SE	Mean	SE
Fish						
Acanthopagrus australis	2	70 - 240	-18.04	0.26	11.79	0.80
Arrhamphus sclerolepis	1	150	-13.32		6.74	
Drepane punctata	1	420	-16.17		11.40	
Gerres subfasciatus	5	65 – 90	-16.57	0.52	10.56	0.49
Herklotsichthys castelnaui	3	70 – 125	-19.55	1.12	9.04	0.28
Hyporhamphus quoyi	2	90-95	-15.51	0.67	7.47	0.51
Leiognathus equulus	9	30 – 75	-16.27	0.59	9.51	0.59
Sillago ciliata	1	70	-16.58		11.63	
Valamugil georgii	11	110 - 300	-14.57	0.31	7.94	0.46
Crustaceans						
Fenneropenaeus merguiensis	4	15 – 35	-19.97	0.53	7.76	0.27
Oratosquilla stephensoni	3	-	-19.33	0.21	10.61	0.09
Penaeus esculentus	1		-18.73		8.40	
Scylla serrata	2	130 - 150	-19.17	1.71	6.54	1.20

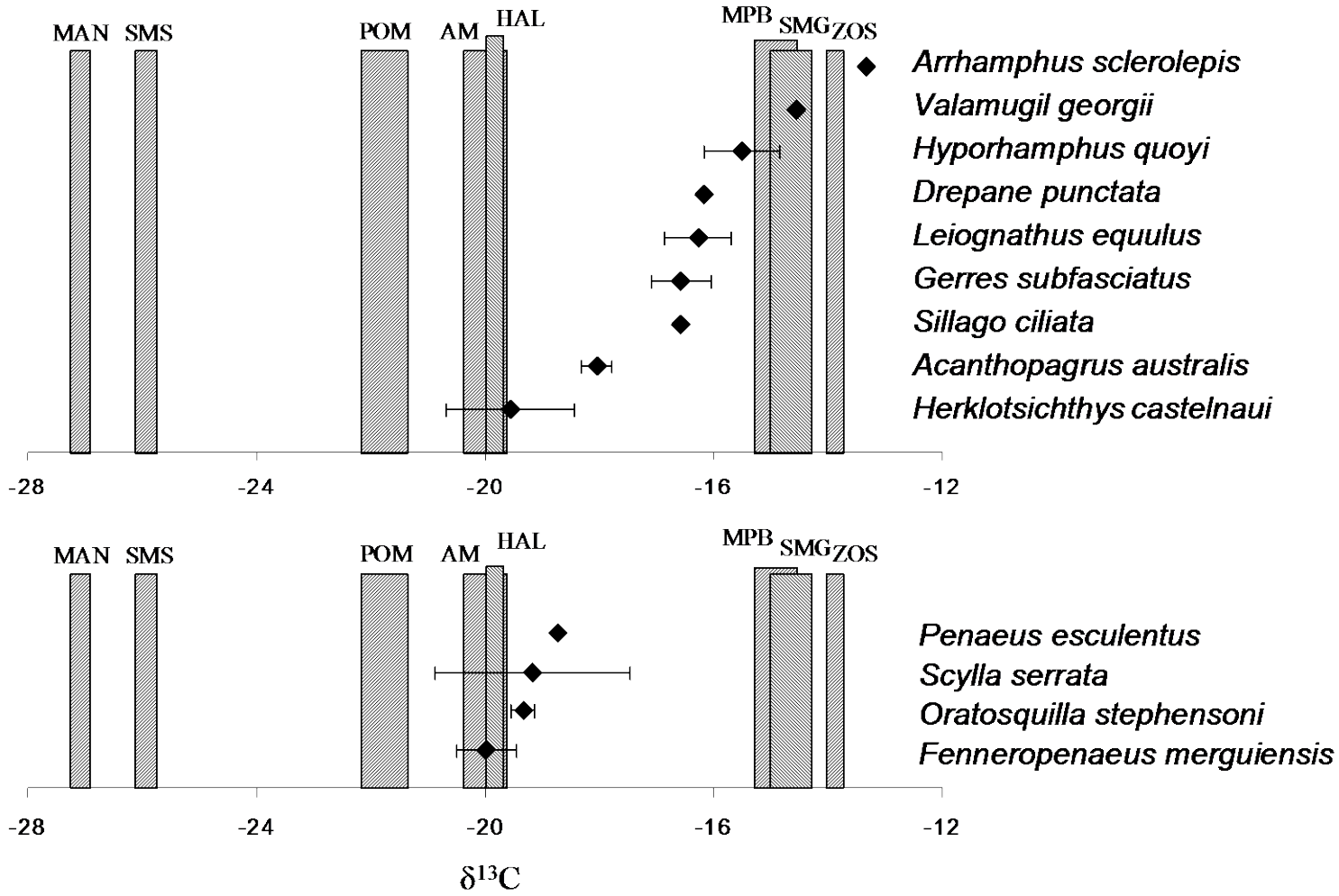


Figure 2: Mean $\delta^{13}C$ (‰) values of fish and crustaceans overlaid on autotroph values. Values are mean ± SE for animals and autotrophs. (Algal mat – AM; Zostera – ZOS; mangroves – MAN; microphytobenthos – MPB; particulate organic matter – POM; saltmarsh grass – SMG; saltmarsh succulents – SMS; Halophila - HAL)

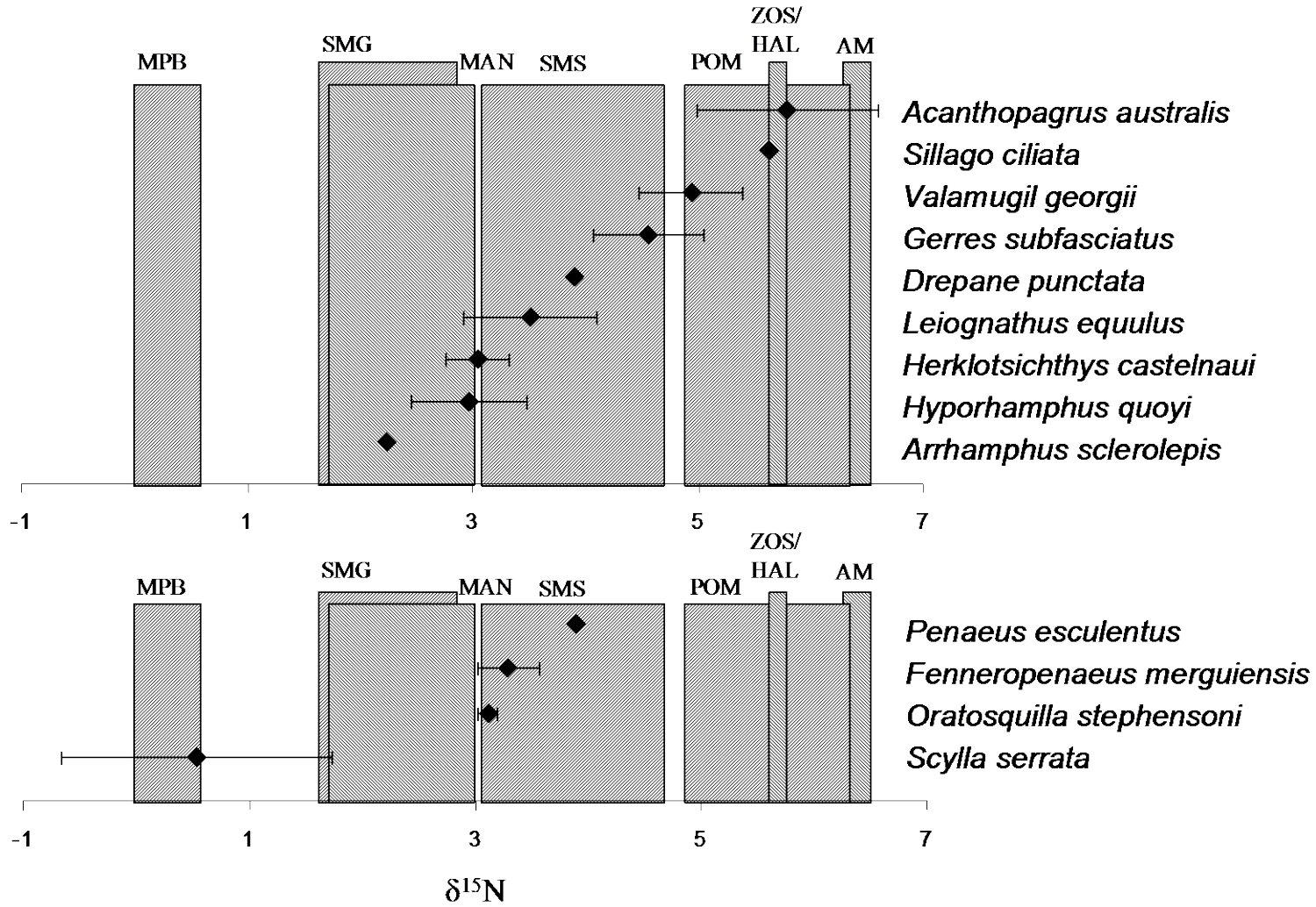


Figure 3: Mean $\delta^{15}N$ (‰) values of fish and crustaceans (adjusted for fractionation) overlaid on autotroph values. Values are mean ± SE for animals and autotrophs. Abbreviations as for Fig. 2.

Table 3: Detailed results of Euclidean mixing model for fish and crustacean species. All values are putative contributions (%). Top three contributors in bold.

	Fish						Crustaceans		
	Acanthopagrus australis	*Gerres subfasciatus*	*Herklotsichthys castelnaui*	*Hyporhamphus quoyi*	*Leiognathus equulus*	*Valamugil georgii*	*Oratosquilla stephensoni*	*Fenneropenaeus merguiensis*	*Scylla serrata*
Algal mat	**17**	14	**17**	8	11	8	**17**	**17**	13
Halophila ovalis	**21**	**17**	**22**	9	13	8	**22**	**22**	**14**
Mangroves	5	5	7	4	5	3	7	7	9
MPB	12	12	10	**17**	**15**	**10**	10	9	**16**
Saltmarsh Grass	12	**18**	11	**37**	**25**	**16**	11	9	**15**
Saltmarsh Succulents	7	6	8	4	5	4	8	9	10
POM	**15**	10	**17**	7	9	6	**16**	**18**	13
Zostera capricorni	11	**18**	9	**14**	**16**	**44**	9	8	10

Table 4: Summary results of the Euclidean mixing model showing top 3 autotrophs for each animal species. Autotrophs are ranked by putative contribution (1, 2 and 3). SMG – saltmarsh grass, POM – particulate organic matter, MPB – microphytobenthos, SG – seagrass.

Species	Autotrophs that contributed most energy			Putative contribution (%)			Standard Deviation about putative contribution		
Fish									
Acanthopagrus australis	Halophila	Algal mat	POM	21	17	15	1.12	0.35	2.03
Gerres subfasciatus	Zostera	SM grass	Halophila	18	18	17	1.14	1.88	1.15
Herklotsichthys castelnaui	Halophila	Algal mat	POM	22	17	17	0.50	0.85	2.14
Hyporhamphus quoyi	SM grass	MPB	Zostera	37	17	14	1.63	1.35	0.79
Leiognathus stephensoni	SM grass	Zostera	MPB	25	16	15	2.24	1.79	2.13
Valamugil georgii	Zostera	SM grass	MPB	44	16	10	1.24	2.31	1.86
Crustaceans									
Oratosquilla stephensoni	Halophila	Algal mat	POM	22	17	16	0.19	0.22	1.64
Fenneropenaeus merguiensis	Halophila	POM	Algal mat	22	18	17	0.55	1.88	0.73
Scylla serrata	MPB	SM grass	Halophila	16	15	14	2.84	2.65	1.70

Euclidean mixing model

Detailed results of the mixing models show that putative contributions of autotrophs vary among animal species (Table 3). When these results are summarised into just the top three contributing autotrophs for each animal species (Table 4), it becomes clear that only a subset of autotrophs are making substantial contributions. For fish, very high putative contributions were recorded for *Zostera* for *Valamugil georgii* and for saltmarsh grass for *Hyporhamphus quoyi*. Mangroves and saltmarsh succulents were not in the top three contributors for any fish species. For crustaceans, putative contributions of the top three autotrophs were very even (i.e. little difference between first and third ranked autotrophs), with *Halophila* the top contributor for *Oratosquilla stephensoni* and *Fenneropenaeus merguiensis*, and MPB for *Scylla serrata*. Mangroves and saltmarsh succulents were not in the top three contributors for crustaceans either.

Relative importance of in situ *production versus outwelled carbon*

In situ autotroph sources, MPB and part of POM, both ranked in the top three putative contributors to fish species (Table 5), with MPB occurring most frequently (three out of six species). Five out of the six fish species had an *in situ* autotroph in the top three contributors (Table 4). Of the outwelled sources, *Zostera* and saltmarsh grass were in the top three autotrophs most often, being involved in four of the six fish species (Table 5). For crustaceans, MPB and POM both occurred in the top three frequently (Table 5), and each of the three crustacean species had *in situ* sources in the top three (Table 4). Of the outwelled sources, *Halophila* was the most prominent for crustaceans (Table 5).

Table 5. Summary of Euclidean mixing model results for each autotroph. Values represent the number of fish species out of six and the number of crustacean species out of three in total for which the putative contribution of a particular autotroph is important, ranked by putative contribution (1, 2 or 3).

Autotroph	Source	Rank 1	Rank 2	Rank 3	Total	%*
Fish (6 species)						
Saltmarsh Grass	outwelled	3	1	-	4	67
Zostera capricorni	outwelled	2	1	1	4	67
Halophila ovalis	outwelled	2	1	-	3	50
MPB	*in situ*	-	1	2	3	50
Algal mat	outwelled	-	2	-	2	33
POM	*in situ*, outwelled	-	1	1	2	33
Mangrove	outwelled	-	-	-	-	-
Saltmarsh Succulent	outwelled	-	-	-	-	-
Crustaceans (3 species)						
Halophila ovalis	outwelled	2	-	1	3	100
Algal mat	outwelled	-	1	1	2	67
POM	*in situ*, outwelled	-	1	1	2	67
MPB	*in situ*	1	-	-	1	33
Saltmarsh Grass	outwelled	-	1	-	1	33
Mangrove	outwelled	-	-	-	-	-
Saltmarsh Succulent	outwelled	-	-	-	-	-
Zostera capricorni	outwelled	-	-	-	-	-

*percentage (%) values are representative of the rankings combined

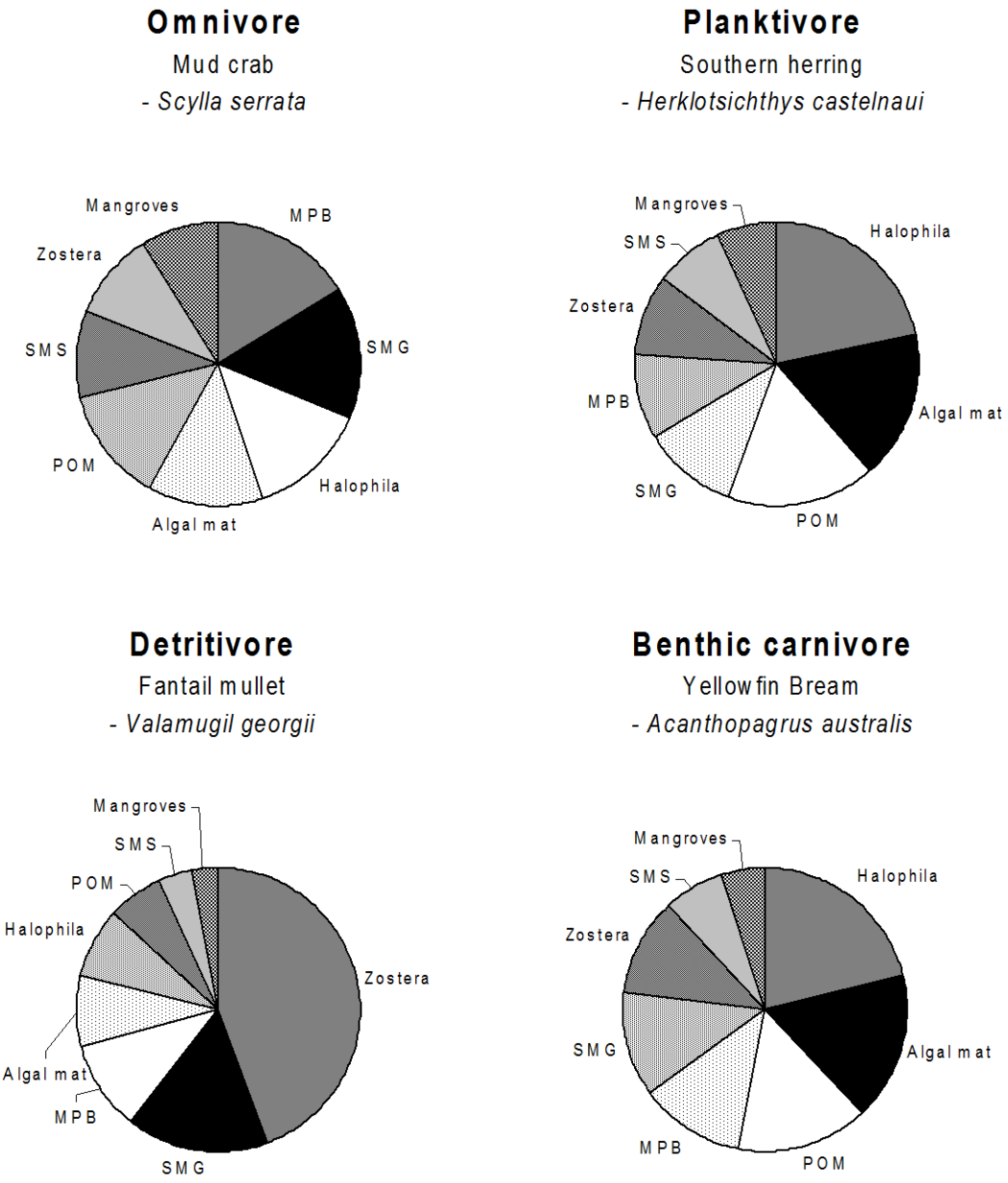


Figure 4: Putative contributions of autotrophic sources to the diet of fish species in Port Curtis displaying different life history types.

5.4 Discussion

Autotroph isotope signatures

Stable isotope signatures for most autotrophs were similar to the values from Moreton Bay (Chapter 4). However, the values of two autotroph taxa were quite different to expectations from Chapter 4. *Halophila ovalis* had more depleted $\delta^{13}C$ than in Moreton Bay (by > 5 ‰), and could not be pooled with *Zostera capricorni* (which itself had values almost the same as in Moreton Bay). Seagrass $\delta^{13}C$ signatures are affected by the amount of light reaching them (related to water depth and turbidity) and probably also exposure to water currents (Grice et al. 1996). *Halophila* tends to occur in deeper water than *Zostera* in Port Curtis. Although this depth differential potentially explains the different $\delta^{13}C$ signatures, *Halophila* is also deeper in Moreton Bay where no difference in $\delta^{13}C$ signatures was found. Some factor other than water depth is presumably involved. Where autotroph species have different signatures in different bays it provides an opportunity to distinguish the importance of the sources to animals, and this is the case with the two seagrass species in Port Curtis and Moreton Bay.

MPB $\delta^{13}C$ values in Port Curtis were > 8 ‰ more enriched than in Moreton Bay. We can find no reports of factors affecting variations in benthic microalgal signatures. Indeed, it is only recently that methods have been developed to obtain relatively pure samples of algae to analyse chemically. Given the extent of mudflats and their MPB in subtropical and tropical estuaries this topic can only become more important.

Isotope variability

As for Moreton Bay, the variability in isotope values among animal species and autotroph groups differed between elements. The variability of $\delta^{13}C$ signatures among fish species was less than that among autotrophs, with values for fish species lying exclusively in the enriched half of the range of autotroph values, and values for crustacean species lying together in the centre of the range for autotrophs. For all fish species this demonstrates that the contribution from the two depleted autotroph sources, mangroves and saltmarsh succulents, is minor. The maximum contribution mangroves could make to fish nutrition can be ascertained by running a $\delta^{13}C$ mixing equation with only two sources, the most depleted (mangroves) and most enriched (*Zostera*). This shows that the mean mangroves contribution ranges from close to zero for fish species having the most enriched signatures (e.g. *Arrhamphus sclerolepis*), up to 43% for *Herklotsichthys castelnaui* (Table 6). For some species, even the 95% upper confidence limits are small (e.g. 29% for *Leiognathus equulus*). For other fish species, however, the variability around animal and autotroph means was high, and confidence limits are wide (e.g. upper confidence limit of 77% for *Hyporhamphus quoyi*). Even for crustaceans, which had more depleted signatures than fish, the mean two-source mixing model contribution of mangroves can be no more than 46% (Table 6), although upper confidence limits are at or above 50% for all crustacean species.

Table 6. Results of a single element (carbon) mixing model for fish and crustaceans, using mangroves and seagrass.

	Mangroves			Seagrass		
	Lower 95% CL*	Mean	Upper 95% CL	Lower 95% CL	Mean	Upper 95% CL
Fish						
A. australis	0.24	0.30	0.36	0.64	0.70	0.76
A. sclerolepis	0.00	0.00	0.34	0.66	1.00	1.00
D. punctata	0.00	0.18	0.88	0.11	0.82	1.00
G. subfasciatus	0.10	0.21	0.31	0.68	0.79	0.90
H. castelnaui	0.06	0.43	0.79	0.21	0.57	0.93
H. quoyi	0.00	0.13	0.77	0.23	0.87	1.00
L. equulus	0.08	0.18	0.29	0.72	0.82	0.92
S. ciliata	0.00	0.21	0.55	0.45	0.79	1.00
V. georgii	0.01	0.06	0.11	0.89	0.94	0.99
Crustaceans						
F. merguiensis	0.33	0.46	0.59	0.41	0.54	0.67
O. stephensoni	0.34	0.41	0.48	0.52	0.59	0.66
P. esculentus	0.03	0.37	0.71	0.29	0.63	0.97
S. serrata	0.00	0.40	1.00	0.00	0.60	1.00

* CL = confidence limit

Mixing models

As explained in Chapter 4, results from two element mixing models that have more than three sources should be interpreted with caution. If material from one or more sources contributes nothing to the foodweb, values for other sources will be higher than those reported here. If the non-contributing endmember(s) have a stable isotope signature similar to that of the heterotroph being analysed, the remaining endmembers contribute significantly more than is reported by the model (Phillips & Gregg, 2001).

A further problem with mixing models (of all types) is the quality of the data used to correct for fractionation. In studies such as these, trophic level must be assigned based on independent information, such as gut content analysis. We used a correction factor of 3 ‰ per trophic level; however, this is a mean (De Niro & Epstein 1981, Minagawa & Wada 1984, Peterson & Fry 1987), around which fractionation levels have been shown to vary considerably (e.g. Vander Zanden & Rasmussen 2001). We recommend experimental work to demonstrate how factors such as food quality and growth rates influence fractionation levels for any one species, in combination with the collection of local data on fish and crustacean gut contents. Such experiments will be necessary for key species, since mixing model results would be sensitive to incorrect trophic fractionation adjustments.

The Euclidean mixing model indicates that across all fish species, *Zostera* and saltmarsh grass are likely to play a substantial role in their nutrition. As argued in Chapter 4, the high putative contributions of saltmarsh grass could simply be a result of this autotroph having a

signature similar to *Zostera*. Although the area of saltmarsh in Port Curtis is very large (larger than Moreton Bay), much of this is unvegetated, and a lot of the vegetated areas consist of saltmarsh succulents. There are no estimates of actual area of saltmarsh grass, but it would be a very small fraction (perhaps as little as 5%) of the saltmarsh area. Given the small area of saltmarsh grass in Port Curtis, and that plants high in the intertidal zone and infrequently inundated are considered to have limited scope for supplying nutrients to deeper waters (Lee 1995), the high putative contribution for this autotroph should be treated with caution. Further work with other biomarkers able to distinguish between the contributions of saltmarsh grass and seagrass are required to resolve their importance to fish.

The mudflats from which fish were sampled are fringed or in one case surrounded by mangrove forests. It is surprising therefore that so little mangrove material is being utilised by fish in Port Curtis. However, this result is consistent with results for fish from Moreton Bay.

In situ production (MPB and part of POM) appears likely to make a substantial contribution to at least some of the fish species occurring over mudflats. Industrial and water transport developments in Port Curtis that require dredging of mudflats may not only affect the amount of habitat available for fish to occupy, but by removing MPB might also reduce autotrophic production sustaining fish production.

Crustaceans caught over mudflats in Port Curtis were relying on different sources to most fish species. In situ production from MPB and phytoplankton (in POM) are important to crustaceans too, but the autotroph material from elsewhere is likely to consist of *Halophila* seagrass rather than *Zostera* (or saltmarsh grass). The contribution from mangroves may be higher to crustaceans than fish, but even species such as *Fenneropenaeus merguiensis* and *Scylla serrata* that are known to have close associations with mangroves probably obtain no more than about half of their nutrition from mangrove production. These two species occur in mangrove forests as well as on adjacent mudflats, and it would be worth examining in the future whether individuals collected from inside and outside mangroves have different levels of utilisation of mangrove material.

Conclusion

The Euclidean mixing model provided a platform to assess the relative importance of outwelling and *in situ* carbon production to estuarine fish and crustaceans in unvegetated areas of Port Curtis. Outwelled carbon from *Zostera* seagrass beds is a major contributor to many fish species. Mangroves and saltmarsh succulents do not make substantial contributions to the species studied, and saltmarsh grass has a high putative contribution but needs to be considered cautiously because its contribution is unable to be separated from that of seagrass. These results for fish are similar to those in Moreton Bay. *In situ* production of MPB and possibly phytoplankton also appears to make a substantial contribution to the nutrition of the fish occurring over unvegetated mudflats in Port Curtis. Seagrass has a high putative contribution to crustaceans, but it is predominantly *Halophila* rather than *Zostera*. The putative contribution to crustaceans of mangroves is higher than for fish, but is still lower than for seagrass or *in situ* sources.

6.0 Spatially explicit analysis of the contribution of estuarine autotrophs to fish over unvegetated mudflats in Moreton Bay

6.1 Introduction

Understanding the role of autotrophs to estuarine foodwebs has important implications for management and conservation. In the past, the relative conservation value of habitats has been determined 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 the different types of habitat. Given the possibly extensive movement of carbon and nutrients in estuarine systems (Odum 1984), consumers may be segregated from the autotrophs upon which they rely (Kneib 2000).

Early foodweb studies used 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 & 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 any other intermediate levels would be required to determine which autotroph(s) are supporting 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 (e.g. 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 & 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 (e.g. 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 & 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. Kitting et al. (1984) found shrimp $\delta^{13}C$ values resembled those of algal epiphytes and not seagrass. Their signature changed concomitantly as epiphyte $\delta^{13}C$ values changed between sites. Similarly, the carbon isotope signature of shrimp, seagrass and epiphytic algae were similar at one site, yet the signatures of shrimp and epiphytic algae, and not seagrass, were elevated at another site (Fry 1984). This spatial tracking of the isotopic signature of primary producers by consumers is evidence that they were assimilating some of the algae.

Mixing models used in previous studies (e.g. Phillips & Gregg 2001) have been restricted to analysing one more autotroph than elements used (refer to Chapter 4). We present a spatial analysis technique that is capable of assessing the relative important of multiple autotrophs. Consumer stable isotope signatures track autotrophs if they contribute consistently across

locations. If a fish species is obtaining some nutrition from an autotroph, and the stable isotope signature of that autotroph differs amongst locations, the signature of the fish should vary in the same way. Previous spatial analysis models have either qualitatively or graphically determined autotroph importance, whereas we have generated probability tests indicating the importance of autotrophs in the nutrition of a consumer.

Whilst there have been many isotope studies that attempt to determine which autotrophs fix carbon used by consumers found over seagrass meadows (Fry 1984; Kitting et al. 1984; Nichols et al. 1985; Fry et al. 1986; Moncrieff & Sullivan 2001), few have assessed this for fish found over unvegetated habitats (but see Herman et al. 2000; Middelburg et al. 2000). Past research in seagrass ecosystems indicates that algal epiphytes may be the primary food source as opposed to the seagrass and the detrital matter they generate (Fry 1984, Kitting et al. 1984, Nichols et al. 1985, Moncrieff & Sullivan 2001). Benthic microalgal production has been found to be an important component of food webs on saltmarshes (e.g. Sullivan & Moncrieff 1990) and in intertidal mangrove forests (Bouillon et al. 2002). Given the high productivity of microphytobenthos (Dennison & Abal 1999), it is possible that algal production is important on unvegetated mudflats. Here we use spatial analysis of stable isotope signatures to attempt to determine which autotrophs provide nutrition to three species of fish that are found over unvegetated habitats in southeast Queensland.

6.2 Methods

Sample collection and processing

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

Mangrove leaves (MAN) were collected from 3 species (*Aegiceras corniculatum*, *Avicennia marina* and *Rhizophora stylosa*), where present, at each of the nine locations. Stable isotope signatures of these three species were pooled because they were similar.

Where present, three species of seagrass (SG; *Zostera capricorni*, *Halophila ovalis* and *H. spinulosa)* were collected from each of the nine locations. Stable isotope signatures for these species were pooled because they were similar. Seagrass epiphytes (EPI) 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 (SMU; *Sarcocornia quinqueflora* and *Suaeda australis*) and saltmarsh grass (SMG; *Sporobolus virginicus*).

Microphytobenthos (MPB) was collected by scraping the surface 1 cm of sediment from mudflats near where collections of fish were made. 100 mL 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 minutes. 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.

Particulate organic matter (POM) was defined as that fraction retained after filtering 100-800 litres of water through 53 μm mesh.

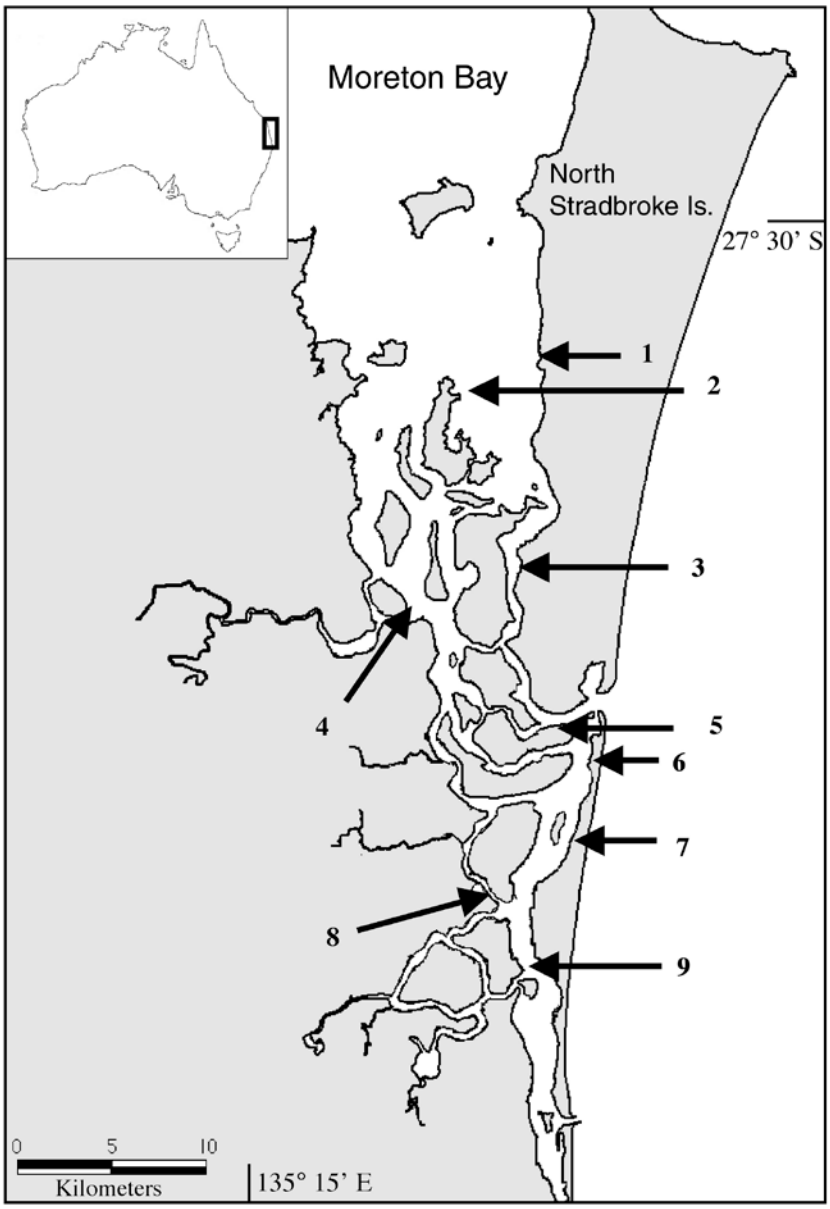


Figure 1. Location of the 9 study sites in southern Moreton Bay.

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; PeeDee belemnite limestone carbonate for carbon).

Spatial analysis

To determine if 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 co-ordinates, 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/autotroph 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:

$$\delta X' = \delta X - (a.FL) \quad \text{..... Equation 6.1}$$

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

6.3 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 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 to 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).

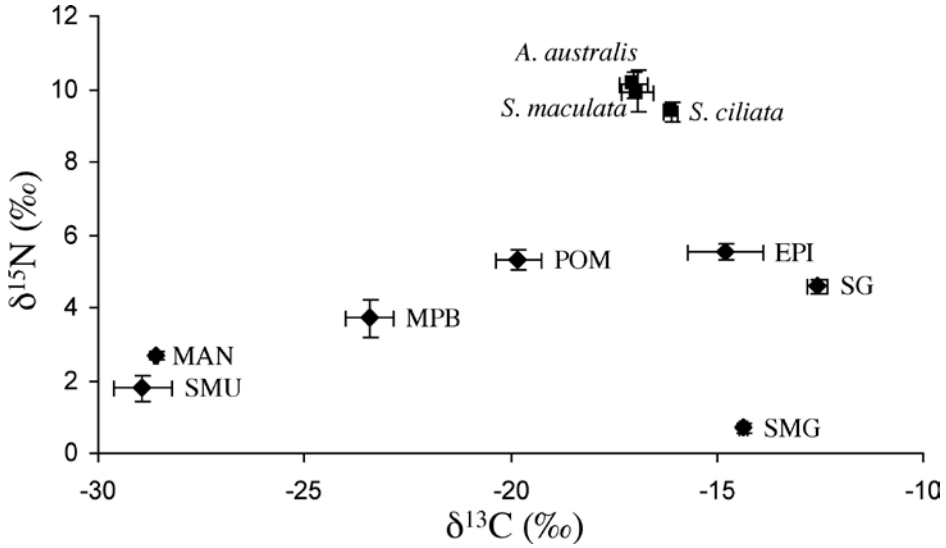


Figure 2. Mean (± SE) carbon and nitrogen isotope values of *Acanthopagrus australis*, *Sillago ciliata* and *S. maculata* and 7 autotrophs (seagrass – SG; seagrass epiphytes – EPI; mangroves – MAN; microphytobenthos – MPB; particulate organic matter – POM; saltmarsh grass – SMG; saltmarsh succulents – SMU).

Size dependent isotopic signatures

There was no correlation between length and $\delta^{13}C$ for any fish species ($p > 0.05$), nor was there for length and $\delta^{15}N$ for *Sillago ciliata* or *S. maculata*. However, there was a positive relationship between length and $\delta^{15}N$ for *Acanthopagrus australis* (Fig. 3). $\delta^{15}N$ signatures of *A. australis* were therefore adjusted (see Eq. 6.1; a = 0.02) prior to the spatial analysis.

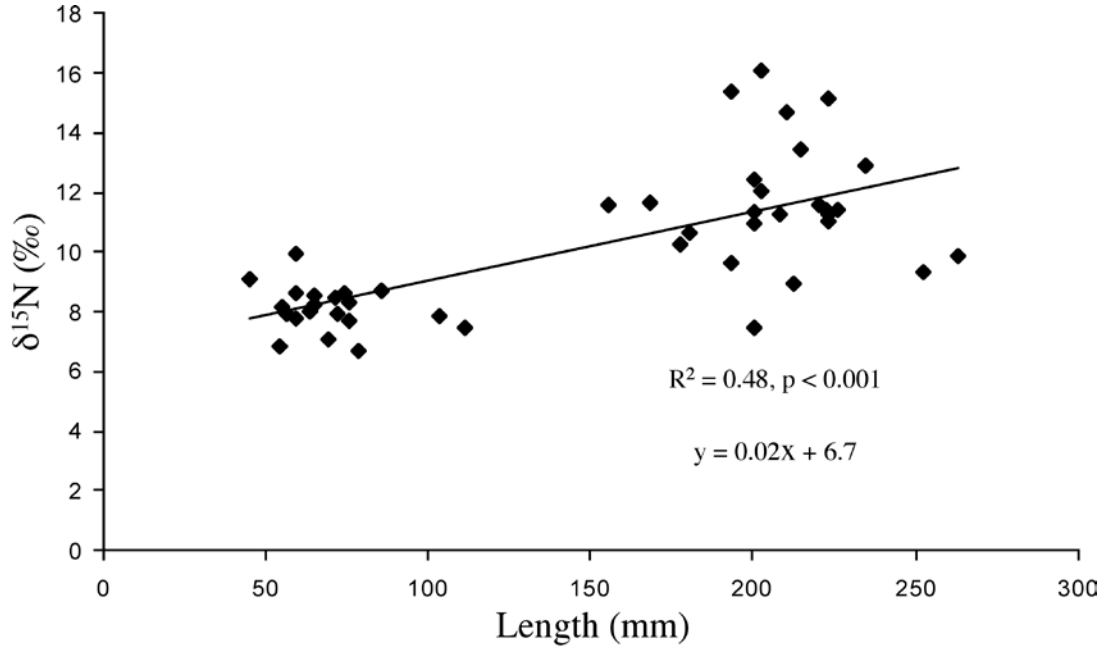


Figure 3. Relationship between fish length (fork length) and $\delta^{15}N$ value for *Acanthopagrus australis*. Each data point represents a single fish.

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. *Acanthopagrus australis* and mangroves; Fig. 4a). 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. 4b), 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 *Acanthopagrus australis* and *Sillago 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.

	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

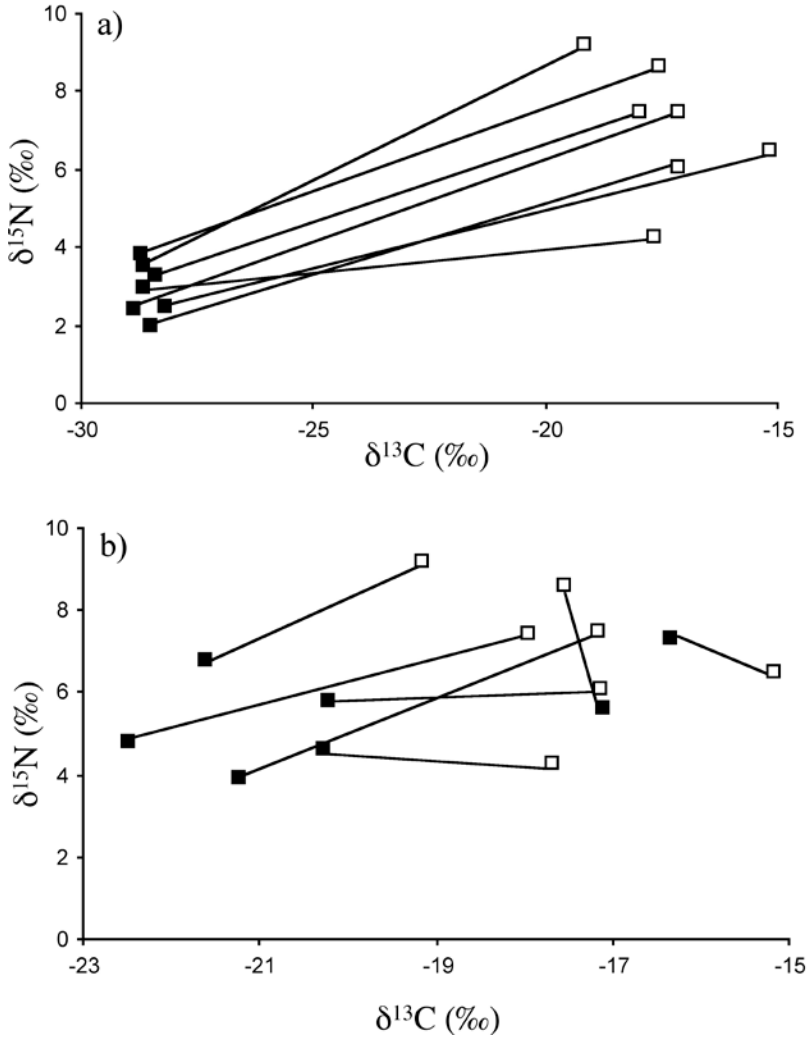


Figure 4. $\delta^{13}C$ and $\delta^{15}N$ of a) *Acanthopagrus australis* and mangroves at 7 different locations, and b) *A. australis* and POM at 7 different locations. Lines join *A. australis* and autotroph from same location. □ = *A. australis*, ■ = mangroves.

6.4 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 (e.g. Fry 1984, Harrigan et al. 1989, Lee 1995, Boon et al. 1997, Bouillon et al. 2002). The $\delta^{13}C$ of MPB were similar to the most depleted values reported in the literature (e.g. Deegan & Garritt 1997). $\delta^{13}C$ values of POM were within the range of previously reported values (e.g. Ogawa & Ogura 1997), representing either a mixture of detritus particles of several autotrophs and/or phytoplankton values.

Autotroph sources for fish

Mangroves and POM isotope signatures were tracked by two of the species investigated in this study. Seagrass, MPB and saltmarsh grass isotope signatures were also tracked. These

results indicate that *in situ* and outwelled sources of nutrition are important for fish occupying unvegetated habitats.

Spatial analysis revealed mangroves, seagrass, POM and saltmarsh grass as important sources of nutrition for *Acanthopagrus australis*. However, as argued in Chapter 4, the contribution of saltmarsh grass to fish in unvegetated areas may be less than indicated by model results. *A. australis* is carnivorous, feeding mainly upon benthic crustaceans and other invertebrates (Morton et al. 1987). Therefore, trophic relay of both *in situ* and outwelled production contributes substantial nutrition to this species.

Both *in situ* and outwelled carbon are important for *Sillago ciliata* which tracked three different autotrophs: mangroves, POM and MPB. *S. ciliata* was the only species to track MPB. Studies in mangrove habitats have also revealed the important role of MPB (*in situ*) carbon for consumers (e.g. Bouillon et al. 2002). Since this species is a benthic carnivore (Burchmore et al. 1988), contribution from autotrophs is most likely from trophic relay.

Sillago maculata was the only species not to track the isotope signature of any autotroph. There are several possible explanations for this result, including site-specific diet selection and low site fidelity. This species is considered a benthic carnivore, feeding mainly on crustaceans and polychaetes (Burchmore et al. 1988, Grant 1993), however, an ontogenetic shift in diet towards crustaceans is common (Burchmore et al. 1988). The diet of this species has been shown to vary with location (Burchmore et al. 1988), and site-specific diet selection is the likely cause for this species not exhibiting locational tracking of any autotroph taxa. Dependence upon different autotrophs (mangroves, phytoplankton, and seagrass) at different locations within an estuary has 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 & Brown 1994). Whilst lack of site fidelity remains a possibility for this species, more detailed fine-scale movement studies would be needed to evaluate this possibility fully.

Importance of mangroves

In assessing the contribution of estuarine autotrophs from a whole estuary perspective (Chapter 4), mangroves appeared unlikely to be a substantial contributor for any fish species analysed. However, the spatial analysis indicates that two out of the three species show locational tracking of the mangrove isotope signature. Hence mangroves must have some importance as a carbon and nitrogen source for fish found over unvegetated habitats. To determine the potential of mangroves as a source, a single element (carbon) mixing model was created for the two species of fish, sourcing carbon from 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% of the carbon sourced by *Acanthopagrus australis* and up to 25% by *Sillago 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.

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

	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

* CL = confidence limit

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).

Size dependent isotopic signatures

There was a positive relationship between length and $\delta^{15}N$ of *Acanthopagrus australis*. Similar results have been found for other fish species (Overman & Parish 2001 and references therein) and are often attributed to either ontogenetic change in diet (e.g. Beaudoin et al. 1999) or differential metabolic fractionation of nitrogen with age (Rau et al. 1981). However, still other studies have found no correlation between length and isotope values (Vander Zanden et al. 2000, Rau et al. 2001). Since there was no correlation between length and $\delta^{13}C$ of *A. australis* it seems more likely that there is differential metabolic fractionation of nitrogen with age and not an ontogenetic shift in diet. Due to the absence of a correlation between length and $\delta^{13}C$ or $\delta^{15}N$ values of *Sillago 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, Overman & Parish 2001). However, in this study neither of the two size classes of *A. australis* were caught at enough sites to analyse separately.

The differential metabolic fractionation of nitrogen with size (age) in *Acanthopagrus australis* could lead to incorrect dietary reconstruction when using stable isotopes, particularly if size classes of *A. australis* display site selectivity. Hence, congregation at particular sites of larger (older) fish, with greater fractionation over their autotroph source, would obscure the relative significance of autotrophs primarily sourced for carbon. 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.

Mixing models often correct for fractionation using a mean value of 3 ‰ per trophic level for nitrogen (e.g. Chapter 4). However, levels of ^{15}N fractionation have been shown to vary considerably about this mean (e.g. Vander Zanden & Rasmussen 2001), being affected by starvation (Hesslein et al. 1991), age (e.g. Overman & Parish 2001) and food quality (Adams & 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 a 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.

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 carbon was important for fish species. Spatial analysis showed that, for *Acanthopagrus australis*, mangroves, seagrass, POM and saltmarsh contribute to their nutrition. For *Sillago ciliata*, mangroves, MPB and POM contribute. The contribution of mangroves to these two species is particularly surprising given the low putative contribution measured using whole estuary analysis. Spatial analysis did not further our understanding of *S. maculata*, either because fish move among locations or because they utilise different sources at different locations.

7.0 Contribution of estuarine and offshore autotrophs to snapper occurring offshore of Moreton Bay

7.1 Introduction

Estuarine autotrophs such as seagrasses, mangroves and saltmarsh plants, are considered to provide crucial nearshore habitat for fish and crustaceans (Bell and Pollard 1989; Kneib 1997; Connolly 1999; Lee 1999; Laegdsgaard and Johnson 2001). They provide protection from predation and episodic disturbance events, and they generally contain higher levels of food compared with areas without vegetation (Klumpp et al. 1989; Blaber et al. 1995; Nagelkerken et al. 2000). Despite the trophic dynamics within these systems being relatively well described via dietary studies and stable isotope analyses (Peterson et al. 1986; Yanez-Arancibia et al. 1993; Loneragan et al. 1997; Sheaves and Molony 2000), we do not know the spatial extent to which these autotrophs contribute to foodwebs in offshore areas adjacent to estuaries.

The transfer of energy and nutrients from nearshore habitats is thought to be important in the productivity of coastal waters. Nutrient fluxes between shallow nearshore habitats and coastal waters can be driven by biotic vectors such as fish, which consume material within estuaries and deposit nutrients in coastal waters (Kneib 1997; Lefeuvre et al. 1999). Nutrients can also be moved offshore by physical processes such as outwelling (Odum 1984), in which organic matter produced in estuaries is transported to adjacent inshore waters (Winter et al. 1996). Tracer methods using stable isotope ratios and other chemical biomarkers suggest that outwelling may, however, be less important than expected, and little is known about the fate of outwelled organic matter on animals, particularly fish, outside estuaries (Lee 1995).

Stable isotope analyses have been used to follow the migration of animals (Hobson 1999) and to trace those autotrophs that are most important as a base for nutritional support (Fry 1984; Hesslein et al. 1991; Hansson et al. 1997). There is generally limited fractionation of carbon and sulphur between trophic levels, so, in the case of carbon, ratios of ^{13}C to ^{12}C in predators reflects that of their prey, and ultimately, the base of nutritional support (De Niro and Epstein 1978). Conversely, the preferential excretion of ^{14}N leads to the enrichment of ^{15}N by 3 ‰ with each consecutive trophic level (Hesslein et al. 1991), and this is relatively consistent between food webs (Hansson et al. 1997). Many autotrophs have proven difficult to separate by their isotopic signatures based on values of $\delta^{13}C$ and $\delta^{15}N$. For example, seagrass and its associated epiphytic algae often have the same isotopic signatures (Moncreiff and Sullivan 2001). Researchers have used alternative isotopes such as $\delta^{34}S$ (Hesslein et al. 1991), which has a lower level of fractionation between trophic levels than carbon, to separate autotrophs that cannot be separated by carbon or nitrogen (Hesslein et al. 1991; Kharlamenko et al. 2001). In fisheries research, stable isotopes have been used to describe the nutritional dynamics of fisheries species (Lindsay et al. 1998), to delineate stocks (Edmonds et al. 1999) and to follow recruitment events (Herzka et al. 2002). They can potentially also be used to evaluate the putative contribution of alternative autotrophs to the nutrition of fisheries species sampled in regions separate from those where the autotrophs actually occur (Jennings et al. 1997).

Snapper (*Pagrus auratus*) forms valuable recreational and commercial fisheries in subtropical and temperate Australia and New Zealand (Kailola et al. 1993). Adult fish frequent large embayments for feeding and spawning, but are common throughout the year further offshore

(MacDonald 1982; Winstanley 1983; Thomas 1985). Juvenile snapper are common in shallow (< 5 m) seagrass habitats within estuaries and small embayments along the eastern Australian coastline (MacDonald 1982), although they also occur in deeper (> 10 m) waters in association with ascidians and red/brown algae (P. Hamer unpublished data). The ecologically sustainable development of local fisheries for snapper depends on understanding which habitats (autotrophs) are important, not simply in the provision of shelter and feeding areas, but also via the provision of nutrition. Stable isotope analyses provide a means of assessing this.

The overall aim of our study was to assess whether the contribution of various autotrophs to the nutrition of snapper changed with distance offshore. In addressing this question we tested among 4 models: 1) the base for nutritional support changes with distance offshore, and the isotopic signatures of fish reflect this change; 2) there is no change in the base for nutritional support, but the signatures of fish change with distance offshore; 3) the base for nutritional support changes with distance offshore, but the changes are not reflected in fish isotope values; 4) there is no change in the base for nutritional support with distance offshore and fish isotope values reflect this. Firstly, we assessed whether the stable isotopic signatures of snapper varied with distance offshore. Secondly, we modelled the putative contribution of each autotroph to the nutrition of snapper. This research improves our understanding of the degree to which autotrophs occurring separately from where fisheries species occur contribute to the nutrition of a fishery.

7.2 Methods

Study site

Our study was done on the southern Queensland coast, Australia, inside the Broadwater (southern Moreton Bay; 27.93 S and 153.43 W) and at 5 and 10 km offshore (Fig. 1), in August 2000 and August 2001. The southern end of Moreton Bay has large stands of mangrove (*Avicennia marina*), saltmarsh succulents (*Sarcocornia quinqueflora, Suaeda australis*) and saltmarsh grass (*Sporobolus virginicus*) intertidally. Patches of seagrass (*Zostera capricorni, Halophila ovalis, H. spinulosa*) and the green alga *Caulerpa racemosa* occur interspersed amongst areas of unvegetated sand in the low intertidal and subtidally. Offshore from the entrance to the Broadwater, the water depth increases to around 30 m at a distance of 5 km from shore, and around 50 m at 10 km offshore. The habitat offshore is rocky reef covered with coralline, red and brown foliose algae and various sponges and ascidians, surrounded by large areas of unvegetated sand. Red algae are increasingly more abundant with distance offshore, and green algae are scarce. Red and brown algae are rare inside the Broadwater, occurring only along the rock wall at the entrance (see Fig. 1).

Collection of samples

Fish were collected at each location (Broadwater, 5 km, 10 km). Samples of each common autotroph (mangrove, saltmarsh succulents, saltmarsh grass, seagrass and epiphytes, red algae, brown algae, green algae, microphytobenthos - MPB) and particulate organic matter (POM - including phytoplankton) were also collected. The larger autotrophs were collected by picking plant material. POM was collected by towing a 53 μm plankton net. Microphytobenthos (MPB) was collected by scraping the surface 1 cm of sediment from mud

banks. Immediately after collection, all samples were placed on ice until they could be frozen.

Sample preparation and stable isotope analysis

Only white muscle tissue immediately ventral to the anterior end of the dorsal fin was used for isotope analysis of fish because there is less variability in this tissue than others (Pinnegar and Polunin 2000). Only the most recent growth (shoots or leaves) in the macro-autotrophs was prepared for isotope analysis. Autotrophs were cleaned of epibionts where necessary using a razor blade.

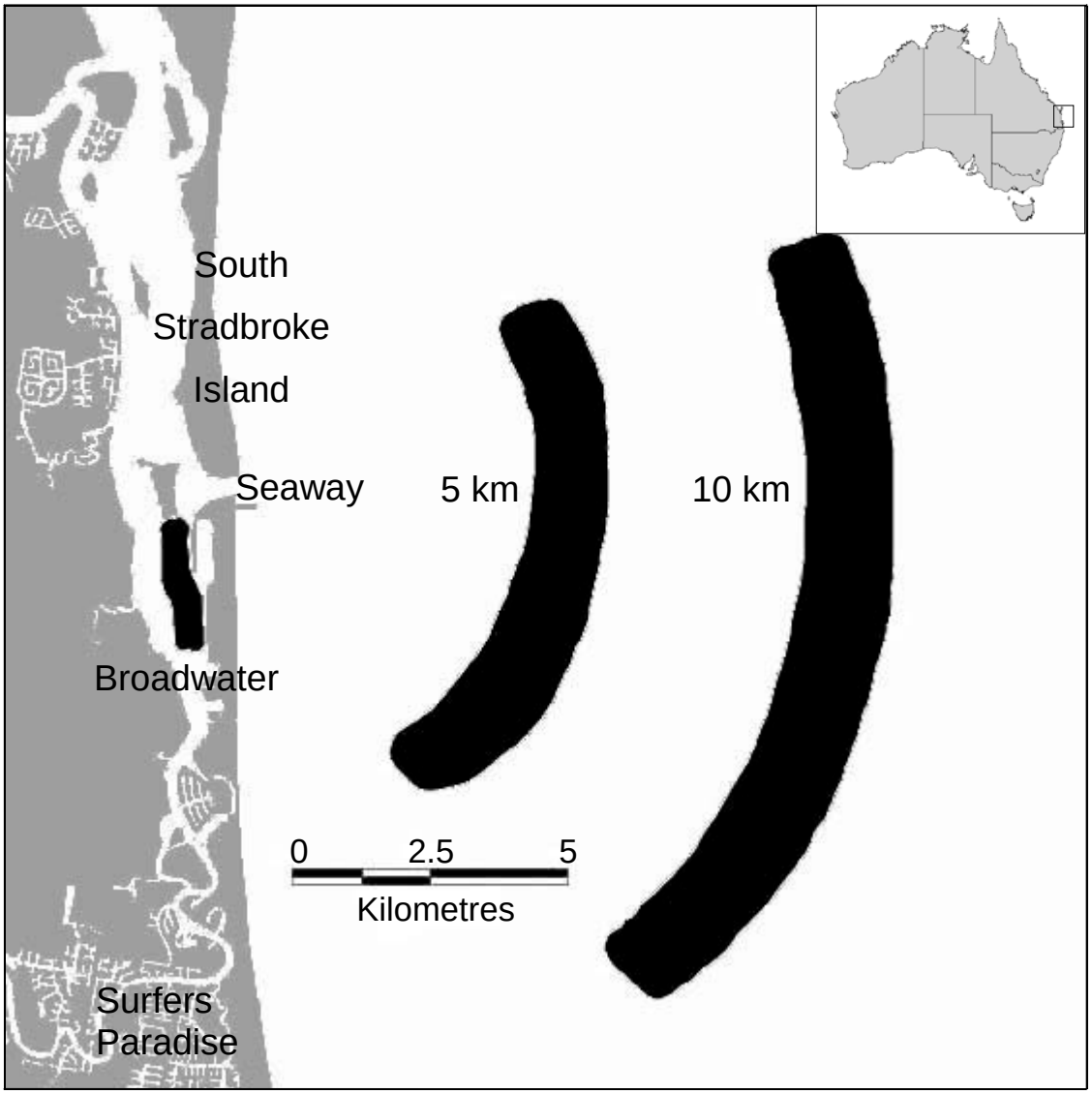


Figure 1. Sampling locations (dark shading) inside the Broadwater and at 2 distances offshore. Inset: Location of study area in Australia.

All samples were washed in distilled water and dried to constant weight at 60°C. Samples were ground to a fine powder using a pestle and mortar. About 2 mg of the fish tissue, and 5 mg of the autotroph samples, were placed into tin capsules for isotope analysis.

MPB was extracted from the mud by washing 100 mL of sediment through a 53 μm sieve. Material passing through the sieve was then washed through a 5 μm mesh. Material retained on this sieve 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 minutes. 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, and the retentate was dried in the same way as the other samples.

Analyses of $\delta^{13}C$ and $\delta^{15}N$ stable isotopes were done at Griffith University, Brisbane, Australia, on an Isoprime isotope ratio mass spectrometer. Samples were combusted and the reaction products were separated by gas chromatography (GC) to give pulses of pure nitrogen and carbon dioxide for analysis of ^{15}N and ^{13}C isotopic contents by mass spectrometer.

$\delta^{34}S$ analyses of biotic samples are less routine, and were done at Iso-Analytical laboratories, UK. For $\delta^{34}S$, a vanadium pentoxide catalyst was added to the sample in the tin capsule. Approximately 30 μg of sulfur was used for analysis. Samples were measured against a reference material of NBS-127 (barium sulfate, $\delta^{34}S_{V\text{-}CDT}$ = +20.3 ‰). For calibration and correction, NBS-17, IAEA-S-1 (silver sulfide, $\delta^{34}S_{V\text{-}CDT}$ = -0.3 ‰) and Iso-Analytical OP-7 (barium sulfate, $\delta^{34}S_{V\text{-}CDT}$ = +11.0 ‰) were used. NBS-1577a (Bovine Liver, $\delta^{34}S_{V\text{-}CDT}$ = +7.6 ‰) and Iso-Analytical OP-7 are calibrated and able to be compared to NBS-127, and samples of these three materials were thus checked to ensure quality control. Analysis was done by Elemental Analysis - Isotope Ratio Mass Spectrometry (EA-IRMS). Samples were dropped into a furnace at 1080°C for flash combustion in the presence of oxygen. As combustion occurred, the temperature in the region of the sample would have been raised to approximately 1700°C. The resulting gases were swept by a stream of helium over combustion catalysts (tungstic oxide/zirconium oxide) and then through a reduction stage of high purity copper wires, producing SO_2, N_2, CO_2, and water. A Nafion™ membrane was used to remove the water and a packed GC column was then used to resolve sulfur dioxide from N_2 and CO_2 at a temperature of 30°C. Upon entry to the ion source of the IRMS, the peak of SO_2 was ionised and accelerated. This ionisation allowed for the subsequent separation of gas species of different mass in a magnetic field. A Faraday cup universal collector array was then used to simultaneously measure the gas species. Analysis was based on monitoring of the mass-to-charge ratios (m/z) of 48, 49 and 50 for SO^+ produced from SO_2 in the ion source.

Isotopic compositions of C, N and S were expressed in terms of δ, as parts per thousand (‰) differences from international standards (Vienna Pee Dee Belemnite for carbon, air for nitrogen, NBS-127 for sulphur):

$$\delta X = [(R\text{sample}/R\text{standard})\text{-}1] \times 10^3,$$

where X is ^{13}C, ^{15}N or ^{34}S and R is the corresponding ratio $^{13}C/^{12}C$, $^{15}N/^{14}N$ and $^{34}S/^{32}S$.

Modeling the putative contribution of autotrophs

Mixing models giving a single solution are restricted to analysing one more autotroph than elements used. These are not useful, however, in nearshore waters where autotrophs are numerous. We therefore used the Euclidean mixing model developed in Chapter 4 that determines the mean putative contribution to a consumer, and variance about this contribution, for multiple autotrophs. We use 'putative contribution' to describe results from

the model as there are multiple solutions for each analysis. Autotrophs were grouped into 9 categories: mangroves, seagrass, seagrass epiphytes, MPB, red/brown algae, green algae, saltmarsh grass, saltmarsh succulents and POM. Mean values were calculated for each isotope for the fish and each of the autotrophs. In a step-wise manner, the Euclidean distance between the fish and each of the autotroph categories for each combination of elements (S & C, S & N, C & S, and S & C & N) were calculated according to:

$$E = [\ (\delta^{x}\text{Asource} - \delta^{x}\text{Afish})^2 + (\delta^{y}\text{Bsource} - \delta^{y}\text{Bfish})^2\ ]^{½}$$

where $\delta^{x}A$ is the mean isotope ratio of one element and $\delta^{y}B$ is the other, with a variance (s^2):

$$s^2 = a \times s^2\ (\delta^{x}\text{Asource}) + a \times s^2\ (\delta^{x}\text{Afish}) + b \times s^2\ (\delta^{y}\text{Bsource}) + b \times s^2\ (\delta^{y}\text{Bfish})$$

where, $a = ((\delta^{x}\text{Asource} - \delta^{x}\text{Afish}) / \text{distance})^2$ and $b = ((\delta^{y}\text{Bsource} - \delta^{y}\text{Bfish}) / \text{distance})^2$. When all three elements were used, a third term was added to both equations.

Based on stomach content analysis (Bulman et al. 2001), snapper were considered to be ≈ 2.5 trophic levels above their autotroph source(s). Assuming a 3 ‰ fractionation per trophic level (Fry et al. 1999), we adjusted $\delta^{15}N$ values by 7.5 ‰ before calculating putative contributions. The Euclidean distance between snapper and an autotroph was inversely proportional to the likelihood of a substantial contribution to the nutrition of the fish, so the distance was inverted and then calculated as a percentage of the total distances for all autotrophs.

Statistical analysis

Univariate

All data were assessed for homogeneity of variances and normality prior to analysis. Data that failed to meet these assumptions were $\log_{10}(x)$-transformed and reassessed. Data analysed using analysis of covariance were assessed to ensure they fulfilled the requirement of homogeneity of slopes.

Variability in the isotope values of each element ($\delta^{13}C$, $\delta^{15}N$ and $\delta^{34}S$) in the samples of fish among the three locations was analysed using a 1-factor analysis of covariance (ANCOVA). Location was treated as a fixed factor and the length of the fish was treated as a covariate. If 1-factor ANCOVA showed that the covariate did not influence the isotopic signatures of fish ($P > 0.05$), the data were re-analysed with a 1-factor analysis of variance (ANOVA). SNK tests were used to assess which of the locations differed from one another. Where the covariate was significant, linear regression was used to assess how isotope values related to fish length. Planned comparisons were used to compare snapper isotope values with autotroph values for each element separately.

Multivariate

Multidimensional scaling (MDS) and analysis of similarity (ANOSIM) (Clarke 1993) were used to assess whether isotopic signatures of fish varied amongst locations using all three elements together. Bray-Curtis dissimilarity matrices were constructed from the untransformed stable isotope data for individual fish for which all 3 isotope ratios had been measured.

7.3 Results

Changes in the isotopic signatures of snapper with distance offshore

When all three elements were considered together, the isotopic signatures of snapper varied only slightly with location (Fig. 2). Multidimensional scaling and ANOSIM showed that fish at the inner location had subtly but significantly different combinations of $\delta^{13}C$, $\delta^{34}S$ and $\delta^{15}N$ to fish sampled 10 km from shore (R statistic = 0.313, $P = 0.048$). There was no difference, however, between fish sampled 5 km offshore and those sampled either inside the Broadwater (R statistic = -0.094, $P = 0.571$) or 10 km offshore (R statistic = -0.081, $P = 0.651$; Fig. 2). Despite the results from the multivariate analyses, when analysed individually, the values of $\delta^{13}C$, $\delta^{34}S$ and $\delta^{15}N$ did not vary statistically amongst locations (Fig. 3).

Little of the variability in values of $\delta^{13}C$ could be attributed to differences in the standard length of fish ($F_{1,49} = 0.632$, $P = 0.430$), so variability in the values of $\delta^{13}C$ in fish amongst locations was analysed with a 1-factor ANOVA. Although $\delta^{13}C$ values for the three locations differed significantly ($F_{2,50} = 3.551$, $P = 0.036$; Fig. 3), means were all within 0.5 ‰ and SNK tests could not differentiate the locations from one another ($P > 0.05$).

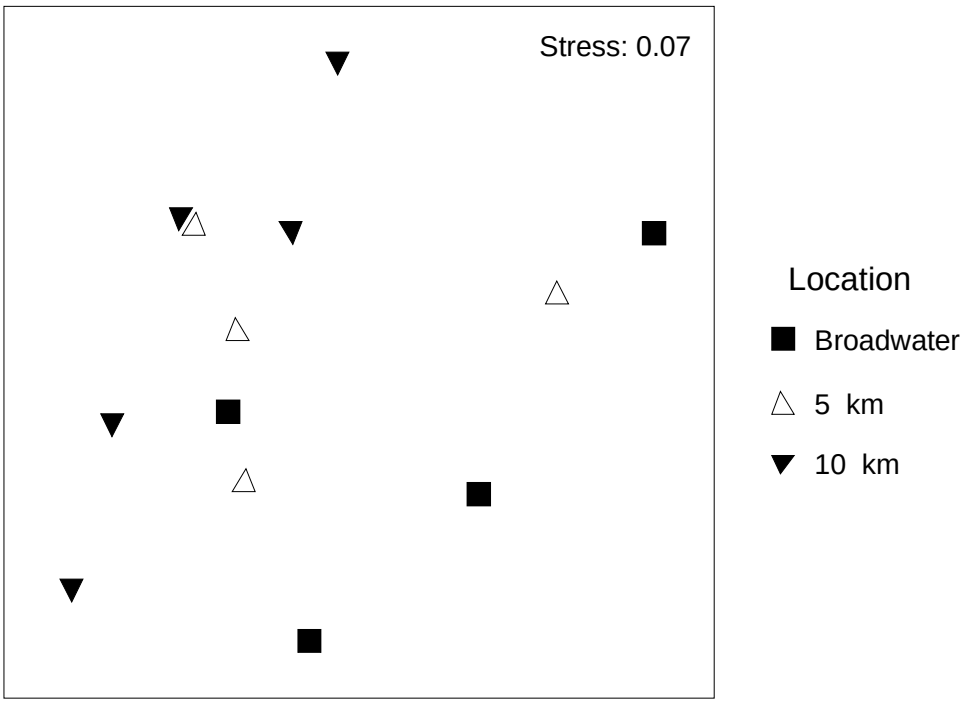


Figure 2. Two dimensional MDS plot of the relative similarity of snapper from each of the three locations (Broadwater, 5 km, 10 km) based on their values of $\delta^{13}C$, $\delta^{34}S$ and $\delta^{15}N$. Points are individual fish. Only individuals for which all 3 elements were analysed are shown.

As in the analysis of $\delta^{13}C$, the length of fish was a poor predictor of variability in the values of $\delta^{34}S$ ($F_{1,15} = 0.167$, $P = 0.690$). Average values of $\delta^{34}S$ in fish differed by less than 1.5 ‰, and a single-factor ANOVA could not differentiate among fish from different locations ($F_{2,12} = 3.389$, $P = 0.068$; Fig. 3).

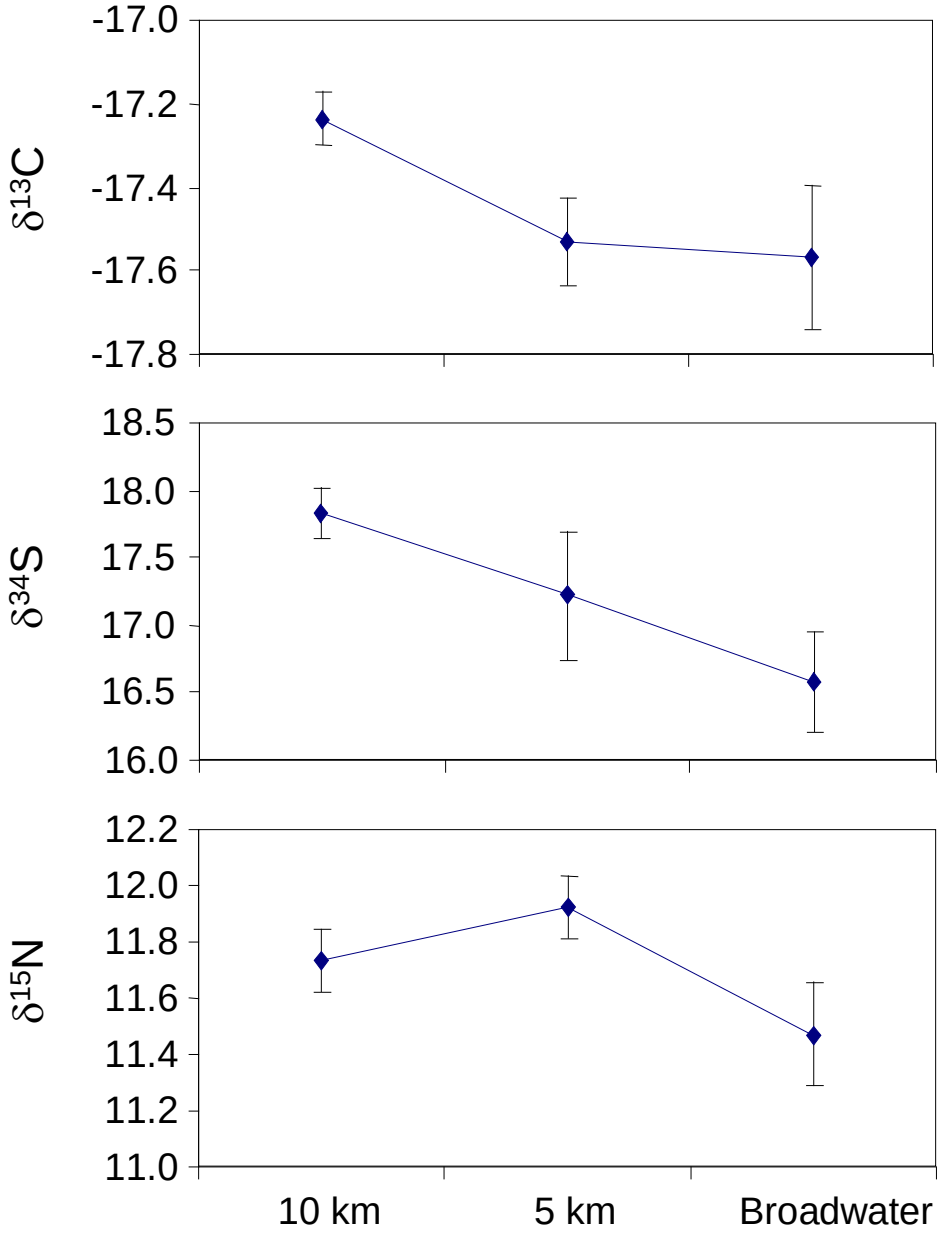


Figure 3. Mean (±SE) values of $\delta^{13}C$, $\delta^{34}S$ and $\delta^{15}N$ in snapper at each location (Broadwater, 5 km and 10 km).

There was a significant positive relationship between fish length and values of $\delta^{15}N$ ($F_{1,49}$ = 22.673, $P < 0.001$; Fig. 4). There was approximately a 2 ‰ enrichment in $\delta^{15}N$ values between the smallest (13 cm) and largest (70 cm) snapper. Values of $\delta^{15}N$ in fish, adjusted for length, did not vary significantly among locations ($F_{2,49}$ = 1.081, P = 0.347). Broadscale temporal sampling (over 2 years) provided us with an opportunity to assess whether the isotopic signatures (C and N) of snapper caught at 5 km offshore varied between consecutive years (2000 & 2001). The lengths of fish explained little of the variability in isotope values of $\delta^{13}C$ in fish between 2000 and 2001 ($F_{1,59}$ = 0.252, P = 0.618), and the carbon isotopes did not vary between years ($F_{1,59}$ = 1.060, P = 0.308). The relationships between fish length and values of $\delta^{15}N$ in each year were dissimilar (assumption of

homogeneity of slopes not met; Fig. 4). Subsequent regressions of fish length on values of $\delta^{15}N$ for each year separately showed that values of $\delta^{15}N$ did not vary significantly with fish length in 2000 ($R^2 = 0.0065$, $P > 0.05$), but varied positively with fish length in 2001 ($R^2 = 0.3159$, $P < 0.05$) (Fig. 4).

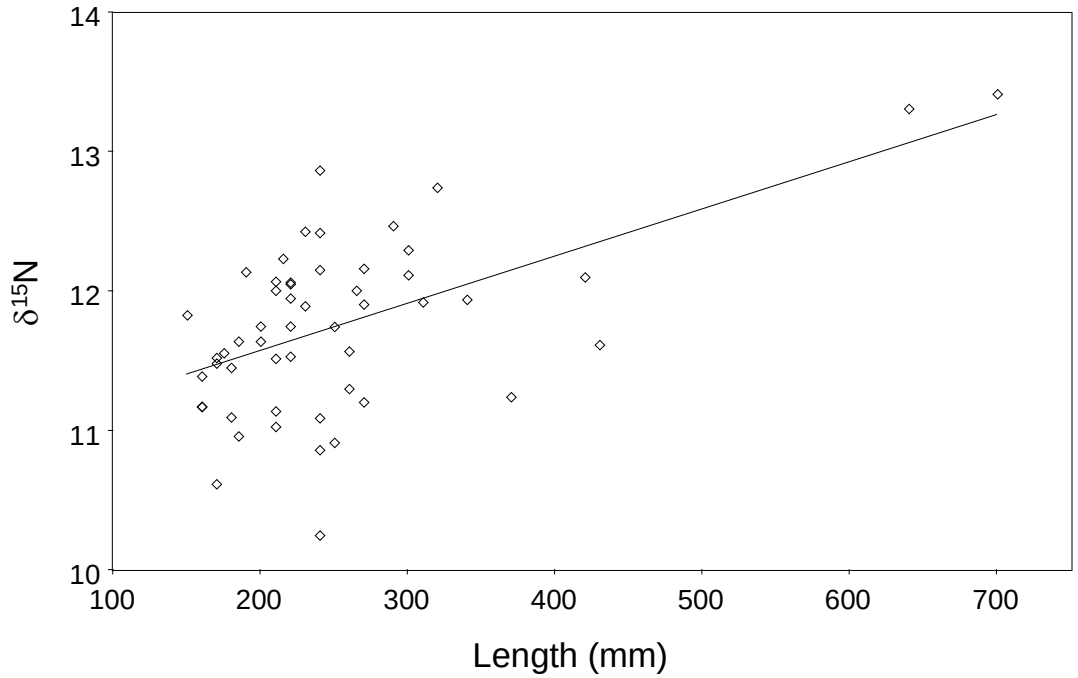


Figure 4. Linear regression of the values of $\delta^{15}N$ with the total length of snapper for fish caught in this study (2000 data only).

Contribution of autotrophs to snapper nutrition

Comparing the isotope values of an autotroph and that of a consumer is generally the first step in reconciling which autotrophs potentially contribute most to the nutrition of an animal (Doucett et al. 1996; Gannes et al. 1997; Eggers and Jones 2000). All of the autotrophs had significantly different values of $\delta^{13}C$ compared with the average value measured in fish (Table 1, Figs 5 & 6). The values of $\delta^{13}C$ in fish were most similar to those of red/brown algae. Mangroves and saltmarsh succulents had $\delta^{13}C$ values furthest from snapper. Only mangroves and epiphytes had values of $\delta^{34}S$ that were statistically different to those of the snapper (Table 1, Figs. 5 & 6). Red/brown algae had the most similar values of $\delta^{34}S$ to snapper. The values of $\delta^{15}N$ in the snapper, adjusted for the trophic position, were significantly different from those in all autotrophs except epiphytes (Table 1, Figs. 5 & 6).

The modelled putative contribution of the various autotrophs to snapper nutrition based on all three elements together (Fig. 7) was largest for red/brown algae (18 %), followed by saltmarsh grass (17 %), green algae (16 %) and seagrass (12 %).

Table 1. Planned comparisons of the mean isotope value of snapper versus each of the autotrophs, for each element. * statistically significant after Bonferroni adjustment (*P* = 0.006).

Autotroph		Element		
		$\delta^{13}C$	$\delta^{34}S$	$\delta^{15}N$
	df	1,94	1,39	1,92
Epiphytes	F	42.7	24.7	7.3
	P	<**0.001***	<**0.001***	**0.008**
Green algae	F	47.7	0.1	22.7
	P	**<0.001***	0.759	**<0.001***
Mangroves	F	227.8	38.1	103.4
	P	**<0.001***	**<0.001***	**<0.001***
MPB	F	39.5	0.4	127
	P	**<0.001***	**0.044**	**<0.001***
POM	F	55.5	1.1	83.1
	P	<**0.001***	0.305	<**0.001***
Red/Brown algae	F	8.9	0.1	28
	P	**0.004***	0.957	**<0.001***
Saltmarsh grass	F	29.3	0.1	243.8
	P	**<0.001***	0.858	**<0.001***
Saltmarsh succulent	F	243.2	0.6	213.7
	P	**<0.001***	0.425	**<0.001***
Seagrass	F	232.8	0.2	168.1
	P	**<0.001***	0.626	**<0.001***

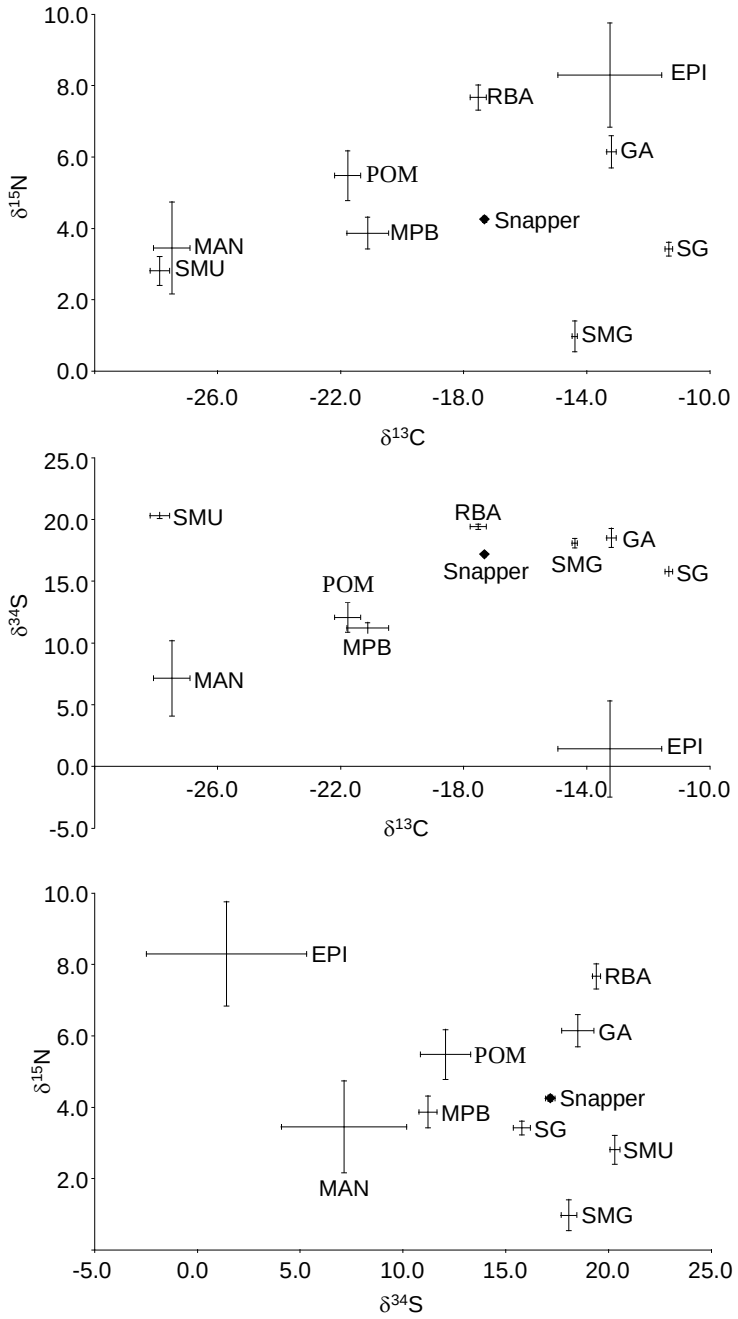


Figure 5. Mean (±SE) values of $\delta^{13}C$, $\delta^{34}S$ and $\delta^{15}N$ in the autotrophs and snapper (averaged across locations), plotted against one-another in a two-element manner. SMG = saltmarsh grass, POM = particulate organic matter, EPI = epiphytes, SG = seagrass, SMU = saltmarsh succulents, MAN = mangrove, RBA = red/brown algae, GA = green algae. Snapper $\delta^{15}N$ values have been adjusted for trophic level fractionation.

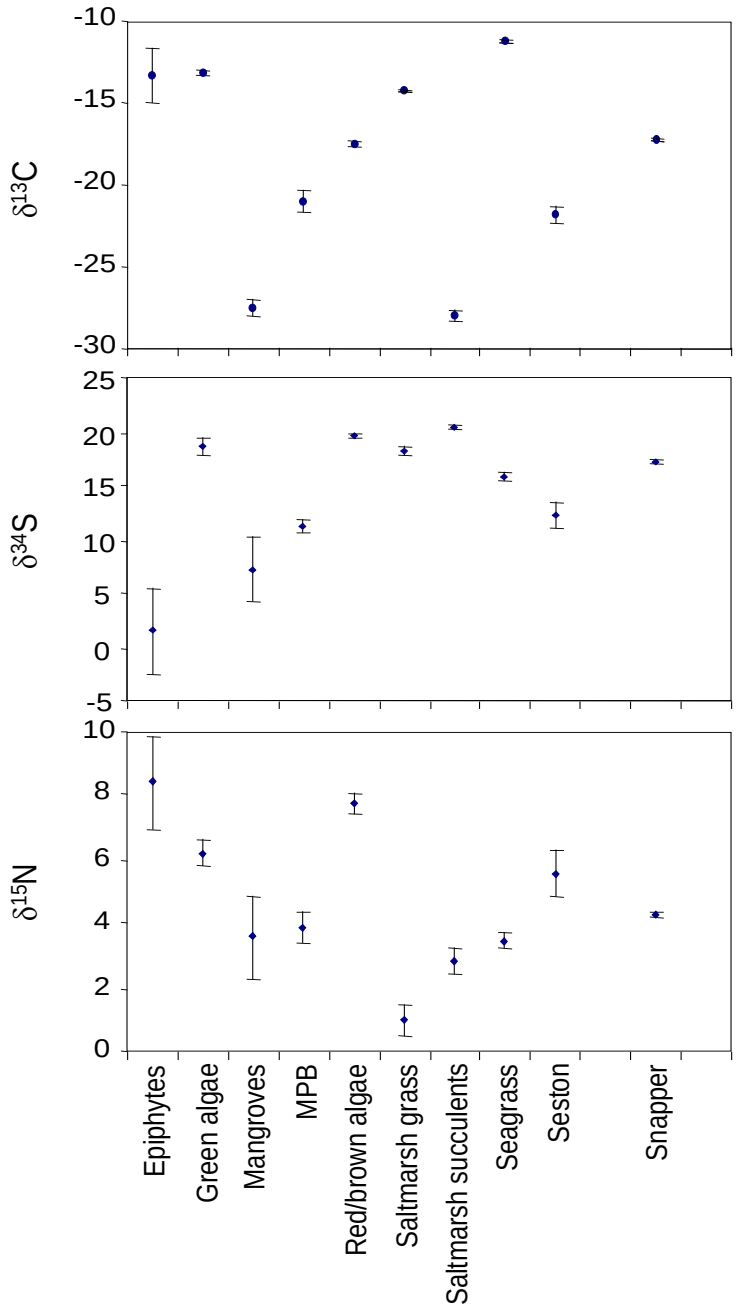


Figure 6. Mean (±SE) values of $\delta^{13}C$, $\delta^{34}S$ and $\delta^{15}N$ in each of the autotrophs and snapper (averaged across locations). Snapper $\delta^{15}N$ values have been adjusted for trophic level fractionation.

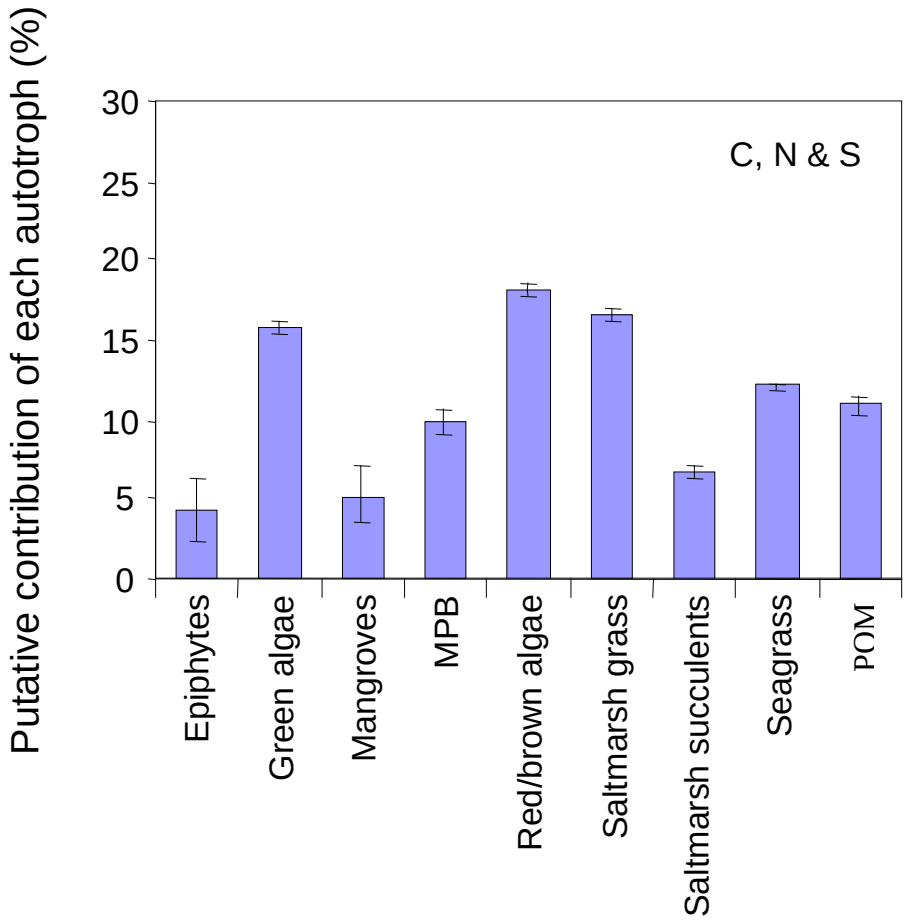


Figure 7. Mean (±SD) putative contribution (% inverse Euclidean distance) of each autotroph to snapper (averaged across locations) for C, N & S.

7.4 Discussion

Nearshore vegetation such as mangroves and saltmarsh is not only thought to be important in the provision of habitat for fish, but also as a base of nutritional support for many fisheries species occurring there (Kwak & Zedler 1987). Although primary production in these intertidal habitats is thought to support high rates of secondary production in deeper waters of estuaries and bays (Odum 1984), there is doubt about how far their influence extends offshore (Rodelli et al. 1984, Lee 1995). Using a multivariate test based on all three elements we could detect a small but significant difference in snapper isotopic signatures between fish inside the Broadwater and those caught 10 km offshore. Examination of individual elements showed, however, that the only significant difference on individual elements was for $\delta^{15}N$, a difference most likely driven by size differences and differential rates of fractionation rather than differences in autotroph sources. Snapper isotope signatures matched most closely those of red/brown algae, and modelling of single sources based on all three elements gave this autotroph the highest putative contribution, at 18%. Given the lack of difference in isotope signatures among locations, the modelling cannot distinguish different putative contributions at different locations. The autotrophs having the next three highest putative contributions, saltmarsh grass, green algae and seagrass, are all restricted to inside the Broadwater. Other estuarine autotrophs such as mangroves and saltmarsh succulents are clearly not the major

contributors to snapper nutrition. The ultimate autotrophic source at the base of the food web supporting snapper may be a mixture of more than one source. The main possibilities are that snapper rely indirectly on: 1) macroalgae alone, 2) a mixture of estuarine macrophytes such as $\delta^{13}C$ enriched seagrass or saltmarsh grass and $\delta^{13}C$ depleted mangroves, or 3) a mixture of estuarine macrophytes and macroalgae. We are unable to distinguish among these possibilities with current data, and recommend the use of alternative chemical biomarkers to examine this further.

The remarkable consistency in snapper isotope values among the 3 locations leads us to reject models 1 and 2 proposed earlier. Rather, the results are consistent with models 3 and 4. Given the lack of difference in snapper values among locations, it is reasonable to conclude that the base for nutritional support does not change with distance offshore (model 4). However, model 3 (different base for nutritional support but similar isotope signatures in fish at different locations) is also plausible, in two ways. Firstly, any difference in autotroph sources could be obscured if, by chance, a combination of autotrophs in one location could give the same isotope signature as a different set of autotrophs in another location. Given that all three elements failed to differentiate fish between locations (apart from the small, size-related difference for $\delta^{15}N$), we consider this possibility very unlikely. Secondly, even if the contribution of autotrophs to fish actually varied at different locations (e.g. high contribution from seagrass inside Broadwater and from red/brown algae offshore), the subsequent location-specific signatures of fish could potentially be masked by movement of fish among locations. Hesslein et al. (1993) demonstrated that the muscle tissue of adult fish incorporates isotope signatures over scales of months. Although movements of snapper in and out of the Broadwater have not been measured, tagging studies of snapper in southeast Australia suggest that these fish can move in and out of bays over such periods (Coutin et al. 2002). Therefore, snapper could potentially move among locations over time scales shorter than those over which isotopic signatures could be incorporated into tissue. This movement would mask any differences among locations in autotrophic sources for snapper. Isotopic analysis of fish tissues such as liver or gut lining, the isotope signature of which responds more quickly to changes in diet (Hesslein et al. 1993), could help to elucidate whether fish movements are masking location-specific sources.

Studies on other fish species have found differences in isotope signatures over similar scales to that used here (e.g. Jennings et al. 1997). Yet snapper values in the present study were very consistent over distance of 5-10 km. The isotope values of snapper in our study ($\delta^{13}C$ = -17.3 and $\delta^{15}N$ = 11.8) were also remarkably similar to those of Davenport and Bax (2002), the only other study to have looked at the stable isotope composition of snapper. They found that snapper (n = 5) caught in the Australian southeast fishery had mean $\delta^{13}C$ values of -16.4 ‰ (range -18.3 to -15.1) and mean $\delta^{15}N$ values of 11.3 ‰ (range 10.9 to 11.6). These results suggest that the base for nutritional support of snapper varies little over spatial scales up to 1000's of kilometres.

The possibility remains that intertidal estuarine autotrophs contribute substantially to food webs on which snapper nutrition depends. Certainly organic matter can be transported offshore (Dittmar & Lara 2001), but although it can potentially be used by animals, there is little empirical evidence to support this (Lee 1995). The contributions that saltmarsh grass and seagrass make to offshore fisheries is particularly interesting. Saltmarsh grass is prevalent on subtropical Australian marshes but is uncommon on the temperate marshes adjacent to the southeast fishery from which Davenport and Bax (2002) sampled; it would be virtually impossible for such a minor component of the marsh vegetation to make a major

contribution to snapper nutrition. If the similarity in isotope signatures in snapper from Queensland and the southeast fishery mean that autotroph sources are the same, it makes it unlikely that saltmarsh grass is a major contributor to Queensland snapper. Seagrass is a more likely source of nutrition for snapper along the whole east coast of Australia, with substantial meadows (including *Zostera capricorni*, the dominant species in the Broadwater) in major estuaries and embayments over the whole region. There is also evidence from another offshore fishery in southern Australia, for blue grenadier (*Macruonus novaezelandiae*), that coastal seagrass provides an almost exclusive source of nutrition for juvenile fish (Thresher et al. 1992).

Bulman et al. (2001) describe the diet of adult snapper in the southeast fishery as consisting predominantly of bentho-pelagic fish, with some benthic fish and benthic invertebrates. The difference in $\delta^{15}N$ signatures between the smallest and largest individuals sampled in our study (2 ‰) is less than the typical shift for a change of one trophic level (Fry et al. 1999). Even though the size range was large (13 to 70 cm), snapper to not appear to change trophic level very much, and even smaller fish are probably piscivorous.

Conclusions

Understanding which autotrophs contribute to the nutritional base of support for fisheries, and how the links vary with distance offshore, is crucial in managing habitats to ensure that fisheries are sustainable. The most likely sources of autotrophic production supporting snapper are red/brown algae associated with rocky reefs, or a mixture of estuarine macrophytes, especially seagrass, with or without macroalgae. No difference in autotrophic source was demonstrated between snapper caught inside or offshore from the Broadwater. Either snapper rely on the same autotrophic source(s) at all locations, or they have location-specific sources but this is not reflected in their isotope signatures because the fish move among locations over the time scale (weeks to months) on which isotope values of muscle tissue are incorporated.

8.0 Benefits

Both commercial and recreational fisheries will benefit from this project through better management of both the quantity and quality of seagrass and other estuarine habitats. Information about the importance of seagrass and other habitats has been related to key fisheries species including whiting, mullet, tailor, flathead, snapper, banana prawns and mud crabs. The project also significantly improves the chances of scientists elsewhere being able to answer previously intractable management questions about the role of different habitats in fisheries foodwebs. This work assists in being able to manage coastal habitats for sustainable fisheries in subtropical Australia. The subtropical fin-fisheries alone are directly worth around $15 million in commercial catch annually, and up to 500,000 recreational fishers use the resource (Williams, L.E. 1997. Queensland's Fisheries Resources QDPI report). Other species included in the current work contribute to the Qld trawl fishery, worth around $120 million annually (excluding scallops).

9.0 Further Development

This project has improved the rigour of isotope analysis in foodweb work. Nevertheless, broad scale surveys such as this are limited by the ability of isotopes, even using multiple elements, to differentiate among certain autotrophic sources (e.g. seagrass and saltmarsh grass).

The work is important to sustainable management of fisheries habitat, and the future clearly lies in:

1. Developing alternative chemical biomarkers that can distinguish between sources supporting fisheries foodwebs. Sterols, fatty acids, hydrocarbons, and compound-specific stable isotope analysis all show promise and have been used in certain situations. They now need development in an hypothesis testing scientific framework.
2. Hypothesis testing through experimental manipulations of isotope signatures of certain autotrophs, and the quantification of the labelled material through the foodweb to fisheries species. This has been achieved on a small scale by the authors in other projects (not funded by FRDC). It now needs expanding to a scale on which fisheries species operate.

10.0 Conclusion

1. Quantify the contribution of seagrass meadows to fisheries species found not in seagrass but elsewhere in estuaries or offshore.

- The contribution of potential autotrophic sources in supporting foodwebs leading to fisheries production was analysed using stable isotope analysis. Isotope analysis has previously been weakened by a failure to identify inconspicuous autotrophs such as microalgae on sediment, and for being qualitative rather than quantitative. We used novel methods for purifying algae from sediment to overcome the former problem. We also developed a mixing model capable of including numerous autotrophs and their spatial variance, which provided a quantitative platform from which to determine putative contributions.
- In Moreton Bay, 22 fish species caught over unvegetated mudflats were analysed using carbon and nitrogen isotopes. Seagrass had the most enriched $\delta^{13}C$ signature of any autotroph, and all fish had values within the enriched half of the range of $\delta^{13}C$ values for autotrophs. The putative contribution of seagrass and its epiphytic algae was very high for most of the 22 species.
- For some fish species (e.g. sea garfish), seagrass was clearly the major source of nutrition, either via export of particulate matter from seagrass to mudflats or via a series of predator-prey interactions (trophic relay).
- Seagrass also appeared to make a substantial contribution to fish nutrition in Port Curtis (Gladstone), with *Zostera* seagrass being the main contributor. For crustaceans such as mud crabs and banana prawns, the likely seagrass source was *Halophila*, which had a $\delta^{13}C$ signature distinct from *Zostera* in this bay.
- The contribution of seagrass to snapper nutrition was examined using isotopes of sulfur, in addition to carbon and nitrogen. Algal sources had the highest putative contribution although seagrass may also contribute to snapper nutrition.
- A model of decreasing contribution of estuarine autotrophs (such as seagrass) to snapper with distance offshore was tested. There was either no change in sources with distance, or any change was masked by movement among locations by snapper over periods of months (the time taken for tissue in adult fish to reflect autotroph source).

2. Determine the ultimate source of primary (plant) productivity sustaining fisheries production of several key species of fish and crustaceans in subtropical Australian waters.

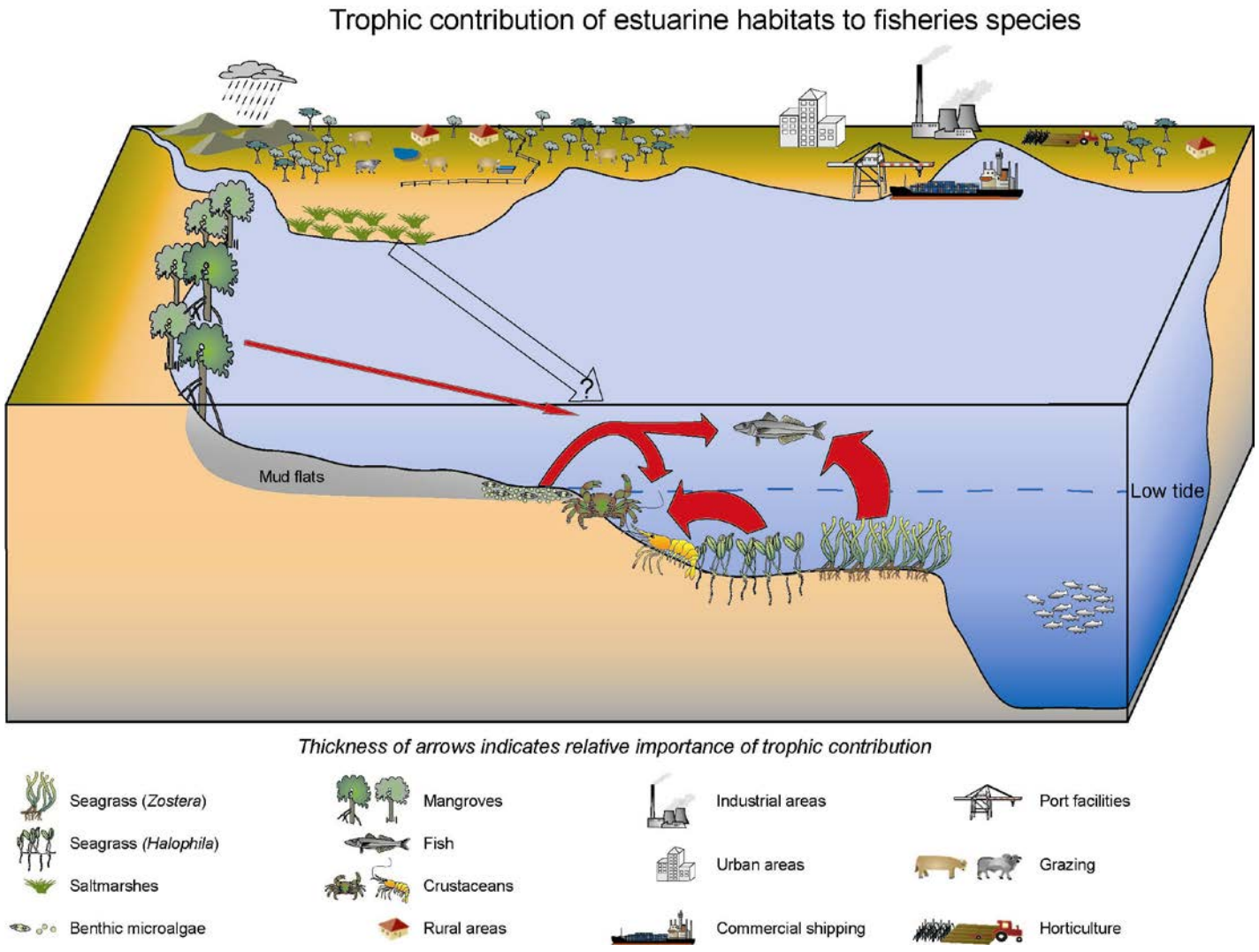
- For fish and crustaceans from Moreton Bay and Port Curtis, we modelled the importance of *in situ* sources (e.g. microalgae on mudflats, or phytoplankton production in the water column) versus sources transported to mudflats (e.g. seagrass, mangroves). Putative contributions for most fish species were largest for seagrass and their epiphytic algae (transported autotrophs), but most species also had an *in situ* source within the top three putative sources.
- Apart from seagrass, saltmarsh grass had high putative contributions to many fish species in Moreton Bay and Port Curtis. We suspect this is a spurious result, reflecting the similarity in saltmarsh grass signatures to those of seagrass. This should be tested in future using alternative chemical biomarkers.
- There was a small contribution to fish nutrition, if any, from mangroves and saltmarsh succulents. This is surprising given the proximity of mudflats to extensive mangrove forests in both study areas.
- A two-element randomisation procedure was developed to test whether animal isotope signatures correlated with autotroph signatures from location to location. This test

provided further evidence of the involvement of autotrophs in fish nutrition – and demonstrated that along with microalgae and seagrass, mangroves do provide nutrition for bream and sand whiting, although with an upper limit on contribution of 33% and 25%, respectively.

- A summary of our understanding of the contributions of various autotroph sources to fish and crustaceans is shown in the conceptual model, below.
- Snapper caught inside and offshore of southern Moreton Bay all had isotopic signatures consistent with either: a) a macroalgae source, or b) a mixture of $\delta^{13}C$ enriched macrophyte sources such as seagrass with a smaller amount of a $\delta^{13}C$ depleted source such as mangroves, with or without a macroalgae contribution. Alternative chemical biomarkers will be needed to distinguish between these possibilities.

3. Ensure that information about the relative importance of seagrass to production in different fisheries is taken to fisheries and other coastal managers to influence future management decisions.

- It is important to disseminate information at two levels: isotope methodological advances and scientific findings about the role of estuarine habitats in fisheries foodwebs, and the management implications flowing from them.
- This project developed quantitative techniques that increase the scientific rigour of isotope studies, and which should be used in subsequent isotope studies. Ultimately the increased scientific rigour will help to more quickly answer management questions about foodwebs and fisheries that have to date been nearly intractable. The new methods were presented in two seminars at the AMSA annual conference, 2001, in Townsville.
- The scientific findings being disseminated are that: a) seagrass is important as an ultimate source of energy and nutrients to fish that are not actually caught over seagrass, and b) mudflats not only act as habitat for certain fish and crustacean species but also support autotrophic production that sustains fish production.
- Management implications are that removal of seagrass or mudflats (e.g. by dredging) will therefore disrupt trophic pathways.
- Results for Moreton Bay have been presented directly to QFS Southern Fisheries Centre, habitat protection section. The main dissemination of results from Port Curtis to coastal managers and other stakeholders is an invited presentation and written paper on estuarine foodwebs and fisheries production to the State of Port Curtis conference (in October 2002).


Trophic contribution of estuarine habitats to fisheries species
?
Mud flats
Low tide
Thickness of arrows indicates relative importance of trophic contribution
Seagrass (Zostera)
Seagrass (Halophila)
Saltmarshes
Benthic microalgae
Mangroves
Fish
Crustaceans
Rural areas
Industrial areas
Urban areas
Commercial shipping
Port facilities
Grazing
Horticulture

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Appendix 1 – Intellectual property

No intellectual property issues have arisen or are expected to arise from this project.

Appendix 2 – Staff

The following is a list of staff that were involved in this project. All are from Griffith University.

Principal Investigator

Dr Rod Connolly

Paid Staff

Rebecca Brown, Rodney Duffy, Troy Gaston, Michaela Guest, Dr Jeremy Hindell, Andrew Melville, Genny Mount, Keith Preston, Bonnie Thomas, Megan Westlake

Appendix 3 – Purifying benthic microalgae for isotope measurement

S. Y. Lee, R. M. Connolly, K. M. Preston

Abstract

A laboratory method following the sieve-and-spin approach for purifying estuarine benthic microalgae for stable isotope signature measurement is described and evaluated. Laboratory trials indicated that a high retention rate of benthic microalgae (mainly diatoms) was achieved by sieving estuarine sediment over a 53 μm mesh, which also minimised contamination by meiofauna and detritus particles. Centrifugation of the filtrate in colloidal silica (1.15 g ml^{-1} density) at 10,000 rpm for 20 minutes separated microalgae from sediment and much of the fine detrital material. A final sieving through 5 μm mesh retained diatoms with minimal contamination from silica and bacteria. Stable isotope analysis of the purified microalgae samples produced a mean $\delta^{13}C$ value of -21.6 ‰ ($\pm$1.5 S.D.), close to the upper limit of those previously obtained using less discriminatory methods, and a mean $\delta^{15}N$ value of 6.3 ‰ ($\pm$0.9 S.D.). Our findings also suggest that methods such as scraping the surface mud, or bulk analysis of the surface sediment will likely result in significant contamination of the microalgae by meiofauna and phytodetritus. Analysis of benthic microalgae sampled using our method from three sites in an estuary in southeast Queensland, Australia, revealed significant variability in carbon isotope values but not in nitrogen isotope values along the tidal gradient and horizontally along the shore.

Introduction

Stable isotopes of carbon, nitrogen and sulphur have been increasingly relied upon as tracers for trophic relationships in estuarine environments. One of the fundamental prerequisites for the stable isotope approach is an accurate database of the signatures of the potential producers in the system of interest (Peterson 1999). Failure to identify important sources of nutrients or the lack of reliable signatures for them seriously compromises the usefulness of the stable isotope approach. While the signatures of macrophytes such as seagrasses and mangroves are easy to obtain, and are thus well known, there is to date little information on the signature of estuarine benthic microalgae. The challenge is to be able to routinely sample microalgae by separating it from potential contaminating materials, such as sediment, infauna, bacteria and detritus.

We report and evaluate a simple method for purifying benthic microalgae from surface estuarine sediment samples for stable isotope analysis, following the sieve-and-spin approach by Hamilton et al. (1992). These authors purified freshwater phytoplankton samples collected from a 53 μm mesh net by centrifugating the detritus-phytoplankton mixture in colloidal silica. Contamination by organic detritus was claimed to be negligible (Hamilton et al. 1992) but no details were given of the assessment. Benthic microalgae represent a potentially important but rarely assessed nutrient source in estuarine environments. This sieve-and-spin approach may not achieve the same degree of efficacy on sediment samples, as the range of potential contaminants is comparatively larger than in planktonic samples. Burgess (2001) successfully used colloidal silica to separate meiofauna from sediment but had difficulty with contamination by detritus where detrital loads were high. In this report, this method was evaluated for its ability to minimise contamination by meiofauna, macrophyte detritus, bacteria and sediment. The method was applied to assess the variability of the signature of

benthic microalgae along the tidal gradient and horizontally along the shore, in an estuary in southeast Queensland, Australia.

Description of the method

Sample Collection

Sediment samples used for determining microalgae extraction methods were collected from the mouth of Loders Creek in the Broadwater (27° 56.9'S; 153° 25.0'E), on the Gold Coast in southeast Queensland. Chlorophyll analysis of sediment cores from Loders Creek showed that in the mid-tide region, at or just before low tide, 70% of microalgae in the top six centimetres were found in the top two centimetres of sediment, and 60% of those (≈40% of total) were contained in the top 0.5 cm of sediment. Loders Creek samples were collected on an afternoon low tide, from the mid-tide region of the mud bank. Each sample consisted of 150 to 250 ml of sediment taken from the top 0.5cm of the mud bank, which was frozen before being processed. The microalgae assemblage at this time consisted predominantly of pennate diatoms, with occasional occurrences of cyanobacteria and dinoflagellates.

Separation of microalgae from other organic material by sieving

Mud samples were thawed and processed as 50 ml sub-samples. Stable isotope analysis on a continuous flow isotope ratio mass spectrometer requires at least 1 mg dry weight of microalgal material (e.g. 10 μg of N), and enough sub-samples must be processed to ensure sufficient dry weight of microalgae is obtained (e.g. 100 ml of sediment for Loders Creek samples). The sediment samples were run through two sieves (125 and 53 μm mesh) to remove most of the animal, detrital, and inorganic matter, while allowing the greatest diatom biomass through the sieves. Meiofauna were deemed to be the most important contaminants in the microalgae samples, and so it was crucial to exclude as many as possible. Size frequency analysis of organisms in the sediment from Loders Creek showed that the majority of the copepods and polychaetes would be excluded using a 53 μm sieve, and although some nematodes passed through the sieve, their biomass and occurrence would be low enough that there would only be negligible contamination of the microalgae samples. All material retained on the 5 μm sieve was kept for centrifugation.

Purification of microalgae by centrifugation

A suspension of density 1.15 g ml^{-1} gave the highest absolute amount of microalgae retained in the useful layer after centrifugation, combined with a high microalgae to phytodetritus ratio. The suspension was centrifuged in 50 ml centrifuge tubes in colloidal silica (LUDOX ™ AM30, initial density = 1.21 $g.ml^{-1}$) at a density of 1.15 $g.ml^{-1}$ for 20 minutes at 10,000 rpm in a Beckman J2-HS centrifuge.

Drying and preparation for stable isotope analysis

Following filtration, material retained on the 5 μm mesh was washed into a large watch glass using distilled water, which was then oven-dried at 60°C until the sample had reached a constant weight. The contents of the watch glass were scraped into pre-weighed tin capsules, such that each sample consisted of 1 ± 0.1 mg of dry weight microalgae material in the capsule. The capsules were then pelletised, and processed for stable isotope analysis.

Evaluating the method

The above method of concentrating and purifying microalgae from mud samples might result in contamination by bacteria. Scanning electron micrographs were used to determine the number of bacteria associated with the microalgal cells recovered from the filtration and centrifugation process. Analysis of the diatoms (the main component of the microalgae) recovered from the extraction methods using scanning electron microscopy showed that there were very few bacteria (< 3) associated with diatoms, and that the volume of bacteria was insignificant relative to that of diatoms. These results indicate that any stable isotope signature acquired from microalgae using this process would not be affected by bacterial contamination.

A single sample of sediment from Loders Creek was processed using the above methods, and analysed for its stable isotopic signature, to ensure the methods were able to generate a suitable sample in terms of biomass required for the analysis. Following this, 25 samples were collected as outlined below to provide information on the spatial variability in the stable isotope signatures of benthic microalgae. From Loders Creek, five sediment samples were taken during a mid-afternoon low tide at the high, mid, and low tide regions of the mud bank. Five samples were also taken from the mid-tide region at two sites located at, respectively, 200 m north and south of the Loders Creek site. These samples were processed using the above methods to obtain an indication of the variability within a tidal height at one location, at three different tidal heights at one location, and at one tidal height between three different locations.

Stable isotope analysis of a single preliminary sample of microalgae extracted from the sediment from Loders Creek returned a carbon content of 12.3%, and nitrogen content of 2.0% (atomic C/N ratio = 7.18), $\delta^{13}C$ of –19.8‰, and $\delta^{15}N$ of 5.9‰. The C/N ratios of the 25 samples ranged from 7.2 to 8.8, with a mean of 8.0±1.1 (S.D.). Results of the samples collected along the tidal gradient at Loders Creek and at nearby locations produced similar results. The mean (± S.D.) values for the mid-shore samples were –20.2±0.6, -21.6±1.3, -22.9±0.8‰ for $\delta^{13}C$, with an overall mean of -21.6±1.5‰. The corresponding values for $\delta^{15}N$ were 6.3±0.7, 6.0±1.2, and 6.7±0.5‰ (overall mean = 6.3±0.9‰). The $\delta^{13}C$ values of the samples were significantly different between the locations (ANOVA, F=7.67, p=0.007), with the values at Loders Creek being significantly lower than those at Runaway Bay (the northern location; SNK test, $p<0.05$). There was no significant difference in the $\delta^{15}N$ values. Samples obtained from different shore levels at Loders Creek also demonstrated significantly different $\delta^{13}C$ values (High shore: 22.2±1.3‰, mid shore: 20.2±0.6‰, low shore: 18.6±1.1‰ ; ANOVA, F=11.45, p=0.002) but not $\delta^{15}N$ values (High shore: 5.3±1.8‰, mid shore: 6.3±0.7‰, low shore: 5.2±1.0‰ ; p=0.411). High shore samples had significantly more negative (depleted) $\delta^{13}C$ values than the other two shore levels (SNK test, $p<0.05$).

Discussion

Signatures of benthic microalgae

The stable isotope signatures of the benthic microalgae obtained using our sieve-and-spin protocol are considerably more depleted in ^{13}C than those recorded in the literature. The range of $\delta^{13}C$ values obtained for estuarine benthic microalgae is large (-12.7 to -21.6, overall mean=-17.2±2.6 excluding cases where only bulk sediment samples were analysed) compared

to those of other estuarine producers such as mangroves. Currin et al. (1995) reported a mean of -14.9±3.1‰ for benthic microalgae in their review, which is about 30 % more enriched than the mean value obtained in our study. Environmental conditions (e.g. temperature, primary nutrient source) and the composition (e.g. dominance of nitrogen-fixing forms) of the benthic microalgal assemblages are expected to contribute to differences in stable isotope values. However, the large difference between the mean $\delta^{13}C$ value reported by Currin et al. (1995) and our value suggests that some additional factors may have contributed to the difference. The data compiled by Currin et al. (1995) already comprise samples with a wide range of taxonomic composition (from cyanobacteria to diatoms) and considerable variation in latitudinal position (Arabia to Nova Scotia). Contamination by meiofauna and/or vascular plant detritus, which seem to have dissimilar stable isotope signatures from benthic microalgae, could be an important factor.

Evaluation of the method

Stable isotope signatures have been reported for benthic microalgae in trophic studies of estuarine ecosystems, but none of the earlier reports provided a simultaneous assessment of the degree of reliability of the methods used. Some methods suffer from obvious drawbacks. For example, Newell et al. (1995) obtained benthic microalgae samples from the stomach contents of a known herbivore, the mudskipper *Boleophthalmus boddarti*. Secretions from the fish, meiofauna, bacteria and detritus are potential contaminants of the 'microalgae' sample. Direct scraping of surface mud most likely would result in contamination by all other forms of organic matter present, e.g. meiofauna, vascular plant detritus, and bacteria.

The process also seems to be able to reduce bacterial contamination, as the density of bacteria as revealed by scanning electron microscopy was small (<3 cells per diatom) compared to the much larger microalgal biomass. The mesh size (53 μm) that is most efficient in eliminating meiofaunal contaminants while allowing most diatoms to pass through is the same as that recommended by Hamilton et al. (1992) in their attempt to purify freshwater phytoplankton samples for stable isotope analysis.

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Appendix 4 - Mangrove sample processing and spatial variability

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Introduction

Recent years have seen much interest in determining energy flows in foodwebs in estuarine systems worldwide. Pioneering work on the contribution of saltmarsh plants on the east coast of North America led to the outwelling hypothesis. This paradigm postulates that movement of carbon from intertidal areas drives much of the secondary production in nearby subtidal creeks and bays. Whilst there are saltmarsh plants on the east coast of Australia the intertidal biomass is dominated by mangrove forests.

Stable isotopes of carbon (C) and nitrogen (N) are often used to determine which primary producers drive food webs in aquatic ecosystems (Peterson and Fry 1987). However, as stable isotope signatures of some primary producers can exhibit spatial variation, it is necessary to obtain replicate samples from primary producers that represent the range of values from the area that may be supplying consumers of interest (Boon and Bunn 1994, France 1996; Peterson 1999). Several studies have been done to determine the contribution of mangroves to food webs, both in coastal (Rodelli, 1984; Zieman et al. 1984; Newell et al. 1995; Loneragan et al. 1997; France 1998) and offshore waters (Harrigan et al. 1989; Newell et al. 1995). Whilst some studies have been done using replicate samples of mangrove leaves (Newell et al. 1995; Loneragan et al. 1997; France 1998) others have been less rigorous (Zieman et al. 1984; Harrigan et al. 1989). If stable isotope signatures of mangroves vary from place to place, sufficient spatially segregated samples need to be taken to accurately determine the range of isotopic values of mangroves that may be contributing carbon to the consumer species being studied. Here we test the variability of mangrove stable isotopic signatures over a large range of spatial scales.

Preparation of samples before analysis in a mass spectrometer has received some attention in the literature (Bunn et al. 1995; Bosley and Wainright 1999). Samples of mangrove leaves are traditionally ground to a fine powder before being analysed for stable isotope signatures of carbon and nitrogen (Rodelli et al. 1984; Peterson and Howarth 1987; Newell et al. 1995; Loneragan et al. 1997; France 1998; Lee 2000). Grinding of samples substantially increases processing time, and should therefore only be done if it increases the accuracy or precision of isotope analysis. We tested whether grinding affects the reported stable isotope signatures of mangrove leaves.

Mangroves, like many plants, routinely shed leaves (Larcher 1995). Chlorophyll and other compounds are broken down and removed from the leaf prior to shedding (Larcher 1995). This causes the leaf to change from green to yellow, as the leaf dies and dries it turns brown. If the removed compounds have different $\delta^{15}N$ and $\delta^{13}C$ signatures to those remaining in the leaf, the $\delta^{15}N$ and $\delta^{13}C$ signatures of green mangrove leaves will not accurately represent the stable isotope signatures of nitrogen and carbon entering the food web. We tested for differences amongst stable isotope signatures of green, yellow and brown leaves.

Methods

All samples used in this study were collected from nine locations in southern Moreton Bay, southeast Queensland, Australia (Fig 1).

To test the effect of grinding on the reported stable isotope signatures of mangrove leaves, one dried *Avicennia marina* leaf was divided into 5 sections by cutting perpendicular to the long axis of the leaf. Each section was divided in two along a line parallel with the long axis of the leaf. One half of each pair, selected at random, was ground to a fine powder, flecks of the other were broken off to obtain appropriate weigh for analysis. All samples were placed in tin capsules for stable isotope analysis. Differences between ground and unground samples were compared using a paired sample t-test.

In the field, leaves of *Avicennia marina* were classified into three colour groups, green, yellow and brown. Measurements of $\delta^{15}N$ and $\delta^{13}C$ were taken from five leaves of each colour from one tree and a one way ANOVA was used to test for differences in isotopic signatures amongst the colours.

To test for differences among leaves within one tree, stable isotope signatures were measured for five samples from each of five haphazardly selected leaves of one *A. marina*. Data were analysed with a one way ANOVA.

To test for differences in carbon and nitrogen isotopic signatures of mangrove leaves over various spatial scales, green leaves of the mangroves *A. marina* and *Rhizophora stylosa* were collected from four locations (Coomera Island, Never Fail Islands, Macleay Island and The Bedroom) in the southern Broadwater. Locations were separated by 1000's of metres. Within each location there were four (*A. marina*) and three (*R. stylosa*) sites separated by 100's of metres and within each site three trees separated by metres. Five leaves were measured from each tree. Data were analysed with a nested ANOVA, tree nested within site, site nested within location.

For all isotopic determinations, samples were frozen immediately after sampling, until further processing when they were oven dried at 60°C for 24 to 48 hours. About five milligrams of sample was then weighed, placed into tin capsules and analysed in an Isoprime GC Mass Spectrometer.

Results

There was no significant difference between the $\delta^{15}N$ and $\delta^{13}C$ signatures of ground and unground leaves (paired sample t-test $p = 0.212$ and $p = 0.212$, respectively) (Table 1). Unground leaves gave slightly less precise estimates of $\delta^{13}C$ but more precise estimates of $\delta^{15}N$. Unground leaves were used for all further tests.

There was no significant difference between the $\delta^{15}N$ and $\delta^{13}C$ signatures of green, yellow and brown leaves of *Avicennia marina* (one way ANOVA, $p = 0.525$ and $p = 0.412$, respectively) (Fig. 2). As green leaves are more abundant and therefore more efficiently obtained, they were used for all further tests.

Table 1. Differences between ground and unground treatments of a leaf of *Avicennia marina*. Means (± SE) n = 5 and precision (SE / mean)

	$\delta^{13}C$	SE	Precision	$\delta^{15}N$	SE	Precision
Unground	-27.56	(0.11)	0.004	3.45	(0.10)	0.029
Ground	-27.35	(0.09)	0.003	3.69	(0.15)	0.040

The different leaves from the one *A. marina* tree had significantly different $\delta^{15}N$ and $\delta^{13}C$ signatures ($p < 0.001$ and $p < 0.001$, respectively) (Fig. 3). The range of means from individual leaves was 2.07 ‰ for nitrogen and 3.5 ‰ for carbon.

Avicennia marina trees had significantly different $\delta^{15}N$ and $\delta^{13}C$ signatures among trees ($p < 0.001$, for both) and between sites for $\delta^{15}N$ ($p < 0.001$) but not for $\delta^{13}C$ ($p = 0.66$). While there was no significant difference in $\delta^{15}N$ signatures among the four locations ($p = 0.61$), there was a significant difference in $\delta^{13}C$ signatures among the different locations ($p = 0.03$). *Rhizophora stylosa* had significantly different $\delta^{15}N$ and $\delta^{13}C$ signatures among trees ($p < 0.001$ and $p = 0.003$, respectively) and among sites ($p < 0.001$ and $p = 0.01$, respectively). $\delta^{15}N$ and $\delta^{13}C$ signatures were not significantly different at the four locations tested ($p = 0.45$ and $p = 0.09$, respectively). Percent variance explained at each level is given in Table 2.

Table 2. Percent variance of stable isotope signatures explained at different levels.

	Avicennia marina		*Rhizophora stylosa*	
	$\delta^{13}C$	$\delta^{15}N$	$\delta^{13}C$	$\delta^{15}N$
Location	14	0	18	0
Site	0	50	18	90
Tree	56	14	15	5
Error	30	37	49	5

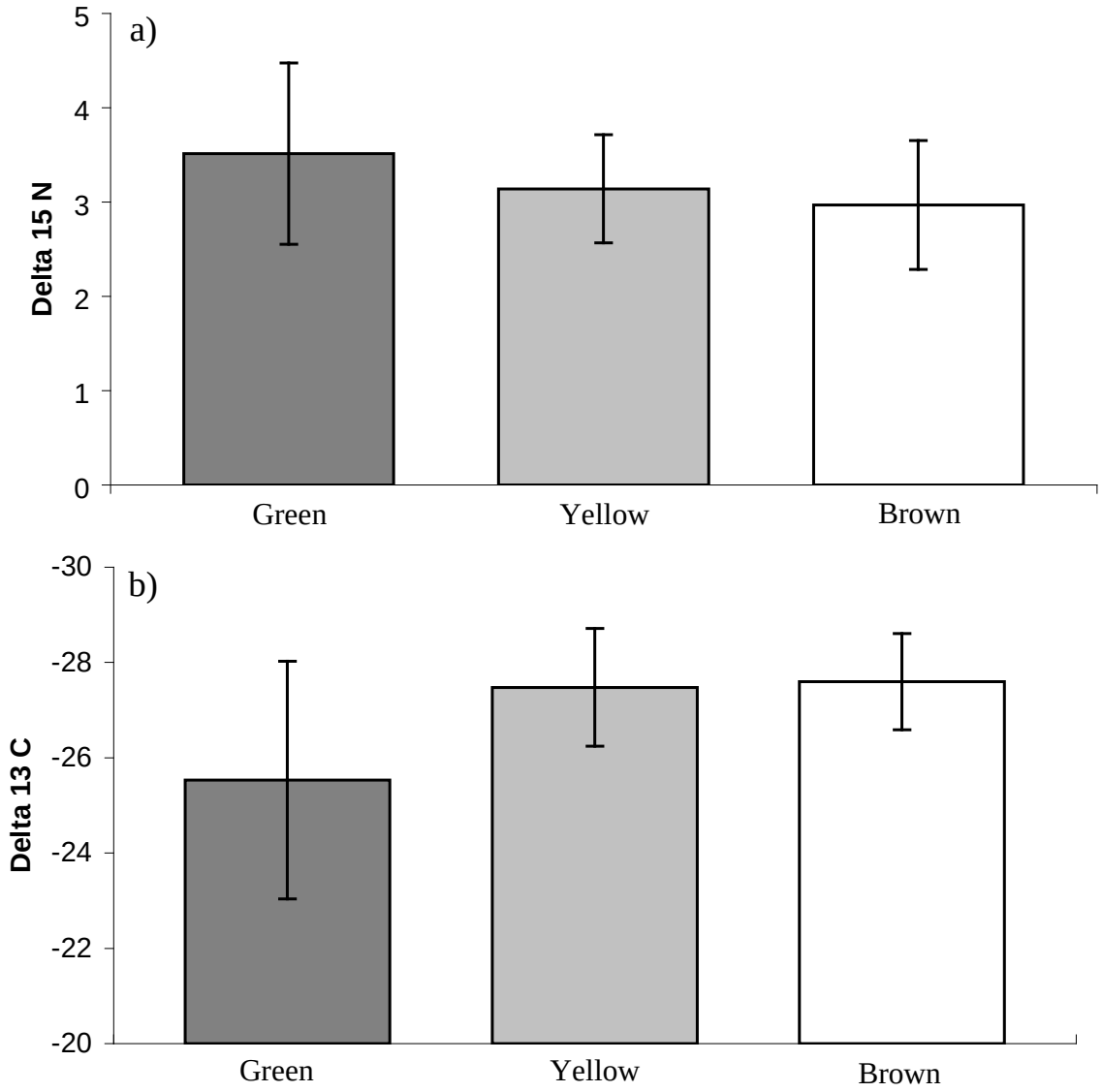

Figure 2. (a) Differences in $\delta^{15}N$ ratios of green, yellow and brown leaves of *Avicennia marina*. (b) Differences in $\delta^{13}C$ ratios of green, yellow and brown leaves of *Avicennia marina*. Bars are means ± 1 SE.

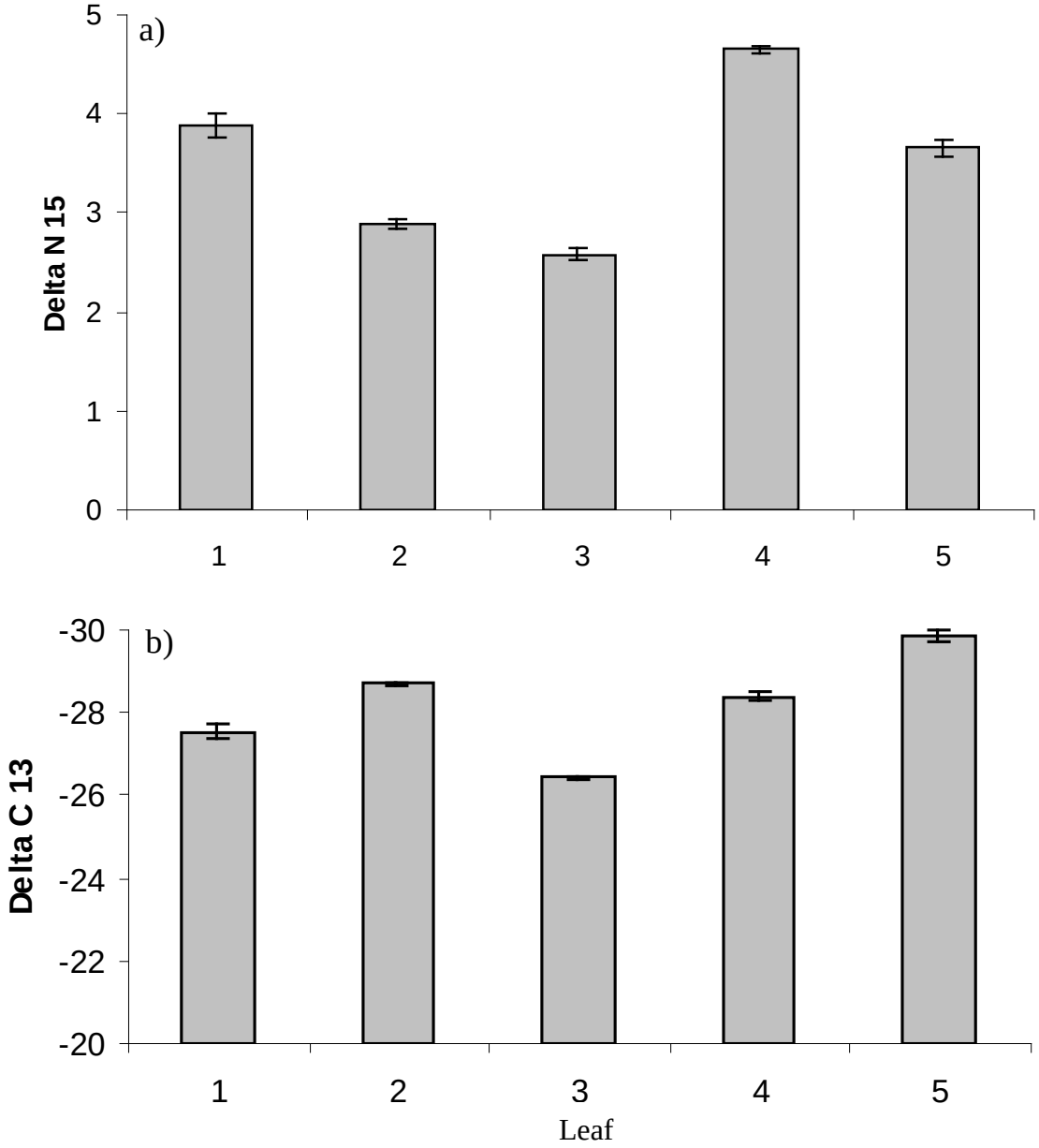

Figure 3. (a) Differences in $\delta^{15}N$ in leaves from one *Avicennia marina* tree. (b) Differences in $\delta^{13}C$ in leaves from one *Avicennia marina* tree. Bars are means $\pm$ 1 SE.

Discussion

Leaf flecks should be completely combusted in a mass spectrometer, provided combustion of occurs for sufficient time at a sufficient temperature. As grinding mangrove leaves has no effect on the accuracy or precision of stable isotope signatures, the decision to grind or not should be made on an outcomes basis. Unground samples are more quickly and therefore more cheaply processed; however, grinding allows aggregation of samples. Aggregating samples allows a mean to be calculated for trees from a specified area using one sample, at the cost of losing an estimate of variability, which may be useful (Peterson 1999).

We found no difference in the stable isotope signatures of green, yellow and brown leaves of *Avicennia marina*. Harrigan et al. (1989) and Lee (2000) found differences between the carbon and nitrogen stable isotope signatures of green and yellow leaves of mangroves. However, as there was no replication of samples, no statistical tests of any potential difference was possible. Currin et al. (1995) found no differences between the $\delta^{13}C$ signatures of standing dead (brown) and live (green) leaves of *Spartina*, however, they found $\delta^{15}N$ signatures had a difference of nearly 1‰, we found no similar difference. This result indicates that the carbon and nitrogen stable isotope signatures of the compounds recovered from leaves as they senesce are not different from those that remain. Although mangrove leaves seen on the forest floor were mostly yellow or brown, they were not often found on the trees in the study area. As green leaves are more abundant, easily collected and still accurately represent the stable isotopic signature of brown and yellow leaves entering the food chain, they were used for the rest of the study. We recommend that future workers either collect replicate leaves from each tree or grind and aggregate replicate leaves from each tree.

The differences in $\delta^{13}C$ signatures found in the leaves within one mangrove tree may be caused by differences in shading and hence CO_2 demand. Shading reduces the demand for CO_2 within the boundary layer around the leaf, causing the main enzyme in the Calvin cycle, RUBISCO, to discriminate in favour of ^{12}C (Fry 1996). Demand for nitrogen within a leaf is affected by growth rate (Larcher 1995), which may be affected by rates of photosynthesis. This may in turn affect the rate of discrimination of $\delta^{15}N$ by enzymes within the leaf.

Causes of differences in $\delta^{15}N$ and $\delta^{13}C$ between sites are less obvious. Differences that may exist in soil salinity between sites can affect productivity (Larcher 1995) and hence CO_2 demand (Ball and Farquhar 1984), which, in turn, affects carbon stable isotope discrimination. Boundary layers around leaves are affected by wind speed. If wind speed differs among sites or trees the thickness of the boundary layer around leaves at those sites and trees will differ, reducing supply of CO_2. This reduces the ability of RUBISCO to discriminate in favour of ^{12}C (Fry 1996). Differences in $\delta^{15}N$ may be caused by different levels of mycorrhizae between sites and trees, which can affect the isotope signature of nitrogen that a tree uptakes from the soil (Schulze et al. 1994). Carbon and nitrogen isotope signatures of mangroves from both locations (Coomera Island and Never Fail Islands) were not significantly different, suggesting that differences in the causes of variation act over smaller spatial scales than 1000's of metres.

The results presented here are in accordance with those of Boon and Bunn (1994) and Jennings et al. (1997) who found variation in the stable isotope signatures of both carbon and nitrogen of primary producers. Results from this survey indicate that samples should be taken over a wide range of spatial scales to accurately estimate the values and variance of $\delta^{15}N$ and $\delta^{13}C$ of mangroves that may be contributing to the foodweb under investigation.

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