Stable isotope analysis in fisheries food webs

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Abstract

Stable isotope analysis has been used as a technique to analyse fisheries food webs for a quarter of a century, and remains the principal method for determining energy and nutrient pathways from primary producers to consumers in aquatic ecosystems. Carbon isotope analysis has been used to distinguish autotrophs at the base of inshore and offshore fisheries food webs. The combination of nitrogen and carbon isotope analysis has established the contributions of different food items to the energy and protein requirements of fish. Methodological issues with stable isotope analysis are being solved using new laboratory and mathematical modelling techniques. For example, compound-specific isotope analysis of phytol (the side-chain of chlorophyll), can be used to obtain a carbon isotope signature of benthic microalgae without interference from contamination. Advanced mixing models help distinguish among sources even in situations such as estuaries where potential sources are numerous. The addition of sulphur analysis can help to separate the contribution of sources indistinguishable using carbon and nitrogen. Ultimately, when natural abundance isotopes cannot separate sources, the addition of enriched isotope material in pulse-chase tracer experiments is effective in testing among alternate food web models.

Keywords: Stable isotopes, food webs, fish diets

Introduction

Analysis of fish diets is a necessary part of fisheries science for two reasons. Firstly, knowledge of the dietary requirements of harvested (wild or cultured) species is important for management of harvestable stocks. Secondly, the provision of organic matter to food webs and its assimilation at different trophic levels is fundamental to sustainable management of fisheries in an ecosystem context (Connolly *et al*. 2005a). From both these perspectives, it is often more important to know what is assimilated, rather than what is merely ingested, and these are mostly not the same thing.

Stable isotopes provide an efficient and useful means of analysing assimilation of energy (carbon) and nutrients in fisheries food webs. Stable isotope analysis is based on the ratio of naturally occurring isotopes of key elements such as carbon, nitrogen and sulphur that are ubiquitous in aquatic environments and are essential to the nutrition of all animals. These elements all have a common, light isotope and a rarer, heavier isotope, in which the atom has an additional neutron (i.e. $^{13}C/^{12}C$, $^{15}N/^{14}N$, $^{34}S/^{32}S$). Different autotrophic sources (often) have different ratios of the heavy and light isotopes, because they use different sources of nutrients (e.g. water for algae, air or sediment for mangroves) or have different photosynthetic pathways (e.g. saltmarsh grass, mangroves). The signatures of different autotroph sources are taken on by primary consumers, and ultimately animals at higher trophic levels. Diets and food web structure can therefore be determined by collecting and analysing plant and animal isotope ratios and using the isotope signatures as tracers (Lajtha and Michener 2006; Fry 2006).

Although analysis of stomach contents of aquatic animals can provide information useful in the interpretation of isotopes, as a stand alone technique its limitations are that it: a) demonstrates ingestion of plant or animal material but does not demonstrate assimilation; b) underestimates or fails to detect consumption of food items that leave no conspicuous presence (e.g. soft-bodied prey such as nematodes, microbes too small to observe, and plant material that is difficult to identify once consumed); and c) cannot be used on very small organisms, such as microbes (e.g. only chemical analysis of bacteria can determine nutrient sources).

In this paper I first provide examples of harvested marine species for which stable isotope analysis have proven effective at determining food web structure, including distinguishing utilisation of different food items for energy and protein requirements. I then provide potential solutions to the key

challenges for aquatic isotope analysis of: 1) measuring inconspicuous sources, 2) analysing contributions from multiple sources using mixing models, and 3) overcoming lack of differentiation among potential food sources.

Effective stable isotope analysis

Carbon isotopes in an offshore food web

Growth rates of larval *Macruronus novaezelandiae* (blue grenadier) in waters offshore of western Tasmania are higher after periods of strong southerly winds. Thresher *et al*. (1992) used stable isotope analysis to demonstrate that although adult blue grenadier rely on a pelagic marine food web driven by plankton, larvae depend on seagrass detritus. The stable isotope evidence helped Thresher *et al*. (1992) develop a model explaining the correlation between larval growth and wind patterns. Periods of strong southerly winds drive detrital mats of seagrass matter from the shallow waters of Bass Strait into western Tasmanian waters. Blue grenadier larvae feeding on microbes associated with the mats grow faster than larvae unable to access seagrass, and ultimately contribute a greater proportion of juveniles recruiting to inshore waters.

Energy and protein sources for fish in artificial waterways

Massive canal developments built to provide waterfront living opportunities in southeast Queensland provide hundreds of kilometres of artificial estuarine habitat, either replacing or in addition to natural coastal wetlands (Waltham and Connolly 2006). Several harvested species occur in the canals, including *Arrhamphus sclerolepis* (snub-nosed garfish). In natural wetlands, snub-nosed garfish feed on live seagrass material during the day and on crustaceans at night. Seagrass is absent from canals, and garfish instead consume microalgae and macroalgae, although during the night rather than the day (Waltham and Connolly 2006). They prey on a variety of animals during the day, including terrestrial insects accidentally entering the water. Garfish obtain the bulk of their energy (carbon) from algae, and the carbon isotope signature of their tissue therefore matches that of algae (mean -19‰). The nitrogen isotope signature of garfish, however, does not match that of algae (after adjusting for fractionation), but sits approximately where it would be expected if the majority of nitrogen is obtained from animals. Isotope analysis therefore provides evidence of the different roles food items have in the nutrition of this species, and demonstrates a plasticity in feeding strategies that allows garfish to flourish in artificial and natural waterways.

Nutrition of wild juvenile prawns

Stable isotopes have been used successfully to investigate the relative importance of mangrove and seagrass organic matter in the nutrition of juvenile penaeid prawns in Queensland (Loneragan *et al*. 1997). Although in theory mangrove leaf litter can form the basis of food webs in adjacent waters (Lee 1995), isotope analysis of prawns show that they derive their nutrition from organic matter in seagrass meadows. The transfer might be either through direct consumption of epiphytic algae and seagrass or via animal intermediaries in a detrital pathway. The study by Loneragan *et al*. (1997) also provides an excellent example of the extent of spatial and temporal variation in isotope signatures, and how to measure variation at multiple scales.

Measuring isotopes of inconspicuous sources

Potential sources that have low biomass, and are therefore inconspicuous, but have high productivity are often overlooked in isotope studies. For example, even the best studies in estuaries (e.g. Loneragan *et al*. 1997) have difficulty obtaining enough benthic microalgae from sediment to obtain an isotope signature. Attempts to extract microalgae from the matrix of sediment, algae, detritus, meiofauna and microfauna usually lead either to a degree of contamination or a failure to extract all algal types (e.g. depends on motility, cell size and density). Centrifuge extraction relies on algae having different densities to other particles in the sediment (Hamilton *et al*. 2005) and is particularly useful where algal biomass is high relative to detrital load. Where algal biomass is relatively low, however, a stable isotope signature for algae is best obtained using a novel compound-specific method (Oakes *et al*. 2005). Phytol (the side-chain of chlorophyll), in marine sediments derives almost exclusively from microalgae, and the compound-specific analysis of carbon isotopes of phytol therefore provides an accurate isotope signature of microalgae without the need to physically extract cells from the sediment matrix.

Advanced mixing models to analyse multiple sources

The commercially important sillaginid fish, *Sillago schomburgkii* (yellowfin whiting), of southern Australia inhabits sheltered, shallow waters supporting large areas of seagrass, mangroves, saltmarsh and unvegetated intertidal flats. Although yellowfin whiting sometimes occurs over seagrass it is more common over unvegetated habitat (Connolly 1994), and the highest densities have been recorded in tidal creeks surrounded by extensive stands of mangroves and saltmarsh (Connolly and Jones 1996). Stable isotopes were used to determine whether yellowfin whiting production was supported by a food web based on seagrass, mangroves and saltmarsh, or algae.

In situations such as these open embayments, where potential autotroph sources are numerous, attempts to use isotopes to distinguish among sources have been hampered by the lack of a unique result in mixing models. Recently developed mathematical modelling of source mixtures helps elucidate important sources in such situations. The IsoSource model of Phillips and Gregg (2003) calculates feasible combinations of autotrophs that could explain the consumer signature. The method examines all possible combinations of each autotroph's potential contribution (0 - 100%) in defined increments (e.g. 1%). Combinations that add almost exactly to the consumer signature are considered feasible solutions. Results are reported as the distribution of feasible solutions for each autotroph. For yellowfin whiting, modelling of feasible source mixtures showed that seagrass and epiphytes were the most important contributors to the nutrition of fish, but their relative importance varied between seasons (Figure 1). The median contribution of other sources was < 10%.

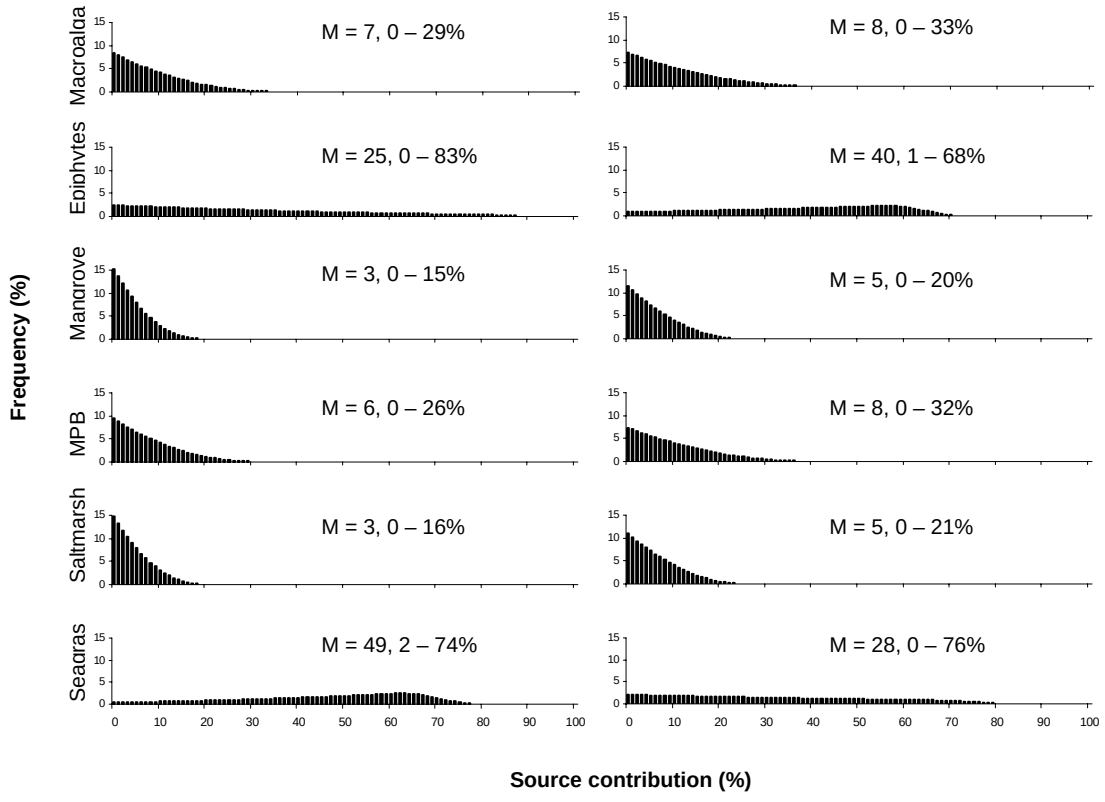

Figure 1: Distributions of feasible contributions of the 6 potential autotrophs to yellowfin whiting based on IsoSource modelling of stable isotope data (after Connolly *et al*. 2005). M = median, the ranges are 1%ile and 99%ile values.

In many cases, sources can logically be pooled into natural groupings (e.g. saltmarsh succulents and mangroves both occur high on the shore and have depleted carbon isotope signatures). Output from the initial IsoSource analysis can be re-processed using the smaller subset of grouped sources (Phillips *et*

al. 2005). This pooling has been used to generate a more informative, narrower range of possible contributions for the grouped sources (Melville and Connolly 2005).

The spatial variability in isotope signatures of fish and potential sources can provide further evidence of source contributions. Correlation between site to site variation in isotope signatures of a consumer and site to site variation in isotope signatures for any of the sources, implies a contribution from that source. For most studies this is best done as a single test on carbon and nitrogen isotopes together, for which a two-dimensional correlation test in Euclidean space has been developed (Melville and Connolly 2003). For yellowfin whiting, the spatial correlation test combined with IsoSource showed that seagrass and epiphytic algae provided most nutrition, and that other algae made a minor (< 10%) contribution. Yellowfin whiting rely on inwelling of organic material from seagrass meadows rather than outwelling from mangroves and saltmarsh (Connolly *et al*. 2005b).

Overcoming lack of differentiation among potential food sources

Where isotopes of the most common elements cannot separate potential sources, additional elements can be used. Sulphur is the most likely candidate in marine systems, since the source and therefore the isotope signature of sulphur utilised by different autotrophs varies strongly. In a review of marine isotope studies that use the three elements (C, N, S), Connolly *et al*. (2004) showed that the mean difference in isotope signature between any two pairs of autotrophs was greatest for S, followed by C and N (Figure 2). The automation of S isotope analysis of ecological samples is increasing both the breadth of food web studies in which S can be employed and the levels of replication that can be used. However, sampling and analysis artefacts are less well understood for S than for C or N. Improved preparation and analytical techniques (e.g. Hsieh and Shieh 1997; Fry *et al*. 2002) are being developed but need to be more widely tested and used to give rigour to the use of sulphur in food web studies.

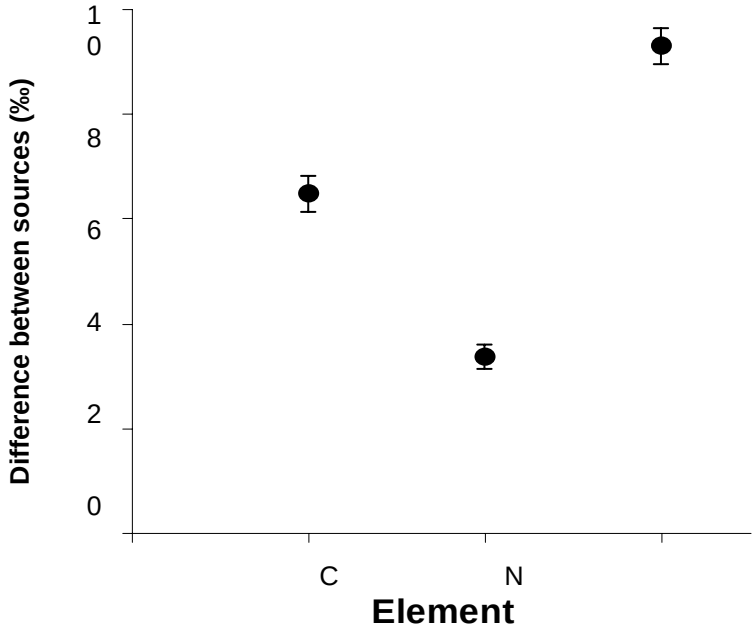

Figure 2: The average isotopic separation between any pair of potential autotrophic sources in all marine food web studies using the three elements carbon (C), nitrogen (N) and sulphur (S), drawn from data within Connolly *et al*. (2004).

Where natural abundance isotopes cannot separate sources, even when using an additional element such as sulphur, pulse-chase tracer experiments are required to distinguish the contribution of different sources. Stable isotope analysis of marine food webs has made major advances through the manipulative enrichment of source signatures using the addition of artificially enriched isotopes (e.g. Gribsholt *et al*. 2005). Such experiments increase discrimination between the roles of potential sources and can therefore provide more rigorous tests of hypotheses about food webs. Although some of these experiments have been on large scales, there has not yet been a focus on fisheries species.

Winning *et al*. (1999) showed how sources that cannot be separated naturally might be distinguished using manipulative experiments. Loneragan *et al*. (1997) had been unable to separate seagrass and its epiphytes using natural abundance isotopes of carbon (both -11‰) and nitrogen (both 4‰). Working in the same system, Winning *et al*. (1999) added potassium nitrate artificially enriched in ^{15}N to seagrass mesocosms. After just 15 minutes of exposure to enriched nitrogen, the two sources were able to be easily separated. After enrichment, seagrass nitrogen isotopes values averaged about 100‰, while epiphytes values averaged about 400‰ (Figure 3). This separation was maintained for up to a month, enough time to show that prawns added to the system relied on seagrass material itself and not just, as theory would predict, the productive epiphytes (Winning 1997).

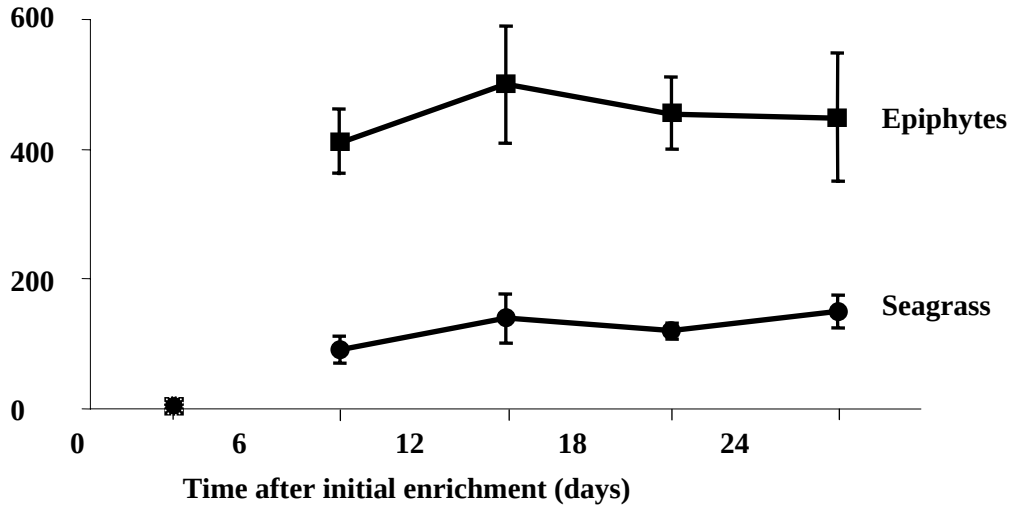

Figure 3: Separation of source (seagrass, epiphytic algae) isotope values using manipulative enrichment, prior to pulse-chase tracer experiment to determine prawn nutrition (data redrawn from Winning *et al*. 1999). Values for seagrass and epiphytes prior to enrichment (time = 0) were both 4‰.

Conclusion

When used well, stable isotope analysis allows rigorous testing among different food web models. Recent developments in the laboratory and in modelling procedures have advanced isotope analysis rapidly, making the technique suitable for many fisheries related situations.

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