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Application of Stable Isotopic techniques to wetlands conservation

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Abstract

Identification of food chain linkages between high trophic order species (particularly those of commercial and recreational importance) and different wetland resources (e.g. saltmarsh, mangrove and seagrass) is fundamental to resource management. The source of energy and trophic connectivity among species in the ecosystem can be quantified using stable isotopic techniques. Stable nitrogen isotopes can be used for tracking of pollutant derived from urban effluent or other anthropogenic sources that contribute to eutrophication and other management issues in aquatic environment. Analysis of non-radioactive, naturally occurring carbon and nitrogen isotopes is one of the most powerful techniques that can be considered in clarifying management questions related to wetland conservation.

Introduction

Analyses of naturally-occurring stable isotopes have emerged as powerful techniques for addressing research and management related questions in ecology. Understanding of linkages between sources of primary production, and how these fuel lower and higher trophic-order consumers and energy pathways is fundamental to conservation decisions. Carbon and nitrogen stable isotope ratios ($^{13}C/^{12}C$ and $^{15}N/^{14}N$) provide an important tool to model food chain connectivity between aquatic species and wetland resources.

What are Stable Isotopes?

Isotopes are different forms of the same element (for example, carbon-12, carbon-13 and carbon-14) which contain the same number of protons, but different numbers of neutrons in the nucleus. Each isotope of an element has a specific mass because of the different numbers of neutrons. Carbon-12 is literally lighter than carbon-13 and carbon-14. An isotopic value is the ratio of the isotopes in a sample. There are two naturally occurring stable atomic forms of carbon (^{13}C and ^{12}C) and nitrogen (^{15}N and ^{14}N). Some naturally occurring isotopes are unstable, gradually changing from one form to another (for example, carbon-14 decays to produce carbon-13). Isotopes can therefore be divided in two major types, stable and unstable (radioactive). Isotopes that do not decay over time (that is, they are not radioactive) are termed stable isotopes.

What is the Technique?

Over the last decade, stable isotopes have been increasingly used in environmental studies. Plants and animals assimilate both stable forms of C and N, and the ratio of ^{13}C to ^{12}C and ^{15}N to ^{14}N compared to a reference standard can be determined by an analysis of tissue on an isotopic ratio mass spectrometer (IRMS). In the laboratory, very small amounts of plant, animal and sediment organic matter samples (microgram to milligram level) are oven dried at 60°C for 48 hours then ground to a fine powder. Powdered and homogenised tissue samples are loaded into tin capsules, and are analysed with a continuous flow isotope ratio mass spectrometer (CF-IRMS) to obtain the isotopic ratios of the sample. Analyses of naturally occurring stable isotope ratios of carbon (C) and nitrogen (N) are commonly used to trace the source of organic matter (Peterson and Fry 1987) in an aquatic food chain.

Why is food chain connectivity important?

Every animal and many plant species depend to some extent on another plant or animal species for their survival. Food chains are the vehicles of transfer of energy and nutrients from one level to another. Photosynthesis is only the beginning of the food chain. In the grazing food chain, food energy and organic compounds are transferred from the plants to the herbivore animals consuming them and subsequently to the carnivores or omnivores preying upon the herbivores (Krebs 2009). In a detrital food chain, dead organic matter (Smith and Smith 2009) of plants and animals is broken down by decomposers, (e.g. bacteria and fungi) and moves to detritivores and then carnivores. An understanding of food chain relationships among species and their habitat resources can be fundamental for resource management. Without detailed knowledge of how materials (including pollutants) and nutrient flows in an ecosystem, it is not possible to appreciate the functioning of ecosystem, or predict changes that might result from any natural or man-made intervention (Blaber 2000).

How are isotopes used to determine food-chain connectivity?

Ecological applications of stable isotope analyses rely on prey species' specific isotope ratios which are transferred to the consumer species upon consumption. There is an increase in the relative proportion of carbon-13 content ($^{13}C/^{12}C$ ratio) and nitrogen-15 content ($^{15}N/^{14}N$ ratio) in the consumer animal compared to the prey due to selective metabolic loss of the lighter isotopes during assimilation, excretion and growth. An organism is typically enriched in heavier (^{13}C and ^{15}N) isotopes relative to its diet by approximately 0-1 for $\delta^{13}C$ and 3-4 parts-per-mil ($^{0}/_{00}$) for $\delta^{15}N$ (De Niro and Epstein 1978; Minagawa and Wada 1984; Peterson and Fry 1987). This process is called trophic discrimination, or fractionation. The relatively low fractionation of carbon isotope values between consumer and diet make this ratio useful to trace the sources of organic nutrition (Hecky and Hesslein 1995), whilst the higher fractionation of nitrogen isotope ratios make this useful in determining the relative trophic position of organisms (Cabana and Rasmussen 1996; Vander Zanden and Rasmussen 2001) in the ecosystem. Comparing both carbon and nitrogen isotopic values of a broad range of ecosystem occupants can be used to construct food chain

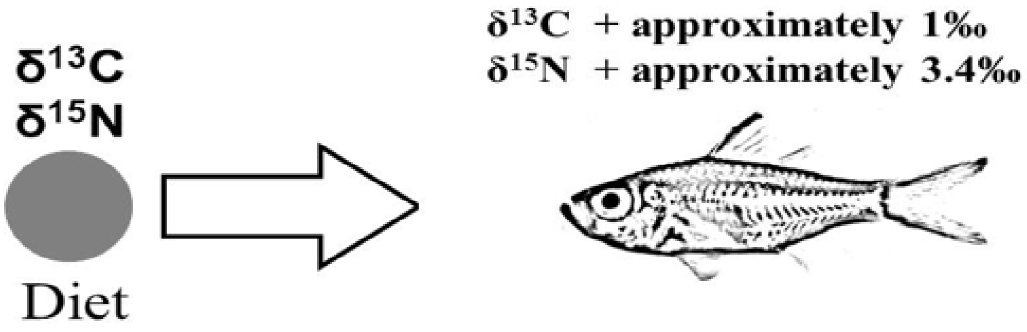

Figure 4.3.1. Conceptual diagram of trophic transfer of carbon and nitrogen from diet to consumer fish.

linkages (Vander Zanden *et al*. 1997; Post 2002) among species and their trophic connectivity with wetlands.

For example, suppose the $\delta^{13}C$ value of a diet item is -24.4‰. If a glassfish exclusively eats that diet item and if that diet is assimilated into muscle tissue, the approximate $\delta^{13}C$ value of the consumer glassfish would be approximately -23.4‰. Accordingly, if the diet $\delta^{15}N$ value is 6‰ then the approximate $\delta^{15}N$ value of glassfish muscle after assimilation of diet would be predicted to be 9.4‰, which is 6‰ + trophic increment 3.4‰ (Figure 4.3.1).

An example of energy flow from coastal wetlands would begin with the autotrophs such as benthic algae, saltmarsh, mangrove and seagrass that accumulate energy from the sun through photosynthesis. Benthic herbivores, such as crabs and snails, eat algal and plant materials and thus energy moves from autotrophs to herbivores (plant eater). Omnivores such as yabbies subsequently feed on the herbivores and, finally, other carnivores, such as fish, prey on the omnivores. The unique carbon and nitrogen isotopic values of ecosystem components (e.g. autotrophs, herbivores, omnivores and carnivores) thus move from the bottom to the top of the food chain (Figure 4.3.2).

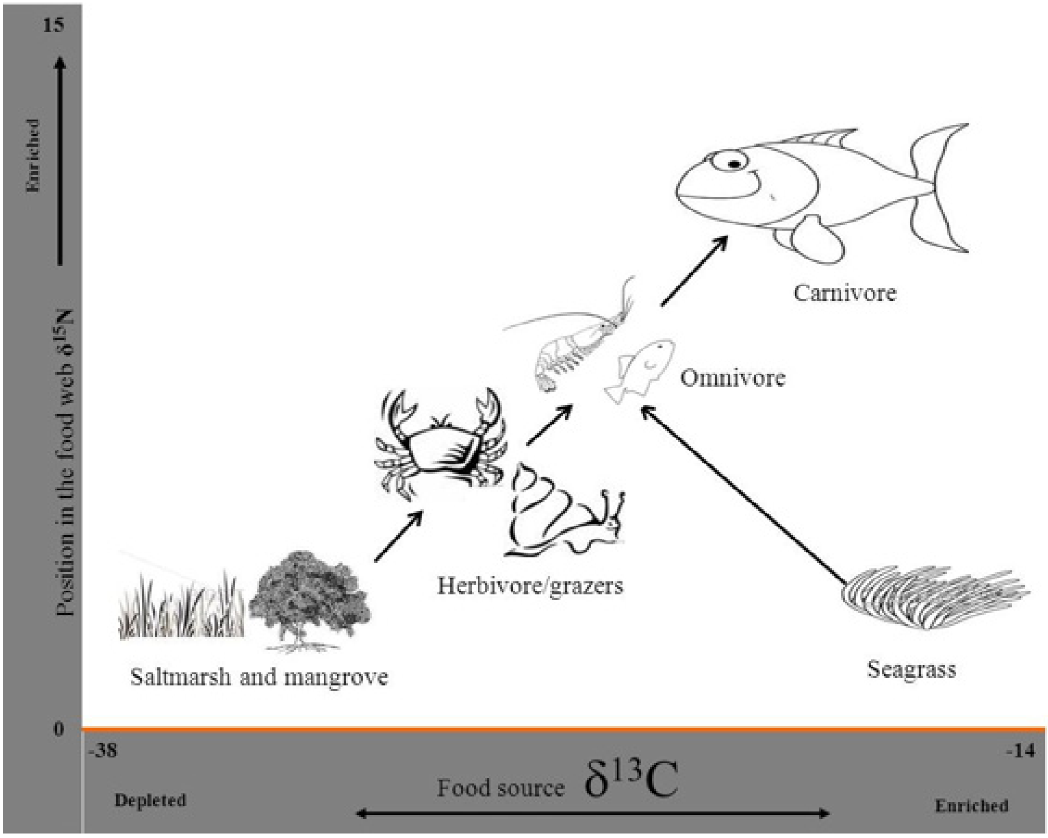

Figure 4.3.2. Postulated food chain connections between wetland resources and consumer species.

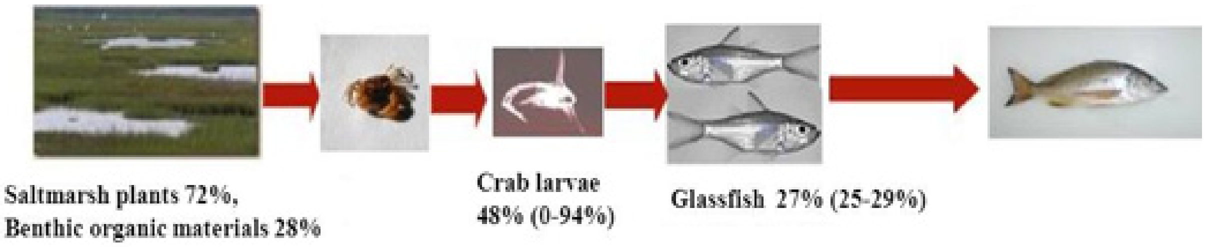

Figure 4.3.3. Food-chain connectivity between estuarine fish and wetland resources.

Carbon and nitrogen isotopic values of ecosystem components are usually used to develop food web "bi-plot" which serves as a conceptual model of ecosystem structure (Figure 4.3.2). In this carbon and nitrogen bi-plot, the X-axis represents the $\delta^{13}C$ values of components using to identify source of diet of an organism. The Y-axis represents $\delta^{15}N$ values of components relating to the trophic level of organisms in the food pyramid. Using trophic discrimination values of organisms for both carbon and nitrogen, we can identify food source and trophic level of organisms in the food web and their trophic linkages with wetland resources.

Recent studies in saltmarsh in NSW found that many fish species, including those of commercial and or recreational value visit saltmarshes during spring high tide (Mazumder *et al.* 2005, 2006). Crabs living in the saltmarsh release large quantities of larvae on spring high tides and these larvae are carried by the ebb tide and enter to estuary food chain (Mazumder *et al.* 2006). To understand food chain connectivity between habitats and species, carbon and nitrogen stable isotope ratios ($\delta^{13}C$ and $\delta^{15}N$) were measured (Mazumder *et al.* 2011) in a variety of plants, crustaceans, benthic macro-invertebrates and fish collected from the mangrove, saltmarsh and seagrass of Towra Point, in New South Wales, Australia. The stable isotope analyses clearly found food-chain connectivity between estuarine fish and wetland resources (i.e. autotrophs, crab larvae).

Estimate the contributions of different food sources to the diet of a consumer animal can be calculated numerically using IsoSource mixing model (Phillips and Gregg 2003). In the mixing model, the $\delta^{13}C$ and $\delta^{15}N$ values of consumer species and their potential food sources are used with appropriate trophic enrichment correction to estimate the feasible contributions of foods to the diet of consumer species. Isotopic studies in Towra Point (Mazumder *et al.* 2011) found crabs living in the saltmarsh feeding on locally available autotrophic material (Saintilan and Mazumder 2010), derive approximately three-quarters of their dietary carbon from saltmarsh autotrophic materials. The contributions of saltmarsh autotrophic materials vary spatially. Alderson *et al.* (2013) study in the Empire Bay and Davistown saltmarshes at Brisbane Water found micro-phytobenthos and fine organic matters in the saltmarsh were the major contributors to the diet of crabs and saltmarsh plant contribution was small (20-36%). The larva produced by crabs is a source of food for itinerant fish including glassfish (Mazumder *et al.* 2006). In a study of the Towra Point wetland, crab larvae contribute approximately half of the dietary carbon of glassfish. Glassfish subsequently contribute approximately one quarter of the dietary carbon to bream and thus energy in the form of carbon compounds moves from the wetland plants to top of the food chain (Figure 4.3.3).

The finding of their study highlights the significance of mangrove and saltmarsh wetlands as a feeding habitat for resident grazers such as crabs and itinerant fish and illustrates the potential significance of trophic relay between the intertidal wetlands and the broader estuarine environment (Kneib 1997). Such findings strengthen the case for coastal wetland conservation as a pillar of sustainable fisheries management, but also potentially inform the monitoring of wetland restoration programs. The aim of wetland restoration is to re-establish the physical, chemical and biological conditions at degraded wetland sites that still possess characteristic wetland features (Weinstein *et al.* 2001). A major concern with wetland restoration works depends how successfully the ecological functionality of the restored wetland are replaced. Key indicators such as vegetation structure, faunal assemblages and functional attributes of the restored wetland would be similar to pre-degraded conditions. Ecological indicators such as flora and faunal assemblages, tidal amplitudes can be measured through conventional surveying tools but measuring ecological functionality (Currin *et al.* 2003; Moseman *et al.* 2004) require isotopic techniques. Analysing carbon and nitrogen isotope values of

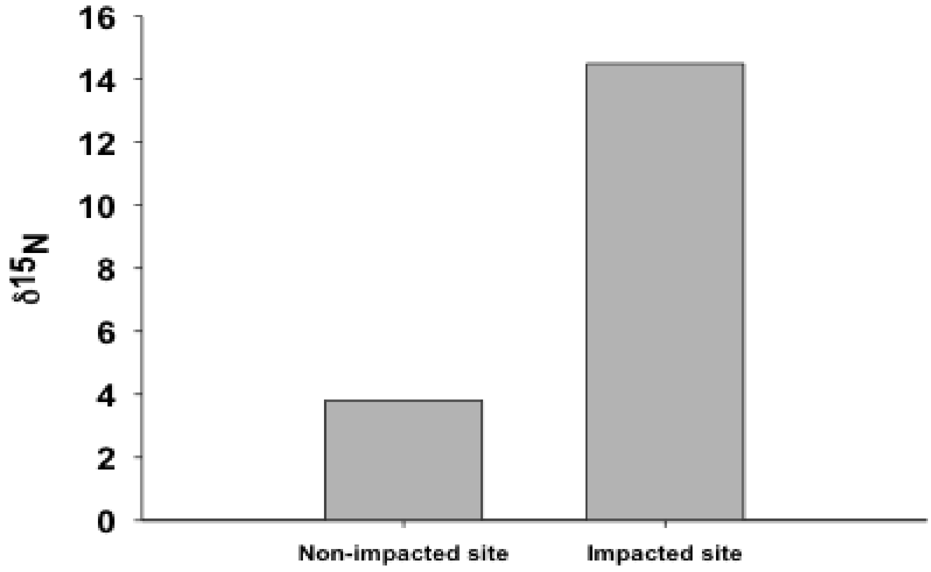

Figure 4.3.4. Example of stable nitrogen isotope ($\delta^{15}N$) values of algae from swage affected sites.

soil, vegetation, benthic fauna and visiting fish in the restored wetlands compared to reference sites would inform resource managers of the extent of food chain connectivity (i.e. ecological functionality) between nekton and autotrophs that may have been planted in the wetlands.

How to track pollutants in the food chain?

Increasing urbanisation puts ever-greater stresses on estuarine waters to dilute and dissipate waste material from point as well as diffuse sources. Waste materials are a rich source of dissolved nutrient and particulate organic matter that can disrupt the natural balance of coastal systems and change the composition of benthic communities (Roberts 1996; Savage *et al.* 2002) and fish assemblages (Smith and Suthers 1999). Difficulties in distinguishing nitrogen from different sources (e.g. sewage, industrial) can lead to management problems associated with identification of the various/specific contributors. The stable isotope ratios of carbon and nitrogen are useful in this regard, as they can be used to trace the movement and biological assimilation of nutrient and organic matter (Fry and Sherr 1984; Post 2002; Davenport and Bax 2002; Melville and Connolly 2003).

Agricultural fertilisers, animal faeces and treated urban effluent are major sources of nitrate in coastal environments (Macko & Ostrom 1994; Cloern 2001; Costanzo *et al.* 2001). The $\delta^{15}N$ of these nitrogen sources are isotopically distinct from each other, therefore nitrogen-isotope analysis is proving to be one of the most robust proxies of urban effluent pollution in coastal waters (Peterson 1999; Costanzo *et al.* 2001, 2003). The $\delta^{15}N$ of treated sewage nitrogen generated from human and animal wastes is approximately 10‰ to 22‰. (McClelland *et al.* 1997; Macko and Ostrom 1994).). Synthetic fertilisers have low $\delta^{15}N$ values ranging from -8‰ to 7‰ (Macko and Ostrom 1994). Much of the nitrogen passing through a sewage treatment plant will be derived from human waste. As humans are at the top of most food chains, the nitrogen released by humans will also tend to be relatively highly enriched as a consequence of having passed through many trophic levels between autotrophs (plants) and primary and secondary consumers prior to human consumption.

For example, the levels of $\delta^{15}N$ in various ecosystem components in coastal systems with sewage impacted watercourses are typically higher compared to non-impacted watercourses. By analysing the $\delta^{15}N$ of algae, plants, and animals from impacted and non-impacted sites, assimilation of urban effluent derived N in the food chain can be traced (Figure 4.3.4).

Land use changes due to urbanisation, agricultural and other developments in the catchment affect the quality of stormwater runoff and increase nutrient flow. Flora and fauna in the wetlands at the end of these systems assimilate these nutrient and subsequently pass them to other animals in the aquatic food chain. Effects can also be measured at local scales in association with point-source discharges. In a study at Coombabah Lake, Southeast Queensland Lee *et al.* (2006) reported the impact of urban effluent on a mangrove wetland. Their study analysed the $\delta^{15}N$ values of mangrove (*Avicennia marina*) leaves and a common crab (*Australoplax tridentata*) with tissue collected from point sources (0m) and 250m from the discharge areas of a creek draining a Sewage Treatment Plant (STP), and stormwater sites draining urban influx into Coombabah Lake. Results of their study found significant anthropogenic nitrogen input from both sources.

Conclusions

Stable isotopes offer an effective natural tracer to investigate the trophic linkages between organisms and wetland resources. Dietary studies (who is eating what?) have traditionally depended on gut content analysis, which involves collecting and dissecting a broad range of organisms to determine food chain links. However, gut content analysis has several inherent limitations: they provide only a short-term (hours to days) dietary snapshot of recently ingested items (Hyslop 1980); not all ingested materials are necessarily assimilated (Michener and Schell 1994); and ingested materials are digested quickly and are therefore rarely found in the stomach (Gee 1989). Stable isotope analyses provide a complementary, and in many cases, more powerful tool for tracing food sources and analysing food chain links than gut content analyses

By using a small amount of sample (microgram level), stable isotope ($\delta^{13}C$ and $\delta^{15}N$) analyses provide chemically validated data from which mathematical quantification can be derived. Isotopic dietary tracer records provide fundamental information about where an organism has been and what it has been eating (Hobson and Wassenaar 1997). Analysing isotopic values of bird feathers or tiny amounts of muscle tisues of other important species such as bell frog, the movement of birds and habitat affiliation of bull frog can be traced preciously.

Coastal wetlands offered many important ecosystem services including habitat and food sources for aquatic organisms (Lee 1999; Clynick and Chapman 2002; Mazumder *et al.* 2006, 2011) and deliver several direct and indirect services to the local population (Bird 1984). Urbanisation puts significant pressure on the structure and function of coastal wetlands, through modifying the hydrological regimes and the dynamics of nutrients and chemical pollutants. Application of isotopic techniques would help to quantify the extent of these stressors affecting ecological functionality of wetlands and work as an important monitoring tool in urban wetland management and restoration.

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