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# The magnitude of spatial and temporal variation in $\delta^{15}N$ and $\delta^{13}C$ differs between taxonomic groups: Implications for food web studies

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Understanding variability in stable isotope abundance is essential for effective hypothesis testing and evaluating food sources, trophic levels and food web structure. The magnitude and sources of variability are likely to differ among taxonomic and functional groups. We aimed to quantify variability of $\delta^{13}C$ and $\delta^{15}N$ for 16 species representing seven distinct taxonomic groups of benthic invertebrates and autotrophs in a marine ecosystem. We quantified the magnitude of variability among individuals or shoots separated by metres, among eight sites separated by kilometres, and between two survey occasions separated by months. $\delta^{13}C$ varied by as much as 7‰ for primary producers, 4‰ for consumers, while $\delta^{15}N$ varied by as much as 9‰ and 2‰ respectively. Variation in $\delta^{15}N$ of seagrass was largely accounted for by differences among sites, while variation in $\delta^{13}C$ was mainly attributable to shoots collected a few metres apart. Compared to seagrasses, variation in macroalgae was mainly explained by differences between the two survey occasions for $\delta^{15}N$ and among individuals collected a few metres apart for $\delta^{13}C$. Variation was generally lower for consumers and typically explained by differences among individuals for $\delta^{15}N$ but displayed inconsistent patterns for $\delta^{13}C$. Dual isotope Bayesian mixing models showed that the potential contributions of food sources for herbivorous consumers varied among sites and between survey occasions, and also that there was high variability or uncertainty in the contributions of sources within sites. The relative consistency in the main sources of variation among broad taxonomic groups in autotrophs suggests that aspects of physiology that are phylogenetically conserved might be important influences on variation in natural abundances of stable isotopes. In comparison, the sources of variability were less consistent within and among broad consumer groups, suggesting complex interactions between consumers and their food sources.



## 1. Introduction

Natural abundances of stable isotopes, especially $\delta^{15}N$ and $\delta^{13}C$, are frequently used to identify the sources of production that sustain consumers (e.g. Moncreiff and Sullivan, 2001; Adin and Riera, 2003; Melville and Connolly, 2003; Hyndes and Lavery, 2005). Inferences about sources of production are based on the assumption that stable isotope ratios display relatively predictable discrimination between diet and consumer (e.g. DeNiro and Epstein, 1978; Minagawa and Wada, 1984; Michener and Schell, 1994). This assumption has been tested with numerous experiments (see reviews by Vanderklift and Ponsard, 2003; Caut et al., 2009). However, less consideration has been given to quantifying the magnitude and causes of intra-species variability in stable isotope ratios, despite the profound influence that this variability can have on the inferences derived from stable isotope data.

Spatial and temporal patterns in light, temperature, and nutrient concentrations can all alter rates of productivity and therefore $\delta^{13}C$ of autotrophs (Hemminga and Mateo, 1996). Furthermore, spatial and temporal variation in $\delta^{15}N$ of ammonium and nitrate in seawater are likely to be reflected by patterns in $\delta^{15}N$ in autotrophs (Short and McRoy, 1984; Hemminga et al., 1999). It is perhaps not surprising that evidence of spatial and temporal variation in stable isotope ratios is particularly pronounced in estuaries (Hemminga and Mateo, 1996; Boyce et al., 2001; Antonio et al., 2012), which typically exhibit steep spatial gradients and extreme temporal fluctuations in environmental conditions, such as salinity, temperature and nutrient concentrations. For consumers, patterns of variation might be even more complex, because intra-species variation might reflect spatial and temporal variation in food

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sources, as well as variation due to different individuals feeding on resources with different stable isotope ratios (Bearhop et al., 2004). In addition, variation among individuals can result from different resource use by different life history stages, as well as differences in sex and individual physiology (Bearhop et al., 2004; Matthews and Mazumder, 2004; Barnes et al., 2008).

The ability to quantify sources of intra-species variation in stable isotope ratios is important for our ability to use patterns in stable isotopes to effectively test ecological questions: the way ecologists design surveys and experiments to characterise trophic pathways depends on the magnitude of these sources of variation. For example, variation among individuals can provide insights into individual dietary specialization and niche width (Bearhop et al., 2004). Similarly, understanding spatial patterns in stable isotope ratios increases the ability to quantify animal movements (Hobson, 1999) and determine the origin of organic matter (Melville and Connolly, 2005). Recent advances in stable-isotope mixing models, using a Bayesian approach, allow variation and uncertainty in stable isotope data to be incorporated into mixing model calculations (Moore and Semmens, 2008; Parnell et al., 2010), and therefore be considered when making inferences regarding sources of production for consumers and food webs. Variation among samples collected from different places or at different times is likely to increase the uncertainty in any conclusions derived from analyses of stable isotope data, so understanding the sources of variation will help ecologists to design surveys in ways that minimise the variation, and therefore reduce uncertainty.

Several studies have shown spatial and/or temporal variability in stable isotopes in marine systems, but most have focused on one or only a few species of autotrophs, particularly seagrasses (e.g. Hemminga and Mateo, 1996; Papadimitriou et al., 2005). Even those that have examined autotrophs and consumers (Jennings et al., 1997; Guest et al., 2010) were restricted to a small range of species. In this study, we aimed to quantify variation in $\delta^{15}N$ and $\delta^{13}C$ of 16 species representing seven broad taxonomic groups of autotrophs and benthic invertebrates in a marine ecosystem on the temperate west coast of Australia. We hypothesised that variation will be explained by the distances among points of collection (ranging from metres to kilometres), and/or between sampling occasions in a year (months representing austral autumn and spring). We then compare the sources of variation across species and broader taxonomic groups. We examine the influence of variability in $\delta^{15}N$ and $\delta^{13}C$ on conclusions regarding the importance of different food sources in food webs, by estimating the potential contribution of food sources to several species of herbivorous invertebrates, using Bayesian mixing models.

## 2. Materials and methods

### 2.1. Study area and sample collection

The study area encompassed a spatial extent of 8.5 km (east/west) by 26.5 km (north/south; Fig. 1) within the Jurien Bay Marine Park (30°17.3′ S, 115°02.5′ E), on the west coast of Australia. The area is part of the Central West Coast marine bioregion (Commonwealth of Australia unpubl. data), which is a broad (600 km in length) biogeographical transition zone between tropical and temperate ecosystems, with an associated Mediterranean-type climate (Morgan and Wells, 1991). The region is characterised by a series of limestone islands and reefs <20 m in depth that run parallel to shore, and provide shelter to shallow (<10 m) lagoons alternating with large sandbars. Extensive seagrass meadows are interspersed with subtidal limestone reefs, limestone pavement and unvegetated sand (Sanderson, 2000).

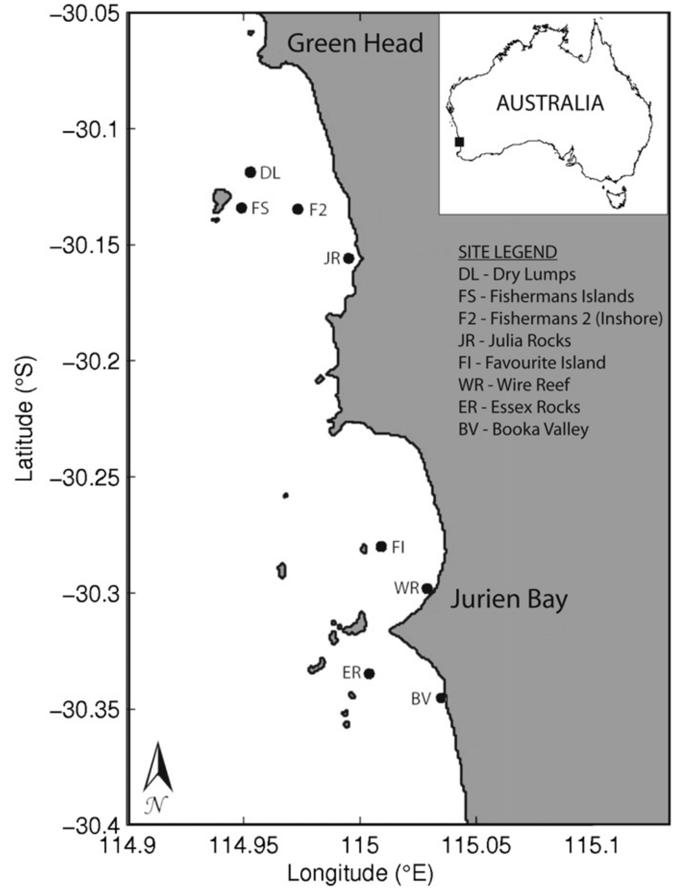


**Fig. 1.** Location of the eight sites surveyed in this study.

Surveys were undertaken twice at eight sites: once during autumn (April/May) and once during spring (October) 2005. For convenience, we refer to these survey occasions as ‘autumn’ and ‘spring’ but the design was established to estimate the magnitude of temporal variation relative to spatial variation, so we do not intend to infer a seasonal pattern. Sites were chosen to represent the mosaic of limestone reef, seagrass and bare sand across the length (long-shore) and breadth (cross-shore) of the region, ensuring that sites were separated by at least 1 km. At each site, three individuals of each of the dominant species of benthic autotrophs and invertebrates (Table 1) were collected by hand by divers. Individual samples were collected randomly and were separated by distances of metres to tens of metres. Samples were frozen immediately after collection and stored at −20 °C until laboratory analysis. Since stable isotope ratios can vary within individuals and among different life history stages or sizes, samples from seagrass and macroalgae were restricted to similar sections (e.g. young leaves of seagrass, upper thalli of kelp or whole epiphytic algae), while samples from consumers were restricted to muscle tissue from the same location and from similar-sized individuals within each species. Seagrass and macroalgae were rinsed with deionised water, and cleaned of epiphytes if necessary. Muscle tissue was taken from the tail of rock lobsters, the foot of gastropods, the Aristotle’s lantern of sea urchins, and the body wall of sea cucumbers and ascidians.

### 2.2. Stable isotope analysis

Each sample was dried at 60 °C, homogenized to a fine powder using a ball mill and weighed into a tin capsule. Subsamples of